Toxic and genotoxic studies of wood dusts; an *in vitro* assessment

A Dissertation

Submitted on the 22nd day of September, 2010

In Partial Fulfillment of the Requirements

of the School of Public Health and Tropical Medicine

of Tulane University for the Degree of

Doctor of Philosophy

[Dept. of Environmental & Health Sciences]

By

Mark J. Wilson M.S.P.H.

Approved by

Charles A. Miller III, Ph.D

(Chair)

Roy J. Rando, Sc.D

Astrid Engel, Ph.D

Thomas Wiese, Ph.D

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

Table of Contents

List of Figures

List of Tables

List of Equations

List of Abbreviations

6-TG	6-Thioguanine
ACGIH	American Conference of Governmental Industrial Hygensists
AhR	Arylhydrocarbon receptor
ARNT	Arylhydrocarbon receptor nuclear translocator
β-NF	Beta-naphthoflavone
CYP1A1	Cytochrome p450 1A1
DMSO	Dimethyl sulfoxide
DRE	Dioxin response element
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
gpt	Guanine xanthine phosphoribosyl transferase
HAT	Hypoxanthine aminopterin thymidine
HGPRT	Human guanine xanthine phosphoribosyl transferase
IARC	International Agency for Research on Cancer
INDELS	Insertion and deletion mutations
LD_{25}	25% lethal dose
LD_{50}	50% lethal dose
LOEL	Lowest observed effect level
MDED	Minimum determined experimental dose
MDF	Medium density fiberboard
MeAQ	2-methylanthraquinone
MMR	Mismatch repair
NIOSH	National Institute of Occupational Safety and Health
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PBS	Phosphate buffered saline
PEL	Permissible exposure limit
REL	Recommended exposure limit
SDS	sodium dodecyl sulfate
SD-trp	Synthetic dextrose medium minus tryptophan
Sgal-trp	Synthetic galactose medium minus tryptophan
TE	Tris EDTA
TLV	Threshold limit value
TNF-α	Tumor necrosis factor alpha
Tris	2-Amino-2-hydroxymethyl-propane-1,3-diol

Acknowledgements

I would like to thank my committee members for the time and effort they invested in this study. I thank my advisor Dr. Charles Miller for his expertise and continued guidance throughout my time in his laboratory. I thank Dr. Roy Rando for the opportunity to get hands on exposure assessment experience. I thank Dr. Gabrielle Sabbioni and Dr. Anoop Kumar for graciously supplying the equipment and expertise for the chemical analysis of my samples. I am also grateful for the cooperation and interest of Dr. Thomas Wiese and Dr. Astrid Engel. I thank my wife, Rachel, and my family for their support and advice.

ABSTRACT

Wood dusts are known human carcinogens. Increased risk for nasal adenocarcinoma has been established in numerous epidemiologic studies but the chemical agents and molecular mechanisms responsible for this disease remain unclear. We employed a panel of *in vitro* assays to test for toxic and genotoxic endpoints in extracts of hardwood, softwood, and wood product dusts. Wood dusts were extracted with methanol or culture media to liberate active components from the particles. We identified specific wood types associated with activities that included aryl hydrocarbon receptor (AhR) activation and genotoxic effects. Teak wood extract was the most active in all these assays. AhR activation from treatment with methanol and medium extracts was observed by immunobloting for the inducible p450 CYP1a1, an AhR regulated gene, in HepG2 cells. One chemical, 2-methylanthraquinone, was identified as a major active constituent of teak dust, but was not present to a large degree in other wood types. 2-methylanthraquinone is a potent AhR agonist and was found to be mutagenic. Three independent samples of teak wood extracts were analyzed with LC-MS, and levels of 2-methylanthraquinone were similar, averaging 0.20% $^{+/-}$ 0.09% by weight. Teak dust extract was more efficacious AhR ligand than 2-methylanthraquinone when compared on a per weight basis, indicating that additional toxic substances are in teak. A significant three-fold increase in large deletion mutations was caused by treatment with teak wood extracts and 2-methylanthraquinone compared to the untreated control. Further analysis of the amplicons recovered from treated cells revealed small deletions,

insertions, and point mutations. Multinucleated g12 cells were observed following treatment with teak extracts in colony formation assays. The toxic activities of various woods types identified in this study suggest wood workers are at variable risk, depending on their exposure levels and the wood types involved. Teak wood is expected to be especially hazardous to wood workers.

BACKGROUND AND SIGNIFICANCE

Chronic exposure to wood dust(s) increases risk for nasal adenocarcinoma. The first published reports date to the 1960's, when excess nasal cancer risk was identified in a group of workers involved in hardwood furniture manufacturing(Acheson et al., 1968). This association between wood dust exposure and nasal cancer risk has been observed in numerous studies in several countries (reviewed in IARC, 1995). Lifetime cancer risk odds ratios as high as 45.5-fold for individuals with high chronic exposure have been reported(Demers et al., 1995). It was this relationship between exposure and disease history led to the classification of wood dust as a carcinogen by IARC and NTP, in 1995 and 2002, respectively, but the mechanism(s) and actual chemical carcinogen(s) that cause the adenocarcinomas are still unknown. Furthermore, it is unknown whether one particular type of wood dust may be more genotoxic than another. It is impossible to conduct human exposure studies with foreign agents such as wood dusts because of ethical considerations, but occupational and epidemiologic studies provided the evidence to identify nasal cancer in wood workers as a disease of environmental origin. There are presently no good animal models for studying wood dust carcinogenicity, but even if they did exist the costs associated with testing multiple doses of different wood dust types would take several years and would cost millions of dollars. Thus, a reasonable alternative is to characterize wood dusts using a group of in vitro assays to serve as a foundation and guide to direct future investigations.

Wood dusts are chemically complex organic mixtures that exist in particulate form. It is important to consider that a piece of lumber is the dried remains of an organism that employed various chemical defenses against fungal and bacterial infections during its life. The various chemical constituents of the wood may retain biologic activity and could play a role in the initiation of carcinogenesis. Woods contain numerous bioactive compounds such as quinones, terpenes, tannins, and flavones, and chronic exposure may allow some of these bioactive compounds to exert toxic or genotoxic effects on the nasal epithelium.

Chronic inflammation has been linked with the process of carcinogenesis (reviewed in Kundu and Surh, 2008). Induction of inflammatory responses by wood dusts through expression of specific chemokines, cytokines, and proinflammatory enzymes like cyclooxygenase 2 (COX-2) have been reported (Holmila and Husgafvel-Pursiainen, 2006; Maatta et al., 2005). Teak and spruce wood dust induced increased levels of TNF-α protein in a mammalian cell culture study(Maatta et al., 2006). Induction of inflammatory markers interleukin-6 and 8 (IL-6 and IL-8), was shown to increase at the mRNA level after treatment with wood dusts, and teak was the most potent followed by medium density fiberboard (MDF), spruce, and beech(Bornholdt et al., 2007). A mammalian in vitro assay showed that the metabolic enzymes cytochrome p4501A1 and aldehyde dehydrogenase levels increased in response to acetone extracts of pine and spruce wood dusts(Torronen et al., 1989). Both of these enzymes can be induced via the AhR signaling pathway, and it is possibile that activation of the AhR signaling pathway mediates the inflammatory response and generates genotoxic metabolites(Marlowe and Puga,

2005; Vogel et al., 2007). The role of activated AhR signaling in response to wood dust treatment has not been studied.

A number of carcinogenic chemicals activate AhR signaling, which subsequently causes inflammation as an intermediate step toward the generation of cancer. Might wood dusts activate AhR signaling as a mechanism of carcinogenesis? To test this idea, I assessed the activity of wood dust extracts in two separate bioassays to determine the presence of AhR ligand(s) in wood dust extracts. The first bioassay was a transgenic yeast bioassay with a human AhR/Arnt expression cassette and a lacZ reporter gene (Miller, 1999). The second was expression of CYP1A1 protein, an AhR regulated metabolic gene, in human hepatoma cells. The use of these two systems allowed for the identification of ligands in both methanol and aqueous medium extracts. The latter hepatoma cell line is metabolically competent and may activate or inactivate AhR ligands.

Cancer is a disease that is associated with genetic changes. Relatively little is known about the genetic changes that occur in wood dust induced cancers. Altered regulation of tumor suppressor and cell cycle checkpoint proteins were reported in nasal tumor samples from workers with a history of wood dust exposure(Saber et al., 1998). Two groups reported G:C to A:T transitions were present in the K-ras and p53 genes from tumor samples in comparison to healthy control tissue (Perrone et al., 2003; Saber et al., 1998). Ethmoidal mucosal cells taken from healthy wood workers overexpressed the p53 protein (Valente et al., 2004). Overexpression of p53 can indicate the presence of mutations in the p53 gene, but also may indicate that cellular repair mechanisms have been activated. These types of tumor sample

studies are important, but must be considered carefully as it is difficult to determine whether such tumorigenic changes are directly related to wood dust exposure, or are late events that occur during tumor progression and are independent of exposure. Some wood dusts have been linked with genotoxic effects. In particular, the teak wood dust and the wood product medium density fiberboard (MDF) caused DNA strand breaks in the human lung epithelial cell line A549 as detected by the comet assay (Bornholdt et al., 2007). Although wood dust was shown to be genotoxic in limited studies, no mutational spectrum has been described for wood dust(s) to date. In studies described below I report the first mutagenic spectrum of teak dust using the g12 hamster fibroblast cell line as a mutagenesis model (Klein et al., 1994a; Klein et al., 2002).

Variations in ploidy are common in cancers and are detectable by current imaging technologies. Some natural chemicals are able to induce alterations in ploidy of cells. Taxol, taken from the pacific yew tree, and cytochalasins from fungi are examples of natural compounds that alter ploidy. It is possible that such a bioactive chemical is present in wood dusts, and could contribute to alterations of cellular ploidy. g12 cells are an immortalized line that maintains consistent ploidy and is useful for mutagenesis studies (Klein and Rossman, 1990). If aneugens are present in wood dust, then exposure of g12 cells should alter ploidy. Indeed, the first evidence that wood dusts have effects on cell ploidy, as detected using colony assays, is provided in this report.

Taken together, the current body of literature indicates that wood dust is a carcinogen, and that exposure can result in cytotoxicity, genotoxicity, and

inflammation. The mechanisms, chemical constituents, and wood types involved in reaching these toxic endpoints remain unclear. The present study used a series of *in vitro* experiments and chemical analyses to determine if cells treated with extracts of wood dusts a) modulate AhR signaling, b) cause mutations, and c) alter ploidy. Additionally, the toxic features of specific wood types and wood products were defined in this study. I have demonstrated that different wood types exert different toxic effects and pose distinct risks to wood workers.

LITERATURE REVIEW

Wood dust particles are generated when wood is sheared or shattered by mechanical means. Dusty wood working operations, like finish sanding or joinery, generate a range of aerosols with particle sizes between 0.1 to 10 μm that varies greatly with work type (IARC, 1995). Chronic wood dust exposure has been associated with adverse health outcomes such as respiratory irritation, asthma, and chronic obstructive pulmonary disease. These effects are the basis of the occupational permissible exposure limit (PEL) promulgated by the Occupational Health and Safety Administration (OSHA). The current exposure limit treats all wood dusts, with the exception of western red cedar (a contact sensitizer, allergen), as nuisance dusts with an allowable airborne dust concentration of 15 mg/m^3 for total inhalable dust and 1 mg/m^3 for the respirable fraction.(29 CFR 1910.1000, Table Z-3) Both the National Institute of Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) give regulatory guidance related to wood dust exposure levels. NIOSH gives a recommended exposure level (REL) for wood dust of 1 mg/m^3 for all wood dusts. This REL is based on increased risk of pulmonary dysfunction due to wood dust particulate exposure. ACGIH publishes threshold limit values (TLV) and recommends 1 mg/m^3 for certain hardwood dusts and 5 mg/m^3 for softwoods. The TLV for hardwood dust is based on increased risks of nasal cancer, and the TLV for softwood is based on increased risk of dermatitis and upper respiratory disease. Of the three agencies, OSHA is the only one with regulatory authority. OSHA can levy

penalties against specific sites or companies that do not comply with a permissible exposure limit (PEL). The PEL is a regulatory value based upon consideration of the excess risk of pulmonary dysfunction, not increased cancer risk, as the primary endpoint. Wood dust particles or the chemical constituents of the dusts may actually pose an increased risk for nasal cancers below the levels deemed acceptable considered protective against pulmonary dysfunction by OSHA at this time.

Woodworking operations can generate an aerosol cloud with a range of particle sizes. Particle size is a disease-determining factor since the larger particles typically comprising wood dusts will be intercepted within the upper respiratory tract. A report of a recent field study indicated inhalable particles account for the majority of the mass of the total ambient dust levels in industrial wood working operations (Rando et al., 2005). The laws of classical physics govern the behavior of aerosolized particles (DiNardi et al., 2003). A particle moving under its own momentum moves in a straight path unless it is acted upon by another force. Large (5 to 30 μm) inhalable particles are generally impacted in the upper airways due to the high air velocity and frequent direction changes present in the upper airways. Humidity increases with penetration distance in the respiratory tract, so as a hygroscopic particle moves deeper into the airways it will pick up water and further increase its likelihood of interception (Casarett et al., 2001). Particles that are impacted in the nasal cavity can be cleared via two mechanisms that are location dependent. Extrinsic forces, such as blowing or wiping, can remove particles in the anterior portion of the nasal cavity. This portion of the respiratory tract is composed of dry non-ciliated epithelium. Particles that are deposited further within

the sinus cavity can be cleared via action of the mucocilliary escalator, which moves the particles to the throat where they can be swallowed and cleared (Casarett et al., 2001). Previous studies have shown that people with a history of chronic exposure to wood dusts have decreased rates of mucocilliary clearance (Tian et al., 2007). A longer residence time in the nasal cavity would permit more delivery of bioactive constituents of wood to the target cells.

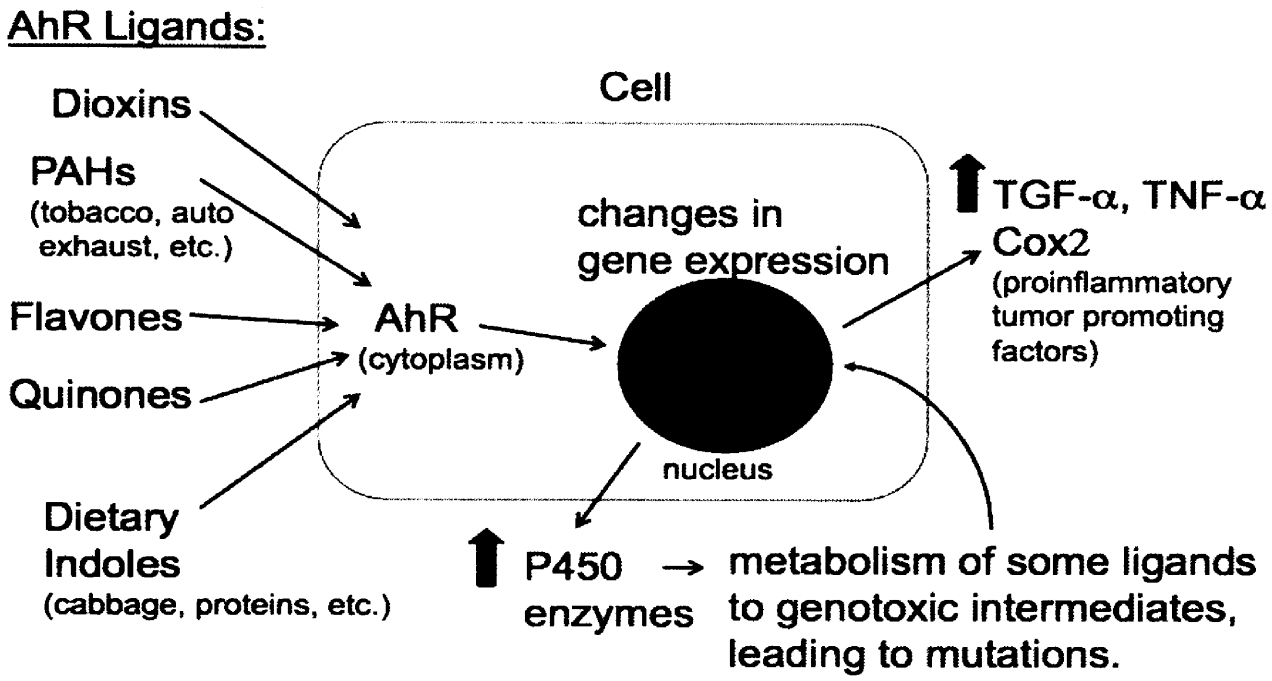
The bulk of the dry weight of wood consists of cellulose, polyoses, and lignins. Cellulose consists of glucose units linked in 1-4 β–acetyl linkages (Sjöström, 1993). Polyoses, also known as hemicelluloses, consist of oligosaccharides that have subunits other than glucose linked via 1-4 β–acetyl linkages. Cellulose and hemicelluloses play a critical role in the structure of the plant cell wall. Lignin is another biopolymer that is found in significant quantity in wood. Lignin's structure is unknown because delignification reactions destroy the structure of the polymer. It is known that lignin content can vary greatly among tree species and that the primary unit of the lignin polymer is phenyl-propane molecules. Lignin acts as cellular glue; via forming numerous hydrogen bonds it holds plant cells together. The remaining small percentage of compounds, called extractives, consist of low to mid molecular weight organic compounds that include tannins, terpenes, flavonoids, quinones, fatty acids, and alcohols. Although the extractive content of wood is relatively low when viewed as percent of dry weight, these molecules may be able to interact with the cellular environment within the nasal passages and may contribute to carcinogenesis.

The non-structural molecules in wood, termed extractives, are thought to play a role in fighting fungal and bacterial infection, and are involved in cellular respiration and metabolism (IARC, 1995). Many of these chemicals have some degree of water solubility, as water is the vascular currency of plants. Quinones are family members of a group of chemicals called redox cyclers. In mitochondria and chloroplasts they serve to pass electrons and protons between large molecular complexes that are generally tethered to or are part of a cellular membrane. Quinones are able to play this role because they are lipid soluble molecules that are free to move about the cellular environment. Generation of reactive oxygen species has been identified as a possible mechanism of inflammation due to wood dust exposure (IARC, 1995; Maatta et al., 2006; Maatta et al., 2005). The presence of quinones in wood dust could play a role in this effect. Tannins are another class of bioactive molecules that are present in wood dusts. There are two classes of tannins, hydrolysable and condensed. Hydrolysable tannins usually contain a sugar molecule with ester linked gallic or egallic acid moieties. When the ester bond is hydrolyzed, it frees the phenolic acid group of the tannin from the sugar base unit and allows interaction with the surrounding environment. Tannic acid, used in the tanning of leather, is an example of a plant derived hydrolysable tannin. Tannic acid is bioactive and is reported to induce apoptosis and cause arrest in G1 phase of the cell cycle (Nam et al., 2001). Condensed tannins are polymers of flavonoid subunits that have covalent carbon-carbon bonds. This class of tannin is not hydrolysis labile, but many condensed tannins are water soluble and their presence in nasal gavage samples from wood workers has been proposed as a marker of exposure

(Mammela et al., 2002). Some flavonoids are known to inhibit topoisomerase II, resulting in resulting in chromosome alignment errors during mitosis (Cantero et al., 2006). AhR agonist and antagonist activity has been reported for flavonoid compounds, and their ability to act as agonists or antagonists varies with cell context (Zhang et al., 2003). The role that wood dust derived flavones plays in activating AhR signaling in human nasal epithelial cells is not known, and some condensed tannins may activate AhR signaling or be metabolically activated via mechanisms that involve AhR mediated signaling. It is known that some polyphenolic phytochemicals are able to modulate AhR signaling and that the structure and quantity of these chemicals can vary between tree species and even within a single organism (IARC, 1995). Some of these compounds may play a role in early events associated with the development of cancer.

It is clear that there are bioactive compounds present in wood dust and their presence and activity might play a role in carcinogenesis. Some of these compounds might be able to modulate AhR signaling. The AhR signaling system plays multiple roles in chemical carcinogenesis. Two mechanisms relevant to this discussion are the generation of toxic or reactive intermediates through metabolic activation and alteration of gene regulation. The signaling pathway is depicted in Figure 1.

Figure 1. The AhR Signaling Pathway



The AhR exists in the cytoplasm complexed with a heat shock protein 90 (hsp90) dimer. When the receptor binds to a ligand it causes a conformational change that exposes a nuclear localization signal (NLS) on the AhR. The ligand bound receptor moves through a nuclear pore into the nucleus where it associates with the aryl hydrocarbon nuclear translocator (ARNT). Once the AhR/ARNT heterodimer is formed it is capable of interacting with dioxin response elements (DREs) that are found in the regulatory regions of dioxin responsive genes. Cytochrome p4501A1 (CYP1A1) is a dioxin responsive gene. The CYP1A1 enzyme can generate reactive metabolic intermediates as well as detoxify xenobiotics. Other genes, such TNF-α and other inflammatory cytokines, can also be induced through the activated AhR signaling pathway.

Signaling through the pathway induces expression of metabolic enzymes and can result in genotoxic reactive intermediates; such as benzo[a]pyrene, whose toxicity depends on a metabolic activation step that results in the generation of a toxic intermediate. Proinflammatory mediators such as COX-2 and TNF-α are also increased when AhR signaling is activated. Chemokines, cytokines, and prostaglandins play a role in chronic inflammation that can lead to cancer through generation of reactive oxygen and nitrogen species, genomic instability, resistance to apotosis, and alteration of epigenetic status (Kundu and Surh, 2008).

Generation of wood dusts

Wood samples were obtained from local New Orleans lumberyards, a plywood manufacturing plant, and P.J. Murphy forest products. The wood and wood products used are shown in Table 1.

Table 1. Woods and wood products examined

Hardwood	Softwood	Wood product
Teak	Untreated yellow pine	Treated (quaternary amine and copper, Osmose) yellow pine
Mahogany	Cypress	Medium Density Fiberboard
Walnut	Spruce	Plywood
Poplar	Cedar	P.J.Murphy sani chips (animal bedding)
Red Oak		

The hardwoods, softwoods, and medium density were chosen because they represent wood types that are frequently used in furniture and cabinetry manufacturing. The plywood dust was collected from an industrial setting. Treated pine is frequently used in outdoor carpentry applications, and the animal bedding material (wood chips) was chosen because the manufacturer has carried out a detailed chemical analysis to ensure that no exogenous chemicals are present in the material.

Wood dusts from the hardwoods, softwoods, treated pine and MDF were generated using a radial bench sander with medium grit paper in a modified glove box. The plywood dust was collected at an industrial plywood manufacturing facility, and as such it represents an actual exposure material. Extracts of wood chip bedding for animal cages (P.J. Murphy, Inc. Montville, NJ) were also prepared and, due to it already being small particles, it was extracted directly rather than sanded. 1 gram of each type of the wood particles were extracted overnight in a 15 ml conical tube in 6 ml of HPLC grade methanol (Fisher, Fair Lawn, NJ) in a rolling incubator at 25°C. The laboratory bedding and plywood dusts were extracted directly. The following morning the extractions were gravity filtered with a standard Whatman paper filter (Fisher, Fair Lawn, NJ) followed by filtration with a Fisher 0.2μm nylon filter using a syringe to push the solution through (Fisher, Ireland). The filtrate was dried at ambient room temperature in pre-weighed tubes. The mass of the dried extract was determined gravimetrically using an analytic balance after the solvent evaporated. The dried extract was suspended in filter sterilized 99.5% dimethyl sulfoxide (DMSO) (Sigma, St.Louis, MO) as stock solutions of 60 mg/ml for treatment in the methanol extract *in vitro* assays. Table 2 shows % yield per gram of wood dust of dried methanol extracts. Medium extractions were done with one gram of wood dust in 30 ml of medium. The same incubation and filtration procedures outlined above were followed. Fresh medium extracts were prepared the day before the assays.

Table 2. Yield of dried methanol extracts of wood dusts

Hardwood extract % yield		**Softwood extract % yield**		**Wood product extract % yield**	
Teak	2.10 %	Yellow Pine	1.47 %	Treated pine	2.17 %
Mahogany	2.06 %	Cypress	4.8 %	MDF	7.8 %
Walnut	2.01 %	Spruce	2.1 %	Plywood	5.2 %
Poplar	1.70 %	Cedar	2.4%	Sani chips	2.2 %
Red Oak	2.65 %				

Dried extracted wood dust is stored at -20°C until needed for use. Fresh stocks were prepared for each assay.

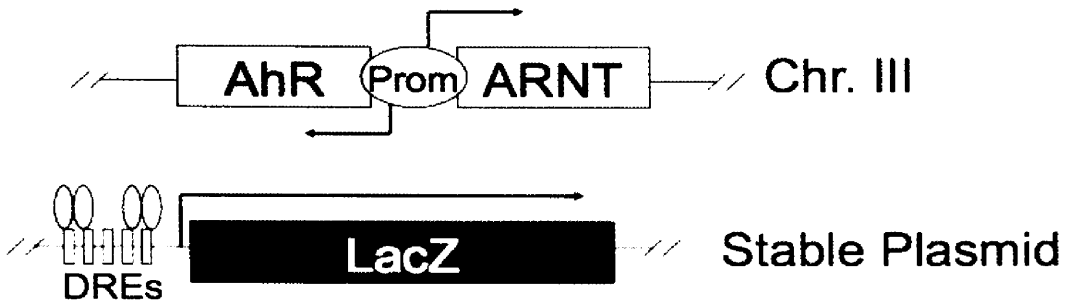
Aryl hydrocarbon receptor activation experiments

Methods

Screening methanol extracts of wood dust and wood products with a yeast bioassay

The transgenic yeast strain called YCM3 was developed to report chemicals that activate aryl hydrocarbon receptor signaling. YCM3 has been described previously (Miller 1999). The system expresses the human aryl hydrocarbon receptor and aryl hydrocarbon nuclear translocator proteins and contains a dioxin responsive reporter plasmid as depicted in Figure 2. This yeast strain was used to assess ligand activity of wood dust extracts.

Figure 2. Detection of AhR ligands using the YCM3 yeast strain.



The S. cerevisiae strain W303a (genotype: MATa, ade2-1, can1-100, his3-11,15, leu2-3, 112, trp 1-1, ura3-1) was used for these experiments. The expression of the AhR and ARNT transgenes is under the control of a bi-directional galactose induced promoter stably integrated into chromosome III. It also contains the reporter plasmid pTXRE5-Z, a LacZ reporter plasmid containing the TRP1 gene as a selectable marker. This plasmid has a ß-galactosidase (LacZ) gene regulated by a minimal cytochrome c promoter and 5 upstream dioxin response elements (DREs). This plasmid reports AhR activation when a ligand is present.

Initially the yeast were grown to saturation overnight in a small volume of-synthetic glucose medium plus adenine, histidine, leucine, and uracil minus the amino acid tryptophan (SD-trp). Table 3 lists the components of the medium used in this assay. The following morning the cell density was determined using a bench top spectrophotometer (Spectronic, Rochester, NY) at 600 nm wavelength and the cells were diluted in SD-trp to a density of 2×10^6 cells/ml. The yeast were grown for an additional 4 to 5 hours until a cell density of 1×10^7 cells/ml was reached. The culture was diluted to 4×10^5 cells/ml in 30 ml of minimal medium minus tryptophan with galactose as the inducing sugar. Cultures were added to 96 well plates at a volume of 200 μl per well to all but the top row. For the assays involving the methanol extracts, the wells on the top row were filled with 300 μl of the yeast culture and a small volume, < 3 μl, of extract dissolved in DMSO, was added to the top row of the plate. The dose range was then generated by serial dilution. For the assays involving medium extracts, yeast were suspended in 100% medium extracts at 4×10^5 cells/ml and the top wells were filled with 300 μl of medium and a dose range was generated by serial dilution. The 96-well plates were incubated 18 hours (overnight) at 30^0C and removed from the incubator the following morning. The cell number was determined by first pipetting the cells up and down to suspend them followed by reading the Abs 600 nm in a BioRad Benchmark Plus microplate spectrophotometer (BioRad, Hercules, CA).

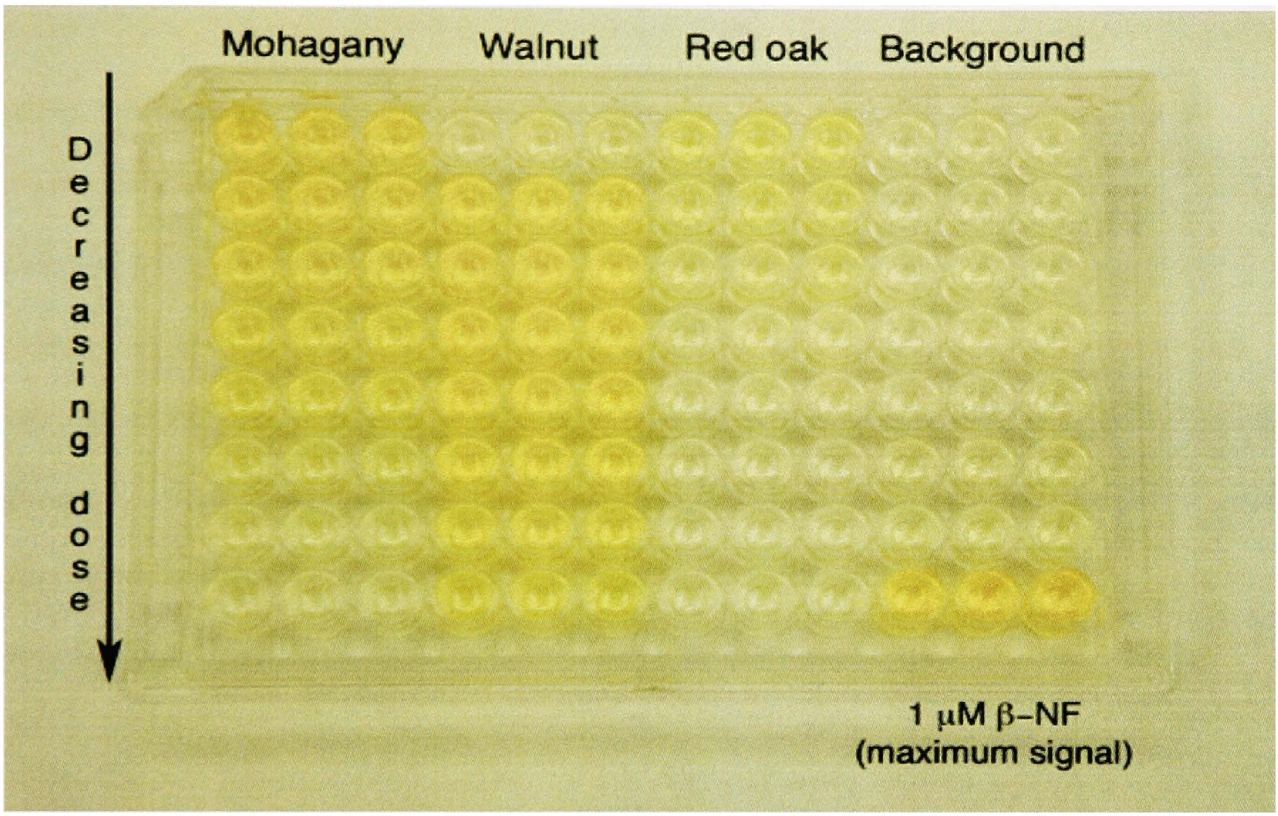
Table 3. Synthetic minimal dextrose and galactose yeast growth medium components for the yeast bioassay.

Synthetic dextrose medium minus tryptophan (SD-trp)	**Synthetic galactose medium minus tryptophan (Sgal-trp)**
2% glucose 20 g/L	2% galactose 20 g/L
0.67% yeast nitrogen base	0.67% yeast nitrogen base
adenine 20 mg/ml	adenine 20 mg/ml
histidine 20 mg/ml	histidine 20 mg/ml
leucine 100 mg/ml	leucine 100 mg/ml
uracil 20 mg/ml	uracil 20 mg/ml
lysine 30 mg/ml	lysine 30 mg/ml

Dextrose and galactose were obtained from Fisher Scientific, Fair Lawn, NJ, yeast nitrogen base from DIFCO, Detroit, MI, and amino acids and bases were from Sigma-Aldrich Chemical Co., St. Louis, MO.

The activity of the reporter gene was determined as follows. First, 25 μl of the yeast culture was replica plated into a new 96 well plate using a multi channel pipette. 225 μl of "Z buffer" was added to each well to lyse the cells. Z buffer consists of 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 1mM $MgCl_2$, 10 mM KCl, 2 mM dithothreitol, 0.2% sarkosyl, and 0.4 mg/ml o-nitrophenol- β-D-galactopyranoside (ONPG) and is the substrate for the β-galactosidase reporter gene. The replica plate was incubated at 37°C for 5 to 10 minutes by floating in a water bath. The time of the reaction was noted and was stopped when a yellow color appeared. β-galactosidase activity frees nitrophenol from the galactopyranose and results in the formation of a yellow color. The yellow color is formed proportionally to the amount of β-galactosidase present in the well. The reaction was stopped by adding 100 μl of 1M sodium carbonate. The addition of the sodium carbonate will intensify the yellow color. Figure 3 shows a representative assay plate for reference.

Figure 3. Yeast AhR reporter assay dose response following treatment with wood dusts extracts.



The top row is the highest dose in each group. Cytotoxicity was responsible for the lack of signal in the highest dose for the walnut extract treatment and it was visually evident the yeast did not grow in this treatment group. The background represents the top nine wells in the last three columns. Each wood extract treatment had a corresponding background group with a vehicle control volume equal to the volume of extract added to the highest dose. The maximum signal is determined as the average response of the 1μM β–NF treatment group.

The response of the reporter system was quantified by normalizing the data to the signal from the 1 μM β–NF treatment group as 100% and comparing the level of induced Lac-Z activity for each of the wood types. LacZ units were calculated using equations one and two. Experiments were carried out on the entire group of methanol extracts using the YCM3 yeast bioassay. The data was generated and Lac-Z activity was calculated as described. The normalized data was divided into categories of hardwood, softwood, and wood product then analyzed with Prism statistical software. First a non-linear regression (sigmoidal) dose response model, shown in the following equation, was fit to each individual data set. Each data set was then assigned to a category of hardwood, softwood, or wood product and the average maximum response value for each of the dose response curves were grouped and calculated. A one-way ANOVA was performed to identify which group(s) showed the significant activity when compared to the background signal for the YCM3 yeast (Figure 17). The AhR activity associated with each wood type was summarized in Table 32.

Equation 1. Lac-Z unit calculation

Lac-Z units= (absorbance at 405nm of sample – background) × 1000÷(time of assay (min) × volume (ml) × absorbance at 600 nm)

Equation 2. Normalization to 1 μM β-NF as 100% of maximum signal

Normalized value = (Lac-Z unit of sample/Lac-Z unit of 1 μM β-NF)×100

Equation 3. Non-linear regression sigmoid dose response model.

Y= Bottom + (Top-Bottom)/1+10^((LogEC$_{50}$-X))

Y= response expressed as % of 1 μM β–NF signal

Bottom = lowest average response for each data set

Top= highest average response for each data set

LogEC$_{50}$= Log10 of the calculated EC$_{50}$ or effective concentration where 50% of the top value is reached.

X = Log10 of the X axis value

AhR signaling in yeast from methanol or medium extracts of wood or wood product dust

Experiments were conducted to compare the response induced by treatment of the yeast with medium extracts and methanol extract of the wood types. The 96-well plates were set up as described and the LacZ activity was determined in the same fashion as for the methanol extract experiments. The difference in the assays was the top row of the plate was filled with 300 μl of yeast suspended at 4 x 10^5 cells per ml in 100% wood dust extract medium and the rest of the dose series was accomplished by three-fold serial dilutions down the plate. The resulting data set was analyzed by fitting dose response curves to the individual wood types then assigning the wood types into hardwood, softwood and wood product categories. The categorical data was analyzed with a one-way ANOVA (statistical tables in supplementary data).

The individual data sets from both the methanol extracts and medium extracts were compared after plotting each on a single graph allowing for comparisons of each pair of dose response curves.

AhR signaling in yeast from methanol or medium extracts of teak wood and 2-methylanthraquinone

Experiments were carried out with three separate teak wood samples and 2-methylanthraquinone to determine if AhR activity correlated with 2-methylanthraquinone content in the separate teak samples. 96 well plates were set up and analyzed as previously described for the wood dust extract treatments. A 2 mg/ml stock of 2-methylanthraquinone dissolved in DMSO was prepared and used to treat the yeast in the same fashion as for the wood dusts. Medium extraction of the teak samples and 2-methylanthraquinone was carried with one gram of dust or chemical per 30 ml of yeast medium as previously described.

Immunological detection of cytochrome p450 CYP1A1 induction following exposure to teak wood dust extract or 2-methylanthraquinone in HepG2 cells.

HepG2 cells were maintained at 37°C in humidified air with 5% CO_2 in 25 cm^2 cell culture flasks in DMEM medium with 1 mM L-glutamine (HyClone Laboratories, Logan UT) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan UT) 100 units penicillin and 100 μg streptomycin per mL (Atlanta Biologicals, Lawenceville GA).

A near confluent (~80%) T-25 flask of cells was treated with trypsin /EDTA solution (Cellgro, Herndon VA) and the culture was collected and counted. The culture was diluted appropriately to allow for seeding 2.5 x 10^5 cells per 60 cm^2 tissue culture dish (Corning, Corning NY). The cells were allowed to attach overnight. The following day the medium was removed and replaced with 5 ml of DMEM medium with 10 or 30 μg/ml of methanol wood dust extracts or 0.3% to 100% of teak wood dust medium extract, 2-methylanthraquinone and β-NF for 24 hrs. After treatment the medium was aspirated away and the cells were washed in ice cold phosphate buffered saline solution (PBS) and 0.25 ml of 2X SDS-PAGE buffer, consisting of 4% SDS, 20% glycerol, 0.1 M Tris, 0.03% bromophenol blue, and 200 mM DTT, pH ~7,was placed on the plate.

The lysis resulted in a viscous liquid that was collected in microcentrifuge tubes and placed on ice. The samples were placed in boiling water and then pulsed with an ultrasonic blast to shear the genomic DNA, thus reducing the viscosity. The samples were loaded into two 4-12% Bis/Tris Criterion XT precast gels (Bio-Rad,

Hercules, CA) along with protein standards. One gel was used for the western blot to detect CYP1A1 (a specific cytochrome P450) levels and the other was placed in Coomassie stain (10% methanol, 10% glacial acetic acid, 0.1% brilliant blue) to determine the relative amount of protein in the samples. The gels were placed in the Criterion gel apparatus and the chamber was filled with chilled running buffer. Running buffer consisted of 50 mM MOPS, 0.1% SDS, and 1 mM EDTA, pH 7. Protein separation was accomplished with 200 volts for 50 min. After electrophoresis, the gels were removed from their cases and one was placed in Coomassie stain, shaken for 2 hours, and then destained to reveal total proteins. The stained gel was put in 20 mL destain buffer, which consists of 25% ethanol, 10% acetic acid, and 65% ultrapure water, and shaken. The destain buffer was changed every thirty minutes until the proteins in the sample and marker lanes of the gel were easily visualized.

The proteins in the second gel were transferred to a nitrocellouse membrane with the Criterion cell transfer apparatus. The gel sandwich was assembled as described in the manufacturer's instructions.

The gel sandwich was placed in the Criterion cell and the chamber was filled with ~1.5 L of chilled transfer buffer. Transfer buffer consists of (6.06g tris base, 28.8 g glycine, 400 mL methanol, and water to a final volume of 2 L. An ice block was placed in the transfer chamber to keep the buffer cold. The transfer was accomplished with 100V for 30 minutes. Following the transfer the gel sandwich was opened, the nitrocellulose membrane was removed, and probed with antibodies to detect levels of CYP1A1 expression following treatment with teak wood dust extracts.

Antibody detection occurred as follows. The membrane was washed in TBST buffer which consists of 25 mM tris base 0.9% NaCl, 0.1% Tween 20, and adjusted to pH 7 with HCl. A blocking solution was made by adding 2.5g of dehydrated milk to 50 ml of TBST. The membrane was covered with 15 ml of the blocking solution and shaken for at least two hours. The primary antibody solution was prepared as follows. Fifteen ml of the blocking solution was placed in a 15 ml conical tube and 15 μl of the primary antibody, rabbit polyclonal anti-serum to cytochrome P450 1A1 was added to the solution (Abcam, ab80318). The blot was washed for 5 minutes with TBST and 15 ml of the primary antibody solution was used to cover the membrane. The membrane was shaken for 1 hour then washed three times for 5 minutes each with TBST. A secondary antibody solution was prepared by adding 1.5 μl of the Licor goat polyclonal anti-rabbit anti-serum to 15 ml of blocking solution. The secondary antibody solution was placed on the membrane and protected from light. The secondary antibody is linked to a chemical dye, IRDye-800, that has an emission wavelength of 795nm. The membrane was shaken for one hour and washed four times for five minutes each with TBST. The blot was then scanned with the Licor imaging scanner in the Tulane Cancer Center core facilities to detect the 51 kDa band representing the cytochrome P450 1A1 protein.

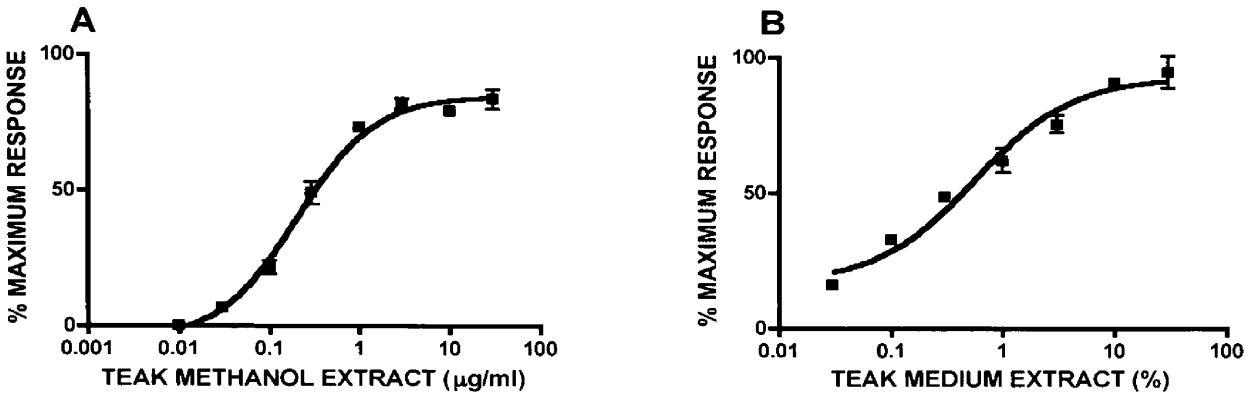
Results

Activation of AhR signaling in a yeast bioassay by hardwood dust extracts

Teak wood dust

Treatment with either methanol or medium extracts from teak dust produced robust AhR signaling. The methanol extract of teak wood dust produced a detectable signal at 0.03 μg/ml and showed a maximum response of 84% of the control signal. Treatment levels greater than 30 ug/ml were found to be toxic. Teak dust medium extract treatment levels of 0.03% produced a signal of 20% relative to control and 30% extract gave a maximum signal of 91% of the control. Yeast incubated with 100% medium extract did not grow.

Figure 4. Effects of teak extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of teak dust treatments ranging from 0.01 to 30 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal. Each assay was conducted in triplicate, and repeated a minimum of three times. Symbols reflect the mean of these experiments, and error bars indicate the standard error of the mean.

B) Yeast were incubated with medium extracts of teak wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 4. Teak dust methanol extract dose response parameters and corresponding treatment levels

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	6%	25%	50%	84%
treatment level (µg/ml)	0.01	0.03	0.1	0.3	1

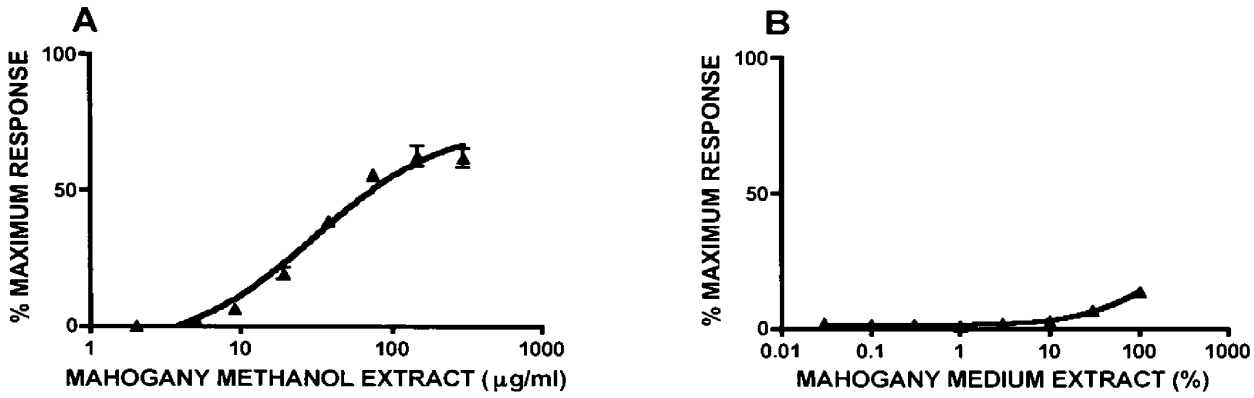
Table 5. Teak dust medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	20%	25%	50%	91%
treatment level (% extract)	-	0.03	0.07	0.45	10

Mahogany wood dust

Incubation with methanol extract of mahogany dust produced a robust signal while incubation with medium extract of mahogany wood dust resulted in some activation at high treatment levels. Treatment with the methanol extract produced a detectable signal at 10 μg/ml and showed a maximum response of 65% of the control signal. No toxicity was observed associated with any treatment level. Treatment with 10% medium extract produced a detectable signal. The maximum signal of 13% relative to the control was observed following treatment with 100% medium extract. No toxicity was noted associated with any treatment level.

Figure 5. Effects of mahogany extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extracts of mahogany dust treatments ranging from 3 to 300 μg/ml. The log base 10 of the methanol extract concentration is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM, or 0.27 μg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of mahogany wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM β-NF control signal.

Table 6. Mahogany wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	11%	25%	50%	75%
treatment level (µg/ml)	3	10	21	74	150

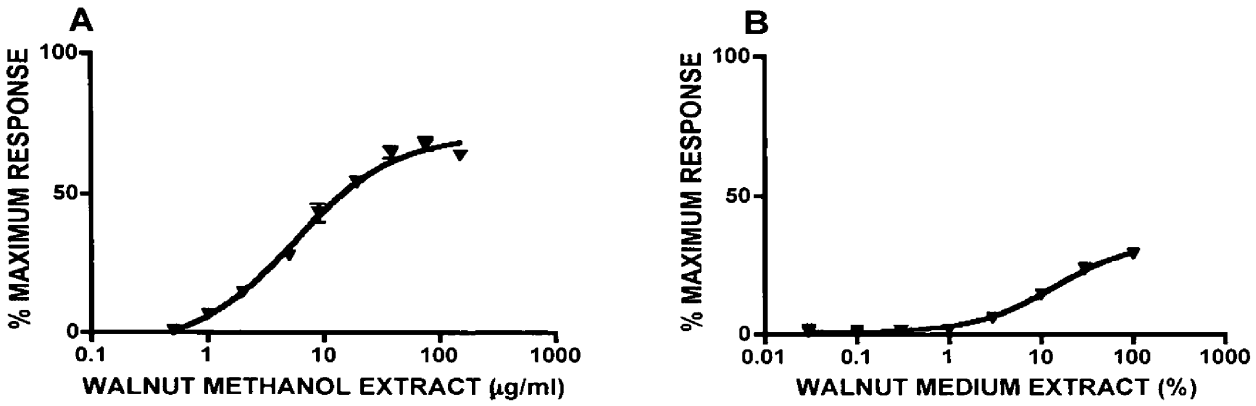
Table 7. Mahogany wood medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	6%	25%	50%	13%
treatment level (% extract)	10	30	-	-	100

Walnut wood dust

Incubation with methanol extract of walnut dust induced a strong signal while incubation with walnut medium extract produced moderate activation of AhR signaling. Treatment with methanol extract of walnut produced a detectable signal at 1 μg/ml and showed a maximum response of 71% relative to the control. Treatment levels higher than 150 μg/ml were found to be toxic, preventing testing at higher doses. Treatment with 3% of the walnut medium extract produced a detectable signal. Yeast grown in 100% walnut medium extract produced a signal 34% of the control. No toxicity was associated with any medium extract treatment level.

Figure 6. Effects of walnut extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of walnut dust ranging from 0.25 to 150 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of walnut wood dust ranging from .03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 8. Walnut wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	6%	25%	50%	71%
treatment level (µg/ml)	0.5	1	4	14.5	100

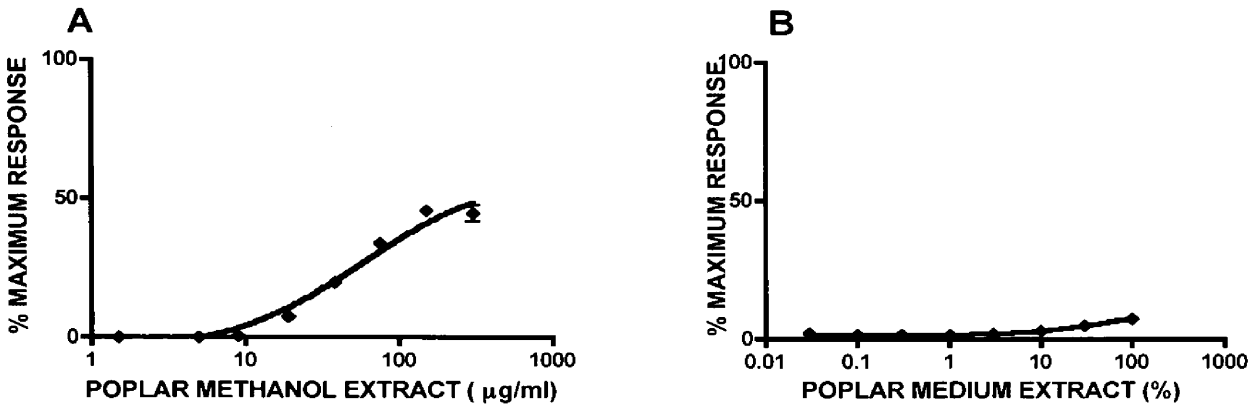
Table 9. Walnut wood medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	6%	25%	50%	34%
treatment level (% extract)	1	3	38	-	100

Poplar wood dust

Incubation with methanol extract of poplar dust resulted in moderate activation of the AhR signaling system while incubation with medium extracts of poplar displayed weak activation at high treatment levels. Treatment with 20 μg/ml poplar methanol extract produced a detectable signal and a maximum signal of 45% relative to the control was observed at the 150 μg/ml treatment level. Treatment with 10% poplar medium extract produced a detectable signal and a maximum signal of 7% relative to control was observed when yeast were grown in 100% poplar medium extract.

Figure 7. Effects of poplar extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of poplar dust ranging from 1.5 to 300 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of poplar wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 10. Poplar wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	7%	25%	50%	45%
treatment level (µg/ml)	10	20	45	-	150

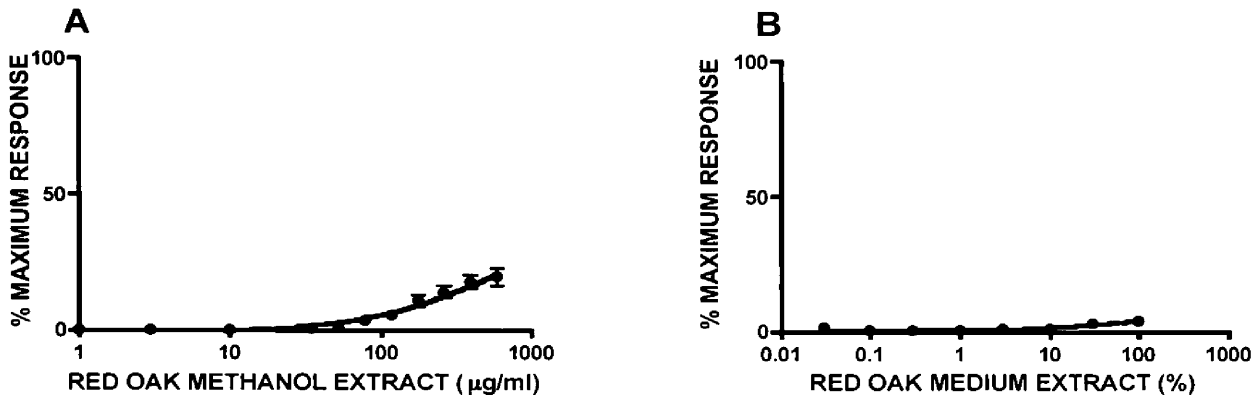
Table 11. Poplar wood medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	2%	25%	50%	7%
treatment level (% extract)	3	10	-	-	100

Red oak wood dust

Incubation with methanol extract of red oak dust produced a weak signal, with a maximum of 18% of the control. Treatment with 5 μg/ml red oak methanol extract produced a detectable signal. No toxicity was observed at any treatment level. Treatment with 3% medium extract produced a detectable signal and yeast grown in 100% red oak medium extract produced a signal equivalent to 5% of the control.

Figure 8. Effects of oak extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of red oak dust ranging from 1 to 600 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of red oak wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 12. Red oak wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	5%	25%	50%	19%
treatment level (µg/ml)	50	80	-	-	600

Table 13. Red oak wood medium extract dose response parameters and corresponding treatment levels.

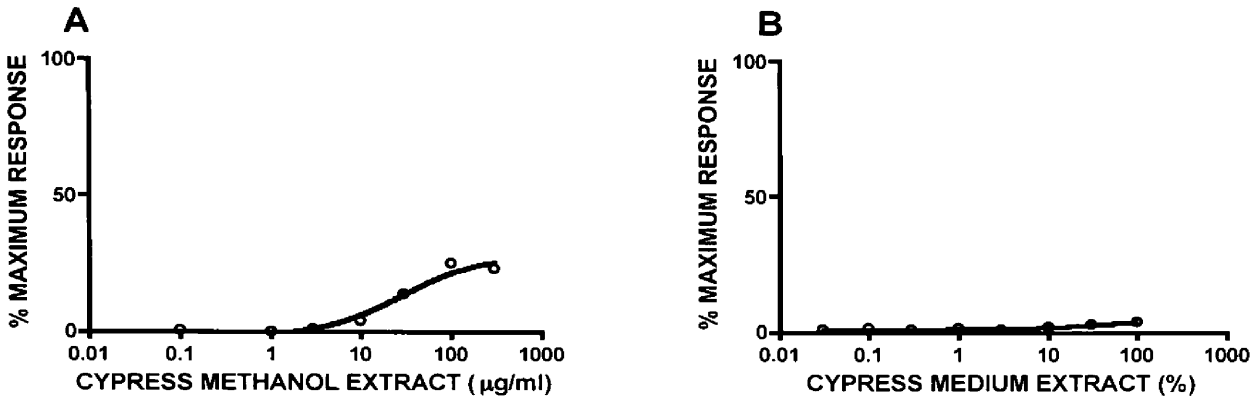
	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	1%	25%	50%	5%
treatment level (% extract)	1	3	-	-	100

Activation of AhR signaling in a yeast bioassay by softwood dust extracts

Cypress wood dust

Incubation with methanol extract of cypress dust produced a moderate signal, with a maximum of 25% of the β-NF control, treatment with 10 μg/ml of the methanol extract produced a detectable signal. No toxicity was observed at any treatment level. Treatment with 10% medium extract produced a detectable signal and yeast grown in 100% cypress medium extract produced a signal equivalent to 3.5% of the control.

Figure 9. Effects of cypress extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of cypress dust ranging from 0.1 to 300 μg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM, or 0.27 μg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of cypress wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM β-NF control signal.

Table 14. Cypress wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	3%	25%	50%	25%
treatment level (µg/ml)	3	10	100	-	100

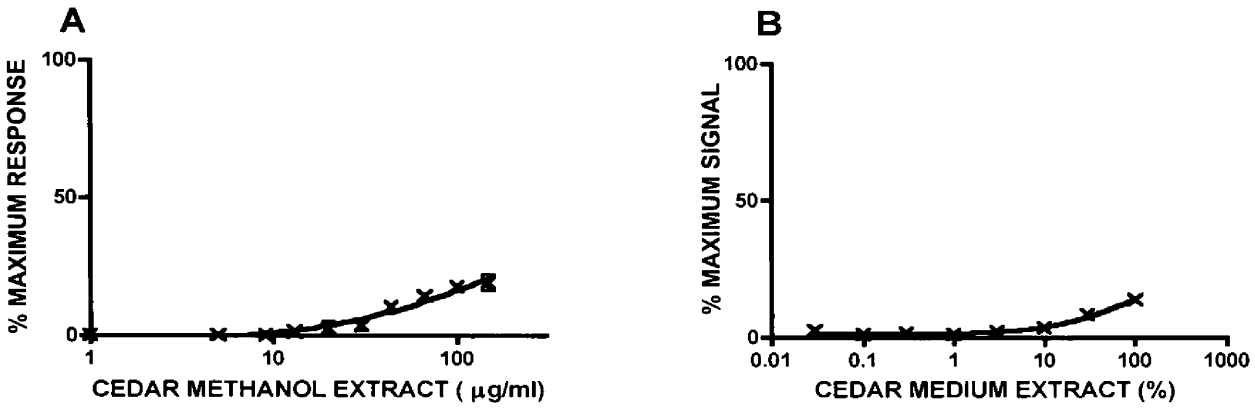
Table 15. Cypress wood medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	2%	25%	50%	3.5%
treatment level (% extract)	3	10	-	-	100

Western red cedar wood dust

Incubation with methanol extract of cedar dust produced a weak signal, with a maximum of 19% of the β-NF control, treatment with 30 μg/ml of the methanol extract produced a detectable signal. No toxicity was observed at any treatment level. Treatment with 10% medium extract produced a detectable signal and yeast grown in 100% cedar medium extract produced a signal equivalent to 14% of the control.

Figure 10. Effects of cedar extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of cedar dust ranging from 1 to 150 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of cedar wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 16. Cedar wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	4%	25%	50%	19%
treatment level (µg/ml)	20	30	-	-	150

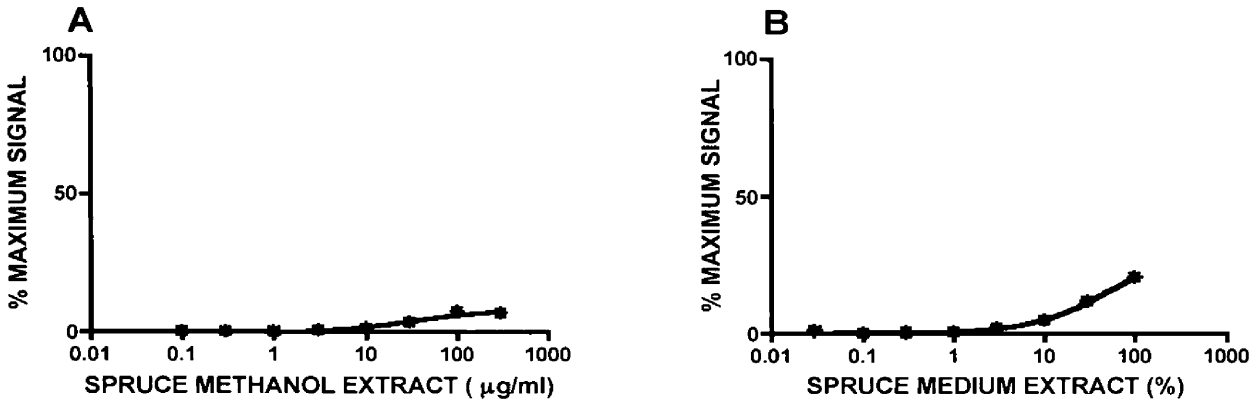
Table 17. Cedar wood medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	3%	25%	50%	14
treatment level (% extract)	3	10	-	-	100

Spruce wood dust

Incubation with methanol extract of spruce dust produced a weak signal, with a maximum of 7% of the β-NF control, treatment with 10 μg/ml methanol extract of spruce wood dust produced a detectable signal. No toxicity was observed at any treatment level. Treatment with 10% medium extract produced a detectable signal and yeast grown in 100% spruce medium extract produced a signal equivalent to 20% of the control.

Figure 11. Effects of spruce extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extracts of spruce wood dust ranging from 0.1 to 300 μg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM, or 0.27 μg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of spruce wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM β-NF control signal.

Table 18. Spruce wood dust methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	1%	25%	50%	7%
treatment level (µg/ml)	3	10	-	-	100

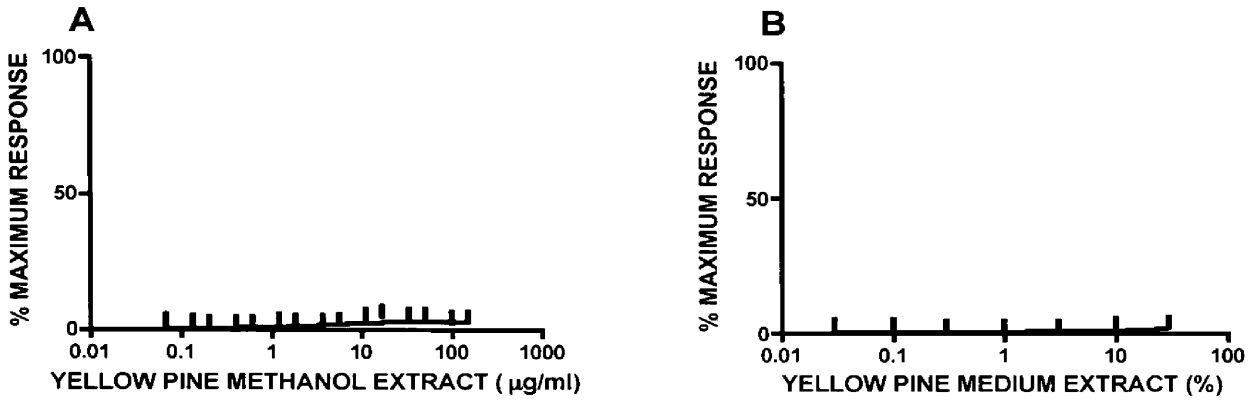
Table 19. Spruce wood dust medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	4%	25%	50%	20%
treatment level (% extract)	1	10	-	-	100

Yellow pine wood dust

Incubation with methanol extract of yellow pine dust extract did not produce a detectable signal. Treatment with 300 μg/ml yellow pine methanol extract was toxic. Treatment with medium extract did not produce a detectable signal and treatment levels higher than 30% of medium extract were found to be toxic.

Figure 12. Effects of yellow pine extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of yellow pine dust ranging from 0.6 to 300 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extracts of yellow pine wood dust ranging from .03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 20. Yellow pine wood methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	1%	25%	50%	7%
treatment level (µg/ml)	3	10	-	-	100

Table 21. Yellow pine wood medium extract dose response parameters and corresponding treatment levels.

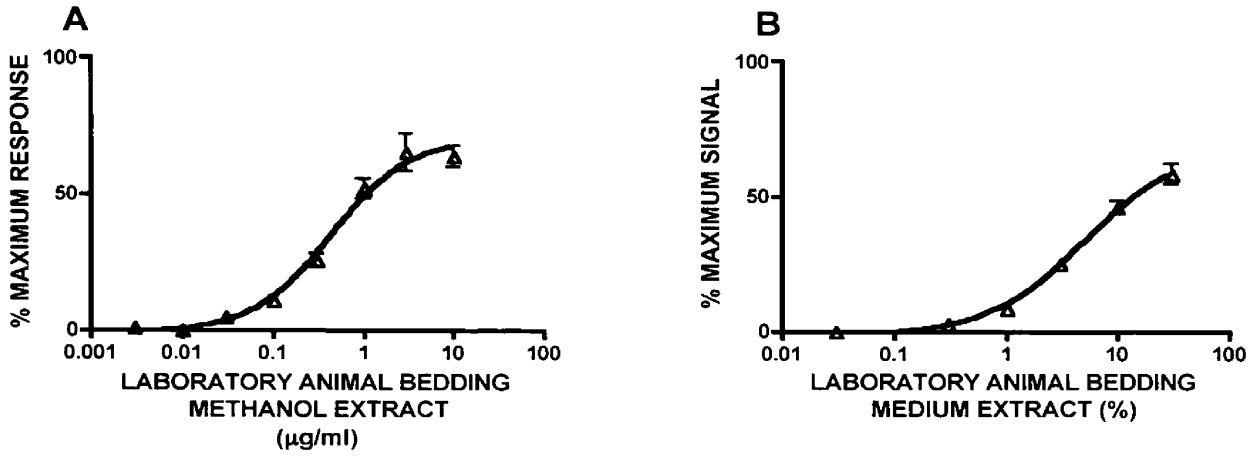
	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	-	25%	50%	-
treatment level (% extract)	30	-	-	-	-

Activation of AhR signaling in a yeast bioassay by wood product dust extracts

Laboratory animal bedding

Incubation with both methanol and medium extracts of laboratory animal bedding induced a strong signal in the AhR bioassay. Treatment with methanol extract produced a detectable signal at 0.03 μg/ml and produced a maximum signal of 71% of the control. Treatment levels higher than 10 μg/ml were found to be toxic. Incubation with medium extract of laboratory animal bedding produced a detectable signal at 1% and reached a maximum of 69% of the control. Treatments higher than 30% were found to be toxic.

Figure 13. Effects of laboratory animal bedding extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of laboratory animal bedding methanol extract ranging from 0.003 to 100 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with laboratory animal bedding medium extracts ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 22. Laboratory animal bedding methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	5%	25%	50%	71%
treatment level (µg/ml)	0.01	0.03	4	1	3

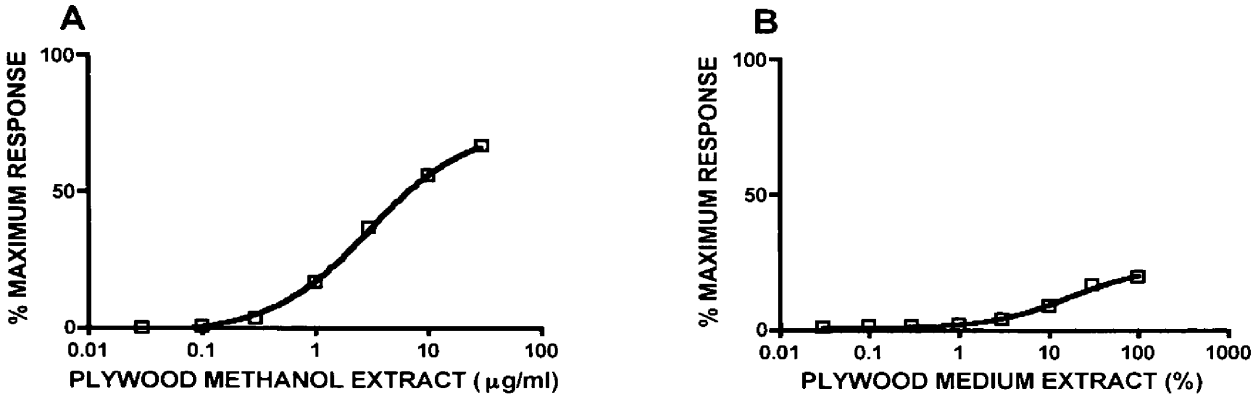
Table 23. Laboratory animal bedding medium extract dose response parameters and corresponding treatment levels

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	3%	25%	50%	69%
treatment level (% extract)	0.1	0.3	3	14	30

Plywood dust

Incubation with methanol extract of plywood dust induced a strong signal in the AhR bioassay. Treatment with methanol extract produced a detectable signal at 0.03 μg/ml and produced a maximum signal of 73% of the control. Treatment levels higher than 30 μg/ml were found to be toxic. Incubation with plywood dust medium extract produced a detectable signal at 3% and reached a maximum of 23% of the control. No toxicity was associated with any treatment level.

Figure 14. Effects of plywood extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of plywood dust ranging from 0.03 to 100 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extract of plywood dust ranging from .03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 24. Plywood dust methanol extract dose response parameters and corresponding treatment levels.

	Methanol extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	3%	25%	50%	73
treatment level (µg/ml)	0.1	0.3	1.6	6.5	30

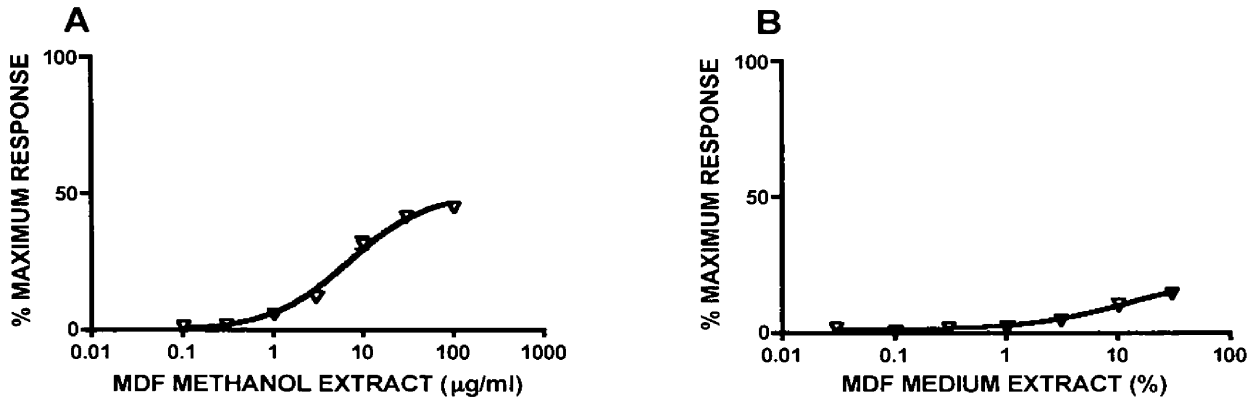
Table 25. Plywood dust medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	1%	25%	50%	23%
treatment level (% extract)	1	3	-	-	100

Medium density fiberboard dust

Incubation with methanol extract of medium density fiberboard dust induced a moderate signal in the AhR bioassay. Treatment with methanol extract produced a detectable signal at 1 μg/ml and produced a maximum signal of 45% of the control. Treatment levels higher than 100 μg/ml were found to be toxic. Incubation with MDF dust medium extract produced a detectable signal at 1% and reached a maximum of 20% of the control. Treatment levels higher than 30% were toxic.

Figure 15. Effects of medium density fiberboard extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of MDF dust ranging from 0.1 to 300 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extract of MDF dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 26. Medium density fiberboard dust methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	6%	25%	50%	45%
treatment level (µg/ml)	0.3	1	7	-	100

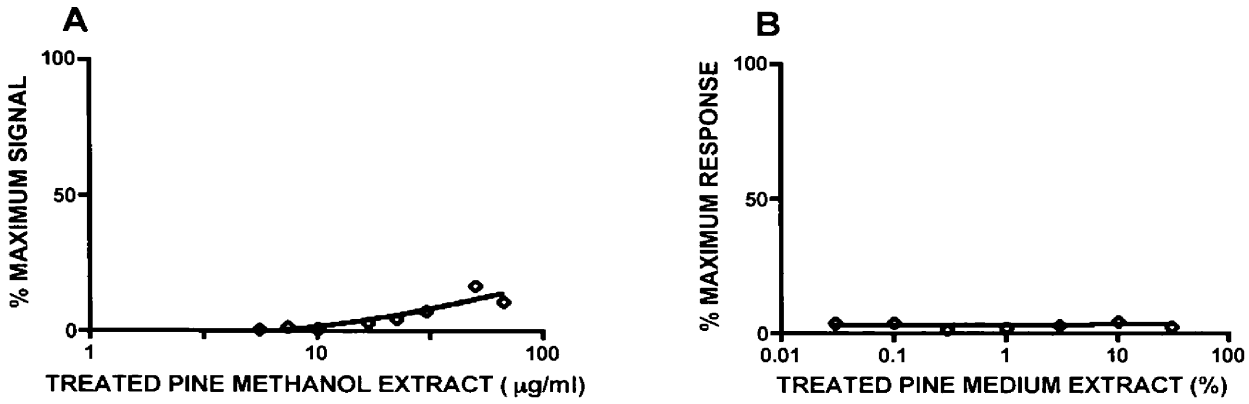
Table 27. Medium density fiberboard dust medium extract dose response parameters and corresponding treatment levels

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	2%	25%	50%	20%
treatment level (% extract)	0.3	1	-	-	30

Treated pine wood dust

Incubation with methanol extract of treated pine wood dust induced a weak signal in the AhR bioassay. Treatment with methanol extract produced a detectable signal at 15 μg/ml and produced a maximum signal of 17% of the control. Treatment levels higher than 50 μg/ml were found to be toxic. Incubation with treated pine wood dust medium extract did not produce a detectable signal. Treatment levels higher than 30% were toxic.

Figure 16. Effects of treated pine extracts on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with methanol extract of treated pine wood dust ranging from 5 to 300 µg/ml. The log base 10 of the concentration of methanol extract is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extract of treated pine wood dust ranging from .03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

Table 28. Treated pine wood dust methanol extract dose response parameters and corresponding treatment levels.

	Methanol Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	2%	25%	50%	17%
treatment level (µg/ml)	5	15	-	-	50

Table 29. Treated pine wood dust medium extract dose response parameters and corresponding treatment levels.

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	-	25%	50%	-
treatment level (% extract)	30	-	-	-	-

Figure 17. Categorical analysis of maximum signal from AhR signaling dose response curves for hardwoods, wood products, and softwoods

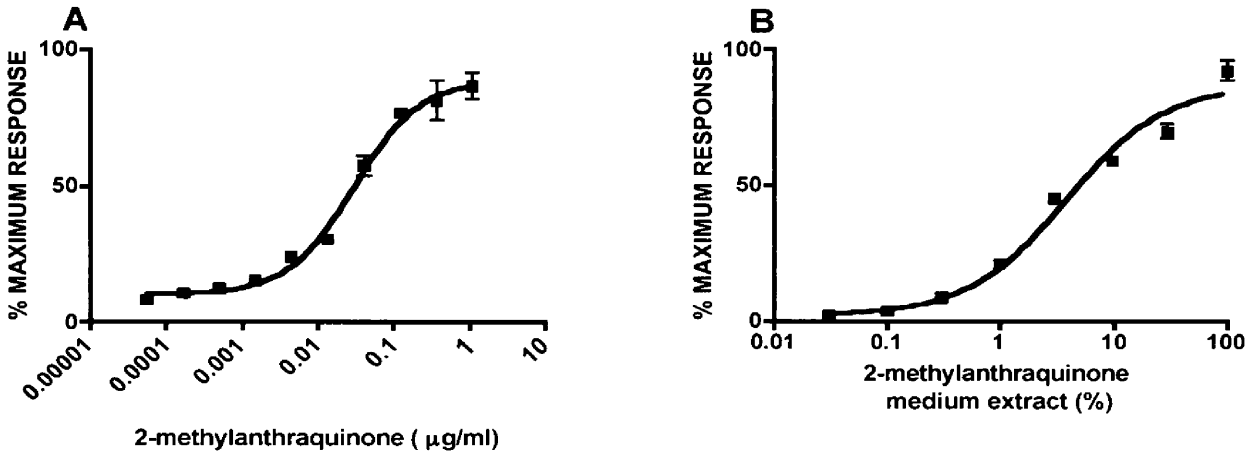
The bar on the left shows the average maximum AhR signal for the hardwood treatment group. The second column shows the average maximum AhR signal for the wood product treatment group, that includes the laboratory animal bedding. The third column shows the average maximum AhR signal for the softwood treatment group. Error bars show the standard error of the mean. The groups were compared statistically with a one-way ANOVA with Dunnett's multiple comparison tests, which compared each group to the background signal. Asterisks indicate statistical significance ($p<0.05$).

Activation of AhR signaling by teak wood dust extracts and 2-methylanthraquinone

Incubation with 2-methylanthraquinone induced a strong signal in the AhR bioassay (Figure 18). Treatment with 2-methylanthraquinone produced a detectable signal at 0.00003 μg/ml and produced a maximum signal of 86% of the β-NF positive control. Treatment levels higher than 1 μg/ml were toxic to the yeast. Incubation with 2-methylanthraquinone medium extract produced a detectable signal at 0.1% and produced a maximum signal of 84% of the β-NF positive control. No toxicity was noted with 100% 2-methylanthraquinone treatment.

Treatment with 2-methylanthraquinone dissolved in DMSO induced AhR signaling more potently than the methanol extracts of three independent methanol extracts of teak wood (Figure 20). The top of the teak extract signaling curves ranged from 86 to 100% of the β-NF control. 2-methylanthraquinone produced a signaling curve with a maximum value of 89% of the β-NF control.

Figure 18. Effects of 2-methylanthraquinone on AhR signaling in a yeast reporter assay.



A) Yeast were incubated with 2-methylanthraquinone ranging from 0.0003 to 1 µg/ml. The log base 10 of the concentration of 2-methylanthraquinone is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM, or 0.27 µg/ml, β-NF control signal.

B) Yeast were incubated with medium extract of 2-methylanthraquinone ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 µM β-NF control signal.

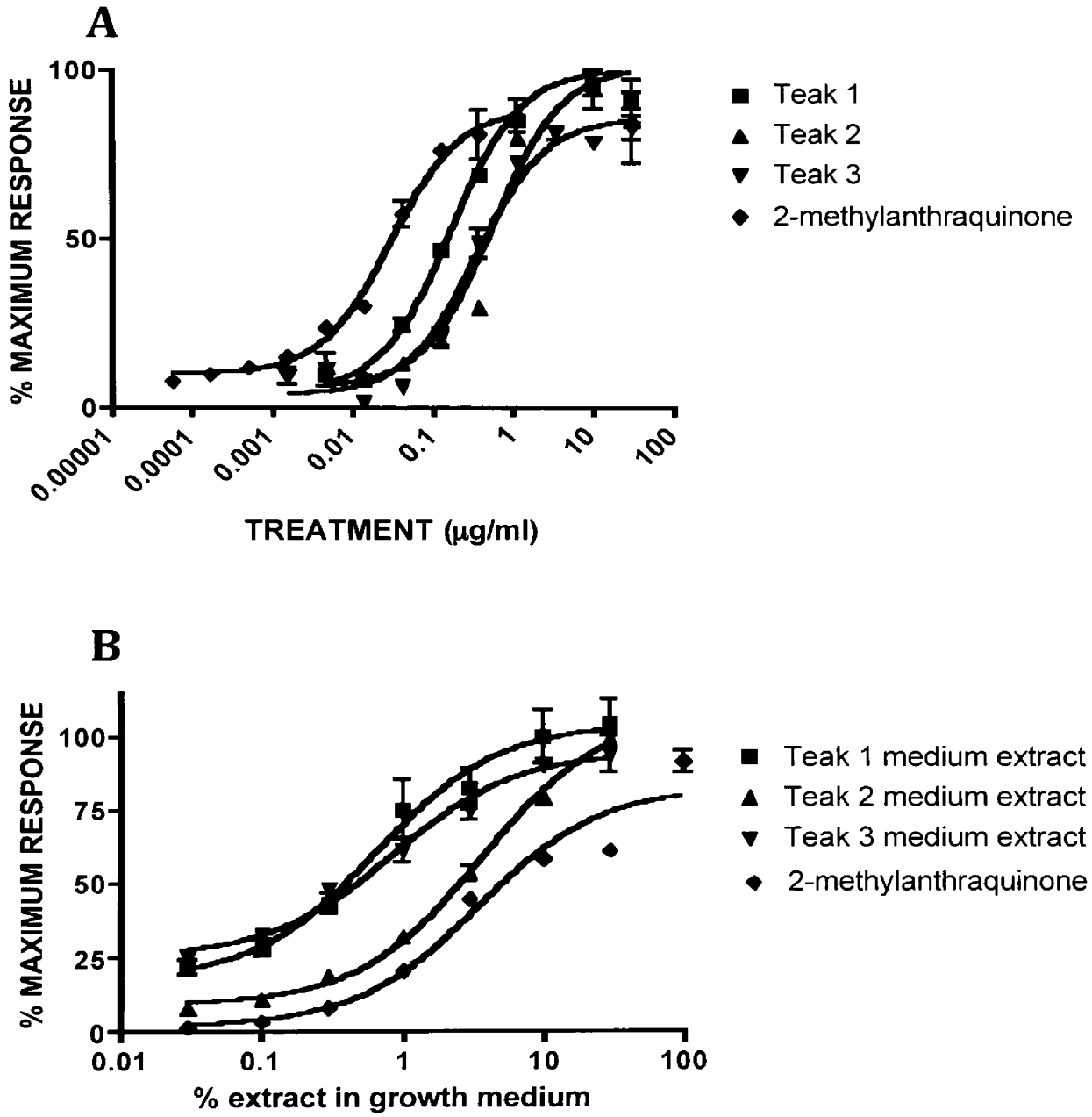
Table 30. 2-methylanthraquinone dose response parameters and corresponding treatment levels

	2-methylanthraquinone				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	10%	25%	50%	86%
treatment level (µg/ml)	-	0.00005	0.007	0.03	0.80

Table 31. 2-methylanthraquinone medium extract dose response parameters and corresponding treatment levels

	Medium Extract				
	NOEL	LOEL	EC_{25}	EC_{50}	Maximum
% response	0%	3%	25%	50%	84%
treatment level (% extract)	-	0.03	1.4	4.8	100

Figure 19. 2-methylanthraquinone and independent teak wood extracts AhR activation



A) Yeast were incubated with 2-methylanthraquinone ranging from 0.0003 to 1 µg/ml or methanol extracts of teak wood from 0.03 to 30 µg/ml. The log

base 10 of the treatment concentration is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM, or 0.27 μg/ml, β-NF control signal.

B) Yeast were incubated with medium extract of 2-methylanthraquinone or teak wood dust ranging from 0.03 to 100% of the total volume of the growth medium. The log base 10 of the percent of the medium extract treatment is plotted on the x-axis. The y-axis shows the percent response relative to the 1 μM β-NF control signal.

Table 32. Summary of AhR signaling bioassay results

Category	Wood or wood product type	Activity in methanol extracts	Activity in medium extracts
Hardwood	Teak	Strong	Strong
	Walnut	Strong	Moderate
	Mahogany	Strong	Weak
	Poplar	Moderate	Weak
	Oak	Weak	Weak
Softwood	Cypress	Moderate	Weak
	Cedar	Weak	Weak
	Spruce	Weak	Weak
	Yellow pine	Inactive	Inactive
Wood product	Plywood	Strong	Weak
	Laboratory animal bedding	Strong	Strong
	Medium Density Fiberboard (MDF)	Moderate	Weak
	Treated pine	Weak	Inactive

The methanol and medium extracts of wood dusts were ranked based on their individual ability to activate AhR signaling into groups of strong, moderate, weak, and inactive based on the ability of the extract treatment to induce AhR signaling in the yeast bioassay. Extracts that induced a signal > 50% of the β-NF control were considered to contain strong agonists for the AhR. Extracts that induced a signal >

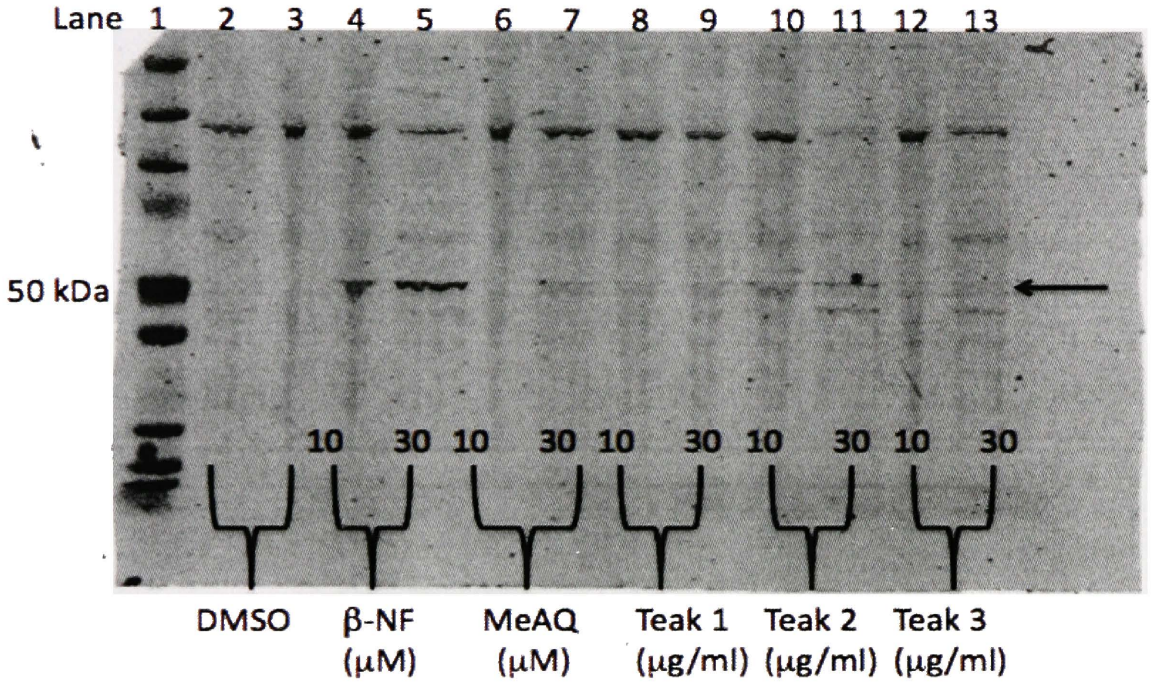
25% but < 50% of the β-NF control are considered to contain moderate agonists for the AhR. Extracts that induced a signal <25% of the β-NF control are considered to contain weak agonists for the AhR. The MDED or minimum detectable experimental dose is reported as the treatment level at which a response > 0 was observed. Extracts that did not result in a MDED >0 are considered to be inactive.

Immunological detection of cytochrome p450 CYP1A1 induction following exposure to teak wood dust extract or 2-methylanthraquinone in HepG2 cells.

Following treatment with 10 or 30 μg/mL of methanol extract of teak wood dust, or 10 or 30 μM 2-methylanthraquinone and β-NF induction of CYP1A1 was detected differentially by western blot (Figures 20 and 21). The strongest induction was observed in the 30 μM β-NF treatment. The 10 μM β-NF also induced CYP1A1 expression but to a lesser degree. 2-methylanthraquinone induced CYP1A1 expression at the 30 μM treatment level. CYP1A1 induction was noted following treatment with 10 and 30 μg/mL of the methanol extract of teak wood sample numbers 1 and 2 but induction was not observed following treatment with teak wood sample 3.

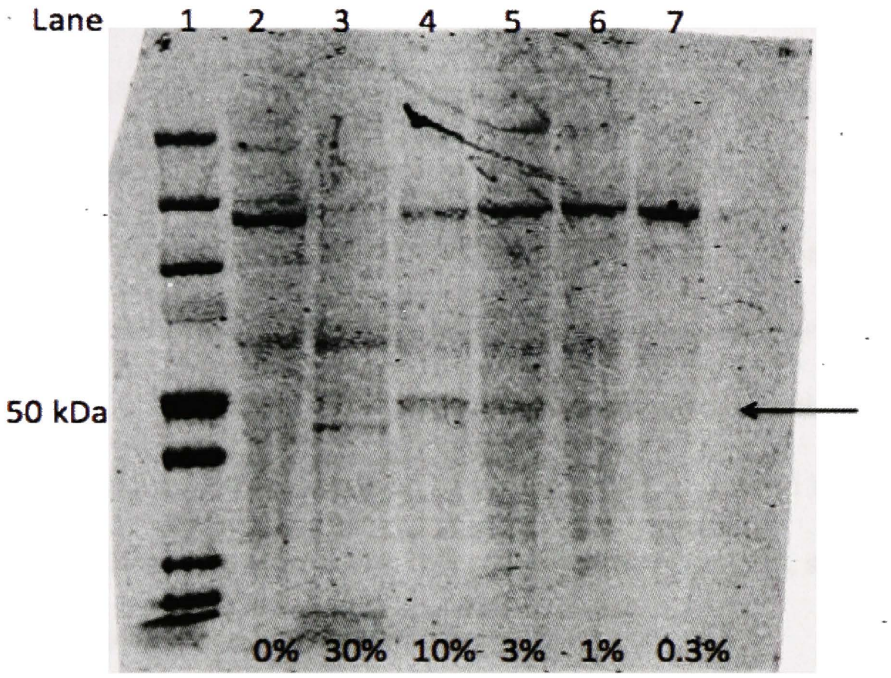
Treatment with different percentages of medium extract of teak wood dust ranging form 0.3 to 100% of the total volume of the medium were probed for CYP1A1 induction by western blot. The 100% treatment was observed to be toxic to the cells while the 30% treatment resulted in roughly 50% of the cells killed. There is CYP1A1 band present at 51 kDa in the 10% and 30% treatment groups indicating that treatment with medium extracts of teak wood dusts induce CYP1A1 activity in HepG2 cells.

Figure 20. CYP1A1 induction in HepG2 cells from 24 hour exposure to β-NF, 2-methylanthraquinone, and three teak wood extracts



Lane 1 is on the left side of the blot image and it contains the protein size marker. CYP1A1 is a 51 kDa protein its expected position is indicated by the arrow on the right. The 50 kDa band in the protein marker is labeled to aid visualization of the appropriately sized protein in the treatment groups. Lanes 2 and 3 represent the solvent negative controls with equivalent volumes to the high and low treatment levels for all samples. Lanes 4 and 5 represent the low and high treatment levels of the positive control, β-NF. Lanes 6 and 7 represent the low and high treatment levels of 2-methylanthraquinone (MeAQ). Lanes 8 to 13 represent the low and high treatment levels for methanol extracts of three independent teak wood dust samples.

Figure 21. CYP1A1 induction in Hepg2 cells treated with medium extracts of teak wood



Lane one is on the left side of the blot image and contains the protein size marker. The 50 kDa band on the marker and the expected position of CYP1A1 in the gel, indicated by the arrow on the right, are identified to help with visualization of the 51 kDa band which should appear if CYP1A1 is induced by the treatments. Lane 2 is the medium alone negative control and lanes 3 to 7 were loaded with the protein from HepG2 cells exposed to 30% to 0.3% medium extract of teak wood treatment.

Discussion

The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor with the ability to upregulate dioxin responsive genes such as cytochrome p450 1A1 (CYP1A1) and NADPH quinone oxidoreductase. Activation of the AhR is required for the clearance of some toxicants. However, activation of AhR signaling can also lead generation of reactive intermediates and altered gene expression that is associated with toxicity. Many AhR ligands are known carcinogens that mediate toxicity via activation of the AhR signaling pathway. We hypothesized that AhR ligands are present in the soluble fraction of wood dusts and used the YCM3 yeast bioassay to test this idea. The woods tested included hardwoods, softwoods, and wood products commonly used in the wood working industry.

Methanol extracts of the different wood dusts showed variable activity when tested with the yeast bioassay. At least one wood or wood product type in each group was able to induce AhR signaling between 25 and 50% of the level produced by the 1 μM β-NF positive control. The hardwoods and wood products groups induced more activity than the softwoods group. The wood types with the highest AhR activity may pose a greater hazard to woodworkers. Teak wood dust extract was the most potent and efficacious activator of AhR. Teak wood is commonly used in furniture and cabinet manufacturing, in decking, and on ships, so the possibility for occupational exposures is considerable. Walnut, mahogany, plywood, and medium density fiberboard are also commonly used in furniture manufacturing, and while their respective AhR activities were less than that of teak dust, extracts from these wood dusts still resulted in AhR activation at relatively low concentrations.

Many planar, multi-ringed chemicals are ligands of the AhR. These ligands include naturally occurring compounds like the flavones found in teas and many vegetables (Ciolino et al., 1999; Fukuda et al., 2007; Zhang et al., 2003). Other naturally occurring ligands include indole derivatives that are generated by metabolism or photoactivation of the amino acid tryptophan (Denison and Nagy, 2003). Thus, it seems likely that wood dusts may contain similar natural ligands. Most of the natural AhR ligands are ultimately metabolized to inert products, and thus they do not activate the AhR for extended periods of time. Often such compounds are reported to have mixed agonist/antagonist activity depending on cell type, and are reported to be competitive inhibitors of other, possibly more toxic, ligands of AhR. In contrast, other AhR ligands such as benzo(a)pyrene are metabolized to a reactive diol epoxide intermediate, which can lead to toxic endpoints that include mutagenesis and carcinogenesis. Whether the AhR ligands in wood dusts are metabolically cleared and whether they are activated to ultimate genotoxic intermediates remain to be determined.

2-methylanthraquinone is a toxicologically important component of teak dust and a potent agonist of AhR, but it does not account for all the AhR activity associated with teak wood dust. 2-methylanthraquinone comprises ~0.3% of the dry weight of teak wood dust. Walnut, mahogany, poplar, cypress, plywood, and MDF extracts all were found to activate the AhR, but contain little or no 2-methylanthraquinone as determined by HPLC fractionation. The observation that other wood dusts that lack appreciable amounts of 2-methylanthraquinone but still

show activity indicates that other potentially toxic AhR activating compounds remain to be identified.

Newspaper is a wood product, and as expected, it has been shown to contain AhR ligands. Indeed, unprinted or "virgin" newspaper from various sources contained variable amounts of soluble AhR agonists (Bohonowych et al., 2008). Activity of newspaper samples was demonstrated by induction of AhR ligand binding in guinea pig hepatic cytosol, and by induction of AhR responsive luciferase reporter genes in recombinant guinea pig and mouse cell lines. Ligand dependent transformation and DNA binding of AhR by DMSO and ethanol extracts of virgin newspaper was reported, but water extracts were inactive. Induction of AhR regulated luciferase reporter genes followed a similar pattern of activity. These effects were observed following treatment with 1% extract of DMSO, ethanol, or water in each of the test systems. A similar trend was noted in our studies. Methanol, which is both a polar and organic solvent, was generally more efficient in extracting AhR ligands from wood dust in comparison to medium (largely aqueous) extractions. The sole exception to the trend was teak dust, which liberated significant AhR ligand activity into medium.

AhR ligands in unprinted newspapers were found to be metabolically labile as indicated by AhR reporter gene activity in hepatoma cells (Bohonowych et al., 2008). Newsprint ligands initially caused increased reporter gene activity and longer exposure times resulted in a decline, consistent with the notion that the ligand(s) was degraded at later treatment times. Workers who are at risk for developing nasal cancers are chronically exposed to AhR ligands in wood dusts.

Thus, while AhR ligands in wood dust may be metabolically labile, daily workplace exposure may lead to chronic activation of the AhR. The net effect of chronic AhR activation in producing cancer and other toxicity is seen in animals and people that are exposed to large concentrations of dioxin.

Paper is a type of wood product, so some of the same compounds found in newsprint may be responsible for the AhR activity observed in our studies. Some of the AhR activity reported by Bohonowhych et al. in newspapers might be due to AhR ligands produced during the pulping and bleaching processes. This possibility of chemical contamination is an important consideration for the plywood and MDF wood products that we tested as well. It is possible that some, if not most, of the activity in these samples was due to a chemical or contaminant that was added or formed during the manufacturing process. However, the activity associated with pure wood dust extracts and virgin paper extracts, excluding contaminates generated during pulping, can only be ascribed to natural soluble ligands in the wood or paper.

Aqueous medium extracts were assessed for AhR activation to better mimic the physiological conditions that wood dust particles encounter when adhering to the sino-nasal epithelium. In general, ligands of the AhR are hydrophobic planar organic molecules, but some compounds with moderately hydrophilic character also activate the AhR. AhR activity observed in the medium extracts should be associated with wood types that pose the greatest risk for sustained AhR activation from chronic exposure. This is an important consideration as the sinus cavity is

both the site where dust is impacted and the site of tumor formation in wood dust associated cancers.

Only two of the wood types tested displayed strong activity in both the medium and methanol extract treatment groups. These were teak and laboratory animal bedding. It is not likely that workers would be exposed to laboratory animal bedding unless they were directly involved in the manufacturing of the product or working in a viviarium. Since the product undergoes extensive chemical characterization by the manufacturer, it serves as a good control to demonstrate that AhR ligands in wood dusts are likely to be endogenous compounds instead of chemical contaminats.

Wood is a natural product and thus its contents may vary according to growth conditions and other factors. To address the issue of sample-to-sample variation in toxic components, we purchased three distinctly different pieces of teak wood and assessed them by chemical analysis and with bioassays. We found that 2-methylanthraquinone was abundant and present in similar concentrations in all the samples. Experiments with the yeast assay showed that 2-methylanthraquinone is soluble in medium as assessed by its ability to activate AhR signaling. 2-methylanthraquinone dissolved in DMSO was more potent in activating AhR signaling and was also more cytotoxic on a per weight basis than the teak methanol extracts. The maximum non-toxic treatment was 3 mg/ml for the 2-methylanthraquinone and 30 mg/ml for the teak dust extracts. Interestingly this trend was reversed in the medium extract treatment groups. The teak dust medium extracts were more potent AhR activators than the 2-methylanthraquinone medium

extract. This difference could be due to the limited solubility of 2-methylanthraquinone relative to the unknown ligands in the teak extract. There was no evident cytotoxicity in the 100% 2-methylanthraquinone medium extract treatment group but levels higher than 30% teak wood medium extract were toxic to the yeast. The reversal of the trend in potency of AhR activation and cytotoxicity suggests that AhR ligands in the teak dust are more hydrophilic than 2-methylanthraquinone, and that 2-methylanthraquinone accounts for some but not all of the AhR activity in teak dust.

We also carried out experiments with teak dust extracts and 2-methylanthraquinone in a metabolically competent human hepatoma cell line. Methanol and medium extracts of 2-methylanthraquinone were used in these experiments. In the methanol teak dust extract groups, 10 and 30 μg/ml, were chosen because they were activating doses in the yeast AhR bioassay. No cytotoxicity was observed with these treatment levels in either the yeast or the HepG2 cells, and activation of the AhR was noted in both systems.

Treatment with 10 μg/ml of teak wood extract produced a response at the top of the dose response curve in the yeast assay but only produced a mild response in the HepG2 cells. The largest induction of CYP1A1 in the HepG2 cells treated with methanol extracts of teak wood dust was at the 30 μg/ml treatment level. It is likely that higher treatment levels would produce a more robust response.

Two major differences exist between these systems, the first being that the HepG2 cells have much greater metabolic capacity for xenobiotics than does yeast. Metabolism should generally be associated with a loss of AhR ligand activity and a

diminution of CYP1A1 activity. Thus, AhR signaling in HepG2 cells might under represent the ligand activity within the teak dust. The second major difference is the HepG2 cells are grown in the presence of 10% fetal bovine serum, while the yeast are not grown in any serum. It is possible that some portion of AhR ligands is effectively sequestered in the medium via interaction with the serum proteins and lipids and is not available to induce AhR signaling. Alternatively, the serum proteins and lipids may act as carriers and could assist in extracting hydrophobic substances from the teak dust. Experiments in the HepG2 cells were also carried out with medium extracts of teak dust. Cytotoxicity was noted at treatments greater than 30% of medium extract of teak dust. Teak extracts made in yeast medium and diluted to 30% were not toxic to the yeast. This result is consistent with the ideas that the HepG2 cell metabolism of teak dust compounds generated cytotoxic intermediates, or that different compounds with different toxicities were extracted in the cell culture and yeast media.

Comparing concentrations of the extracts of teak dust and 2-methylanthraquinone needed to produce effects reveals the plausibility for a role in carcinogenesis. The volume of the human nasal mucosa is approximately ~32 ml in an adult (International Commission on Radiological Protection. Task Group on Reference Man., 1975). This volume is approximately the same as the 30 ml volume of medium used to extract one gram of wood dust or 2-methylanthraquinone. In the HepG2 cells, AhR-mediated CYP1A1 induction was detected at 10% and 3% medium extract treatments. These conditions correspond to 105 mg and 32 mg teak dust, respectively, in the total volume of the nasal mucosa. AhR activation greater than

50% signaling of the positive control in the yeast assay was noted at treatment levels ranging from 0.3% to 30% of medium extract. The maximum signal was reached with 10% teak dust extract. Again, 10% extract corresponds to 105 mg of dust in the total volume of the nasal mucosa while 0.3% medium extract represents 3 mg of dust in the total volume of the nasal mucosa. If exposure is extrapolated by using the OSHA PEL of 15 mg dust/m^3 air and a minute volume of 8 liters per minute during an eight-hour workday as standard exposure assumptions, then a daily exposure of 58 mg dust is expected. If exposure is extrapolated by using the lower ACGIH TLV of 5 mg dust/m^3 air or 1 mg dust/m^3 air, with the same assumptions as before, a total daily exposure of 19 mg or 3.5 mg of wood dust is expected. Reports on particle deposition in the nose show that almost 100% of particles > 10 μm are trapped in the upper airways (Cheng, 2003; Kelly et al., 2004). The kinetics of soluble ligands partitioning out of the dust and into the nasal mucosa should be rapid, and on going work day exposures could lead to sustained activation of the AhR in the nasal tissues of workers exposed to teak dust. The AhR-activating treatments in both the yeast and HepG2 assays are within the realm of reasonable exposures for a worker exposed to teak dusts. Thus, it is plausible that breathing airborne teak dusts produces persistent AhR activation in the nasal sinuses, and that this could contribute to carcinogenesis.

In summary, we provide the first demonstration that AhR activation is induced following treatment with teak dust at doses that are comparable to what wood workers would receive under the current occupational exposure guidelines. We also provides the first evidence that 2-methylanthraquinone is an AhR agonist and

suggest it is a major carcinogenic component in teak dust. 2-methylanthraquinone is not listed as a known or suspected carcinogen, but the closely related compounds anthraquinone and 2-aminoanthraquinone were found to be carcinogenic in National Toxicology Program studies (NTP, 2005). Activation of the AhR signaling pathway has not previously been reported for teak, or other wood dusts, or from the soluble fractions of these dusts. Continual activation of AhR in the nasal sinuses of workers chronically exposed to teak may be involved in carcinogenesis. Carcinogenesis studies using appropriate animal models and human epidemiological studies focusing on teak dust exposures are needed to confirm this hypothesis.

Genotoxicity of wood dust extracts

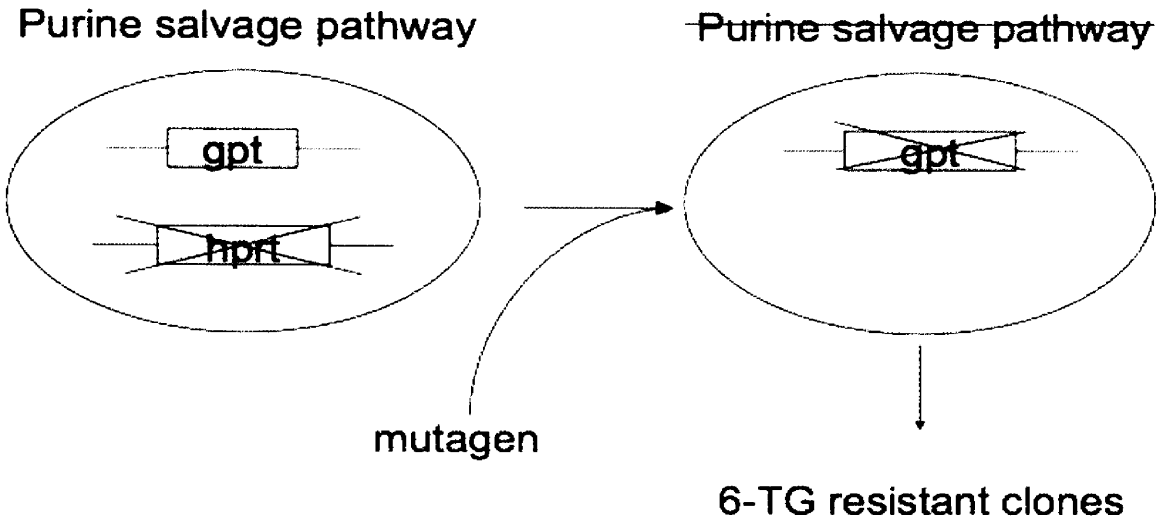
Methods

g12 cell mutant frequency assay

The g12 cell line was derived from the V79 Chinese hamster fibroblast line. It lacks a functional hprt gene, but has a functional, yet much smaller gpt gene from E. coli as shown in figure 22. Initial selection in F12 medium supplemented with 5% FBS and 100 μM hypoxanthine 1 μM aminopterin 100 μM thymidine (HAT) (Invitrogen, Grand Island NY) was carried out to ensure the population of cells at the beginning of the experiment carry the gpt transgene. The gpt gene encodes for xanthine-guanine phosphoribosyltransferase, a functionally equivalent enzyme to the mammalian hprt that is required for the functioning of the purine nucleotide

salvage pathway. Initial selection was carried out by expanding and splitting, at a ratio of 1:30, a freshly ~80% confluent T-25 culture flask into T-75 culture flasks. The ratio used for the split allows for at least three days of growth in the HAT selection medium. One day prior to treatment the medium was aspirated from the flasks and the cells were released from the flask by treatment with trpysin / EDTA solution (Cellgro, Herndon VA) for two to three minutes. The flasks were watched under 40X magnification to determine detachment. Cells were ready for collection when the morphology changed from a flattened adherent cell to a noticeably more rounded shape. The trypsin/EDTA solution was aspirated, replaced with 12 ml of fresh F12 medium and the cell suspension was transferred to a sterile 14 ml round bottom polypropylene Falcon tube (Becton Dickinson Labware, Franklin Lakes, NJ). Culture density, as cells/ml, was determined using a Bright-Line hemacytometer (Hausser Scientific, Horsham PA).

Figure 22. Forward selection assay for 6-thioguanine resistance in g12 cells



g12 cells are hamster fibroblasts in which the hprt gene, required for purine nucleotide salvage pathway, has been ablated and the gpt gene, a functionally equivalent gene from E. coli, is expressed. Initial selection in HAT medium, which inhibits the de-novo purine biosynthesis pathway, ensures the population of cells is gpt+. A population of cells is treated with a prospective mutagenic agent, then mutagenized cells are placed under 6-thioguanine selection. Only cells that do not express the functional gpt protein will survive in the presence of 6-thioguanine (6-TG) in the medium.

Two separate sets of cell cultures were required for the mutagenesis assay. The first was the mutagenesis treatment group consisting of cells plated on three separate 100 mm Cellstar tissue culture dishes (Greiner bio-one, Frickenhausen, Germany) per each treatment dose, and one triplicate group for the positive control. These plates were seeded with 1 x 10^6 cells per plate in 14 ml of non-selective F12 medium and allowed to attach overnight. The second group of plates was needed to determine the cytotoxicity associated with the treatment. Three 60 mm Cellstar tissue culture dishes per each treatment condition were seeded with 100 cells per plate in non-selective F12 medium and allowed to attach overnight.

Equation 4. % Survival determined by colony formation assay

% cells surviving treatment = (Colony number on treatment plate÷colony number on control plate) × 100

The following morning dosing solutions were prepared by adding 60 mg/ml of freshly dissolved wood dust extract in DMSO. In general, doses were set at comparably cytotoxic levels across different dust treatments. The wood dust extract was applied to the cells in the afternoon and removed the following morning (~18 hrs treatment period).

After the 18 hr treatment period, the medium was removed and replaced with fresh F12 medium: 14 ml per 100 mm dish, and 5 ml per 60 mm dish. The cells were allowed to grow for an additional six days. Because the cell number on the mutagenesis plates was high, medium changes were required daily for the 100 mm plates. The cytotoxicity assay plates were removed from the incubator five to six days after treatment. The cytotoxicity assay plates were rinsed in PBS, stained and

fixed with 0.1% crystal violet in 100% ethanol, and then washed with water to remove the excess stain. Colonies were counted on treatment and control plates and the percent of cells surviving the treatment was determined.

On the sixth day after treatment, cells were detached from 100 cm culture dishes using trypsin/EDTA solution and then counted. Four separate 100 cm dishes were seeded with 1 X 10^5 cells per plate for the mutagenesis assay and three 60 cm dishes seeded with 100 cells /plate in order to determine the plating efficiency. Plating efficiency was determined by allowing the cultures to grow for six days in non-selective F12 medium then staining and fixing them as described above. Plating efficiency is defined as described by equation 5.

Equation 5. g12 mutagenesis assay plating efficiency calculation

% plating efficiency = (colonies observed ÷ cell number plated) × 100.

g12 mutagenesis assay with methanol extracts of hardwood, softwood, and wood product dusts

The cells for the mutagenesis assay were put in selective medium, F12 + 10 μg/ml 6-thioguanine, (F12-6TG). Due to preselection in HAT medium as described above, only cells with a functional gpt gene were present at the start of the assay. Only cells that picked up a mutation or otherwise silenced the gpt gene can grow in the presence of 6-thioguanine as it is incorporated as a toxic nucleotide in DNA and RNA if the gpt gene is active. Thus, only gpt mutants or cells in which the gpt transgene has been turned off through some alternative mechanism will be resistant to the 6-TG selection. The mutants were allowed to grow in the selection medium

for fourteen days. Cells in three of the expanded dishes from each treatment condition were used to calculate the mutation frequency at the *gpt* transgene locus and the fourth was used to isolate individual clones. This strategy ensured that each mutant selected for expansion and subsequent genotyping arose from an independent event.

The mutant colonies on three of the expanded culture plates were fixed and stained in 0.1% crystal violet and 100% ethanol as described above. The colonies were counted on all treatment and the control plates. The average colony number per treatment group and standard deviation were calculated and the mutation frequency was determined (Equation 6).

Equation 6. Mutant frequency calculation

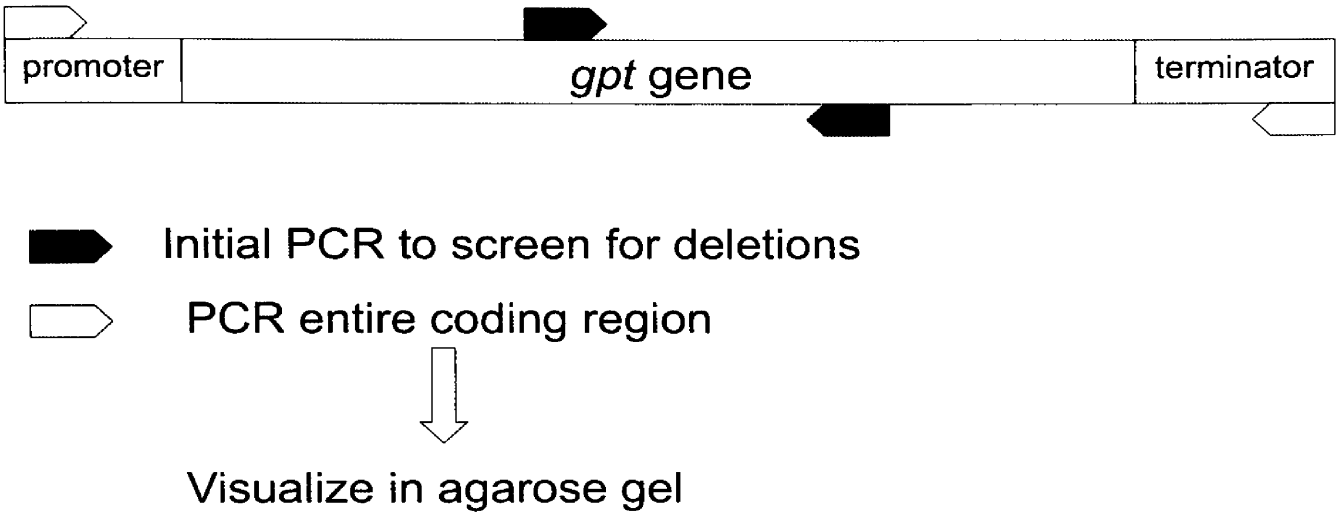
Mutation frequency = (average gpt$^-$ colony # in treatment ÷ average gpt$^-$ colony # in control) ÷ (average of cells surviving treatment ÷ average of cells surviving in untreated control) ÷ (plating efficiency)

Background deletion rate in spontaneous g12 cell mutants

6-thioguanine resistant clones arising spontaneously from untreated cells were expanded into 60 cm culture dishes. Single colonies were detached with one μl trypsin/EDTA. The cells were then seeded into five ml of complete medium in a 60 cm tissue culture dish and allowed to grow to confluence. Genomic DNA was isolated from the individual clones in order to screen for deletions at the *gpt* locus. The cell lysis and genomic DNA preparation were carried out identically for all mutagenesis experiments. Cells were harvested from confluent 60 cm tissue culture plates with a sterile cell scraper then washed in PBS and pelleted in a microcentrifuge tube. The cell pellets were suspended in 0.5 ml of TNES buffer (50 mM Tris, pH7.8-8, 400 mM NaCl,10 mM EDTA, 0.6% SDS) supplemented with proteinase K (10 mg/ml) and incubated 12 -18 hours at 55^{0}C in order to lyse the cells and digest cellular nucleases. The following morning 140 ml of 6 M NaCl was added, and samples were mixed vigorously for fifteen seconds. A microcentrifuge set at 12,000 x g for five minutes at room temperature was used to pellet the cellular debris. The supernatant was transferred to another microcentrifuge tube and an equivalent volume of ice cold isopropanol was added to to precipitate the DNA. The DNA was precipitated at ≥ 15,000 x g in a bench top microcentrifuge for one minute. The supernatant was removed and the DNA pellet was washed in 70% ethanol for five minutes at maximum speed in the benchtop centrifuge. The ethanol was removed and the DNA pellet was allowed to air dry. The pellets were suspended in TE buffer (10 mM Tris HCL, pH 8.0, 1 mM EDTA) and allowed to dissolve for at least an hour before measuring the DNA concentration.

DNA content was measured by reading the absorbance at 260 nm with a Nanodrop 2000C spectrophotometer. 100 ng/μl stocks of the clonal genomic DNA were prepared in 0.1 ml of TE buffer for use as templates in the PCR reactions. First, pcr using the chaperonin P1 primer set was used to determine if the genomic preps were of sufficient quality to screen with pcr (Figure 23). Amplification of the target sequence was assessed by the presence of a band at 1.5kB as determined by agarose gel electrophoresis. Samples that did not amplify were not used in further reactions. The qualified samples were then screened with two rounds of pcr (Figure 24). The first pcr reaction utilized closely spaced primer binding sites within the coding region of the *gpt* gene, and the second reaction primer binding sites that flanked the *gpt* coding region. In order to be considered a deletion mutation, the two *gpt* PCR reactions should yield no amplicon or an amplicon of altered size.

Figure 23. PCR screening strategy to determine deletion mutations in g12 cells.



Samples that did not produce a product for the *gpt* internal and coding region PCRs but did produce a 1.5 Kb amplicon for the chaperonin P1 positive control were considered transgene deletions.

PCR reactions were carried out as described previously (Romac et al., 1989) except the additional internal *gpt* primer set was used. Briefly, primers flanking and within the *gpt* transgene and the chaperonin P1 gene, as a positive control, were obtained (IDT, Coralville, IA). The primers were added to a PCR mix containing either mutant or control purified genomic DNA as a template, reaction buffer 2, and polymerase from the Accuprime PCR kit (Invitrogen, Carlsbad, CA) in a total volume of 15 μL. The 35 cycle PCR reactions conditions shown in Table 33 were carried out in a BioRad MyCycler thermocycler. The resulting amplicons were resolved by agarose gel electrophoresis to determine if the *gpt* transgene locus had undergone deletion or large mutations. The presence of an amplicon in the positive control reaction indicated the DNA was of suitable quality, and in conjunction with the absence of a product from the *gpt* reaction, indicated the presence of a deletion mutation. The sequence of the primer sets and the expected amplicon size is shown in Table 34.

Table 33. PCR conditions for each primer set

Chaperonin P1	Internal *gpt* region	Full *gpt* coding region
95° 2:00 Hot start	95° 2:00 Hot start	95° 2:00 Hot start
95° 0:30	95° 0:30	95° 0:30
56.7° 0:30	54.6° 0:30	48.8° 0:30
72° 1:30	72° 0:10	72° 0:33
35 cycles then hold at 10°	35 cycles then hold at 10°	35 cycles then hold at 10°

Table 34. Chaperonin P1 and *gpt* primer sets and expected amplicon size

Primer pair			**Expected amplicon size**
Internal *gpt*	Forward	5'-cgcactttgtcaccatcttcg -3'	101 bp
	Reverse	5'-atcccacggctgttcaatcc -3'	
gpt coding region	Forward	5'-aacacttttaagccgtagataaa-3'	561 bp
	Reverse	5'-tattgtaacccgcctgaagttaaa-3'	
Chaperonin P1	Forward	5'-aaggaaggctttgagaagatcagcaaa-3'	1500 bp
	Reverse	5'-cccattgcgcccattccagggtc-3'	

The chaperonin P1 primers amplify a fragment from within the chaperonin P1 gene. The internal *gpt* primers bind within the coding sequence of the *gpt* gene and the *gpt* coding region primer set flanks the entire coding region of the *gpt* transgene.

Deletion mutation frequency in g12 cells treated with teak wood dust extract, or 2-methylanthraquinone

Two different strategies were employed to generate multiple independent mutants. The first involved setting up 100 cm tissue culture dishes as described above in the mutant frequency experiments. The treatment was applied to the entire population of cells on the plates for 24 hours. The treatments consisted of five μg/ml of methanol extract from three independent teak wood samples and seven μg/ml of 2-methylanthraquinone. DMSO was used as the solvent vehicle and the doses selected were equally toxic. After the twenty-four hour exposure period, the cells were detached with trypsin/EDTA, collected, and suspended in fifty ml of non-selective F12 medium. A 48-well tissue culture dish was seeded with one ml of the cell suspension per well. Conducting the treatment over this time frame insured that the subsequent mutants were independent. The cells were allowed to recover for twenty-four hours and were then placed under 6-thioguanine selection for fourteen days and then evaluated with a microscope. Wells that contained a single colony were detached with trypsin/EDTA and expanded into individual 60 cm tissue culture dishes. The cells were grown to confluence and processed for recovery of genomic DNA as described above.

The second method employed to generate independent mutants involved treatment, recovery, and selection in 96-well tissue culture plates. g12 cells that were under HAT selection were placed in 96 well plates at 8000 cells per well or 100 cells per well. The plates with 8000 cells per well were treated with five or

seven μg/ml of methanol extract of teak wood dust or seven μg/ml 2-methylanthraquinone. The plate with 100 cells per well was used for the solvent control. The reasoning behind this was that the treatment with 2-methylanthraquinone and the teak extracts was toxic and killed about half of the cells in each well and the surviving subset slowly resumed cell division. The DMSO treated cells had no treatment toxicity and continued doubling every sixteen hours. Thus, the relative cell number per well across the plates was similar after the treatment and growth periods. The cells were treated with medium containing either 2-methylanthraquinone, one of the methanol extracts of teak wood dust, or DMSO vehicle. The exposure duration lasted 24 hours, the treatment medium was replaced with fresh F12 medium. The cells were allowed to grow an additional six days to ensure that any mutants that survived had no functional *gpt* protein. Medium was changed daily during this time. Following the six day expression and recovery period, the cells were placed under selection with complete F12 medium containing 10 μg/ml 6-thioguanine. The cells were incubated for an additional fourteen days with a medium change on day seven. Then each well on each plate was evaluated for single colony growth by viewing with a microscope. Wells that had a single colony were treated with trypsin/EDTA, and the clones were collected and expanded by seeding into five mL of medium in a 60 cm tissue culture dish. The mutant clones were processed for DNA recovery as described above.

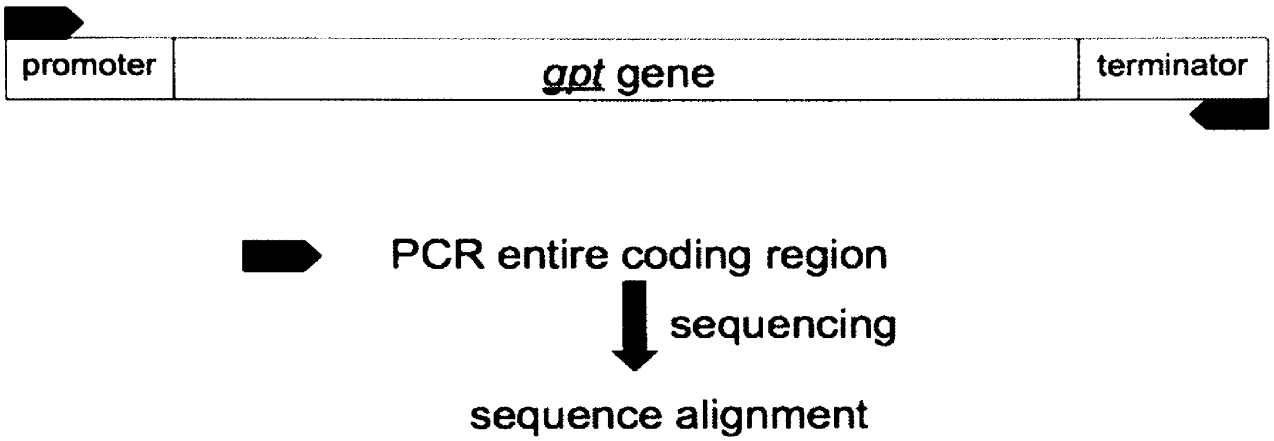
DNA samples from 6-thioguanine resistant mutants from vehicle control and chemically-treated cells were subjected to PCR as described above. The resulting amplicons were resolved by agarose gel electrophoresis to determine if the *gpt*

transgene locus had undergone deletion or large mutations. The presence of an amplicon in the positive control reaction indicated the DNA was of suitable quality, and in conjunction with the absence of a product from the gpt reaction, indicated the presence of a deletion mutation. Figure 23 outlines the PCR screening strategy.

Identification and characterization of small mutations in the *gpt* gene

Full length 561 bp amplicons were sequenced to identify internal mutations (Figure 24). The reactions were carried out using the same primers, conditions, and reagents as described above, but the reaction volume was scaled up to 50 µl. The reactions were checked for amplification by visualization in an agarose gel, and then purified with minelute PCR reaction clean up columns following the manufacturer's protocol. (Qiagen, Valencia CA) The concentration of DNA in the purified amplicons was determined with a spectrophotemter at 260 nm. The amplicons were diluted 50 ng/µL in a total of 10 µL and were sent to SeqWright, Inc. (Houston, TX) to be sequenced. Forward and reverse primers were used to obtain sequence information for both gpt DNA strands. The mutated sequences were compared to the *gpt* reference sequence via a Clustal W Alignment generated by MacVector 9.0 computer software. Mutations in the coding sequence of the gpt gene were translated *in-silico* to identify the effect at the protein level. The sample size of the sequencing experiment is shown in Table 35.

Figure 24. PCR amplification of gpt gene for sequence analysis



Forward and reverse primers (black arrows) bind ~50 nucleotides up and downstream from the start and stop codon of the *gpt* gene. Amplification yields a 561 base pair fragment that contains the 460 base pair open reading frame of the *gpt* transgene.

Table 35. Sample sizes used in sequence analysis

Treatment	**Mutants obtained**	***gpt* amplicons obtained**	**Amplicons sequenced**
DMSO	27	20	20
2-Methylanthraquinone	64	40	26
Teak extract #1	49	32	25
Teak extract #2	60	36	24
Teak extract #3	63	40	31

The first column defines the treatments. The second column lists the total number of observed 6-TG resistant clones per treatment. The third column lists the number of gpt amplicons obtained from 6-TG mutants in each treatment group. The fourth column lists the number of amplicons that provided sequence information.

Results

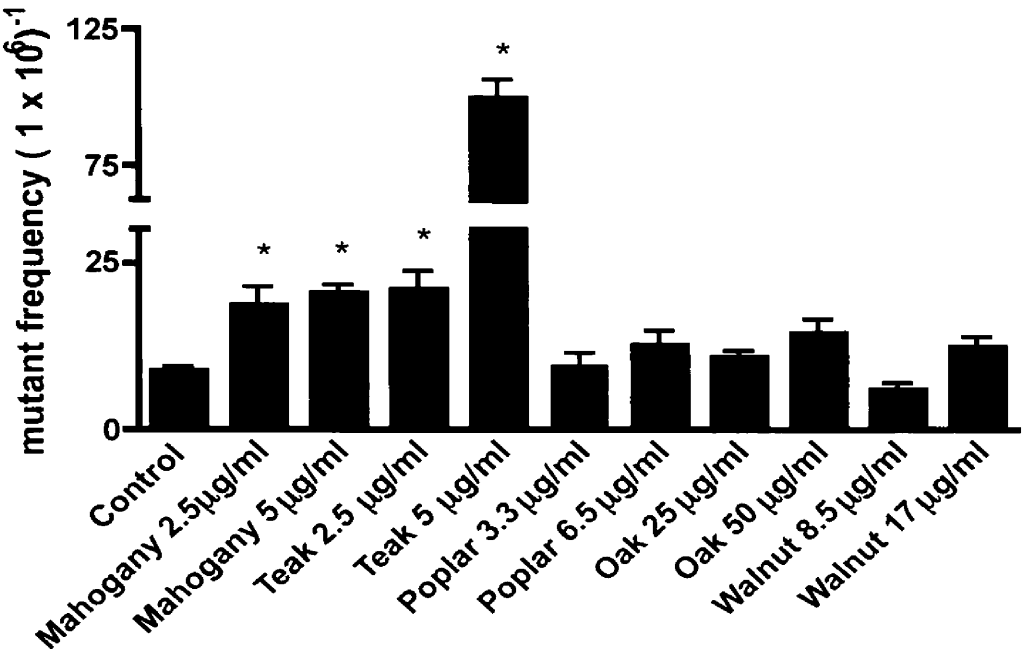
Mutation frequency experiment

Approximately a two-fold increase in mutant frequency was observed following treatment with 2.5 and 5 μg/ml of mahogany wood methanol extract and 2.5 μg/ml teak wood methanol extract (Figure 25). Treatment with 5 μg/ml teak wood extract resulted in a ten-fold increase in mutant frequency relative to control. Approximately a two-fold increase in mutant frequency was observed following treatment with 13.5 μg/ml of cypress wood methanol extract. Treatment with 40 μg/ml spruce wood extract resulted in a three-fold increase in mutant frequency relative to control (Figure 26). Approximately a two- fold increase in mutant frequency was observed following treatment with 3 μg/ml of MDF dust methanol extract and 40 μg/ml hardwood laboratory animal bedding extract (Figure 27).

Table 36. Cytotoxicity of methanol extracts of wood dusts in the g12 cells

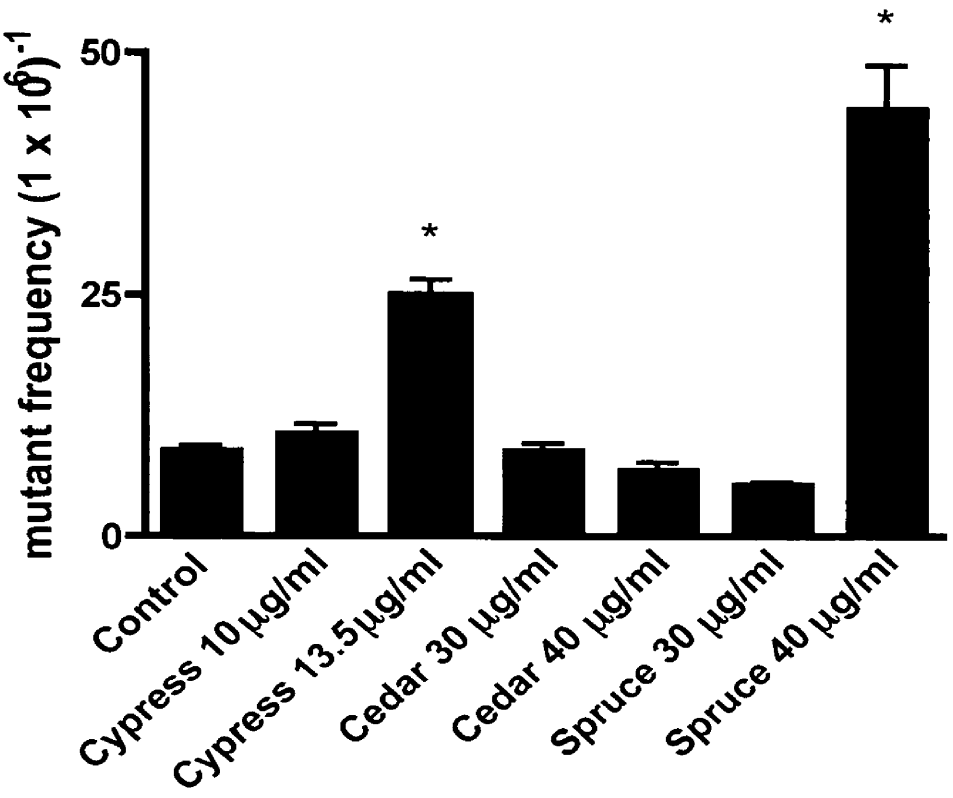
Hardwoods		Softwoods		Wood products	
Species	LD_{50} (µg/ml)	Species	LD_{50} (µg/ml)	Type	LD_{50} (µg/ml)
Teak	5	Spruce	30	Medium density fiberboard	6
Mahogany	8	Cypress	10	Hardwood bedding	30
Red Oak	50	Cedar	40		
Walnut	17	Spanish cedar	30		
Poplar	6.5				

Figure 25. 6-thioguanine resistant gpt mutant frequency observed following treatment with methanol extracts of hardwoods



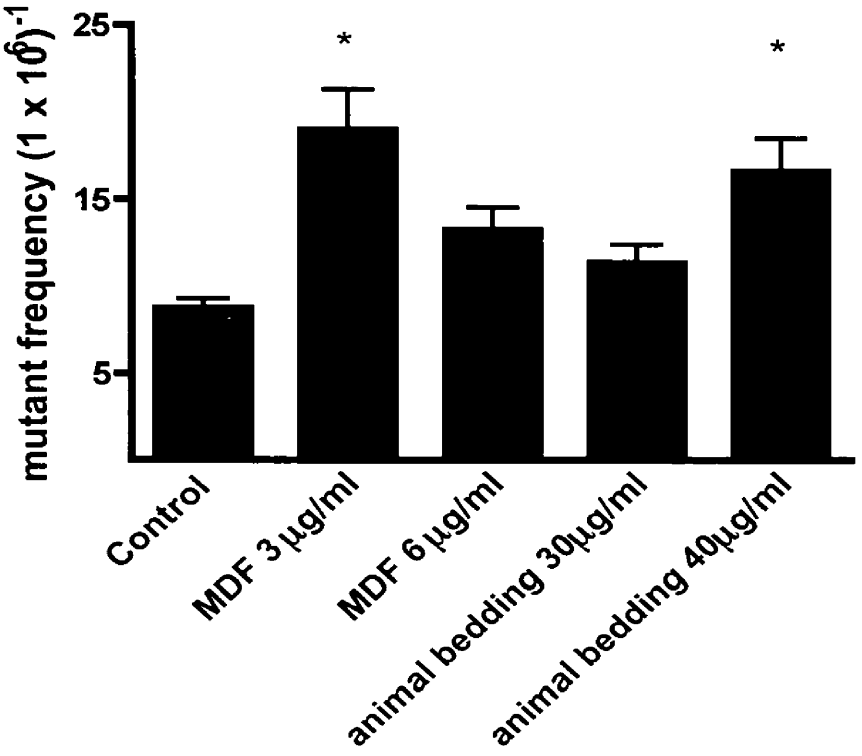
The first bar on the left reflects the mutation frequency determined for the solvent (DMSO) treated g12 cell cultures. Doses of methanol extracts of hardwood dusts were used at LD_{25} (lower concentration) and LD_{50} (higher concentration) levels of cytotoxicity to examine the mutagenic potential of methanol wood dust extracts. Statistical significance of $p < 0.05$, indicated by asterisks, was determined by one way ANOVA followed by Dunnett's post-hoc pairwise comparisons.

Figure 26. 6-thioguanine resistant gpt mutant frequency observed following treatment with methanol extracts of softwoods



The first bar on the left reflects the mutation frequency determined for the solvent (DMSO) treated g12 cell cultures. Doses of methanol extracts of softwood dusts were used at LD_{25} (lower concentration) and LD_{50} (higher concentration) levels of cytotoxicity to examine the mutagenic potential of methanol wood dust extracts. Statistical significance was determined by one way ANOVA followed by Dunnett's post-hoc pairwise comparisons

Figure 27. 6-thioguanine resistant gpt mutant frequency observed following treatment with methanol extracts of wood products

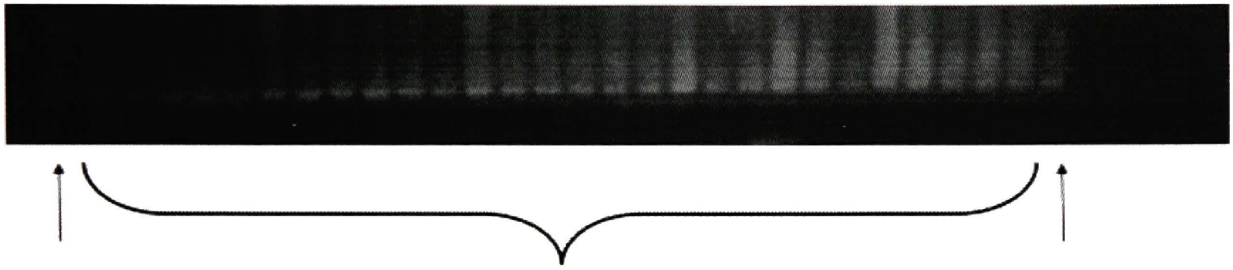


The first bar on the left reflects the mutation frequency determined for the solvent (DMSO) treated g12 cell cultures. LD_{50} treatments of methanol wood product dust extract produced significant differences in mutant frequency relative to control in two of the wood product dust extract treatments. Approximately a two- fold increase in mutant frequency was observed following treatment with 3 µg/ml of MDF dust methanol extract and 40 µg/ml hardwood laboratory animal bedding extract. Statistical significance was determined by one-way ANOVA followed by Dunnett's post-hoc pairwise comparisons.

Background deletion rate in spontaneous g12 cell mutants

Twenty-eight of the genomic DNA preparations isolated from 6-thioguanine resistant control mutants produced the amplicon of the positive control chaperonin P1 gene (Figure 28). This indicated the DNA was of sufficient quality to proceed with determination of the background deletion frequency. The small internal primer set was used to screen the samples for deletion mutations in the gpt transgene locus (Figure 29). Four of the twenty-eight samples did not amplify during this PCR. This indicates the background deletion rate is ~14%. Deletions were verified with a second PCR that revealed the lack of an expected 561 base pair product (Figure 30).

Figure 28. Agarose gel to visualize the chaperonin P1 PCR amplicons

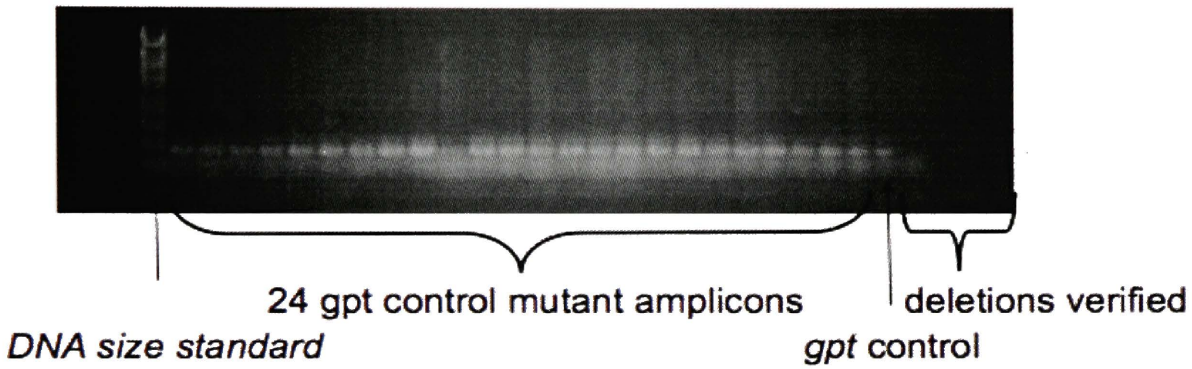


DNA size standard 28 6-TG resistant control mutants *gpt* positive control

Figure 29. Nusieve agarose gel to visualize the small internal *gpt* PCR amplicons

Samples were separated using a 2% Nusieve gel. The arrows indicate deletion mutations. Four out of the twenty-eight spontaneous mutants had deletions of the gpt gene as indicated by the lack of the 101 base pair product.

Figure 30. Agarose gel of 516 bp gpt amplicons

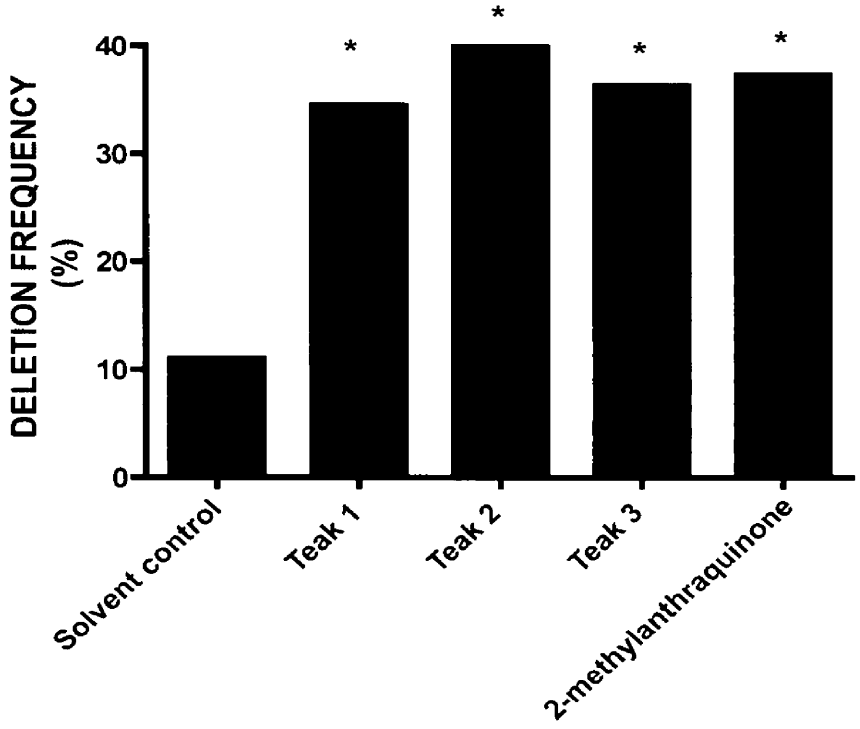


the four deletions shown in figure 29 were confirmed using a second PCR designed to cover the entire gpt gene.

Deletion frequency in g12 cells treated with teak wood dust extract, or 2-methylanthraquinone

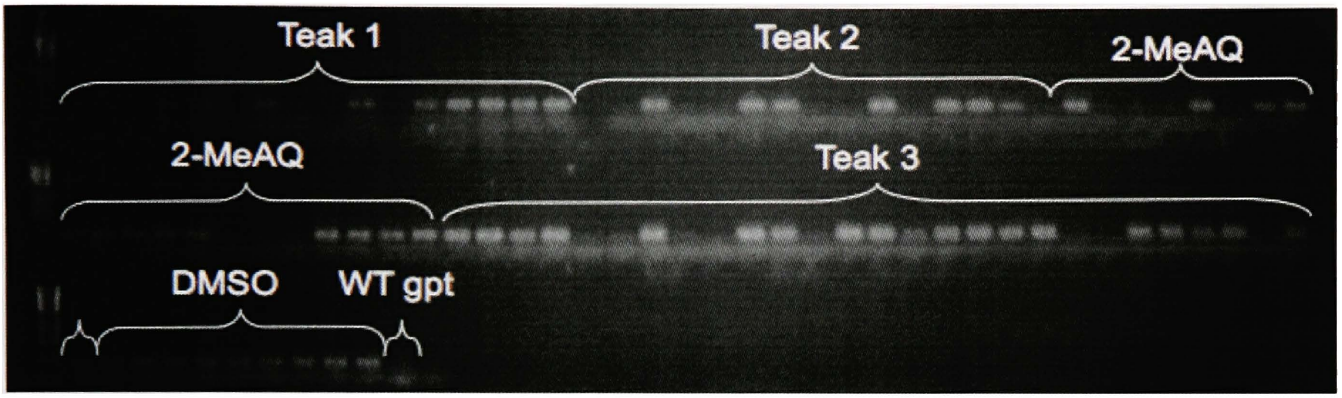
Mutants arising from teak or 2-methylanthraquinone treatments were assessed for deletions as described above. The statistical significance of the deletion frequencies were determined with Fisher's exact test using the Prism software package (Analysis tables in supplementary data). Extracts from three separate teak samples resulted in 35%, 40%, and 37% deletion rates. A similar deletion frequency, 38%, was observed in the 2-methylanthraquinone treated mutants. (Figure 31) All these increased deletion frequencies due to treatments were significantly different than the deletion frequency in the control treatment (Figures 32-35).

Figure 31. Deletion frequency in g12 cells treated with teak wood dust extract and 2-methylanthraquinone



The bar represents the percent of recovered mutants with deletions of the entire *gpt* gene following treatment with teak wood dust extract or 2-methylanthraquinone compared to the solvent, DMSO, control. Asterisks indicate values that were statistically different than the control as determined by Fisher's exact test, with $p < 0.05$.

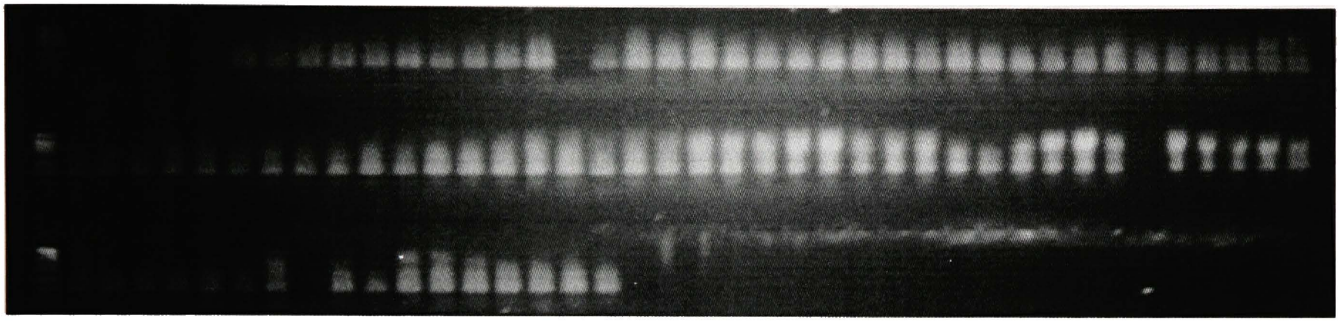
Figure 32. Identification of gpt deletions caused by teak or 2-methylanthraquinone



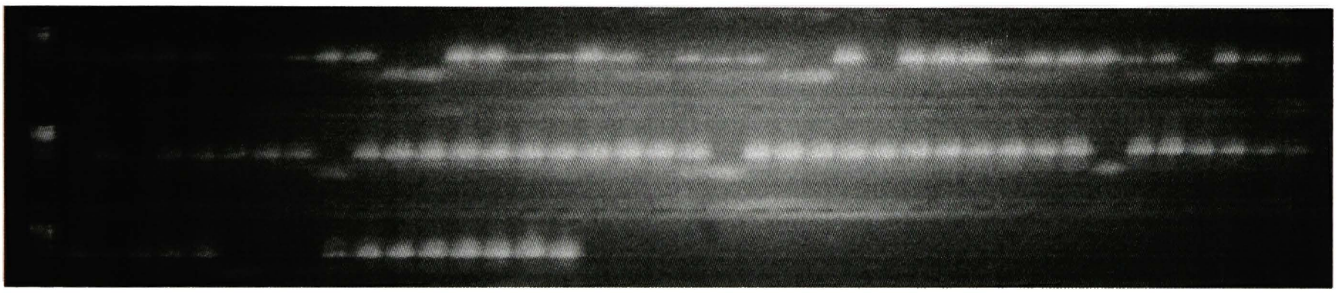
These mutants were isolated using the 48-well protocol described in the methods section. Deletion mutations of the gpt gene (bands absent) are due to treatment with teak wood dust extract (5 μg/ml) or 2-methylanthraquinone (7 μg/ml).

Figure 33 (A-C). 96-well format chaperonin P1 amplification from *gpt* mutants

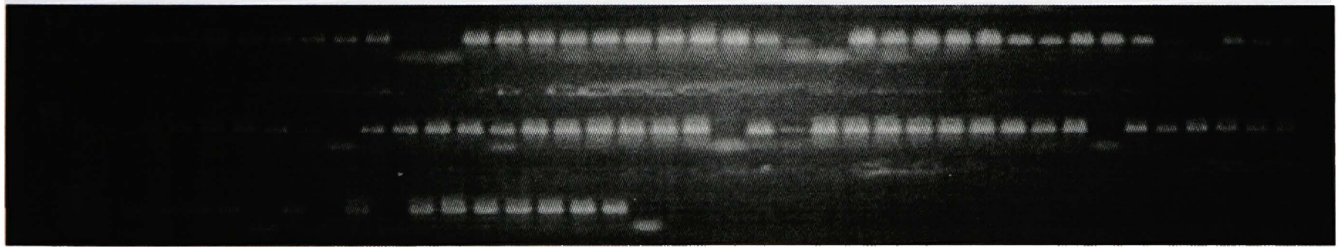
A



B



C



A) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three to twenty are the solvent control treatment. Lanes twenty-one to forty are from the 2-methylanthraquinone treatment group. Lanes two, three, five, and six do not show a product due to technical error. The sample analysis was repeated and all produced the target amplicons in the PCR reactions.

Row two. Lane one is the DNA standard. Lanes two to twenty-five are the from the 2-methylanthraquinone treatment group. Lanes twenty-six to forty are from teak dust extract # 1 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak dust extract # 1 treatment group at five μg/ml.

B) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three to five are the remaining teak dust extract # 1 group at five μg/ml. Lanes six through forty are from the teak dust extract # 1 treatment group at seven μg/ml.

Row two. Lane one is the DNA standard. Lanes two to five are from the teak dust extract #1 treatment group at seven μg/ml. Lanes six through forty are from the teak dust extract #2 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through seventeen are from the teak dust extract #2 treatment group at five μg/ml.

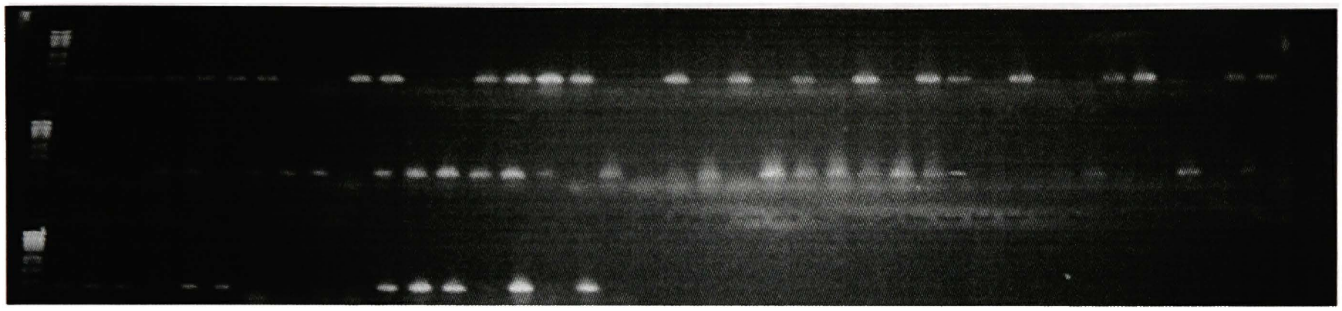
C) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three through thirty-eight are from the teak extract #2 treatment at seven μg/ml. Lanes thirty-nine and forty are from the teak wood dust extract #3 treatment at five μg/ml.

Row two. Lane one is the DNA standard. Lanes two through thirty-seven are from the teak wood extract #3 treatment group at five μg/ml. Lanes thirty-eight to forty are from the teak wood extract #3 treatment group at seven μg/ml.

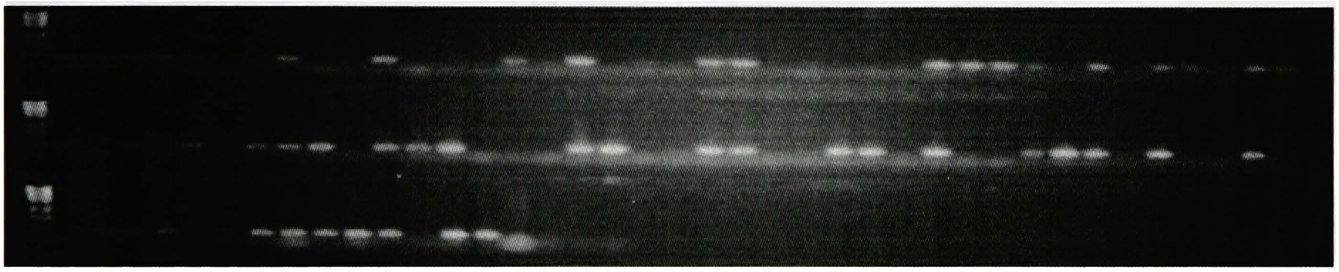
Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak wood extract #3 treatment group at seven μg/ml.

Figure 34 (A-C). Deletion mutations of the full length *gpt* DNA sequence caused by teak extract or 2-methylanthraquinone.

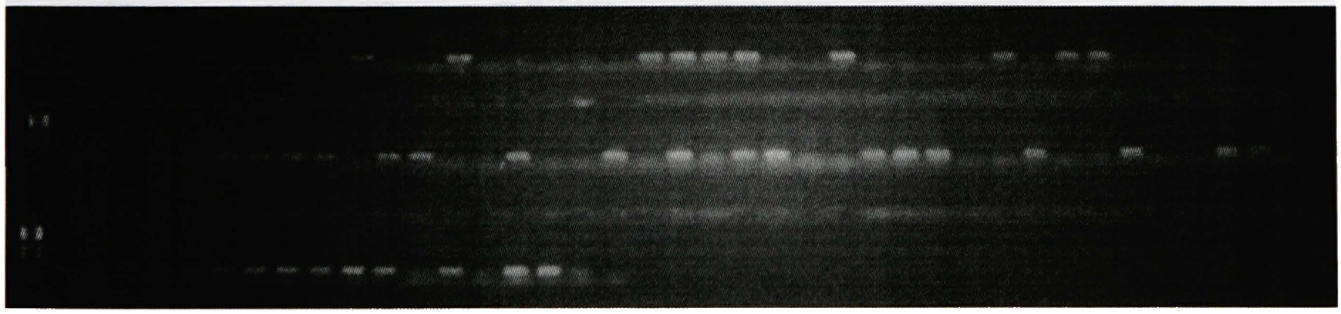
A



B



C



A) Row one. Lane one is the DNA standard. Lane two is the untreated positive control DNA that yields the expected 561 bp pcr product . Lanes three to twenty are PCR reactions from mutants treated with solvent alone. Lanes twenty-one to forty are from the 2-methylanthraquinone treatment group.

Row two. Lane one is the DNA standard. Lanes two to twenty-five are the from the 2-methylanthraquinone treatment group. Lanes twenty-six to forty are from teak dust extract # 1 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak dust extract # 1 treatment group at five μg/ml.

B) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three to five are the remaining teak dust extract # 1 group at five μg/ml. Lanes six through forty are from the teak dust extract # 1 treatment group at seven μg/ml.

Row two. Lane one is the DNA standard. Lanes two to five are from the teak dust extract #1 treatment group at seven μg/ml. Lanes six through forty are from the teak dust extract #2 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through seventeen are from the teak dust extract #2 treatment group at five μg/ml.

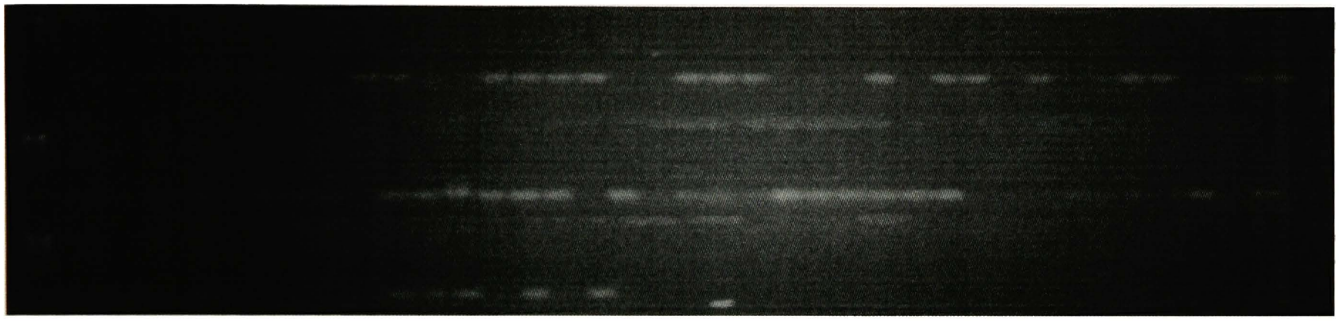
C) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three through thirty-eight are from the teak extract #2 treatment at seven μg/ml. Lanes thirty-nine and forty are from the teak wood dust extract #3 treatment at five μg/ml.

Row two. Lane one is the DNA standard. Lanes two through thirty-seven are from the teak wood extract #3 treatment group at five μg/ml. Lanes thirty-eight to forty are from the teak wood extract #3 treatment group at seven μg/ml.

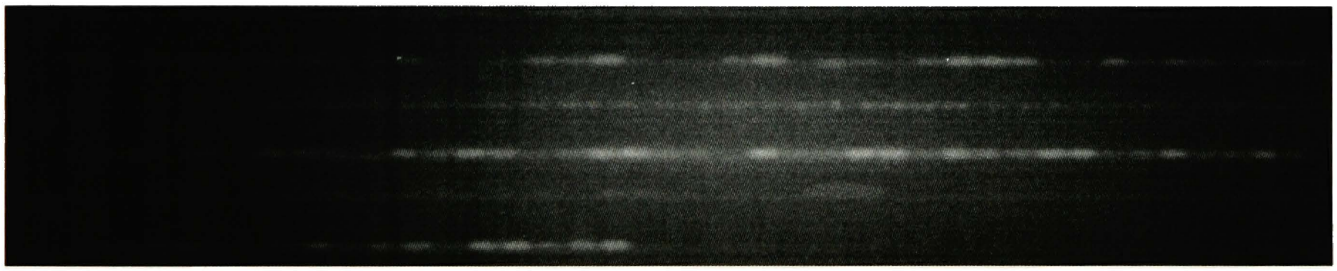
Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak wood extract #3 treatment group at seven μg/ml.

Figure 35 (A-C). Deletion mutations of the small internal *gpt* DNA sequence caused by teak extract or 2-methylanthraquinone.

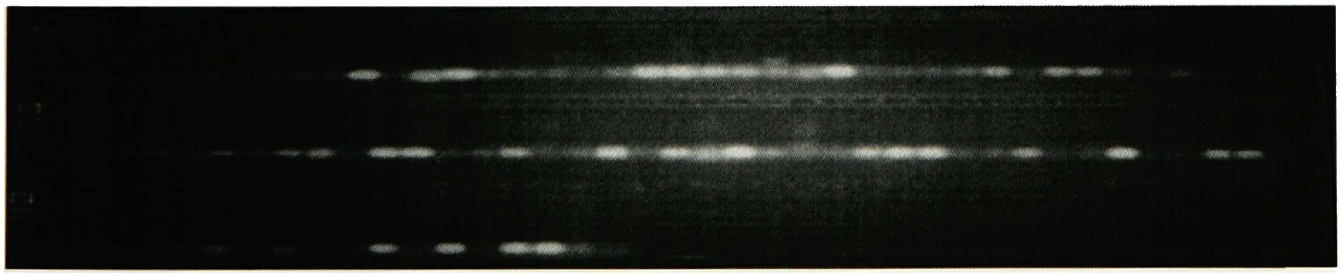
A)



B)



C)



A) Row one. Lane one is the DNA standard. Lane two is the untreated positive control DNA that yields the expected 101 bp product. Lanes three to twenty are the solvent control treatment. Lanes twenty-one to forty are from the 2-methylanthraquinone treatment group.

Row two. Lane one is the DNA standard. Lanes two to twenty-five are the from the 2-methylanthraquinone treatment group. Lanes twenty-six to forty are from teak dust extract # 1 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak dust extract # 1 treatment group at five μg/ml.

B) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three to five are the remaining teak dust extract # 1 group at five μg/ml. Lanes six through forty are from the teak dust extract # 1 treatment group at seven μg/ml.

Row two. Lane one is the DNA standard. Lanes two to five are from the teak dust extract #1 treatment group at seven μg/ml. Lanes six through forty are from the teak dust extract #2 treatment group at five μg/ml.

Row three. Lane one is the DNA standard. Lanes two through seventeen are from the teak dust extract #2 treatment group at five μg/ml.

C) Row one. Lane one is the DNA standard. Lane two is the untreated positive control. Lanes three through thirty-eight are from the teak extract #2 treatment at seven μg/ml. Lanes thirty-nine and forty are from the teak wood dust extract #3 treatment at five μg/ml.

Row two. Lane one is the DNA standard. Lanes two through thirty-seven are from the teak wood extract #3 treatment group at five μg/ml. Lanes thirty-eight to forty are from the teak wood extract #3 treatment group at seven μg/ml.

Row three. Lane one is the DNA standard. Lanes two through eighteen are from the teak wood extract #3 treatment group at seven μg/mL.

Identification and characterization of small mutations in the *gpt* gene

Among the 6-TG resistant clones recovered in the solvent control treatment group, 80% did not have any changes to the *gpt* coding sequence. (Figure 36). Fifteen % of the solvent treated cells had a complete deletion of the full-length *gpt* coding sequence. A single point mutation, the G > A transition at position 241 representing 5% of the mutants, was observed. The sequence changes, mutation types, and effect at the protein level are shown in tables 36-40 for each treatment. The individual sequence alignments for all treatment groups are attached as supplementary data. The sequences from mutants exposed to teak wood dust extract were pooled and compared to the solvent control group and the 2-methylanthraquinone treatment group. The number of 6-TG resistant clones with no change to the *gpt* coding region was 40% for the teak wood dust extract and 48% 2-methylanthraquinone treatment group respectively (Table 37). Deletion of the entire *gpt* sequence was found in 47% of mutants from the teak wood dust extract group, 43% of 2-methylanthraquinone mutants, and 15% in the solvent control mutants. DMSO (5%), teak wood dust extract (6%) and 2-methylanthraquinone (2%) treatments produced a low frequency of transition point mutations in the *gpt* gene. One transition mutation, (G > A at position 241) was observed in the all treatment groups. As such, this should be considered as a background mutation. Transversion point mutations accounted for 1% of observed mutations in the pooled teak wood dust extract treatment group and were not present in the 2-methylanthraquinone or solvent control treatment groups. A single mixed mutation observed in the 2-methylanthraquinone treatment

group (Table 39). This mutation consisted of a repeated AATAAT, followed by a single G, then a single nucleotide deletion. The frequency of these observations is shown in Table 37. Both teak extract and 2-methylanthraquinone treatments produced insertion and deletion (INDEL) mutations, 5% and 4% respectively. Treatment with teak wood produced primarily deletions greater than a single nucleotide, while 2-methylanthraquione treatment was associated with insertions greater than a single nucleotide. The teak treatment group also displayed insertion mutations greater than a single nucleotide, and the 2-methylanthraquinone treatment group had a single deletion greater than a single nucleotide. No INDEL mutations were observed in the solvent control treatment group.

The variability among independent teak wood samples is gauged in Figure 37. The frequency of the individual mutation types for each teak wood treatment is shown in Table 37. The percentages of 6-TG mutants that did not show any sequence differences at the gpt locus were 44% for teak extract one, 33% for extract two, and 43% for extract three. Frequencies of mutants that lost the entire *gpt* gene ranged from 44% to 54%, making this the most commonly type of observed mutation. Transition mutations were found in frequencies of 2% for teak extract 1, 7% for teak extract two, and 10% for teak extract three. Transversion mutations were found only in mutants from teak wood dust extract #2. All of the teak extract treatments produced INDEL mutations ranging from 2% to 9% in frequency. Treatment with teak wood dust extract number 1 produced four INDELS (Table 40). Three mutants contained three-nucleotide deletions and one had a four-nucleotide insertion. Treatment with teak wood dust extract number two produced one three-

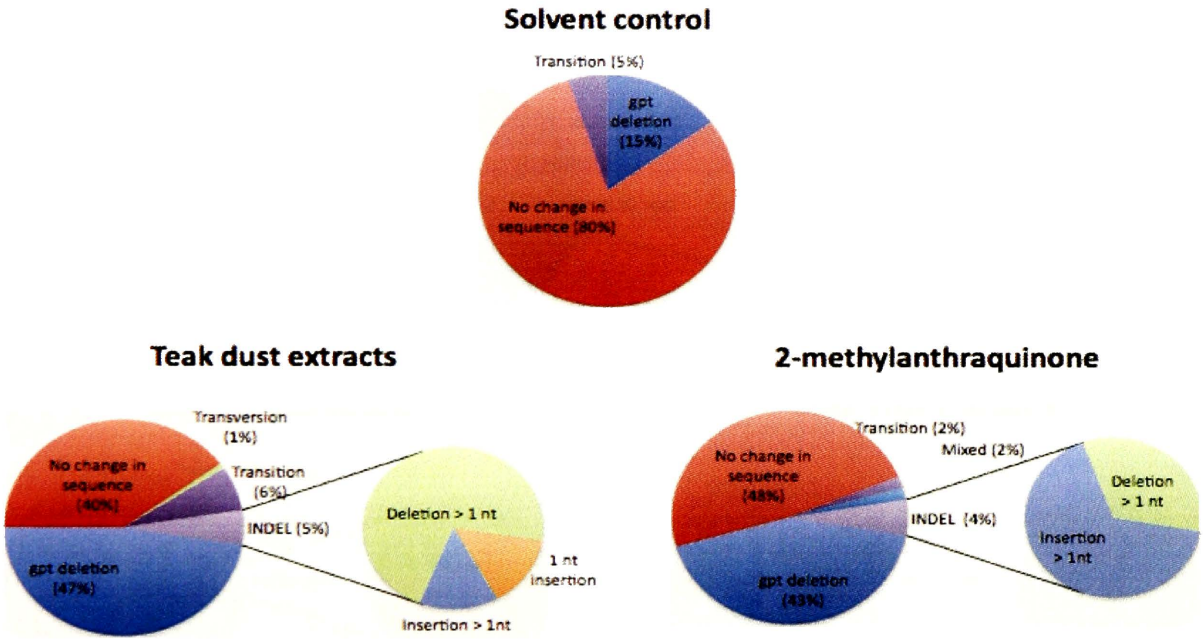
nucleotide deletion and one four-nucleotide insertion (Table 41). Treatment with teak wood dust extract number three resulted in a single six-nucleotide deletion (Table 42).

Table 37. Frequency of observations in sequence analysis

Sequence Change	**DMSO**	**MeAQ**	**Pooled teak samples**	**Teak 1**	**Teak 2**	**Teak 3**
gpt deletion	(3/20)	(24/56)	(64/135)	(17/42)	(24/45)	(23/51)
None	(16/20)	(27/56)	(54/135)	(20/42)	(15/45)	(22/51)
Transversion	-	-	(1/135)	-	(1/45)	-
Transition	(1/20)	(1/26)	(9/135)	(1/42)	(3/45)	(5/51)
Mixed	-	(1/56)	-	-	-	-
INDEL	-	(3/56)	(7/135)	(4/42)	(2/45)	(1/51)
1 nt insertion	-	-	(1/7)	-	(1/2)	-
Insertion > 1nt	-	(2/3)	(1/7)	(1/4)	-	-
1 nt deletion	-	-	-	-	-	-
Deletion > 1 nt	-	(1/3)	(5/7)	(3/4)	(1/2)	(1/1)

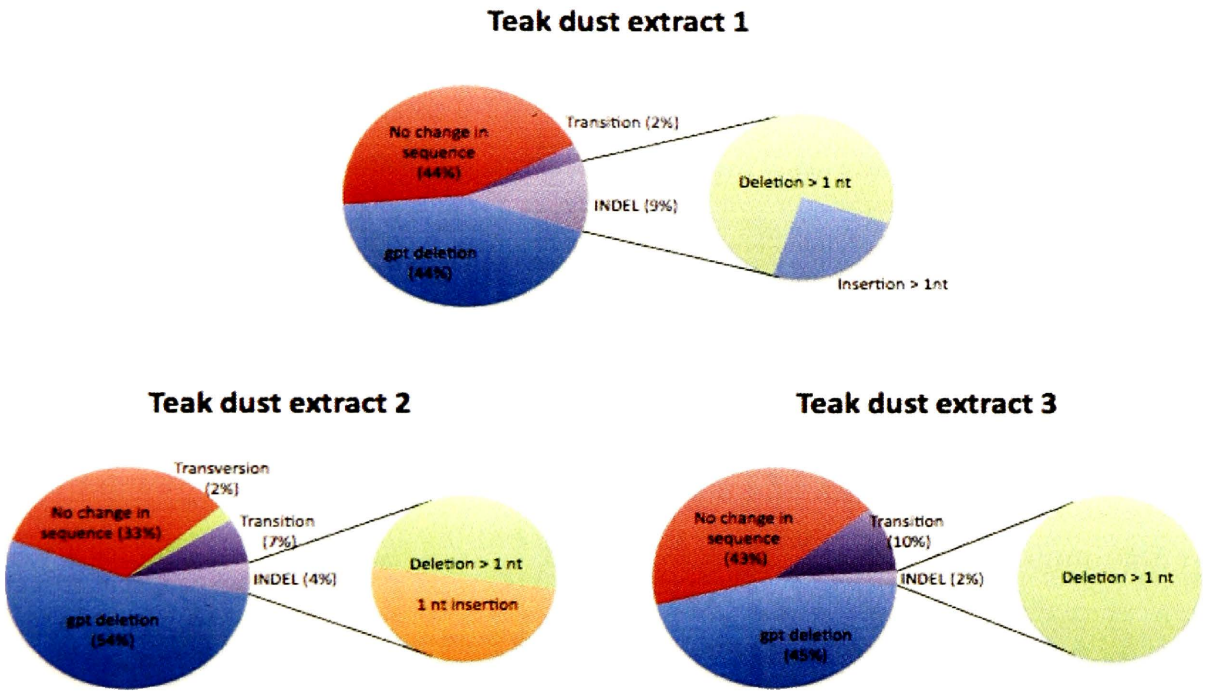
The top row defines each treatment group. The first column lists each of the observed mutation types. The data is displayed as (# of specific mutation events / total number of mutation events). The INDEL category is divided by the types of insertions and/or deletions. The INDEL data is displayed as (type of INDEL / total number of observed INDELs). The pooled teak category represents the sum of the mutations derived from the three teak dust extracts when considered as a single group. This pooling was justified by the similarity of mutations obtained from the individual teak dust extract treatments.

Figure 36. Sequence differences in solvent, teak wood dust extract, and 2-methylanthraquinone treatments



The red portions represent 6-TG resistant clones that did not have a change in the *gpt* gene coding region. The dark blue sections represent large deletions of the full-length *gpt* gene. The dark purple portion represents transition point mutations and the light green represents transversion point mutations. The light purple portion represents small insertion and deletion mutations (INDELs). The types of INDEL mutations are shown in the graphs on the right in the two lower charts. The light green portion represents deletions greater than a single nucleotide, the blue portion represents insertions greater than a single nucleotide, and the orange section represents insertions of a single nucleotide.

Figure 37. Sequence differences following treatment with three teak wood dust extracts



The red portion of the graphs represents 6-TG resistant clones that did not have a change in the *gpt* gene coding region. The dark blue portion represents deletions of the full-length *gpt* gene. The dark purple portion represents transitions and the light green represents transversion mutations. The light purple portion represents insertion and deletion mutations or INDELs. The types of INDEL mutations are shown in the smaller circles. The light green portion represents deletions greater than a single nucleotide, the blue portion represents insertions greater than a single nucleotide, and the orange represents insertions of a single nucleotide.

Table 38. DNA and protein sequence changes in DMSO treatment group.

Mutant ID	**DNA mutation (position)**	**Mutation type**	**Protein change (residue)**	**Result**
DMSO 86	G > A (241)	Transition	G > S (81)	Missense

Table 39. DNA and protein sequence changes in 2-methylanthraquinone treatment group

Mutant ID	**DNA mutation (position)**	**Mutation type**	**Protein change (residue)**	**Result**
MEAQ 41	G > A (241)	Transition	G > S (81)	Missense
MEAQ S-68	CGG inserted (116↓117)	Insertion	G inserted (39)	Residue is next to substrate binding site
MEAQ S-60	C > A (109)	Transversion	R > N (36)	Missense
	G > A (110, 112, 113)	Transition	G > N (37)	Missense Missense
	C > T (114)	Transition	G > X (38)	Missense
	G deleted (116)	Deletion		All changes at substrate binding residues
MEAQ S-38	CACG inserted (33↓34)	Frameshift	STOP (22)	Nonsense Truncated
MEAQ S-55	20 bp deletion (216-236)	Frameshift	STOP (94)	Nonsense Truncated

Table 40. DNA and protein sequence changes in teak wood dust extract #1 treatment group

Mutant ID	**DNA mutation (position)**	**Mutation type**	**Protein change (residue)**	**Result**
Teak 1 S-133	AAG Deleted (233-235)	Deletion	E > G (78) G deleted (79)	Missense 9 and 10 residues from catalytic site
Teak 1 S-126	TGA Deleted (369-371)	Deletion	D deleted (134)	Deletion
Teak 1 S-119	ATT deleted (97-99)	Deletion	V > S (35) S deleted (36)	Missense Deletion S(36) is substrate binding
Teak 1 S-106	AATA Inserted (15↓16)	Insertion	STOP (5)	Frameshift Truncated
Teak 1 S-95	C > T (55)	Transition	L > F (19)	Missense

Table 41. DNA and protein sequence changes in teak wood dust extract #2 treatment group

Mutant ID	**DNA mutation (position)**	**Mutation type**	**Protein change (residue)**	**Result**
S-184	TGA deleted (369-371)	Deletion	D deleted (124)	Deletion
S-200	TATC inserted 387↓388	Insertion Frameshift	STOP (137)	Nonsense Truncated
S-223	A > C (345)	Transversion	K > N (115)	Missense K(115) is catalytic
S-226	G > T (116)	Transversion	G > C (39)	Missense
S-159	C > A (49) G > A (50)	Transversion Transition	R > N (17)	Missense Residue in helix 1

Table 42. DNA and protein sequence changes in teak wood dust extract # 3 treatment group

Mutant ID	**DNA mutation (position)**	**Mutation type**	**Protein change (residue)**	**Result**
S-260	G > A (107)	Transition	S > N (36)	Missense Residue binds substrate
55	G > A (241)	Transition	G > S (81)	Missense Seven residues from catalytic site
S-331	G > A (87)	Transition	STOP (29)	Nonsense Truncated
S-311	6 bp deletion (238-243)	Deletion	D deleted (80) G deleted (81)	Deletion Eight residues from catalytic site
S-301	G > A (92)	Transition	G > D (31)	Missense Five residues from substrate

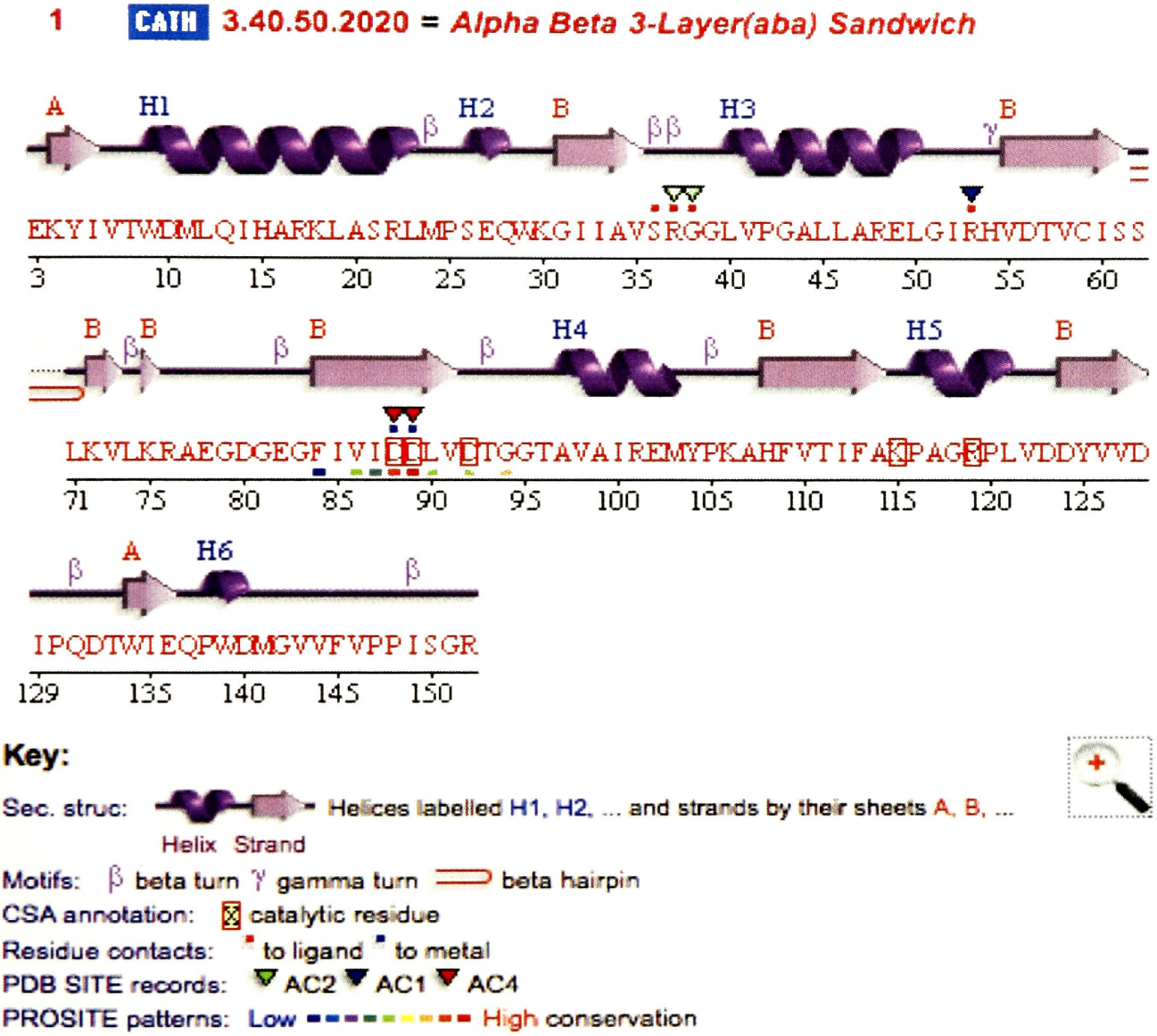
				binding site
S-298	T > C (56)	Transition	L > P (19)	Missense
				In helix 1

Discussion

The *gpt* gene encodes the tetrameric xanthine-guanine phosphoribosyltransferase enzyme that genetically complements the defective purine nucleotide salvage pathway in the g12 cells. The catalytic and ligand binding residues as well as the crystal structure of the enzyme have been identified (Figure 38)(Vos et al., 1997). Compared to the mammalian hprt gene, which it replaces, the *gpt* gene is quite small. The entire coding region is 459 base pairs long. Reasonably high doses of genotoxic chemicals are required to cause detectable levels of mutations in such a small target region. Treatment with teak wood dust extracts and 2-methylanthraquinone at doses between LD_{50} and LD_{75} levels produced mutations in the *gpt* target gene. Other investigators that employed the g12 cell line reported that similar toxicity was required to observe appreciable mutations within the *gpt* gene (Klein et al., 1994a; Klein et al., 1994b; Klein et al., 1997). In our studies we used equally toxic doses of wood dust extracts and 2-methylanthraquinone for comparative purposes.

Klien et al. reports g12 cells display a variable response to mutagens (Table 43) We found that treatment with different wood dust extracts also produced a variable mutation rate based on wood type. Teak wood dust induced the highest mutation rate among the wood dusts tested (Table 44) Teak wood dust extract induced a mutation rate comparable to the known mutagenic agents UV radiation and mAMSA .

Figure 38. Amino acid sequence and structural motifs of the *gpt* protein



The source of secondary structural information for the gpt protein was the EMBL-EBI server: (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=1nul&template=protein.html&r=wiring&l=1&chain=A, accession date 5/15/10)

Table 43. g12 cell mutation frequencies reported by other investigators

Mutagen	**6-TG mutatgenesis (times background)**
X-Ray	18 X
Bleomycin	8 X
mAMSA	10 X
UV	10 X

Table 44. g12 cell mutation frequencies in response to wood dusts

Wood dust	**6-TG mutatgenesis (times background)**
Teak	10 X
Mahogany	2 X
Cypress	2 X
Spruce	3 X
MDF	2 X
Laboratory animal bedding	2 X

Eighty percent of the spontaneous 6-TG resistant clones did not have changes to the *gpt* coding sequence. The spontaneous full-length *gpt* gene deletion rate of 14% to 17% was consistent with the reported composite 20% spontaneous *gpt* deletion rate generated from several previous reports using the g12 cell line (Klein et al., 1997; Lee et al., 1993). Other transgenic *gpt* cell lines, like the CHO-derived AS52 cells, have spontaneous *gpt* deletion rates approaching 80% (Stankowski and Hsie, 1986). The low spontaneous deletion rate indicates the g12 cell assay is a sensitive method for assessing mutagenic potential of chemicals.

In the cells treated with teak extract, the most common mutation was deletion of the entire *gpt* transgene. Treatment with teak wood dust extract produced a three-fold increase in the deletion of the *gpt* transgene compared to the solvent control. In a similar manner, the 2-methylanthraquinone caused deletion of the *gpt* transgene at a frequency that was three-fold greater than the solvent control. The abundance of 2-methylanthraquinone in teak wood dust and its similar pattern of deletion mutations suggest it may account for a significant portion of the mutagenic activity of teak dust. Large deletions are the signature mutation associated with exposure to teak wood dusts, 2-methylanthraquinone and possibly compounds found in other wood dusts. The frequency of *gpt* deletion was comparable to results reported by Klein, et al, for X-ray treated and bleomycin treated g12 cells (Klein et al., 1997). Both of these treatments result in double strand breaks in DNA.

Deletion of the *gpt* gene requires double strand break formation to occur. Some clastogens, like radiation, can cleave DNA directly while others may inhibit enzymes, such as topoisomerase II (topo II), that enzymatically cleave DNA. The presence of compounds in wood dusts that inhibit the topo II enzyme is not surprising as some compounds, such as quercetin and luteolin, found in plants are known to be topo II inhibitors (Cantero et al., 2006). Anthracycline antibiotics, such as doxorubicin, inhibit topo II and share the anthracene nucleus structure with 2-methylanthraquinone (Pommier et al., 2010). Inhibition of the topo II enzyme results in free 5' and 3' ends that may or may not be rejoined correctly. If the ends are joined erroneously then large sections of DNA can be moved from one genomic region to another or lost all together (Khan et al., 2009). There are limited data that address double strand breaks as a genotoxic mechanism of action for wood dusts. One group that examined the effects of teak on human lung epithelial cells found double strand DNA breaks using a neutral comet assay. The double strand breaks were present three hours following treatment, but were not detected six hours after exposure, suggesting repair of the lesion (Bornholdt et al., 2007). The suggestion of teak-induced double strand breaks supports our results on the prevalence of the loss of the gpt gene. Deletion of the gpt gene in the g12 cell system requires that double strand breaks occur first, followed by gene loss, rejoining of DNA ends, and then clonal expansion of the 6-TG resistant mutants.

Loss of tumor suppressor genes has important implications for the onset of carcinogenesis. Consistent with our experimental findings, losses of large sections of DNA were reported in nasal tumors from workers with a history of exposure to

wood dusts (Ariza et al., 2004; Korinth et al., 2005). It is not known whether these losses are important events associated with the initiation of carcinogenesis or if they are "baggage" mutations that resulted from an acquired mutator phenotype in the tumors. The data generated in the current study lacks this ambiguity. Deletions encompassing the full-length *gpt* gene were the primary mutation caused by treatment with teak wood dust extracts and 2-methylanthraquinone.

Frameshift mutations occur when small insertions or deletions (INDELS) of DNA sequences happen in any number other than a multiple of three nucleotides. INDELS that occur in multiples of three nucleotides result in gains or losses of amino acids in the corresponding protein sequence. Both deletions and insertions were caused by the teak wood dust extract treatment. Treatment with 2-methylanthraquinone also produced insertion and deletion mutations in the *gpt* gene. Many of these INDELS altered the *gpt* gene reading frame and caused nonsense mutations. INDEL mutations were not present in the solvent control. The mechanism(s) involved in generating these INDEL mutations remains unclear, but the mutation profiles we obtained suggest teak wood dust extract and 2-methylanthraquinone may involve a common mechanism (or mechanisms) of action.

One possibility is that errors in the mismatch repair (MMR) process lead to these INDEL mutations. When MMR functions properly it corrects misaligned base pairs and mismatches before they result in permanent alterations to the DNA sequence, and when it does not mutations occur.(Hsieh and Yamane, 2008) Another possibility is the genes involved in the MMR pathway were themselves damaged

following treatment with teak extract or 2-methylanthraquinone, or perhaps mismatches, deletions or insertions were generated because the repair system became saturated. If the MMR enzymes were compromised, then the ability to remove errors in the DNA sequence would be diminished or destroyed. If the amount of mismatched base pairs was too great and the MMR pathway was overwhelmed, then some errors may not be repaired.

Another possible explanation for the INDEL mutations is that 2-methylanthraquinone may intercalate into the DNA strand and induce errors in repair mechanisms by altering the physical structure of the DNA during replication (Bolton et al., 2000). Other molecules possessing the anthracene nucleus, such as the antibiotic doxorubicin, are known to intercalate into DNA (Swift et al., 2006). Due to the high probability of a deleterious effect on functional proteins, these INDEL mutations could play a critical role in tumor development. We demonstrated that soluble compounds found in teak wood dust including 2-methylanthraquinone caused these types of mutations. Based on these findings, it is probable that the quinones found in wood dusts contribute to genotoxicity in chronically exposed wood workers.

Previous reports indicate that oxidative stress is induced following exposure to wood dusts (Long et al., 2004). Particulate air pollution has generally been associated with increases in oxidative stress in the respiratory system and has been attributed both to reactive chemicals adsorbed to particles as well as to the particles themselves. The mutagenesis assays demonstrate that soluble chemicals extracted from teak dust may have the potential to cause oxidative damage to DNA that is

independent of the particles. Quinones in wood dusts can be reduced to a non-toxic hydroquinone by the inducible enzyme NADPH-quinone oxidoreductase or to a toxic semiquinone radical via oxidation by NADPH-cytochrome p450 reductase. Semiquinone radicals can damage DNA and proteins directly and also can produce reactive oxygen species (Bolton et al., 2000). Either of these pathways would contribute to DNA damage. The resulting lesions would most likely lead to point mutations if not properly repaired.

Transition point mutations were observed in the treated and untreated groups. The mutated sequences were translated in-silico and compared to the *gpt* reference sequence. These sequences were matched to Figure 38 to determine if mutations were localized to substrate binding, conserved, or structurally important residues within the gpt enzyme. One point mutation, G >A at 241, was observed in the solvent control, 2-methylanthraquinone, and teak extract treated groups. This point mutation resulted in replacement of a glycine with a serine residue. The mutation is positioned seven amino acid residues from the *gpt* enzyme's catalytic site. Because the mutation was found in the treatment and control groups, it was considered a background mutation. All other point mutations found were treatment-related, unique, and altered the *gpt* protein sequence.

In the 2-methylanthraquinone treatment group, a single mutant, MEAQ S-60, was found to contain transitions, tranversions, and a single nucleotide deletion (Table 39). These mutations were proximal to each other and would cause changes in the gpt protein sequence at ligand binding residues. Eight point mutations were observed in the teak treated groups. Seven of these point mutations resulted in

missense mutations that altered residues within or near catalytic or substrate binding sites, or were within the helical structures of the enzyme. One point mutation generated a stop codon, which is expected to result in a truncated protein (Table 39).

Two recent studies by Homila, et al, focus on point mutations in the p53 gene in nasal cancers (Holmila et al., 2010a; Holmila et al., 2010b). The authors report a strong association between point mutations in the p53 gene from adenocarcinomas that developed in workers with wood dust exposures of 24 years or more. PCR was carried out on paraffin embedded tumor samples in order to compare the p53 sequences from tumors to the normal p53 sequence. The most common mutations reported were missense mutations, and the second most common mutations reported were frameshift (INDEL) mutations. There are some important factors to consider when comparing the mutation spectrum of tumor samples with the spectrum from a positive selection mutation assay such as the *gpt* assay. Missense mutation was the most commonly observed class in the p53 studies. In the fixed tumor samples all point mutations, whether silent or not, were detected in the sequence analysis. Codons 248, 175, 179, and 135 of the p53 gene were frequently mutated in the tumor samples. Codons 248, 175, are the most frequently mutated codons in human cancer, and mutations at codon 179 are frequently seen in head and neck cancers. Mutations at codon 135 are less common in human cancers in general but were prevalent in the tumor samples. Other mutations were detected throughout the coding region of the gene. Some of these mutations may have

altered the protein sequence while others may be "baggage" mutations acquired after tumor cells developed a mutator phenotype.

There is a distinct possibility that mutations identified from pcr samples dervived from formalin fixed adenocarcinomas misrepresents the p53 mutation spectrum. It is thought that formalin reacts with cytosine nucleotides and forms crosslinks between two DNA strands (Williams et al., 1999). This crosslinking leads to artificial C > T and G > A point mutations in PCR amplicons. These were the most frequent point mutations observed in the p53 mutation spectrum analysis (Holmila et al., 2010b). Formaldehyde also reacts with amino moieties of adenine, guanine, and cytosine. Missense mutations were observed in the *gpt* assay but only mutations that conferred 6-TG resistance were seen. It is probable that many more point mutations occurred but they were either silent or coded for a similar amino acid that did not inactivate the *gpt* protein. INDEL mutations were reported to be present in the p53 gene in wood dust associated cancer, and they were also observed in the *gpt* assay. Because these types of mutations often lead to non-functional proteins, they may play a critical role in the onset of carcinogenesis.

Another important consideration that complicates data comparisons is that it is unlikely wood workers from Northern Europe who developed nasal adenocarcinomas were chronically exposed to teak wood dusts. The mutation spectra for oak dusts and other wood types expected to be the etiological agent for the cancers studied by Homila, et al has not been determined. Large deletion mutations were not reported in wood dust associated nasal cancer in the p53 gene, but INDEL mutations were present (Holmila et al., 2010b). Large deletions were

reported in studies that analyzed chromosomal rearrangements in nasal tumors from wood workers (Ariza et al., 2004; Korinth et al., 2005). Large deletions, possibly generated by inhibition of topo II or other mechanisms involving double strand breaks, were the most common mutagenic event in the *gpt* assay. We consider this to be the signature mutations for teak wood dust and 2-methylanthraquinone exposures. The secondary INDEL mutations caused by teak dust extract or 2-methylanthraquinone generally have an effect at the protein level and could be involved in tumor development.

Changes in ploidy caused by treatment with wood dust extracts and 2-methylanthraquinone

Methods

Giant multinucleated cells were seen within the colonies of g12 cells treated with teak dust extract, but were not observed in control cells (Figures 39 and 40). In order to determine if this was a general toxic response to teak wood dust extract treatment, a comparison was made between equally toxic treatments of teak dust extract and dichromate, a genotoxic agent with a distinct toxicity (Figure 41). g12 cells were cultured at a density of 100 cells/60 mm culture dish and treated in triplicate with 5 μg/ml of teak dust extract, 3 μM dichromate or DMSO solvent control. The medium was changed the next day, and the cells were grown for five additional days. The cells were then fixed and stained in 0.1% crystal violet dye dissolved in ethanol. One hundred cells per colony from ten colonies per plate were counted and averaged according to treatment (Figure 41). Statistical significance was determined by one way ANOVA with Dunnett's post-hoc comparisons (supplementary data).

g12 cells treated with teak wood dust extracts, 2-methylanthraquinone, or cytochalasin-B were allowed to recover for one or three days and then were collected and analyzed with flow cytometry for cell cycle perturbations or the formation of polyploidy cells. The cell culture conditions and statistical analysis of the results were identical for both experiments. The treatment effects were tested for significant differences with two-way ANOVA followed by Bonferonni post-tests.

With the first method, cells had a three-day post-treatment recovery before harvest. The second method had a twenty-four hour recovery period before the cells were harvested. These two methods allow for analysis of cells with approximately one or four potential cell cycles post-treatment. g12 cells were inoculated into 60 cm tissue culture dishes with 2.5 x 10^5 cells per plate in F-12 medium supplemented with 5% FBS. The cells were allowed to attach overnight. The following morning the medium was removed and replaced with medium that contained 2-methylanthraquinone (7 μg/ml), cytochalasin-B, (1.5 μg/ml), 7 or 10 μg/ml of the teak wood methanol extract for the three-day recovery time experiment. Ten μg/ml of teak wood dust extract was used for the twenty-four hour recovery experiment. The cells were allowed to recover either for one or three days. After the recovery period, the cells were washed in PBS and collected by treating with trypsin/EDTA. The cells were washed in PBS and centrifuged at 200 x g. The cell pellet was resuspended in 1 ml of ice cold PBS, then the cell solution was added dropwise to 9 ml of ice-cold 70% ethanol for fixation and stored in the freezer before staining with propidium iodide (PI).

The same day the as the flow cytometric analysis, the fixed samples were collected by centrifugation at 200 x g, washed twice with PBS, and the pellet was resuspended in PI staining solution. PI staining solution consisted of PBS with 0.1% Triton X-100, 0.2 mg/ml RNAse, (5-Prime, Pittsburg PA) and 20 μg/ml propidium iodide (MP-Biomedicals, Solon OH). The samples were incubated at 37°C for 30 minutes in a water bath and then analyzed for differences in DNA content. Analysis

was carried out in the Tulane Center for Gene Therapy's Flow Cytometry Core Facility with a three laser Becton-Dickinson FACSVantage SE cell sorter.

Two approaches were used to analyze the distribution of cycling cells in each of the treatment groups.(supplementary data) The first approach used the computer program Modfit to identify differences in the distributions at G1, S, and G2 phases of the cell cycle and to determine if a cycling population of polyploidy cells was induced by the various treatments. The second approach was to analyze the manually gated flow histograms for an increase in the total percent of the cell population that had DNA content greater than 4N.

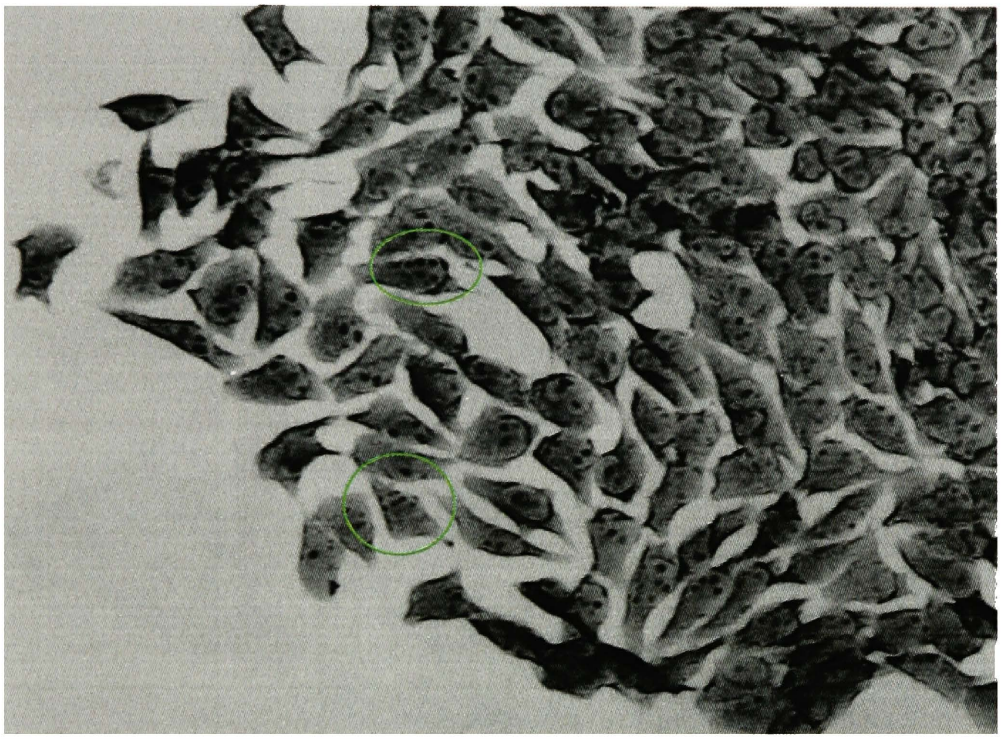
Results

Giant multinucleated cells were evident in the g12 cells treated with teak wood dust extract. To preclude the possibility that the multinucleated cells were a general phenomenon, we included the genotoxic agent dichromate in these studies. Treatment with dichromate at equally toxic doses as those used for teak dust extract did not produce a significant increase in multinucleated cells. Treatment with equally toxic doses of 2-methylanthraquinone did not produce an increase in multinucleated cells while cytochalasin-B treatment resulted in a 6-fold increase in multinucleated cells relative to the solvent control. Treatment with teak wood dust extract resulted in a 8-fold increase in multinucleated cells. This increase was significantly different from that of the untreated control cells as determined by Dunnett's pairwise comparisons and ANOVA. (Figure 41)(Supplementary data)

Analysis of the flow cytometry data produced ambiguous results (supplementary data). Significant differences in ploidy were consistently detected

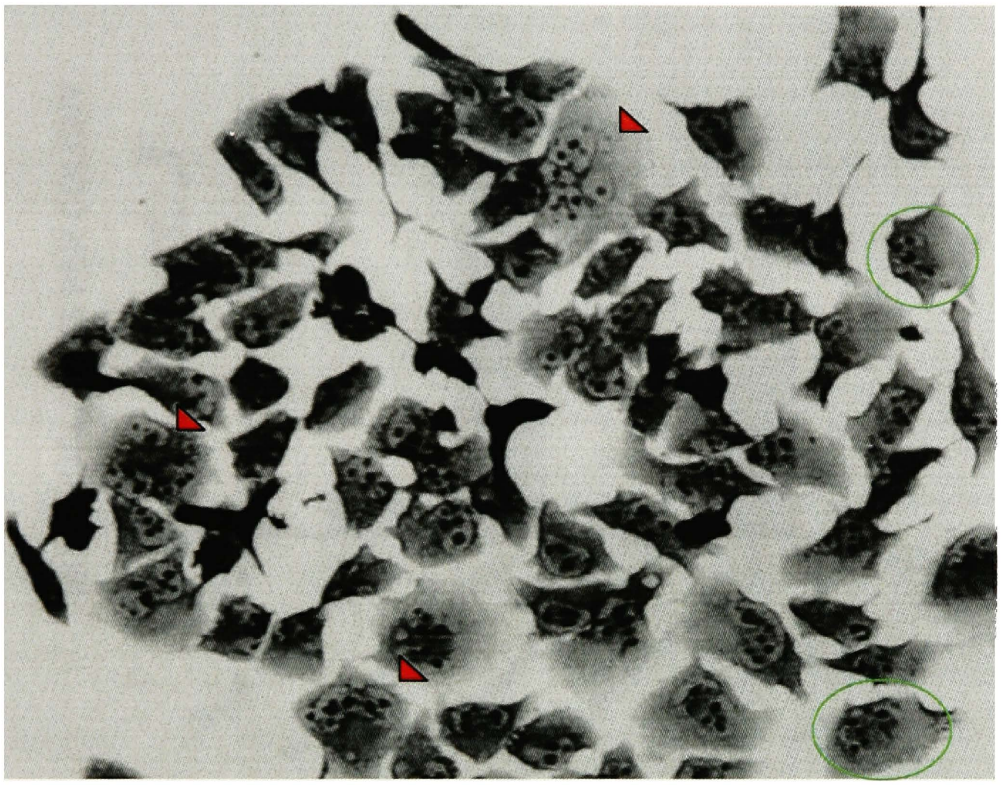
in the cythochalasin-B treated positive control cells following 24 hour or three day recovery via both the Modfit cell cycle analysis and the manual analysis for DNA content greater than 4N. However consistent significant differences were not observed in the teak wood dust or 2-methylanthraquinone treated cells.

Figure 39. Normal morphology of g12 cells



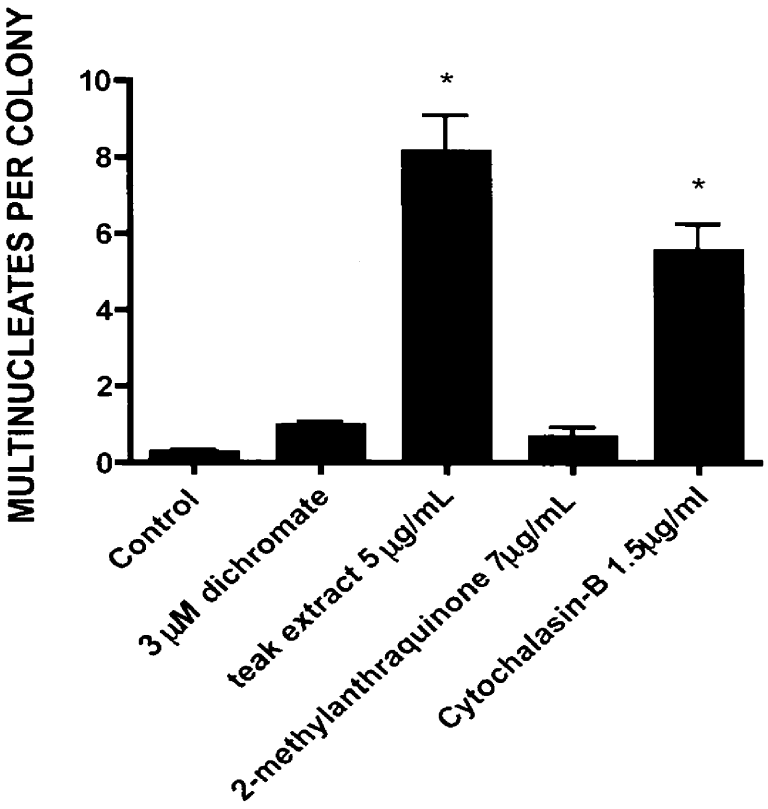
(100X magnification, g12 cells treated with solvent vehicle alone and grown for five days) Some of the cells in the control group have increased number of nucleoli, seen as the dark spots within the cells, present. They are identified in the green circles and may reflect cycling cells. No multinucleated giant cells were observed in the control group.

Figure 40. Morphology of g12 cells treated with teak dust extract



(100X magnification teak wood dust extract treated g12 cells after five days of growth) Some of the cells in the treatment group also display an abnormal number of nucleoli, identified with green circles. The red arrows point to some of the giant multinucleated cells present in the teak dust treatment group.

Figure 41. Multinucleated cells treated with dichromate, teak extract, 2-methylanthraquinone, and cytochalasin-B



The X-axis shows the treatment groups and the Y-axis shows the average number of multinucleated cells identified per 100 cells within individual colonies of g12 cells. The bars show the average multinucleated cells per each group and the error bars show the standard error of the mean. Asterisks indicate statistical significance ($p<0.01$).

Discussion

An increase in multinucleated cells following treatment with teak wood dust extract and cytochalasin-B was observed in a colony formation assay. 2-methylanthraquinone had several activities in common with teak dust extract, but it did not cause the giant multinucleated phenotype. These observations indicate that teak wood dust contains chemicals other than 2-methylanthraquinone that alter ploidy, possibly via a similar mechanism as cytochalasin-B. The compounds in teak wood dusts that produce the giant multinucleated phenotype remain to be identified. Induction of multinucleated V79 cells, the parental cell line of the g12 cells, was reported by Banrud et al following exposure to UV A radiation (Banrud et al., 1995). The Banrud study also found significant dose dependent changes in ploidy with flow cytometry following exposure to UV-A but not UV-B radiation. In contrast to the colony formation assays, no consistent treatment dependent effects on ploidy were found by flow cytometric analysis. This discrepancy between the techniques may be due to the gating technique used in the flow cytometric analysis, or it may indicate that significant polyploidy is not induced after treatment with 2-methylanthraquinone or teak wood dust extracts.

Chemical analysis

Methods

HPLC analysis of methanol extracts of wood dusts

A gram of each of the wood dusts were extracted in ten ml of methanol in fifteen ml conical tubes. The extractions were carried out overnight in a roller incubator at 25^{0}C. The extracts were passed through Whatman paper P2 filters to remove large particles then passed through a 0.2 μm syringe tip filter (Millipore). One-half ml aliquots of each extract were placed in pre-weighed microcentrifuge tubes and the methanol was removed by evaporation in a fume hood. The dried extracts were weighed and dissolved in DMSO at 60 mg extract/ml. A 500 μg/ml standard of 2-methylanthraquinone was also prepared in methanol. A ten μl aliquot from each DMSO extract solutions was diluted in 0.5 ml of methanol, and ten μl of the diluted extract was injected into the HPLC. The 2-methylanthraquinone standard was diluted to 1.5 μg/ml and ten μl of solution was injected into the HPLC. The presence of 2-methylanthraquinone in wood dust extracts was inferred by comparing the peaks on the individual extracts HPLC traces to the 2-methylanthraquinone standard.

Determination of 2-methylanthraquione content in three independent teak wood samples by HPLC

Wood dust extracts and reference samples were analyzed using a Hewlitt Packard 1100 high performance liquid chromatography (HPLC) system with a quaternary pump. Samples were separated with a LiChrosphere 100 RP-18 (125 mm X 4 mm X 5 μM) column with a 17 minute 20-90% methanol gradient in 10 mM ammonium acetate and a flow rate of one mL/minute. The HPLC was coupled to a photo source (λ = 250 nm) and a photodiode array detector was used to determine the relative amount of 2-methylanthraquinone in the teak wood dust extracts. A gram of each wood dust was extracted in methanol, filtered and dried as described above. A one mg/ml stock solution of 2-methylanthraquinone was prepared in methanol and diluted to give a range of standards from 15 to 450 μg/ml. These references were injected in 10 μl volumes to generate a standard curve based on the peak area. The three independent teak samples were analyzed by injecting 10 μl aliquots of extract into the HPLC. The amount of 2-methylanthraquinone was determined by comparing the peak areas from the teak extract HPLC traces eluted at 15.77 minutes to the 2-methylanthraquonine standard curve.

LC-MS-MS identification of 2-methylanthraquinone in teak wood dust extracts

Dust was generated from three independent biological teak wood specimens. One gram of each teak wood dust sample was extracted in ten mL of methanol and filtered as described above to remove particles. A stock solution of 2-methylanthraquinone, one mg/ml, was prepared in methanol. The 2-methylanthraquinone standard and the three independent teak samples were analyzed by injecting five μL aliquots into the LC-MS apparatus. A Shimadzu C18 (50 mm X 4 mm X 5 μM) column with a ten minute 20-90% methanol gradient in 0.1% formic acid and a flow rate of 1 mL/minute fitted to a API 4000 Qtrap LC-MS-MS system equipped with an atmospheric pressure chemical ionization source was used for this analysis. 2-methylanthraquinone, which has a molecular weight of 222.25, was ionized to generate the parent ion, 223.25 amu, and two daughter ions, 152 and 165 amu, were selected for comparison against the LC-MS-MS analysis of the teak wood extracts.

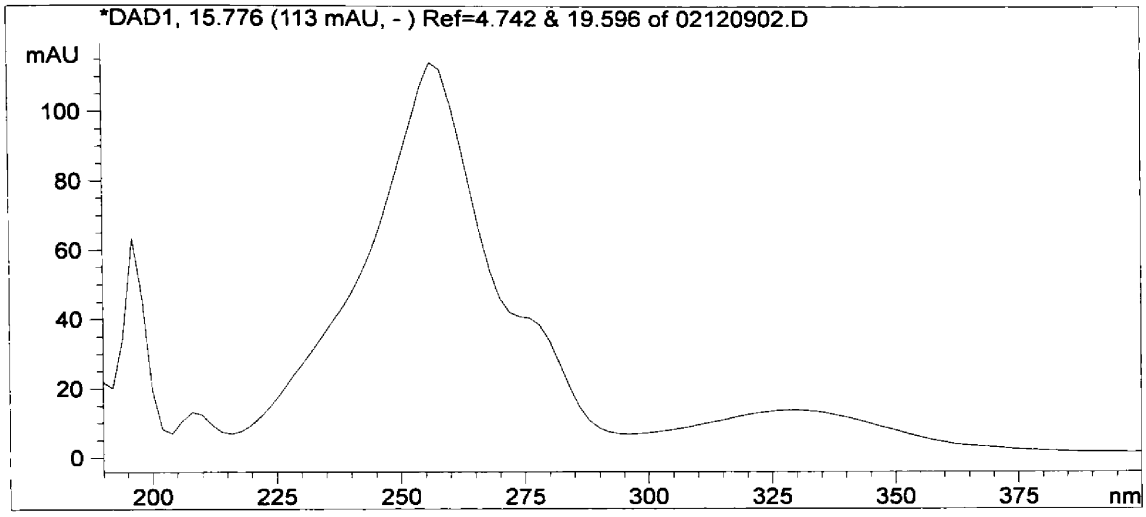
Results

HPLC analysis of methanol extracts of wood dusts

2-methylanthraquinone was abundant in teak wood dust extracts. Teak wood dust extract analysis showed a major peak with an elution time of 15.7 minutes (Figure 45). The fraction eluted from the column at 15.7 minutes from the 2-methylanthraquinone and teak wood dust samples were collected and the UV spectra were compared. Both the teak wood dust and the 2-methylanthraquinone have a strong absorbance at 254 nm (Figures 42 and 43).

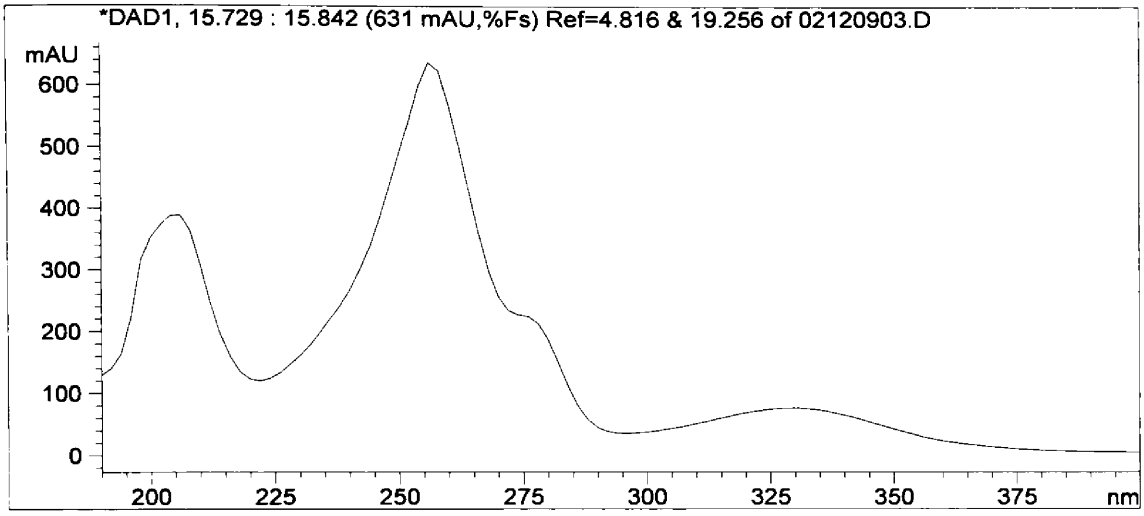
No other wood types tested contained 2-methylanthraquinone in appreciable quantities. However, red cedar and mahogany wood dust extracts displayed small peaks with the same elution time as 2-methylanthraquinone. Comparison of the HPLC traces from all of the wood dust extracts shows that there are extractable organic compounds present in all the wood types tested.

Figure 42. UV spectrum of 2-methylanthraquinone



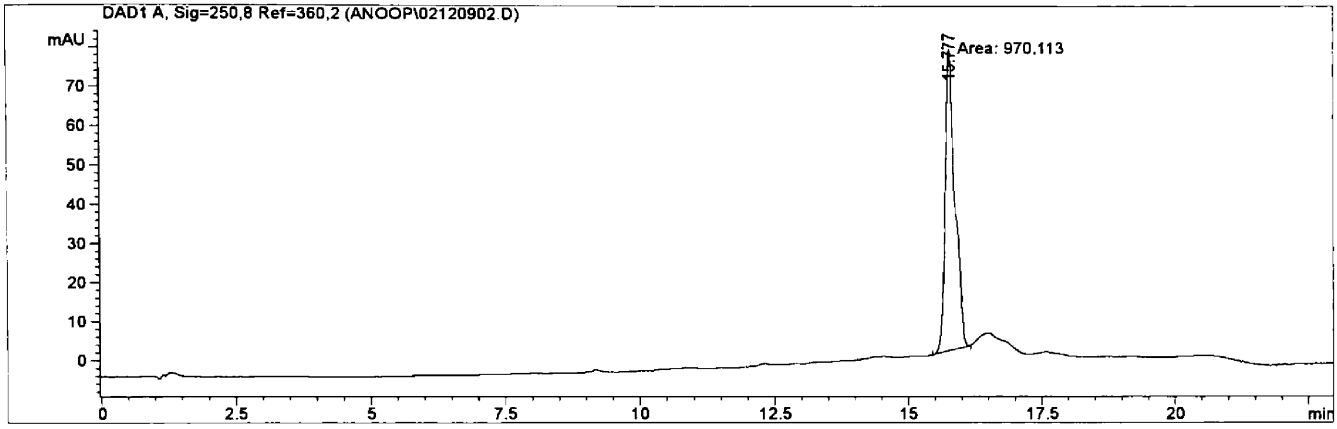
2-methylanthraquinone has a strong UV absorbance peak at 254 nm.

Figure 43. UV spectrum of teak wood extract



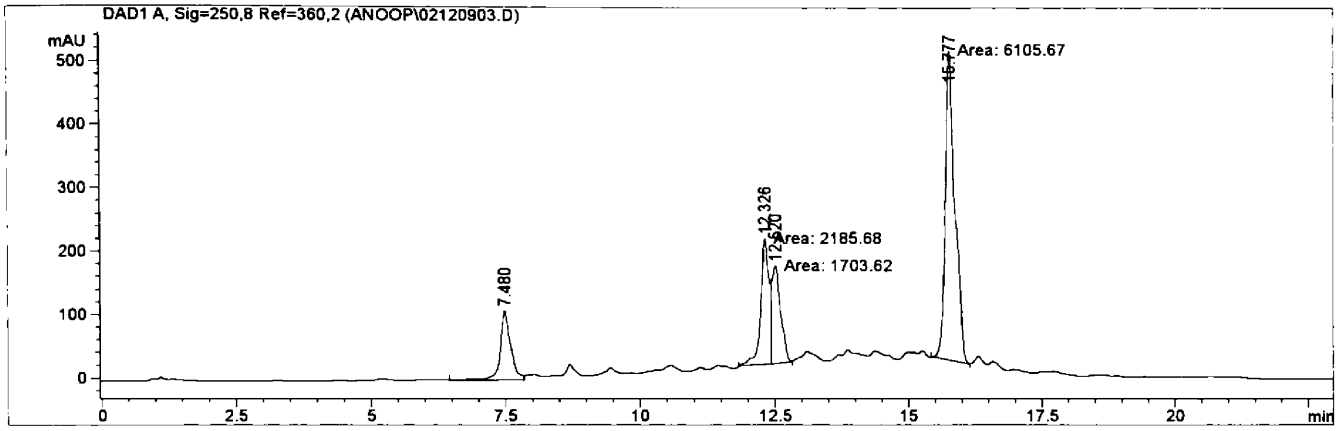
Teak wood dust extract has a strong UV absorbance at 254 nm.

Figure 44. 2-methylanthraquinone in methanol fractionated by HPLC with UV detection



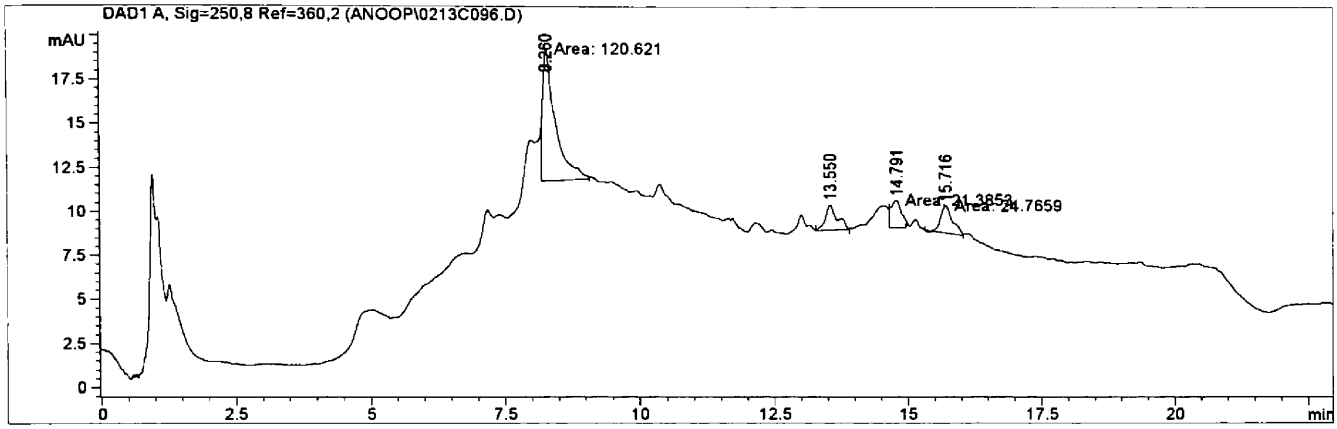
The HPLC profile of 2-methylanthraquinone has one major peak with an elution time of 15.7 minutes

Figure 45. Methanol extract of teak wood dust fractionated by HPLC with UV detection



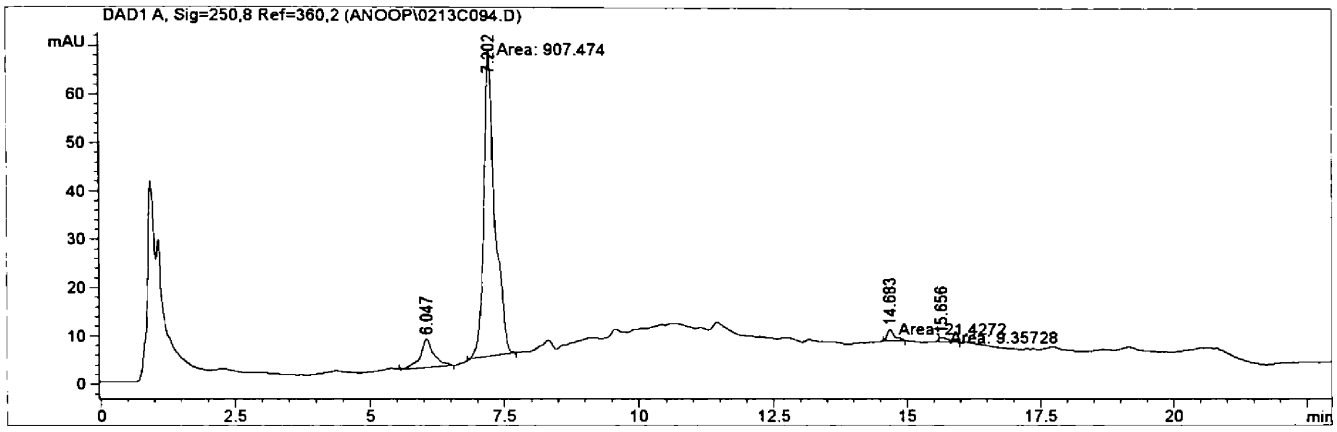
HPLC analysis of teak wood extract showed four clear peaks at elution times of 7.5, 12.3, 12.5, and 15.7 minutes. The major peak eluted at 15.7 minutes.

Figure 46. Methanol extract of mahogany wood dust fractionated by HPLC with UV detection



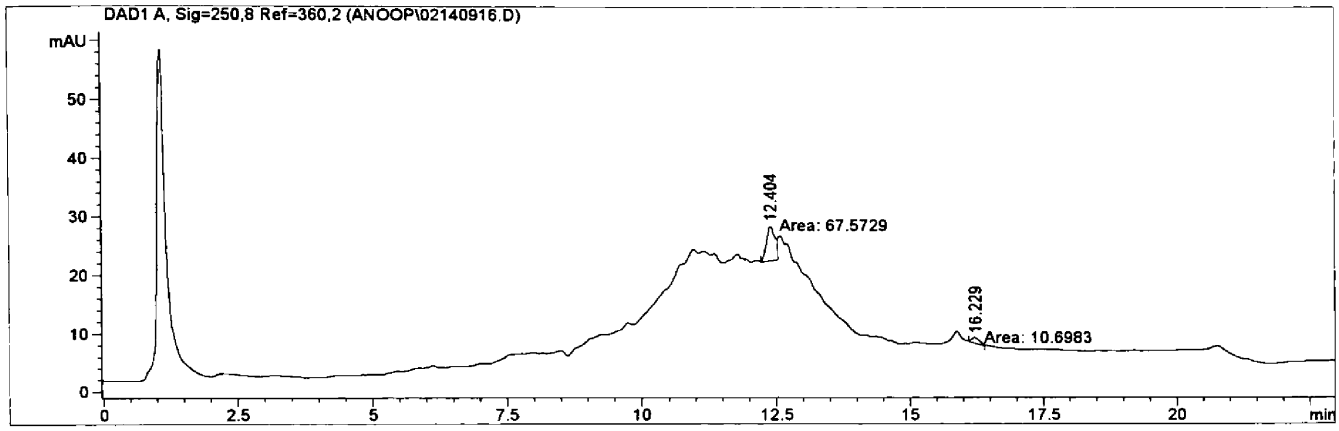
A small peak is present at 15.7 minutes indicating that some 2-methylanthraquinone may be present.

Figure 47. Methanol extract of walnut wood dust fractionated by HPLC with UV detection



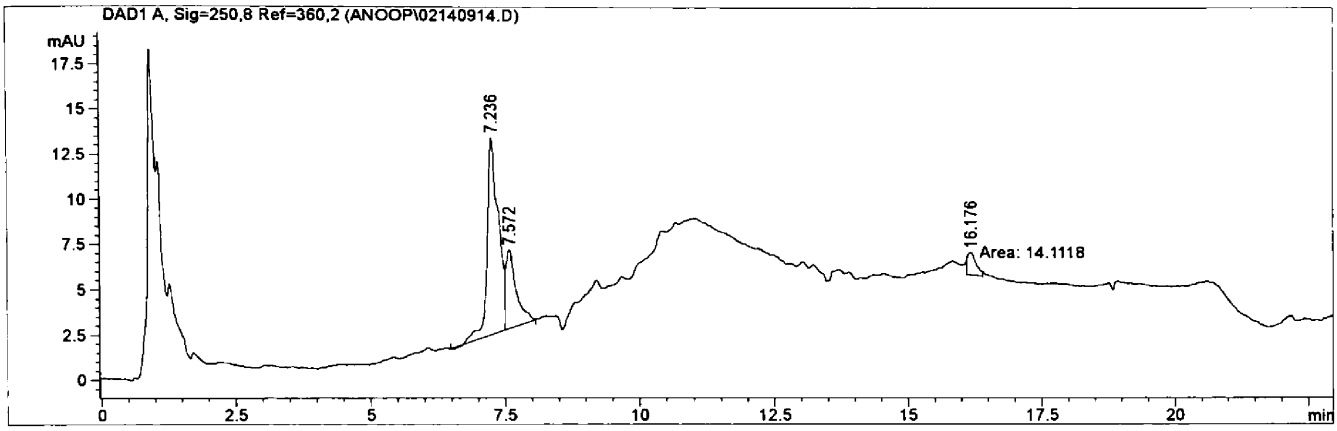
Walnut wood dust extract has a major peak with an elution time of 7.2 minutes and there are three smaller peaks with elution times of 6.0 minutes, 14.6 minutes, and 15.6 minutes.

Figure 48. Methanol extract of poplar wood dust fractionated by HPLC with UV detection



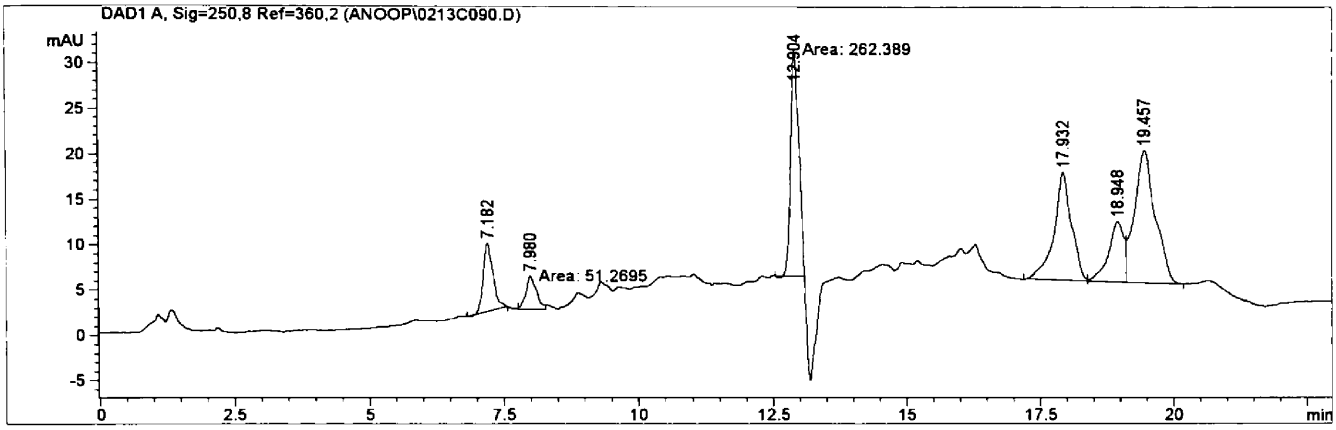
Two peaks are present with elution times of 12.4 minutes and 16.2 minutes.

Figure 49. Methanol extract of red oak wood dust fractionated by HPLC with UV detection



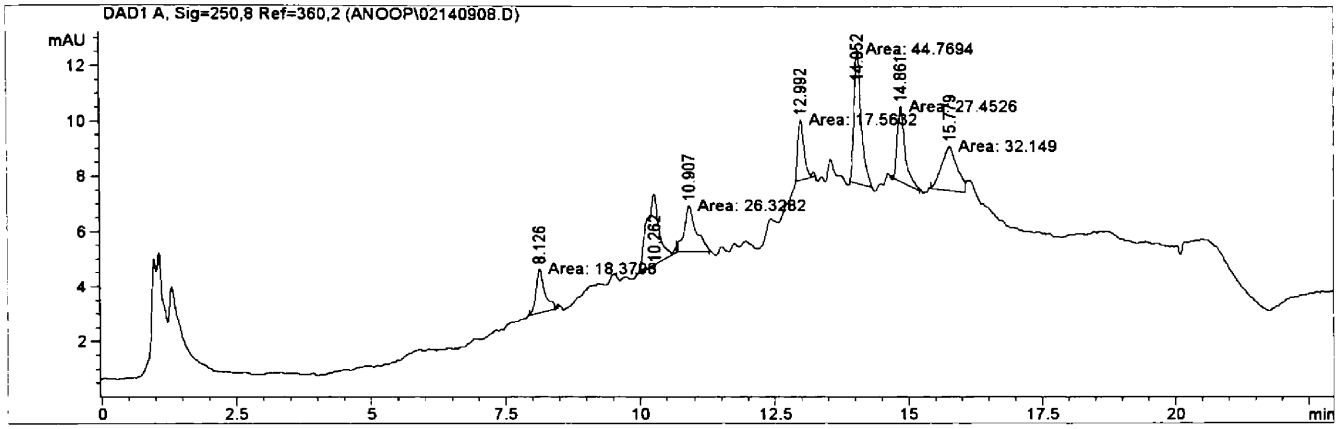
Three peaks are evident in the HPLC analysis of red oak wood dust the major peak was eluted at 7.2 minutes, and two smaller peaks that had an elution time of 7.5 and 16.2 minutes.

Figure 50. Methanol extract of cypress wood dust fractionated by HPLC with UV detection



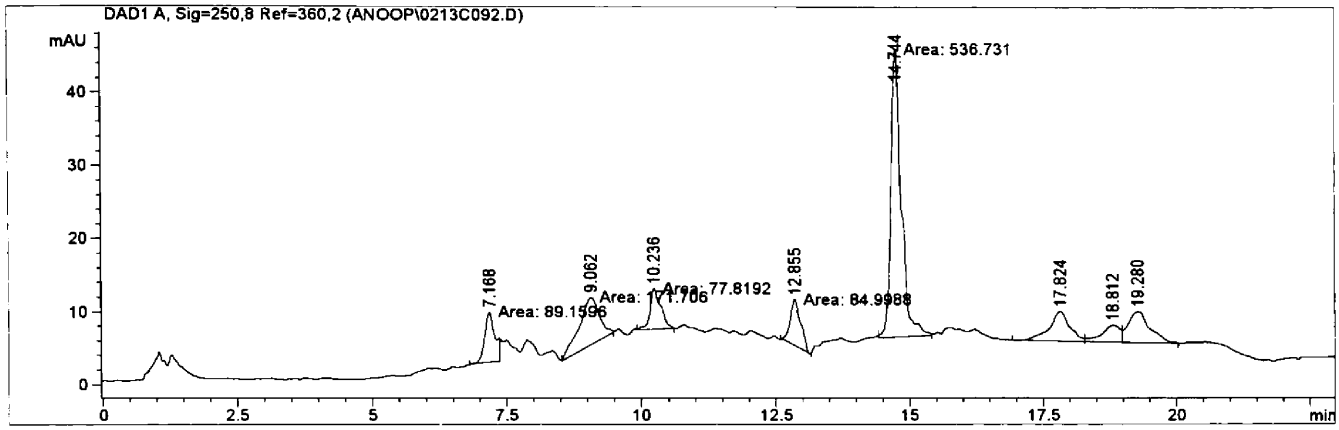
Six peaks are evident in the HPLC trace of cypress wood dust extract. The major peak was eluted at 12.9 minutes. The remaining peaks were eluted at 7.1, 7.9, 17.9, 18.9 and 19.4 minutes.

Figure 51. Methanol extract of red cedar wood dust fractionated by HPLC with UV detection



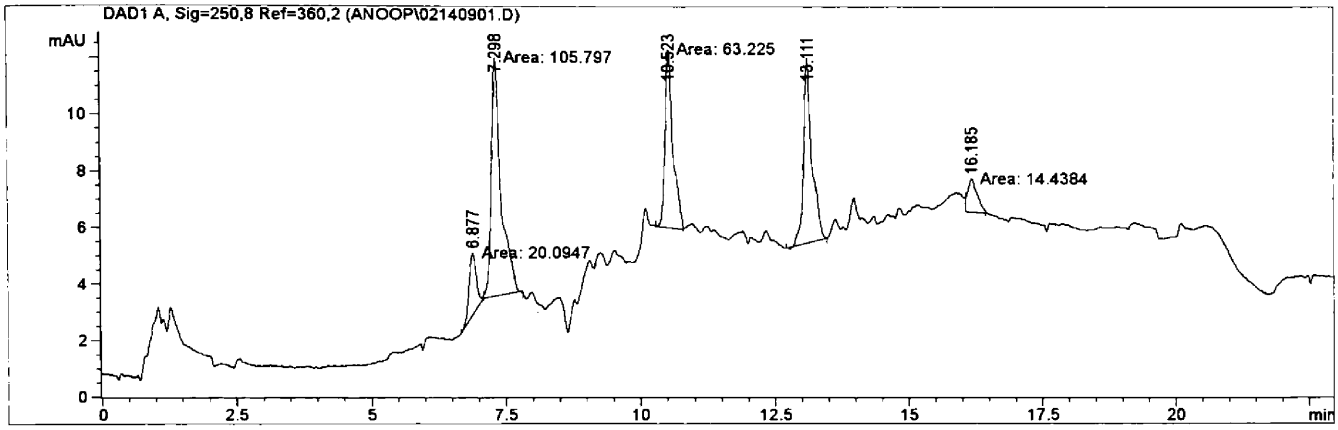
Seven peaks are shown in the HPLC trace of red cedar wood dust. The major peak was eluted at 14.0 minutes. The other six peak were eluted at 8.1, 10.3, 10.9, 12.9, 14.8, and 15.7 minutes

Figure 52. Methanol extract of spruce wood dust fractionated by HPLC with UV detection



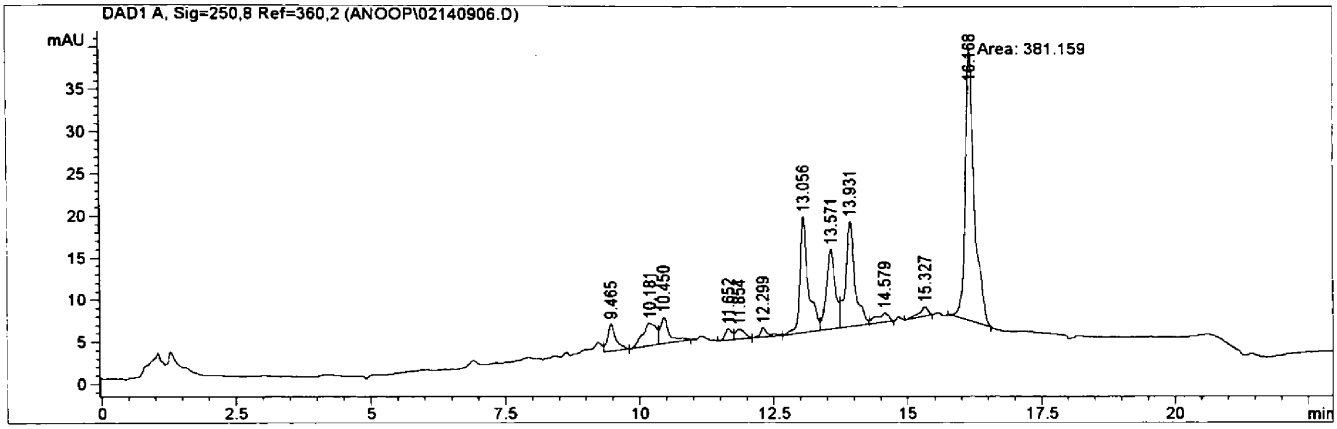
There are eight peaks in the HPLC trace of spruce wood dust extract. The major peak was eluted at 14.7 minutes. The other minor peaks were eluted at 7.1, 9.0, 10.2, 12.8, 17.8, 18.8, and 19.2 minutes.

Figure 53. Methanol extract of yellow pine wood dust fractionated by HPLC with UV detection



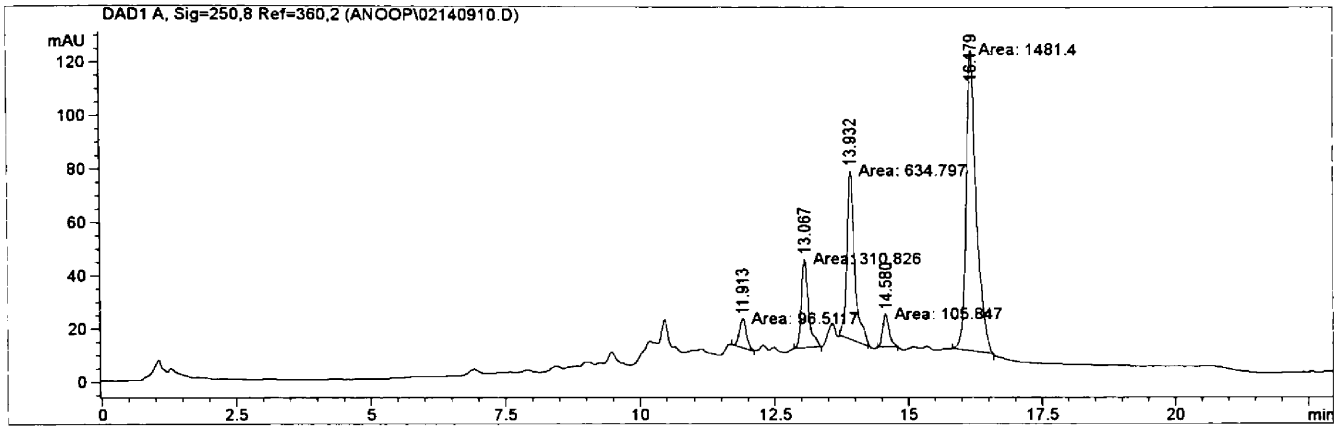
Five peaks were identified in the HPLC trace of yellow pine wood dust extract. Three major peaks were eluted at 7.2, 10.5, and 13.1 minutes. Two minor peaks were eluted at 6.8 and 16.1 minutes.

Figure 54. Methanol extract of plywood dust fractionated by HPLC with UV detection



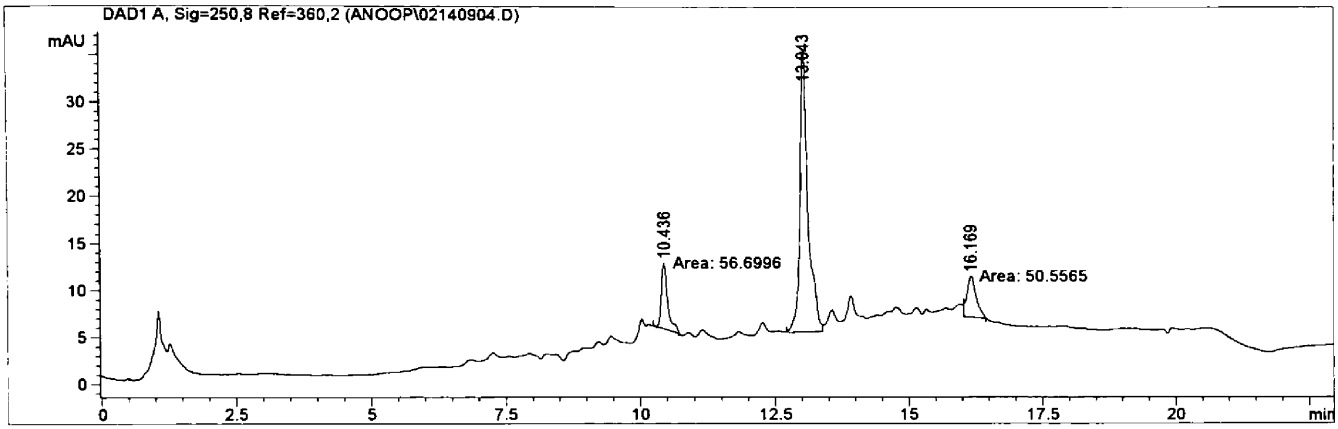
Twelve peaks were identified in the HPLC trace of plywood dust extract. The major peak was eluted at 16.1 minutes. Three moderate peaks were eluted at 13, 13.5, and 13.9 minutes. The remaining minor peaks were eluted at 9.4, 10.1, 10.4, 11.6, 11.8, 12.2, 14.5, and 15.3 minutes.

Figure 55. Methanol extract of laboratory animal bedding fractionated by HPLC with UV detection



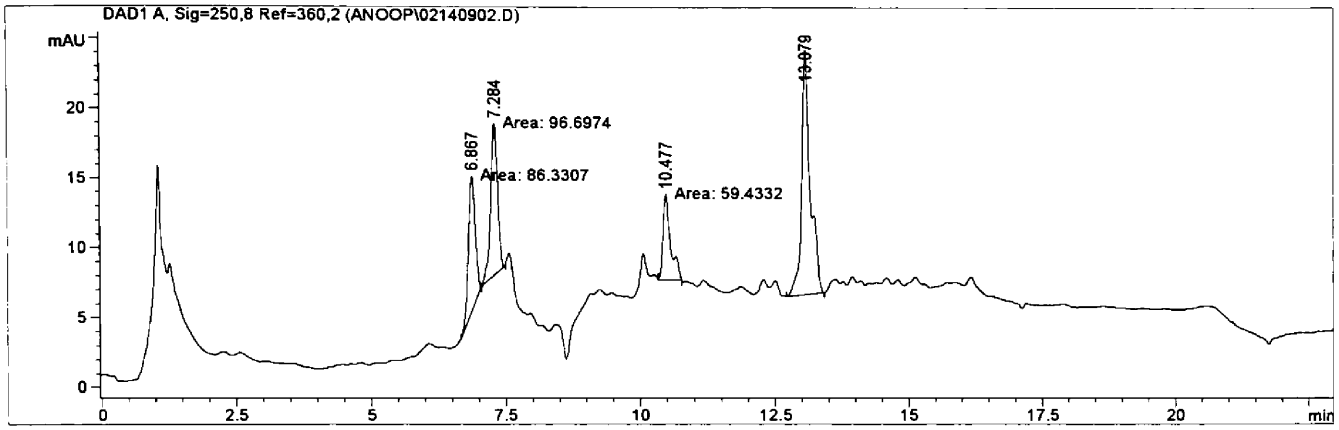
Five peaks were identified in the HPLC trace of laboratory animal bedding extract. The major peak was eluted at 16.1 minutes. Two moderate peaks were eluted at 13.0 and 13.9 minutes and two minor peaks were eluted at 11.9 and 14.5 minutes.

Figure 56. Methanol extract of medium density fiberboard dust fractionated by HPLC with UV detection



Three peaks were identified in the HPLC trace of medium density fiberboard extract. The major peak was eluted at 13 minutes and the two minor peaks were eluted at 10.4 and 16.1 minutes.

Figure 57. Methanol extract of treated pine wood dust fractionated by HPLC with UV detection

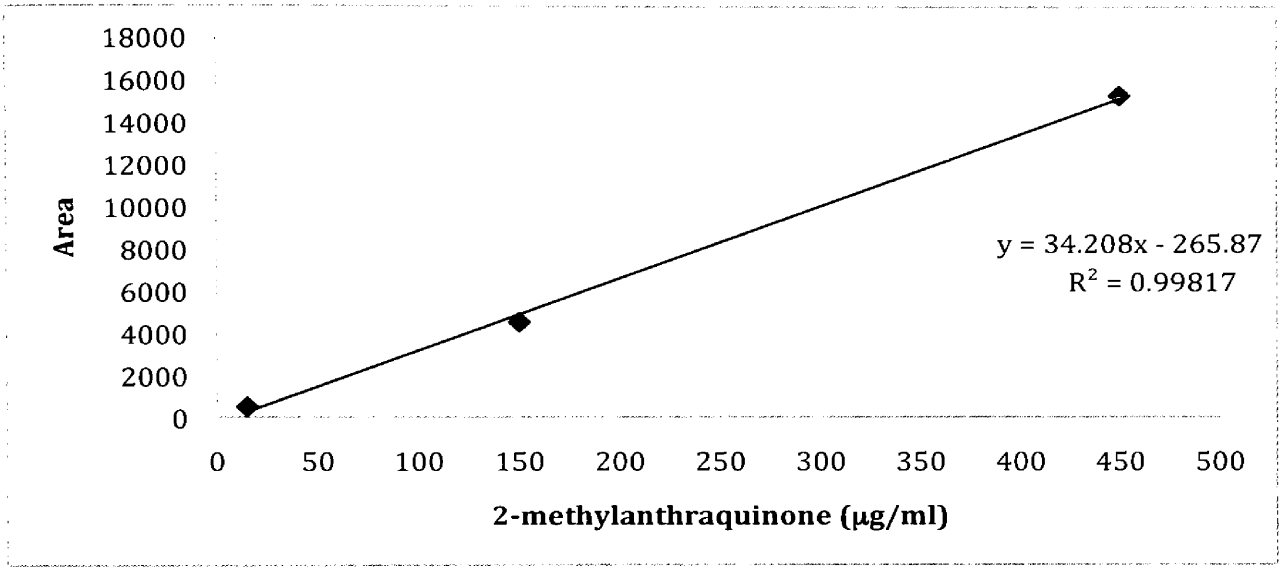


Four peaks were identified in the HPLC trace of treated pine wood dust extract. The major peak was eluted at 13 minutes and the three minor peaks were eluted at 6.8, 7.2, and 10.4 minutes.

Determination of 2-methylanthraquione content in three independent teak wood samples by HPLC

The amount of 2-methylanthraquinone was determined in three separate teak wood samples. The standard curve used to determine 2-methylanthraquinone concentration is shown in Figure 58. Two of the samples had equivalent amounts of 2-methylanthraquinone, and one was approximately a third lower (Table 45).

Figure 58. HPLC Standard curve of 2-methylanthraquinone ranging from 15 to 450 μg/mL



Three standard solutions of 2-methylanthraquinone were used to generate this standard curve. The peak area, eluted at 15.7 minutes, was used to calculate the amount of 2-methylanthraquinone in the teak wood dust.

Table 45. Calculated 2-methylanthraquinone content in teak wood dust

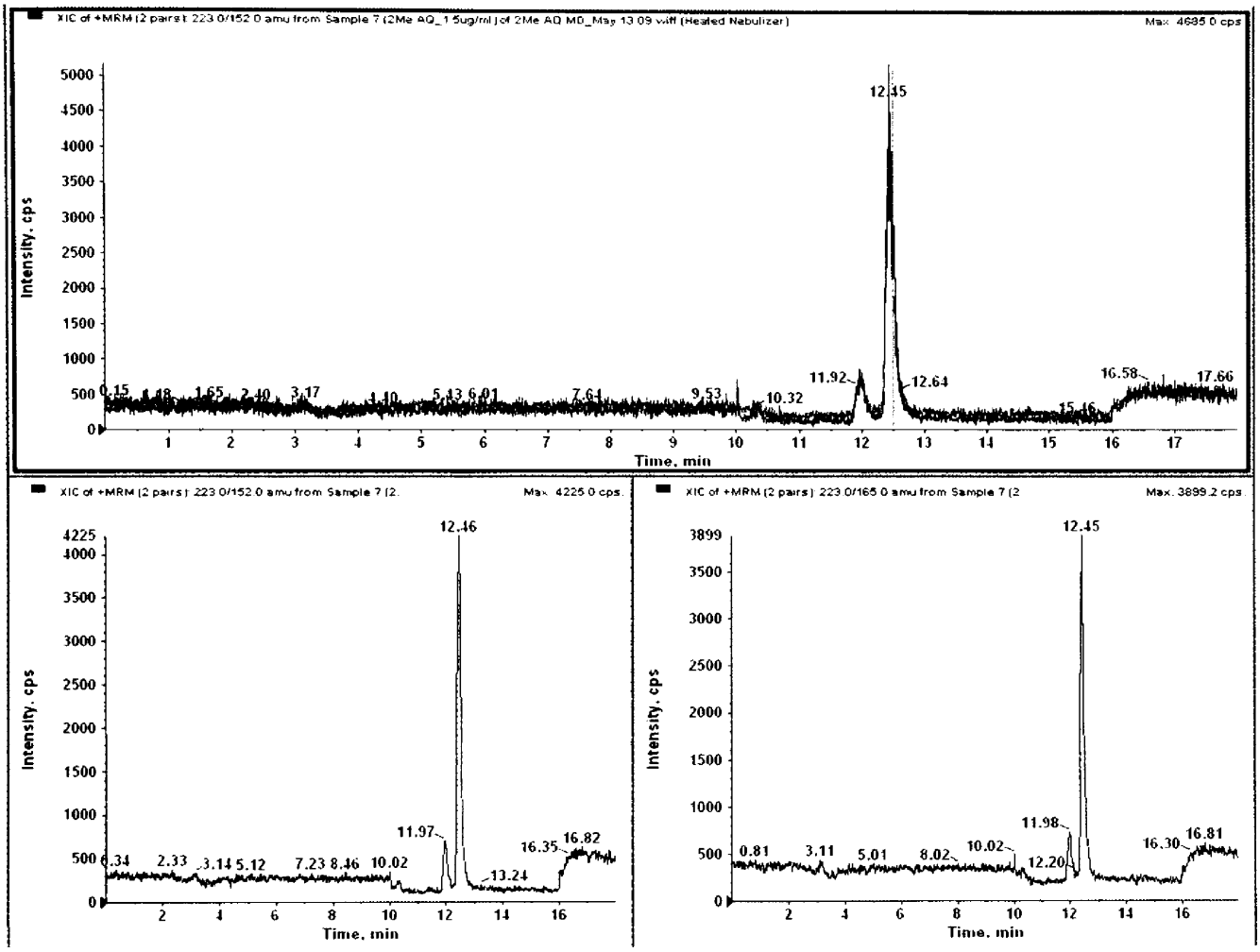
	μg/mL extract	% dry wood
Teak 1	307.777	0.31%
Teak 2	131.109	0.13%
Teak 3	362.583	0.36%

LC-MS-MS identification of 2-methylanthraquinone in teak wood dust extracts

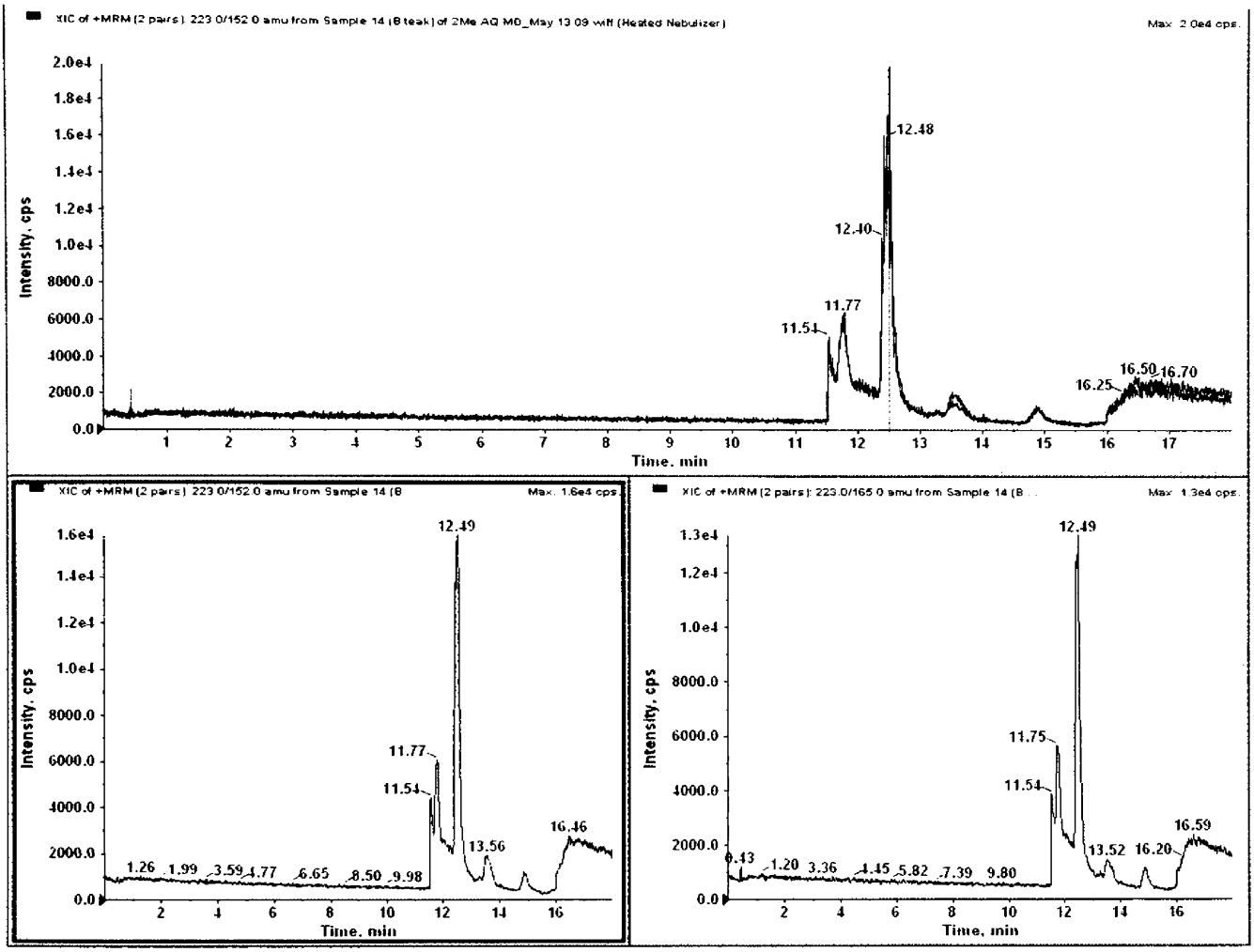
The spectral analysis of the teak wood dust extracts all had the molecular ions of interest present. Thus, the presence of 2-methylanthraquinone in the teak wood dust samples is confirmed (Figure 59).

Figure 59 (A-D). Analysis of parent to daughter ion ratios from 2-methylanthraquinone.

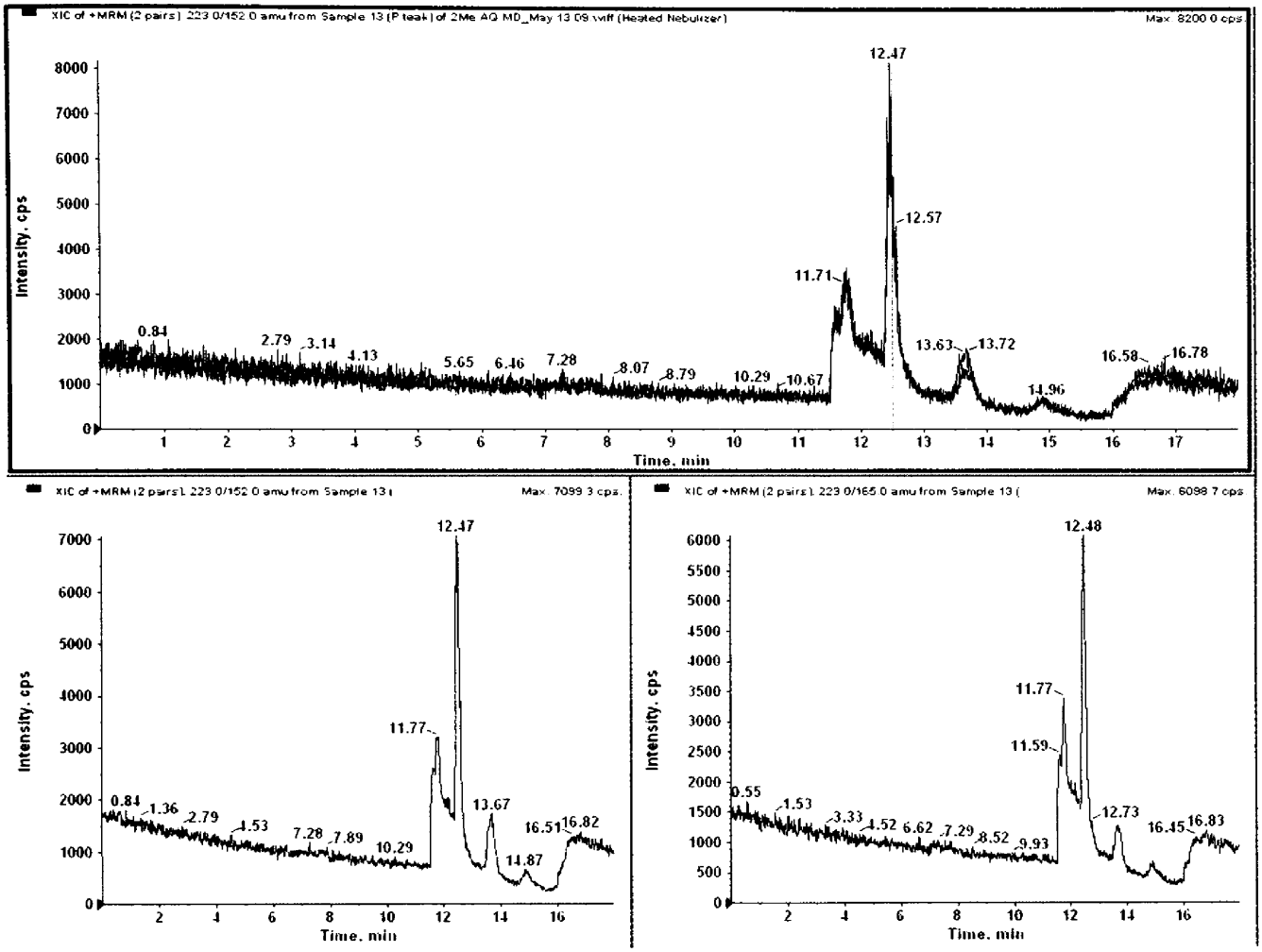
A



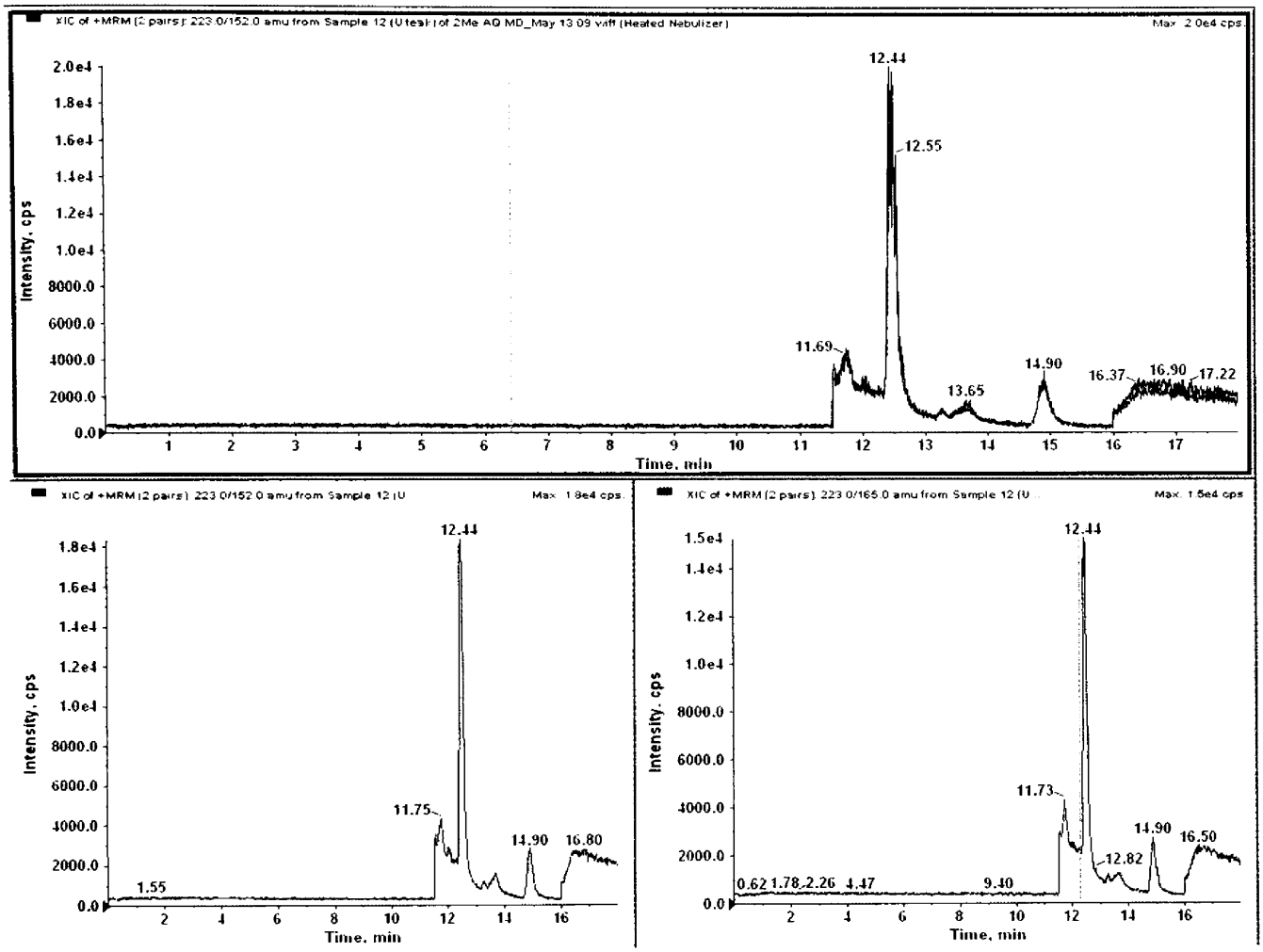
B. LC-MS-MS analysis of teak wood dust extract sample 1



C. LC-MS-MS analysis of teak wood dust extract sample 2



D. LC-MS-MS analysis of teak wood dust extract sample 3



The fractions used in the mass spectral analysis of 2 methylanthraquinone (Fig. 19 A) and teak wood dust samples 1-3 (Fig, 19 B, C and D) were eluted from the chromatography column at 12.48 minutes. The eluted fractions were collected and assayed by mass spectral analysis and shown as the ratio of the mass of the parent ion (223 amu) over the mass of the daughter ions (152 amu and 165 amu). These are the ions used to identify the chemical 2-methylanthraquione.

References

Acheson, E.D., Cowdell, R.H., Hadfield, E., and Macbeth, R.G. (1968). Nasal cancer in woodworkers in the furniture industry. British Medical Journal *2*, 587-596.

Ariza, M., Llorente, J.L., Alvarez-Marcas, C., Baragano, L., Salas, A., Rodriguez Prado, N., Hermsen, M., Suarez, C., and Sampedro, A. (2004). Comparative genomic hybridization in primary sinonasal adenocarcinomas. Cancer *100*, 335-341.

Banrud, H., Stokke, T., Moan, J., and Berg, K. (1995). S phase arrest and induction of multinucleated cells after exposure to ultraviolet radiation. Carcinogenesis *16*, 1087-1094.

Bohonowych, J.E., Zhao, B., Timme-Laragy, A., Jung, D., Di Giulio, R.T., and Denison, M.S. (2008). Newspapers and newspaper ink contain agonists for the Ah receptor. Toxicological Scicience *102*, 278-290.

Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., and Monks, T.J. (2000). Role of quinones in toxicology. Chemical Research in Toxicology *13*, 135-160.

Bornholdt, J., Saber, A.T., Sharma, A.K., Savolainen, K., Vogel, U., and Wallin, H. (2007). Inflammatory response and genotoxicity of seven wood dusts in the human epithelial cell line A549. Mutation Research *632*, 78-88.

Cantero, G., Campanella, C., Mateos, S., and Cortes, F. (2006). Topoisomerase II inhibition and high yield of endoreduplication induced by the flavonoids luteolin and quercetin. Mutagenesis *21*, 321-325.

Casarett, L.J., Doull, J., and Klaassen, C.D. (2001). Casarett and Doull's Toxicology : the basic science of poisons, 6th edn (New York, McGraw-Hill Medical Pub. Division).

Cheng, Y.S. (2003). Aerosol Deposition in the Extrathoracic Region. Aerosol Science and Technology *37*, 659-671.

Ciolino, H.P., Daschner, P.J., and Yeh, G.C. (1999). Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. The Biochemical journal *340 (Pt 3)*, 715-722.

Demers, P.A., Kogevinas, M., Boffetta, P., Leclerc, A., Luce, D., Gerin, M., Battista, G., Belli, S., Bolm-Audorf, U., Brinton, L.A., *et al.* (1995). Wood dust and sino-nasal cancer: pooled reanalysis of twelve case-control studies. American Journal of Industrial Medicine *28*, 151-166.

Denison, M.S., and Nagy, S.R. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol *43*, 309-334.

DiNardi, S.R., DiNardi, S.R., and American Industrial Hygiene Association. (2003). The occupational environment : its evaluation, control, and management, 2nd edn (Fairfax, Va., AIHA Press (American Industrial Hygiene Association)).

Fukuda, I., Mukai, R., Kawase, M., Yoshida, K., and Ashida, H. (2007). Interaction between the aryl hydrocarbon receptor and its antagonists, flavonoids. Biochemical and Biophysical Research Communications *359*, 822-827.

Holmila, R., Bornholdt, J., Heikkila, P., Suitiala, T., Fevotte, J., Cyr, D., Hansen, J., Snellman, S.M., Dictor, M., Steiniche, T., *et al.* (2010a). Mutations in TP53 tumor

suppressor gene in wood dust-related sinonasal cancer. International Journal of cancer *127*, 578-588.

Holmila, R., Bornholdt, J., Suitiala, T., Cyr, D., Dictor, M., Steiniche, T., Wolff, H., Wallin, H., Luce, D., and Husgafvel-Pursiainen, K. (2010b). Profile of TP53 gene mutations in sinonasal cancer. Mutation Research *686*, 9-14.

Holmila, R., and Husgafvel-Pursiainen, K. (2006). Analysis of TP53 gene mutations in human lung cancer: comparison of capillary electrophoresis single strand conformation polymorphism assay with denaturing gradient gel electrophoresis and direct sequencing. Cancer Detection and Prevention *30*, 1-6.

Hsieh, P., and Yamane, K. (2008). DNA mismatch repair: molecular mechanism, cancer, and ageing. Mechanisms of Ageing and Development *129*, 391-407.

IARC (1995). Wood dust. IARC Monogr Eval Carcinog Risks Hum *62*, 35-215.

International Commission on Radiological Protection. Task Group on Reference Man. (1975). Report of the Task Group on Reference Man : a report (Oxford ; New York, Pergamon Press).

Kelly, J., Asgharian, B., Kimbell, J., and Wong, B. (2004). Particle Deposition in Human Nasal Airway Replicas Manufactured by Different Methods. Part I: Inertial Regime Particles. Aerosol Science and Technology *38*, 1063-1071.

Khan, F., Sherwani, A.F., and Afzal, M. (2009). Chromosomal aberration and micronucleus studies of two topoisomerase (II) targeting anthracyclines. Journal of Environmental Biology / Academy of Environmental Biology, India *30*, 409-412.

Klein, C.B., Kargacin, B., Su, L., Cosentino, S., Snow, E.T., and Costa, M. (1994a). Metal mutagenesis in transgenic Chinese hamster cell lines. Environmental Health Perspectives *102 Suppl 3*, 63-67.

Klein, C.B., and Rossman, T.G. (1990). Transgenic Chinese hamster V79 cell lines which exhibit variable levels of gpt mutagenesis. Environmental and Molecular Mutagenesis *16*, 1-12.

Klein, C.B., Su, L., Bowser, D., and Leszczynska, J. (2002). Chromate-induced epimutations in mammalian cells. Environmental Health Perspectives *110 Suppl 5*, 739-743.

Klein, C.B., Su, L., Rossman, T.G., and Snow, E.T. (1994b). Transgenic gpt+ V79 cell lines differ in their mutagenic response to clastogens. Mutation Research *304*, 217-228.

Klein, C.B., Su, L., Singh, J., and Snow, E.T. (1997). Characterization of gpt deletion mutations in transgenic Chinese hamster cell lines. Environmental and Molecular Mutagenesis *30*, 418-428.

Korinth, D., Pacyna-Gengelbach, M., Deutschmann, N., Hattenberger, S., Bockmuhl, U., Dietel, M., Schroeder, H.G., Donhuijsen, K., and Petersen, I. (2005). Chromosomal imbalances in wood dust-related adenocarcinomas of the inner nose and their associations with pathological parameters. Journal of Pathology *207*, 207-215.

Kundu, J.K., and Surh, Y.J. (2008). Inflammation: gearing the journey to cancer. Mutation Research *659*, 15-30.

Lee, Y.W., Pons, C., Tummolo, D.M., Klein, C.B., Rossman, T.G., and Christie, N.T. (1993). Mutagenicity of soluble and insoluble nickel compounds at the gpt locus in G12 Chinese hamster cells. Environmental and Molecular Mutagenesis *21*, 365-371.

Long, H., Shi, T., Borm, P.J., Maatta, J., Husgafvel-Pursiainen, K., Savolainen, K., and Krombach, F. (2004). ROS-mediated TNF-alpha and MIP-2 gene expression in alveolar macrophages exposed to pine dust. Particle and Fibre Toxicology *1*, 3.

Maatta, J., Luukkonen, R., Husgafvel-Pursiainen, K., Alenius, H., and Savolainen, K. (2006). Comparison of hardwood and softwood dust-induced expression of cytokines and chemokines in mouse macrophage RAW 264.7 cells. Toxicology *218*, 13-21.

Maatta, J., Majuri, M.L., Luukkonen, R., Lauerma, A., Husgafvel-Pursiainen, K., Alenius, H., and Savolainen, K. (2005). Characterization of oak and birch dust-induced expression of cytokines and chemokines in mouse macrophage RAW 264.7 cells. Toxicology *215*, 25-36.

Mammela, P., Tuomainen, A., Vartiainen, T., Lindroos, L., Kangas, J., and Savolainen, H. (2002). Biological monitoring of wood dust exposure in nasal lavage by high-performance liquid chromatography. Journal of Environmental Monitoring *4*, 187-189.

Marlowe, J.L., and Puga, A. (2005). Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. Journal of Cellular Biochemistry *96*, 1174-1184.

Miller, C.A., 3rd (1999). A human aryl hydrocarbon receptor signaling pathway constructed in yeast displays additive responses to ligand mixtures. Toxicology and Applied Pharmacology *160*, 297-303.

Nam, S., Smith, D.M., and Dou, Q.P. (2001). Tannic acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis. Cancer Epidemiology Biomarkers and Prevention *10*, 1083-1088.

NTP (2005). NTP 11th Report on Carcinogens. Rep Carcinog, 1-A32.

Perrone, F., Oggionni, M., Birindelli, S., Suardi, S., Tabano, S., Romano, R., Moiraghi, M.L., Bimbi, G., Quattrone, P., Cantu, G., *et al.* (2003). TP53, p14ARF, p16INK4a and H-ras gene molecular analysis in intestinal-type adenocarcinoma of the nasal cavity and paranasal sinuses. International Journal of Cancer *105*, 196-203.

Pommier, Y., Leo, E., Zhang, H., and Marchand, C. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chemistry & Biology *17*, 421-433.

Rando, R., Poovey, H., Mokadam, D., Brisolara, J., and Glindmeyer, H. (2005). Field performance of the RespiCon for size-selective sampling of industrial wood processing dust. Journal of Occupational and Environmental Hygiene *2*, 219-226.

Romac, S., Leong, P., Sockett, H., and Hutchinson, F. (1989). DNA base sequence changes induced by ultraviolet light mutagenesis of a gene on a chromosome in Chinese hamster ovary cells. Journal of Molecular Biology *209*, 195-204.

Saber, A.T., Nielsen, L.R., Dictor, M., Hagmar, L., Mikoczy, Z., and Wallin, H. (1998). K-ras mutations in sinonasal adenocarcinomas in patients occupationally exposed to wood or leather dust. Cancer Letters *126*, 59-65.

Sjöström, E. (1993). Wood chemistry : fundamentals and applications, 2nd edn (San Diego, Academic Press).

Stankowski, L.F., Jr., and Hsie, A.W. (1986). Quantitative and molecular analyses of radiation-induced mutation in AS52 cells. Radiation Research *105*, 37-48.

Swift, L.P., Rephaeli, A., Nudelman, A., Phillips, D.R., and Cutts, S.M. (2006). Doxorubicin-DNA adducts induce a non-topoisomerase II-mediated form of cell death. Cancer Research *66*, 4863-4871.

Tian, Z.F., Inthavong, K., and Tu, J.Y. (2007). Deposition of inhaled wood dust in the nasal cavity. Inhalaltion Toxicology *19*, 1155-1165.

Torronen, R., Pelkonen, K., and Karenlampi, S. (1989). Enzyme-inducing and cytotoxic effects of wood-based materials used as bedding for laboratory animals. Comparison by a cell culture study. Life Science *45*, 559-565.

Valente, G., Ferrari, L., Kerim, S., Gervasio, C.F., Ricci, E., Migliaretti, G., Pira, E., and Bussi, M. (2004). Evidence of p53 immunohistochemical overexpression in ethmoidal mucosa of woodworkers. Cancer Detection and Prevention *28*, 99-106.

Vogel, C.F., Li, W., Sciullo, E., Newman, J., Hammock, B., Reader, J.R., Tuscano, J., and Matsumura, F. (2007). Pathogenesis of aryl hydrocarbon receptor-mediated development of lymphoma is associated with increased cyclooxygenase-2 expression. The American Journal of Pathology *171*, 1538-1548.

Vos, S., de Jersey, J., and Martin, J.L. (1997). Crystal structure of Escherichia coli xanthine phosphoribosyltransferase. Biochemistry *36*, 4125-4134.

Williams, C., Ponten, F., Moberg, C., Soderkvist, P., Uhlen, M., Ponten, J., Sitbon, G., and Lundeberg, J. (1999). A high frequency of sequence alterations is due to formalin fixation of archival specimens. The American Journal of Pathology *155*, 1467-1471.

Zhang, S., Qin, C., and Safe, S.H. (2003). Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. Environmental Health Perspectives *111*, 1877-1882.

Supplementary data

AhR activation

Table 46. Sigmoidal dose-response best fit values for AhR activity inducted by treatment with methanol extracts of hardwood dust

	Teak	Mahogany	Walnut	Poplar	Oak
BOTTOM	-4.008	-17.74	-5.641	-7.782	-4.887
TOP	84.76	72.78	71.14	57.22	30.55
LOGEC50	-0.6787	1.363	0.7412	1.691	2.4
EC50	0.2096	23.09	5.511	49.08	251.1

As a group the hardwood extracts showed the most activity. Particularly the teak wood dust and walnut wood dust extracts. The teak treatment resulted in a signal that was ~85% of the maximum signal with a very low EC_{50} at 0.2 μg extract /ml growth medium. The Walnut treatment resulted in a signal that was 71% of the maximum signal with an EC_{50} at 5.5 μg extract /ml growth medium. This means that teak wood and walnut extracts are the most potent and effective at inducing AhR signaling in the yeast assay. Mahogany and poplar extracts both achieved a signal greater than 50% of the maximum but have much higher EC_{50} values when compared to teak and walnut. Oak did not achieve 50% of the maximum signal.

Table 47. Sigmoidal dose-response best fit values for AhR activity induced by treatment with methanol extracts of wood product dust

	Plywood	Bedding	MDF	Treated Pine
BOTTOM	-3.036	-0.4629	-0.3347	-2.915
TOP	73.28	71.32	50.21	29.25
LOGEC50	0.4509	-0.3512	0.8385	1.767
EC50	2.825	0.4454	6.894	58.48

As a group the wood product dust extracts induced more signaling than the softwoods but less than the hardwoods. It should be noted that the bedding is a hardwood product and the hardwood content in the plywood and MDF treatments is unknown but should not be assumed to be zero.

Table 48. Sigmoidal dose-response best fit values for AhR activity inducted by treatment with methanol extracts of softwood dust

	Cedar	Cypress	Spruce	Yellow Pine
BOTTOM	-4.704	-3.296	-0.4444	0.4801
TOP	32.7	27.63	8.271	3.212
LOGEC50	1.867	1.387	1.456	0.5871
EC50	73.67	24.36	28.58	3.865

As a group the softwood extracts did not induce AhR signaling in a potent or effective manor. Cypress extract has the highest reported value at ~28% of the maximum signal and the EC_{50} for the dose response curve is almost 25 μg extract/ml of medium.

Table 49. ANOVA table with post hoc pairwise comparisons between hardwood, wood product, softwood, and background signal in the YCM3 yeast bioassay

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. Different? (P < 0.05)	Yes
Number of groups	4
F	16.78
R squared	0.7824

ANOVA Table	SS	df	MS
Treatment (between columns)	13040	3	4346
Residual (within columns)	3626	14	259
Total	16660	17	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
background vs Hardwood	-63.29	6.218	P < 0.01	-90.06 to -36.52
background vs Softwood	-17.95	1.663	P > 0.05	-46.35 to 10.44
background vs Wood product	-56.02	5.189	P < 0.01	-84.41 to -27.62

The Hardwood and wood product categories had showed an average maximum response that was significantly different from background while the softwood category was not found to be significantly different from background.

g12 genotoxicity experiments

Mutation frequency ANOVA tables

Table 50. ANOVA table from hardwood mutant frequency comparison

Parameter	Value
Table Analyzed	
Hardwoods	
One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	11
F	104.2
R squared	0.9148

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	90.79
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	64710	10	6471
Residual (within columns)	6025	97	62.11
Total	70730	107	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Control vs Mahogany 2.5 μg/ml	-9.685	3.01	P < 0.05	-18.61 to -0.7556
Control vs Mahogany 5 μg/ml	-11.47	3.566	P < 0.01	-20.40 to -2.544
Control vs Teak 2.5 μg/ml	-12.32	3.829	P < 0.01	-21.25 to -3.391
Control vs Teak 5 μg/ml	-91.17	28.33	P < 0.01	-100.1 to -82.24
Control vs Poplar 3.3 μg/ml	-0.6343	0.1971	P > 0.05	-9.564 to 8.295
Control vs Poplar 6.5 μg/ml	-3.84	1.194	P > 0.05	-12.77 to 5.089
Control vs Oak 25 μg/ml	-1.899	0.5902	P > 0.05	-10.83 to 7.031
Control vs Oak 50 μg/ml	-5.561	1.728	P > 0.05	-14.49 to 3.369
Control vs Walnut 8.5 μg/ml	2.824	0.8778	P > 0.05	-6.105 to 11.75
Control vs Walnut 17 μg/ml	-3.412	1.06	P > 0.05	-12.34 to 5.518

Table 51. ANOVA table from softwood mutant frequency comparison

Parameter	Value
Table Analyzed	
Softwoods	
One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	7
F	68.26
R squared	0.863

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	82.26
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	Df	MS
Treatment (between columns)	11330	6	1888
Residual (within columns)	1798	65	27.66
Total	13130	71	

Dunnett's Multiple Comparison Test	Mean Diff.	Q	P value	95% CI of diff
Control vs Cypress 10 μg/ml	-1.691	0.7878	P > 0.05	-7.352 to 3.970
Control vs Cypress 13.5 μg/ml	-16.34	7.611	P < 0.01	-22.00 to -10.68
Control vs Cedar 30 μg/ml	-0.03161	0.0147	P > 0.05	-5.693 to 5.629
Control vs Cedar 40 μg/ml	1.882	0.8763	P > 0.05	-3.780 to 7.543
Control vs Spruce 30 μg/ml	3.534	1.646	P > 0.05	-2.127 to 9.195
Control vs Spruce 40 μg/ml	-35.54	16.55	P < 0.01	-41.20 to -29.88

Table 52. ANOVA table from wood product mutant frequency comparison

Parameter	Value
Table Analyzed	
Wood products	
One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	11.21
R squared	0.4778
Bartlett's test for equal variances	
Bartlett's statistic (corrected)	19.66
P value	0.0006
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	785.1	4	196.3
Residual (within columns)	858.1	49	17.51

Total	1643	53		

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Control vs MDF 3 μg/ml	-10.24	5.993	P < 0.01	-14.55 to -5.922
Control vs MDF 6 μg/ml	-4.466	2.614	P < 0.05	-8.783 to -0.1500
Control vs L. Bedding 30 μg/ml	-2.594	1.518	P > 0.05	-6.910 to 1.722
Control vs L. Bedding 40 μg/ml	-7.784	4.556	P < 0.01	-12.10 to -3.467

Full-length gpt transgene deletion Fisher's exact test tables

Table 53. Teak wood dust extract #1 deletion frequency analysis

Fisher's exact test

P value	0.0217
P value summary	*
One- or two-sided	One-sided
Statistically significant? (alpha<0.05)	Yes

Data analyzed	Solvent	Teak #1	Total
Deletion	3	17	20
Non-deletion	24	32	56
Total	27	49	76

Table 54. Teak wood dust extract #2 deletion frequency analysis

Fisher's exact test

P value	0.0054
P value summary	**
One- or two-sided	One-sided
Statistically significant? (alpha<0.05)	Yes

Data analyzed	Solvent	Teak #2	Total
Deletion	3	24	27
Non-deletion	24	36	60
Total	27	60	87

Table 55. Teak wood dust extract #3 deletion frequency analysis

Fisher's exact test

P value	0.0116
P value summary	*
One- or two-sided	One-sided
Statistically significant? (alpha<0.05)	Yes

Data analyzed	Solvent	Teak #3	Total
Deletion	3	23	26
Non-deletion	24	40	64
Total	27	63	90

Table 56. 2-methylanthraquinone deletion frequency analysis

Fisher's exact test

P value	0.0091
P value summary	**
One- or two-sided	One-sided
Statistically significant? (alpha<0.05)	Yes

Data analyzed	Solvent	2-methylanthraquinone	Total
Deletion	3	24	27
Non-deletion	24	40	64
Total	27	64	91

Table 57. Pooled teak wood dust extract deletion frequency analysis

Table Analyzed	Pooled teak
Fisher's exact test	
P value	0.0047
P value summary	**
One- or two-sided	One-sided
Statistically significant? (alpha<0.05)	Yes

Data analyzed	Control	Pooled teak	Total
Deletion	3	64	67
Non-deletion	24	108	132
Total	27	172	199

gpt sequences from 6-TG resistant g12 cells

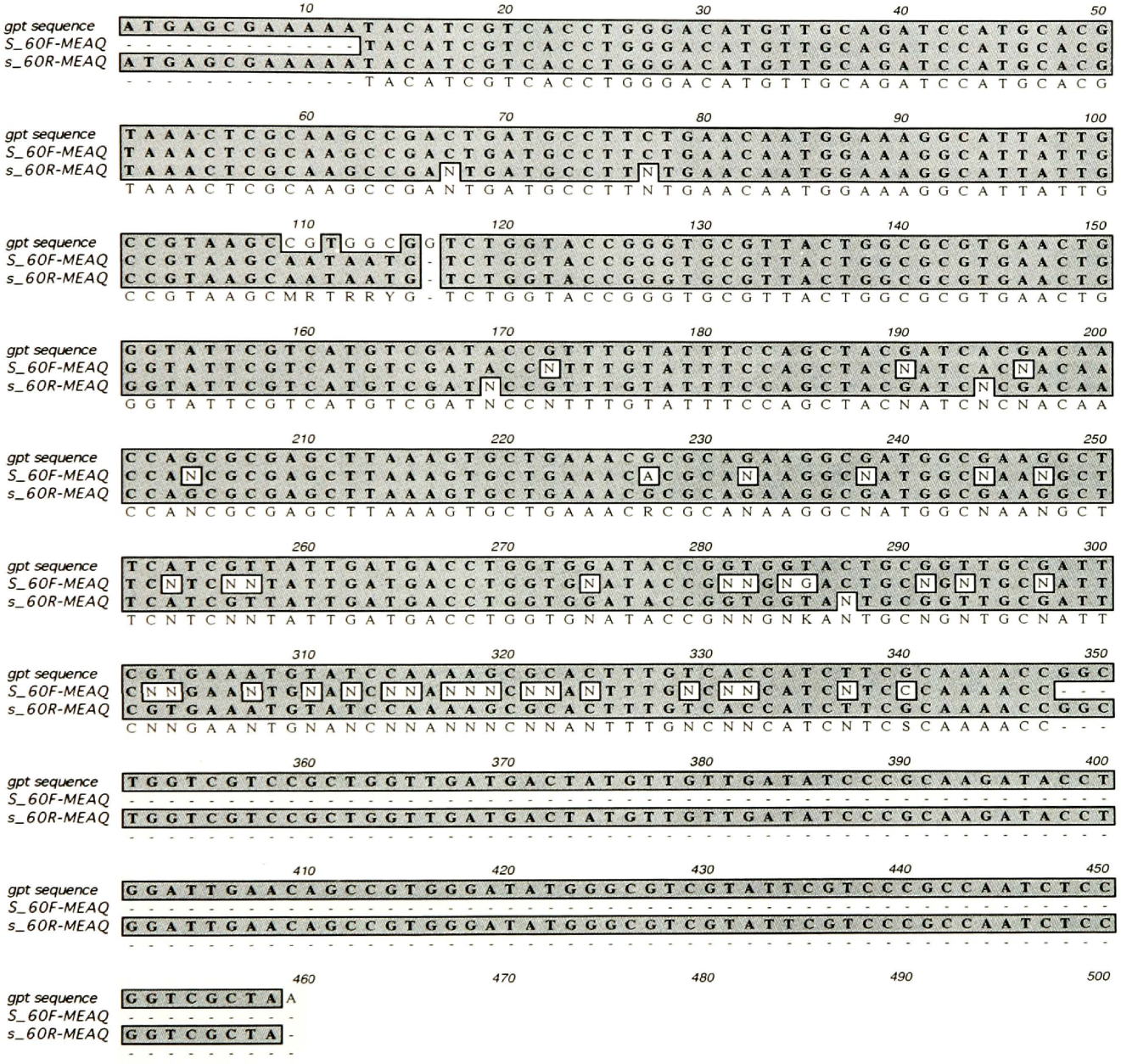
Figure 60. Mutations of *gpt* coding region in spontaneous 6-TG resistant cells

Figure 61. (A-E) Mutations of *gpt* coding sequence in 2-methylanthraquinnone treated cells

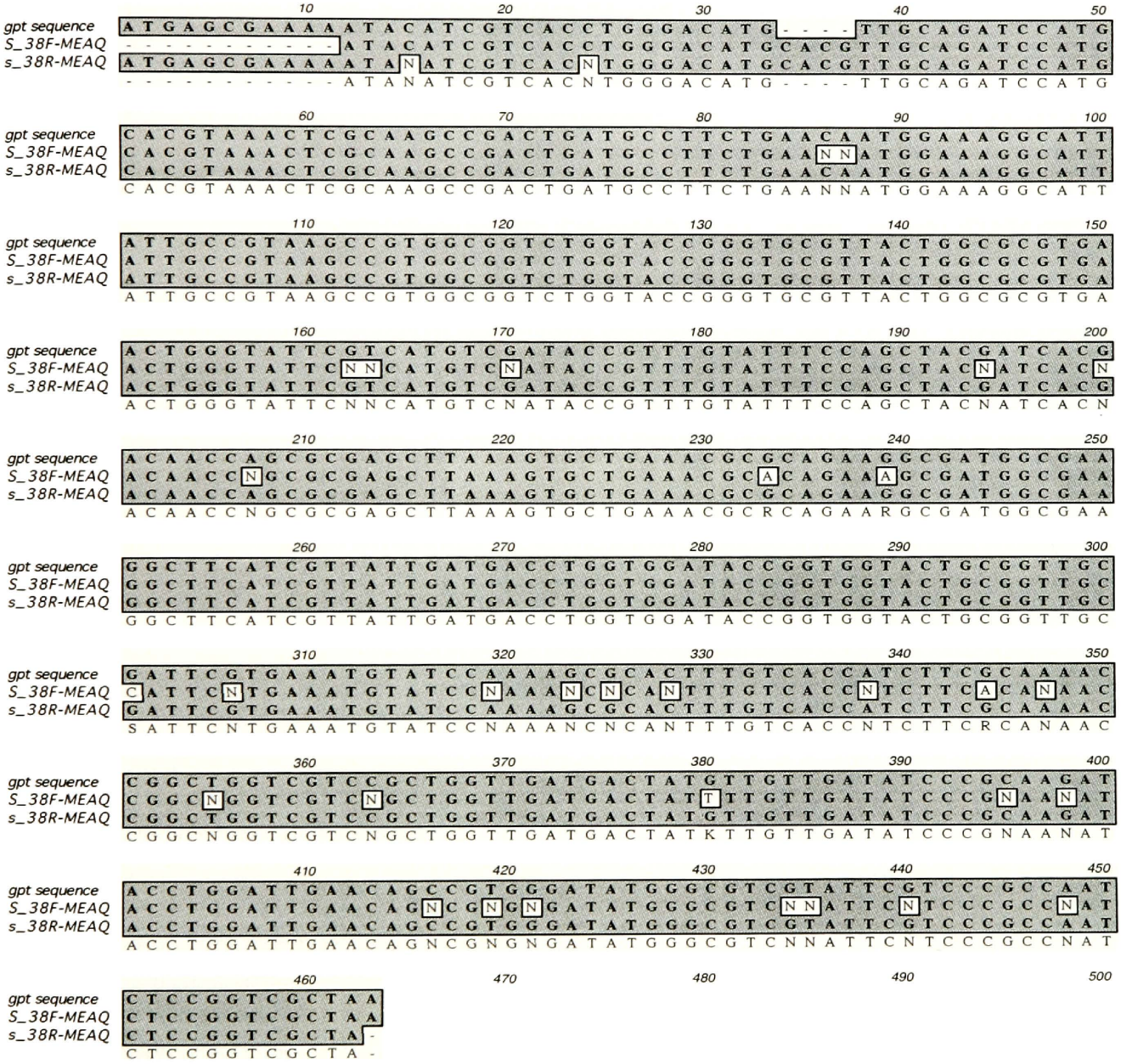
A)

B)

C)



D)

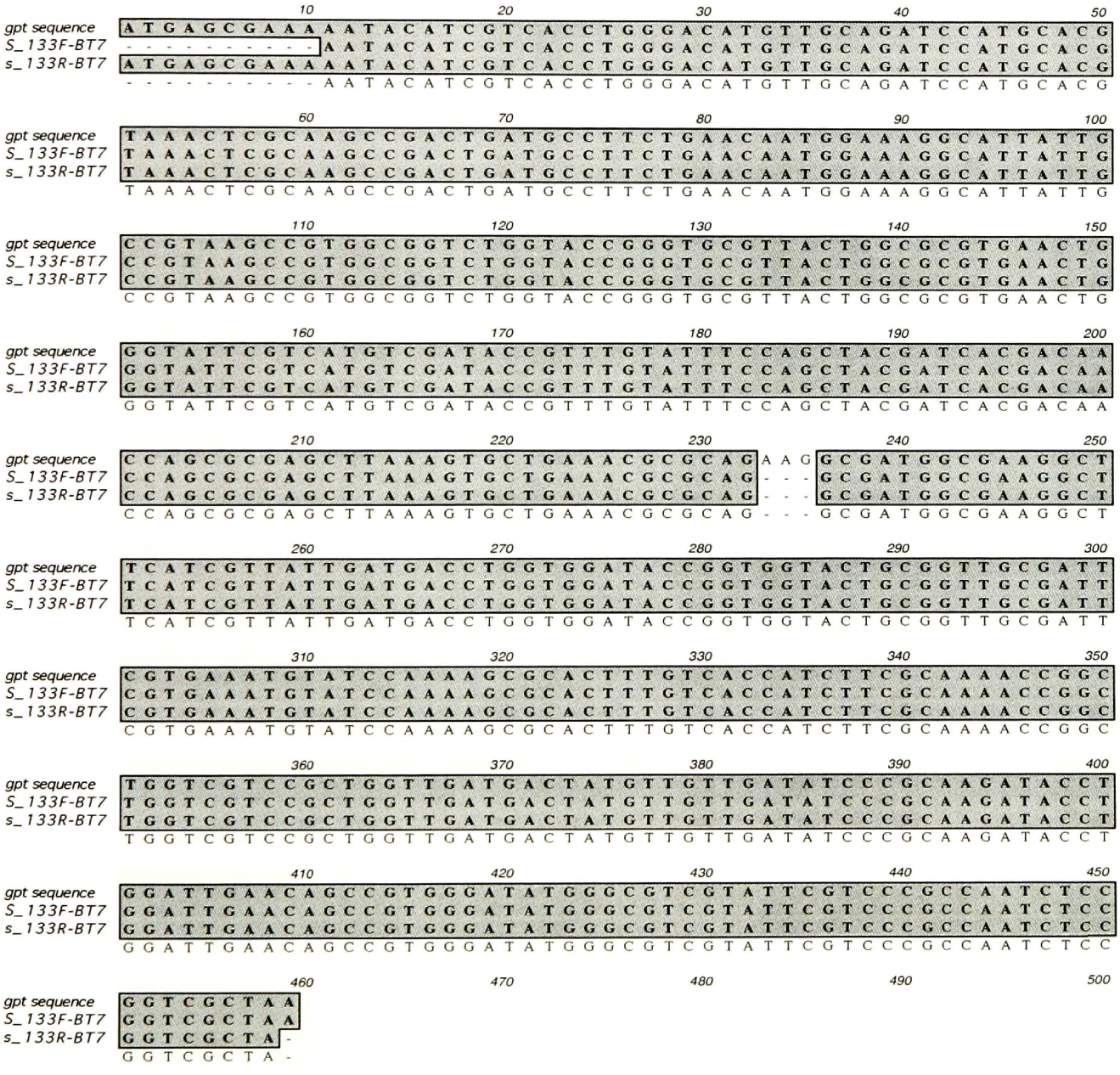


E)

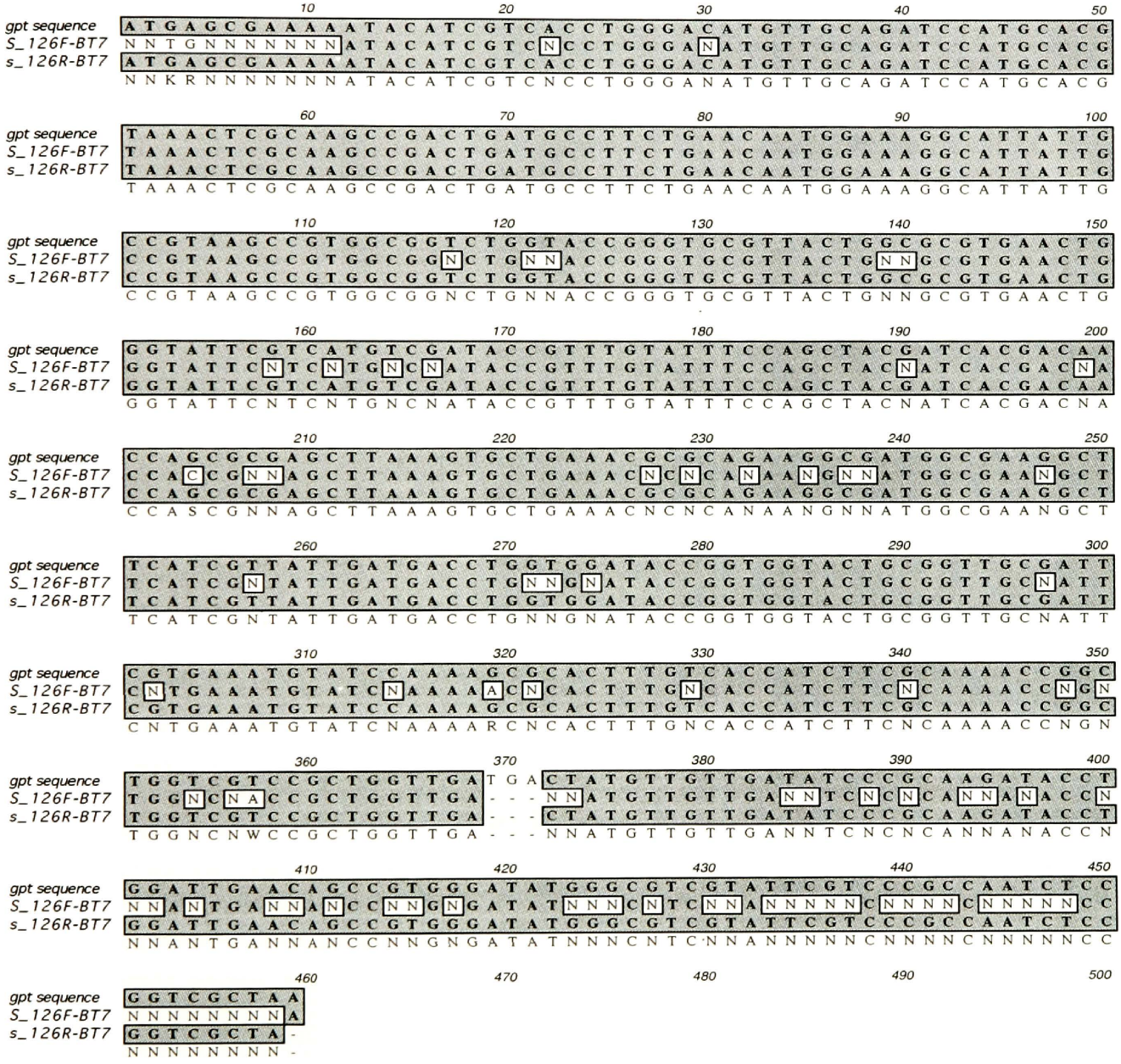

gpt sequence
S_55F-MEAQ
s_55R-MEAQ
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
CCGTAAGCCGTGGCGGTCTGGTACCGGGGTGCGTTACTGGCGCGTGAACTG
GGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGATCACGACAA
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCT
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
CGTGAAAATGTATCCAAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGC
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATCCCGCAAGATACCT
GGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAATCTCC
GGTCGCTAA

Figure 62. (A-E) Mutations of *gpt* coding sequence in Teak wood dust extract #1 treated cells

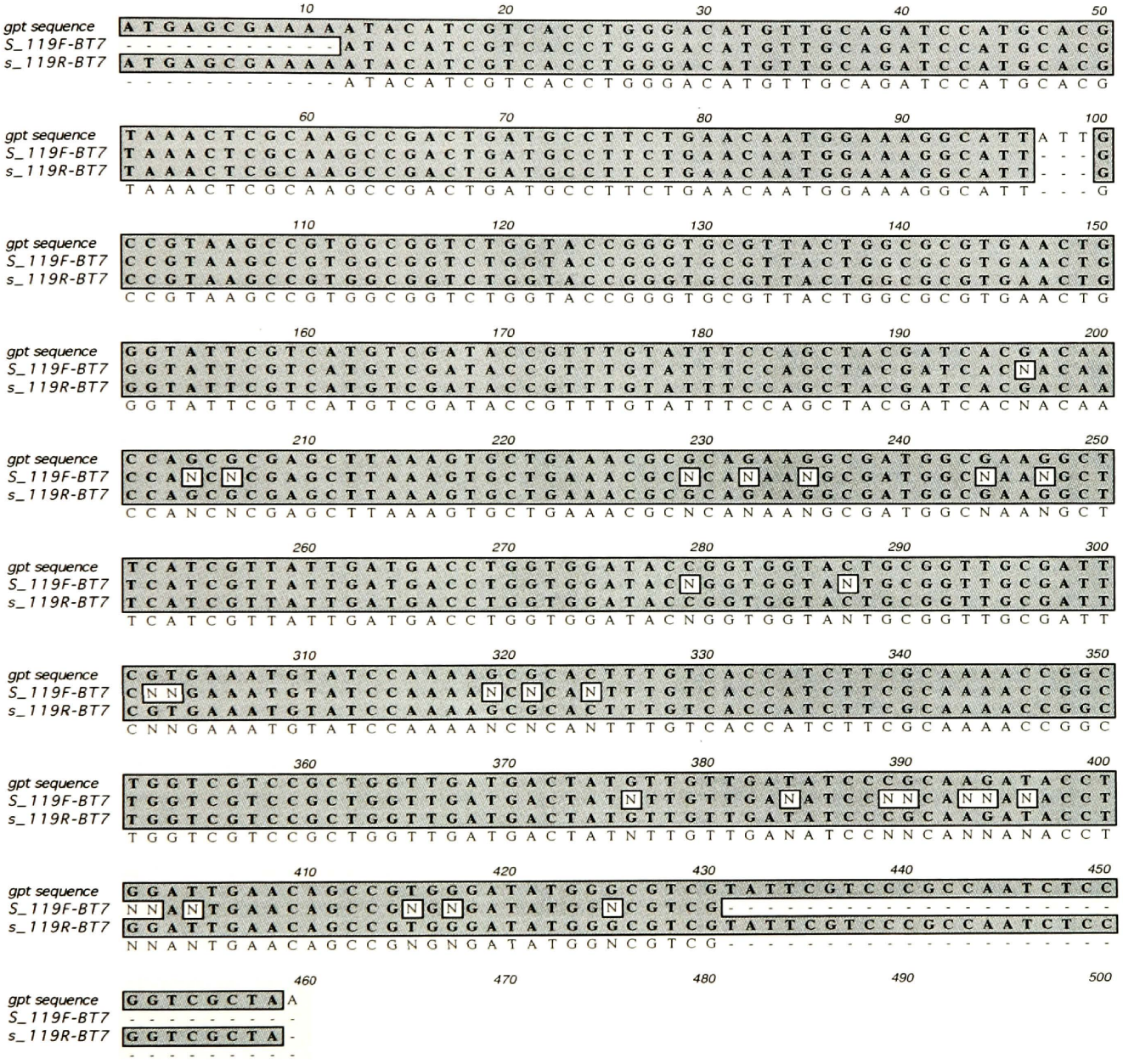
A)



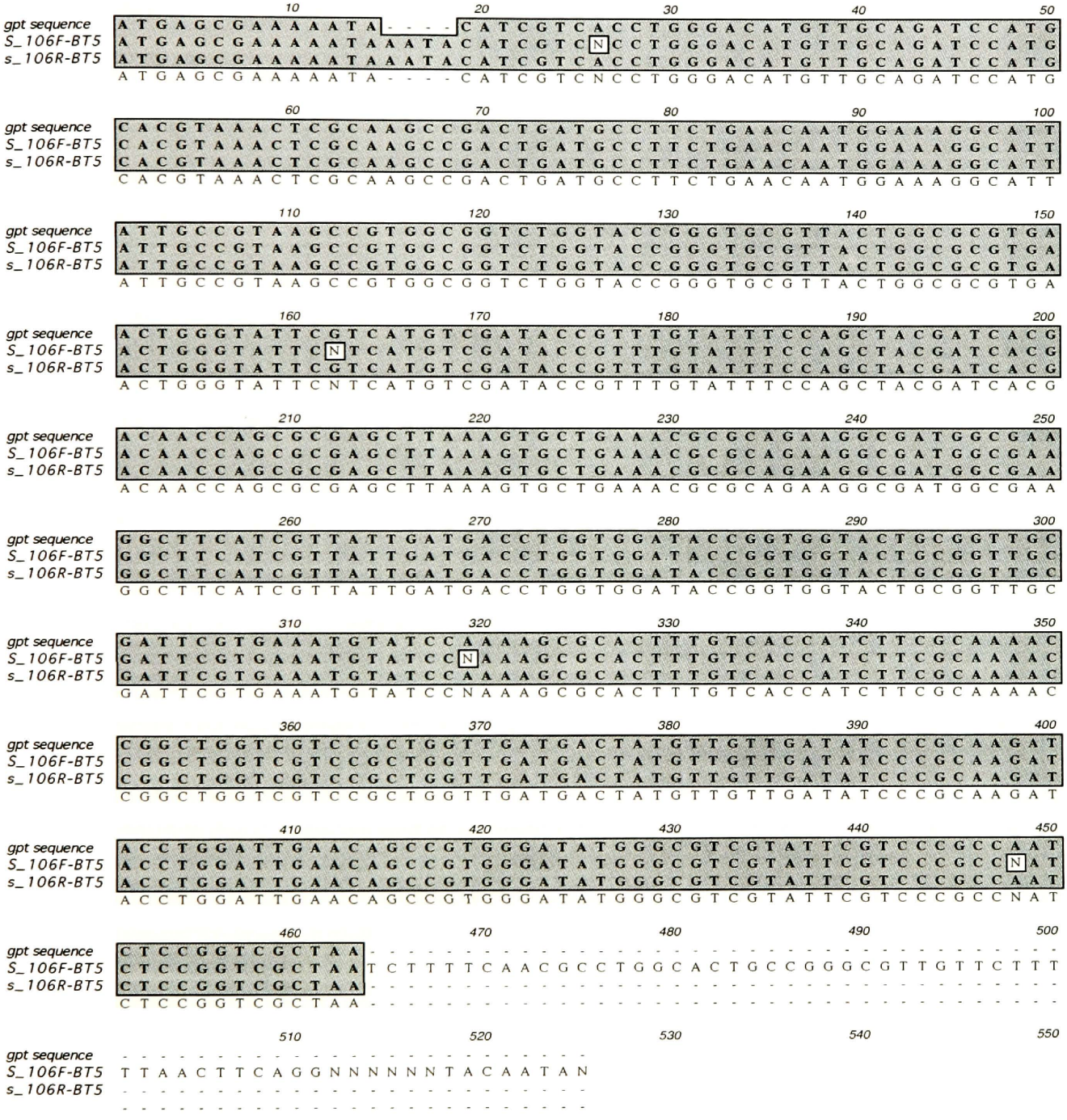
B)



C)



D)



E)

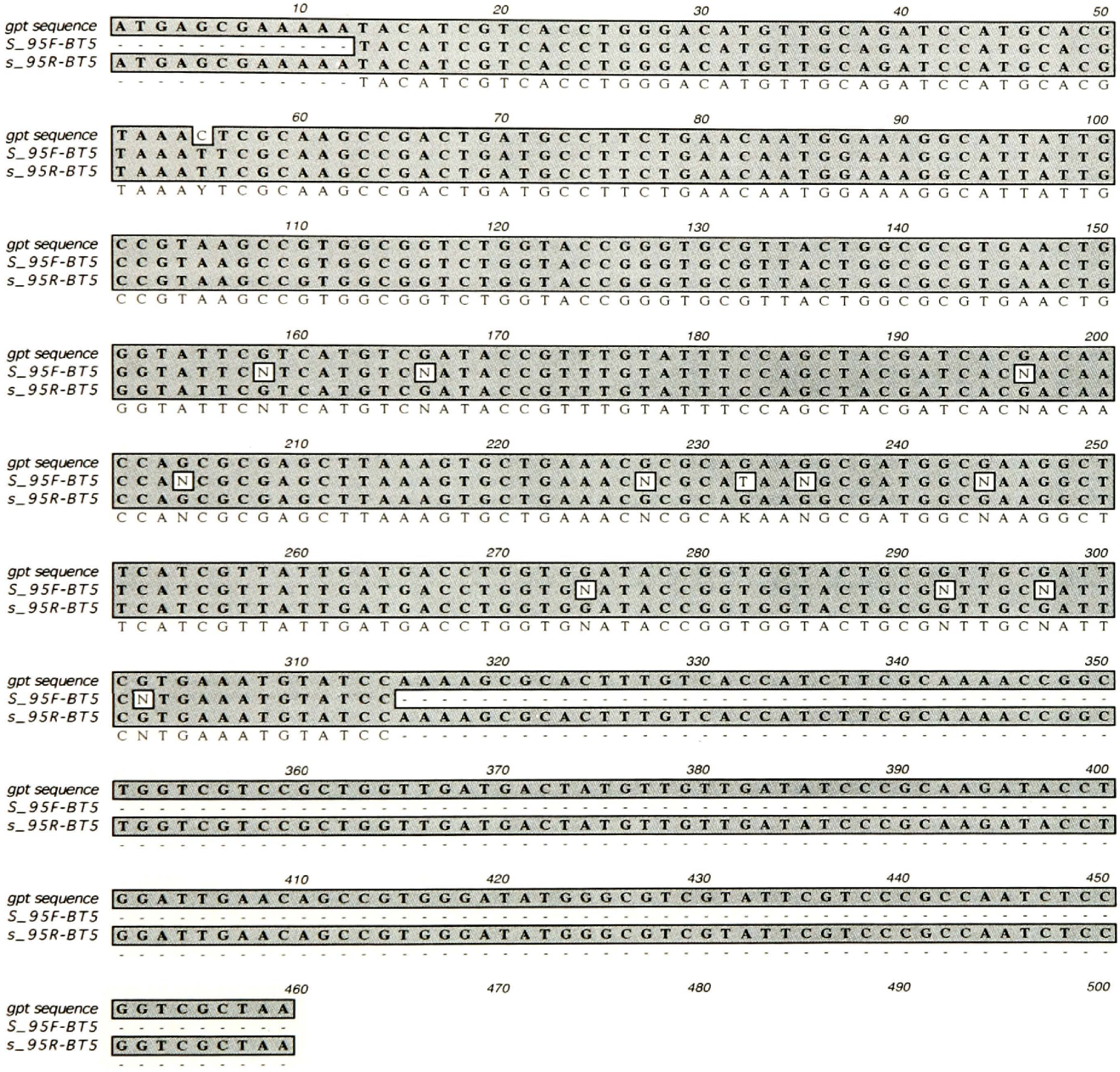
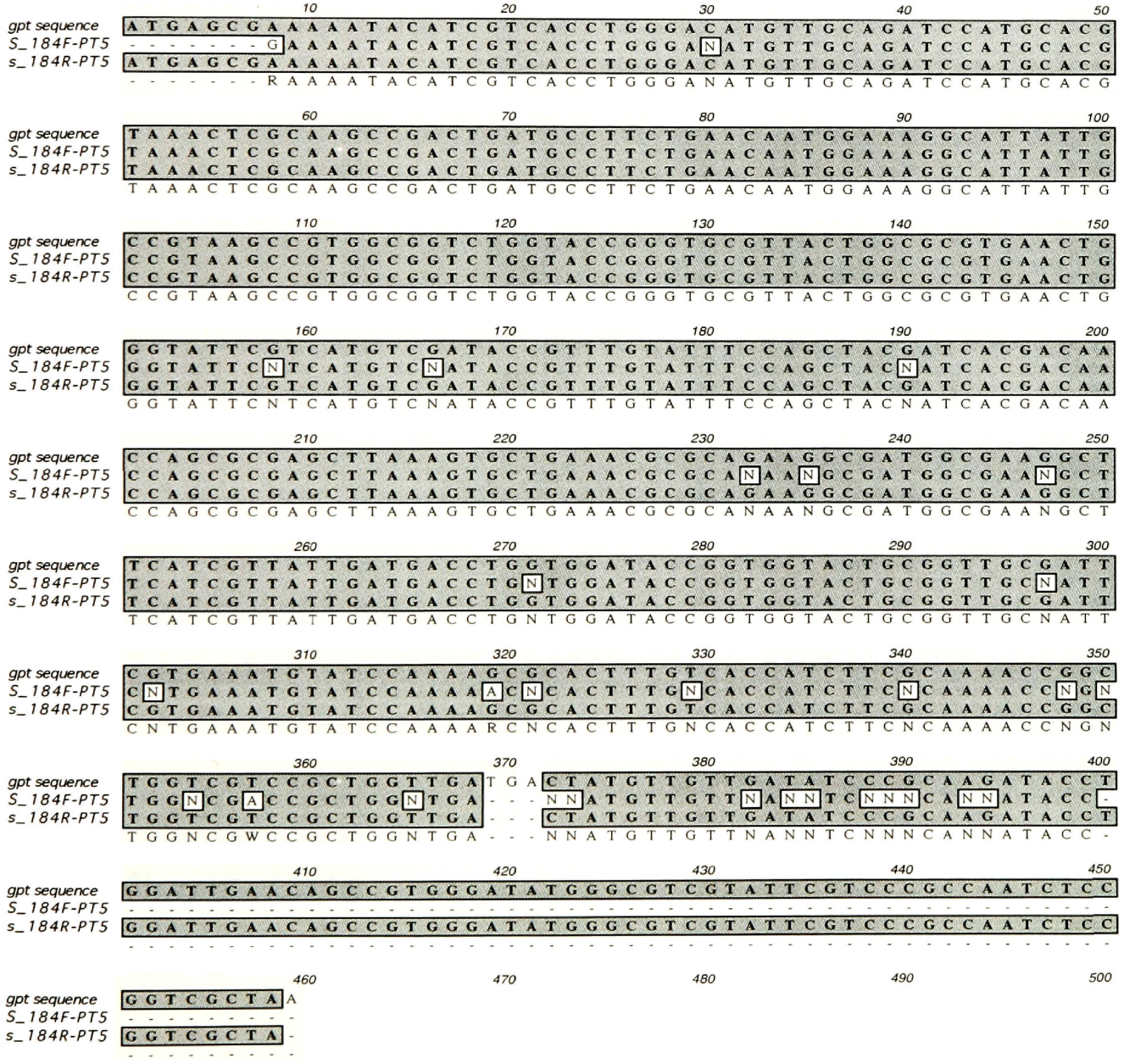
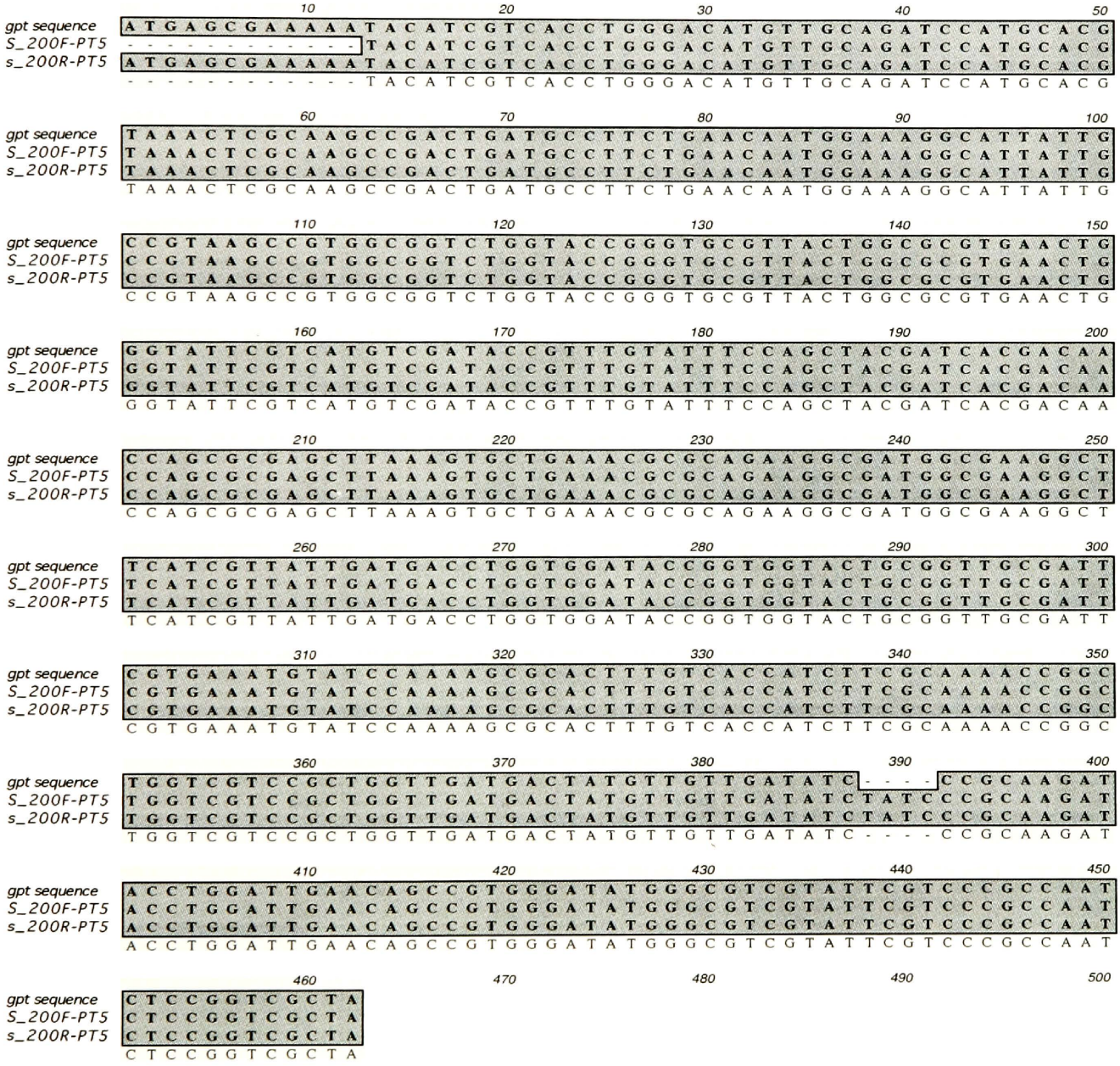


Figure 63. (A-E) Mutations of *gpt* coding sequence treated with teak wood dust extract #2

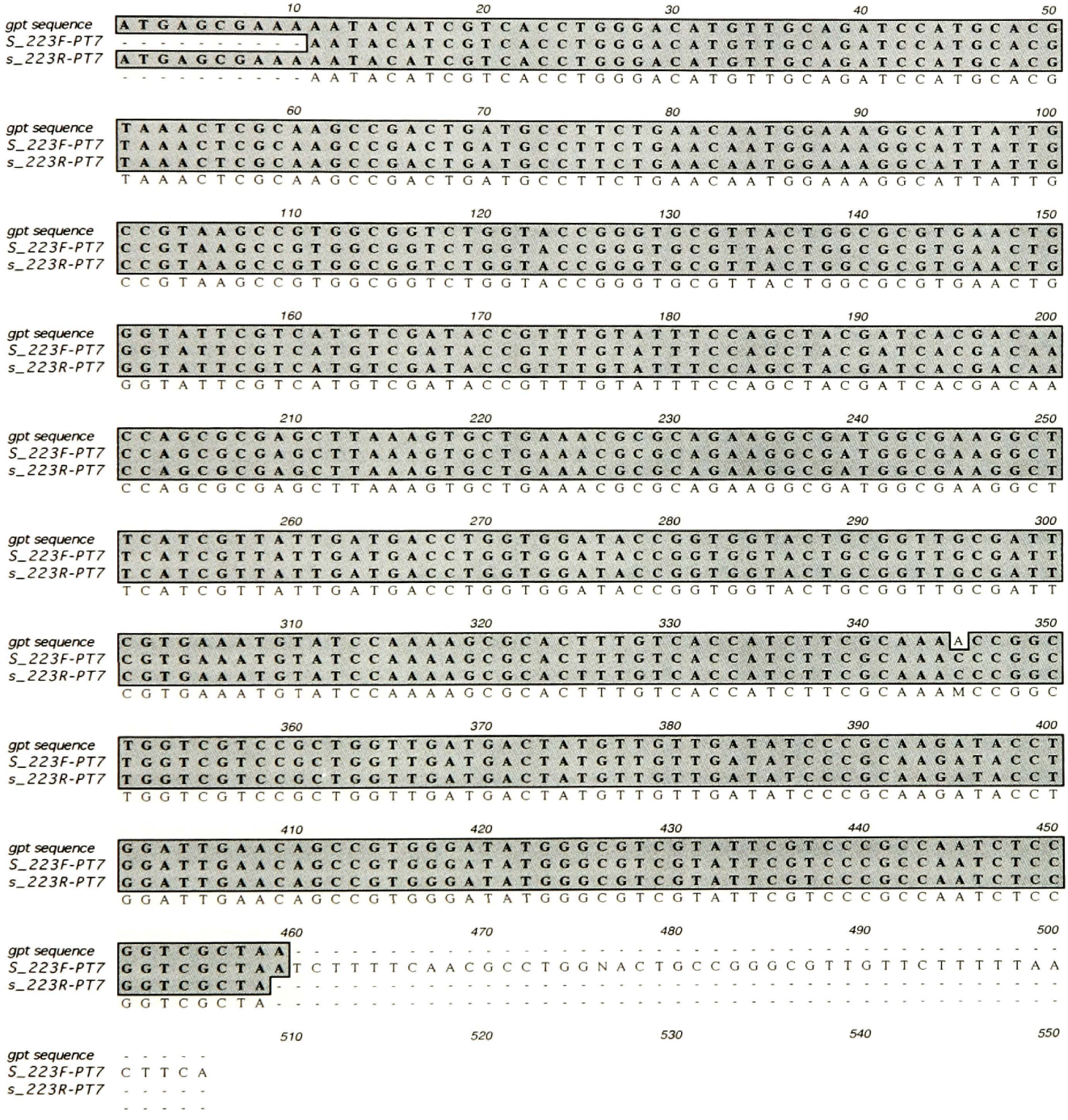
A)



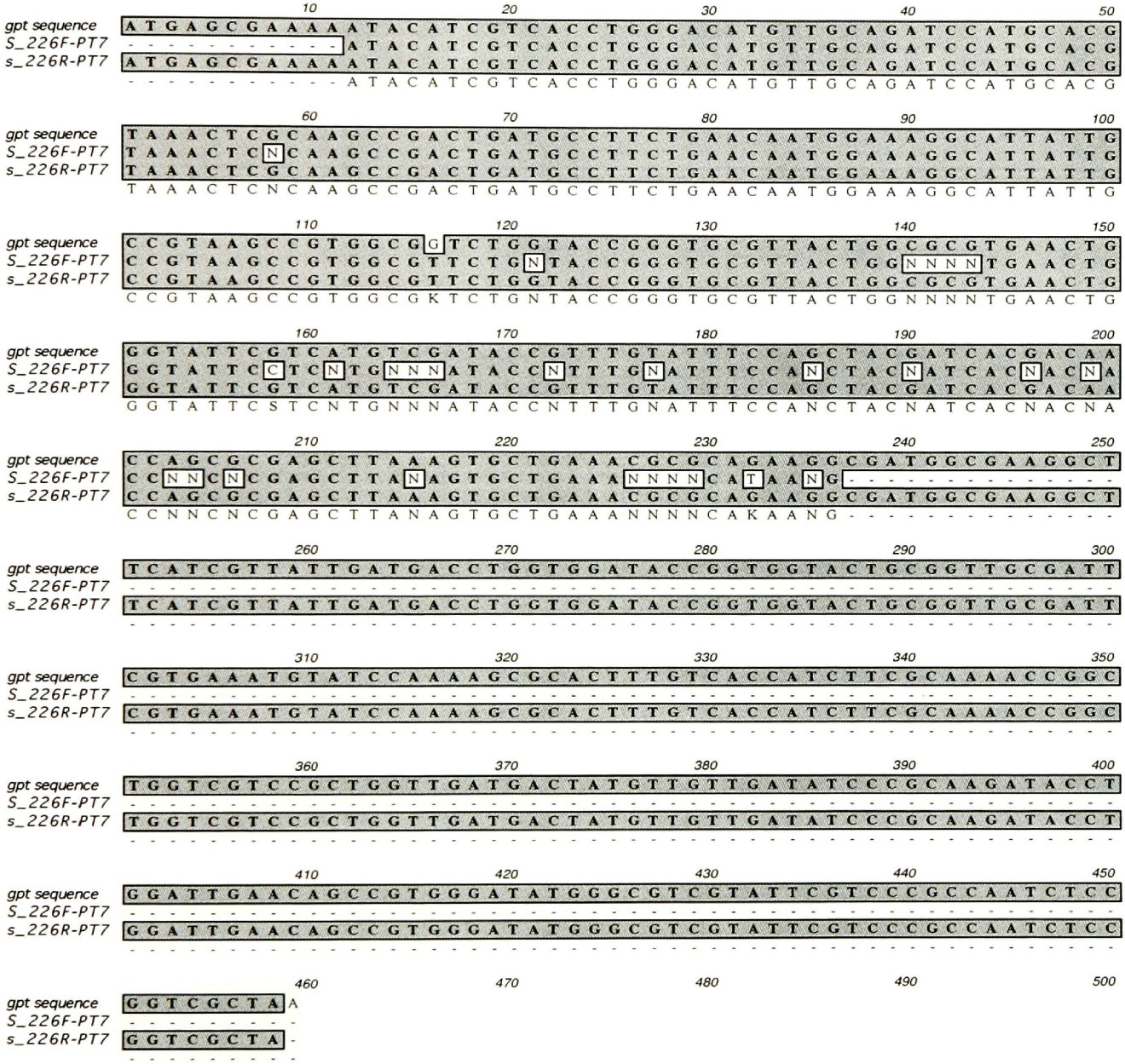
B)


gpt sequence
S_200F-PT5
s_200R-PT5
10 20 30 40 50
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
60 70 80 90 100
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
110 120 130 140 150
CCGTAAGCCGTGGCGGTCTGGTACCGGGTGCGTTACTGGCGCGTGAACTG
160 170 180 190 200
GGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGATCACGACAA
210 220 230 240 250
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCT
260 270 280 290 300
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
310 320 330 340 350
CGTGAAATGTATCCAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGC
360 370 380 390 400
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATC----CCGCAAGAT
410 420 430 440 450
ACCTGGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAAT
460 470 480 490 500
CTCCGGTCGCTA

C)


gpt sequence
S_223F-PT7
s_223R-PT7
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
AATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
CCGTAAGCCGTGGCGGTCTGGTACCGGGTGCGTTACTGGCGCGTGAACTG
GGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGATCACGACAA
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCT
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
CGTGAAATGTATCCAAAAAGCGCACTTTGTCACCATCTTCGCAAAACCCGGC
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATCCCGCAAGATACCT
GGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAATCTCC
GGTCGCTAA
CTTCA

D)



E)

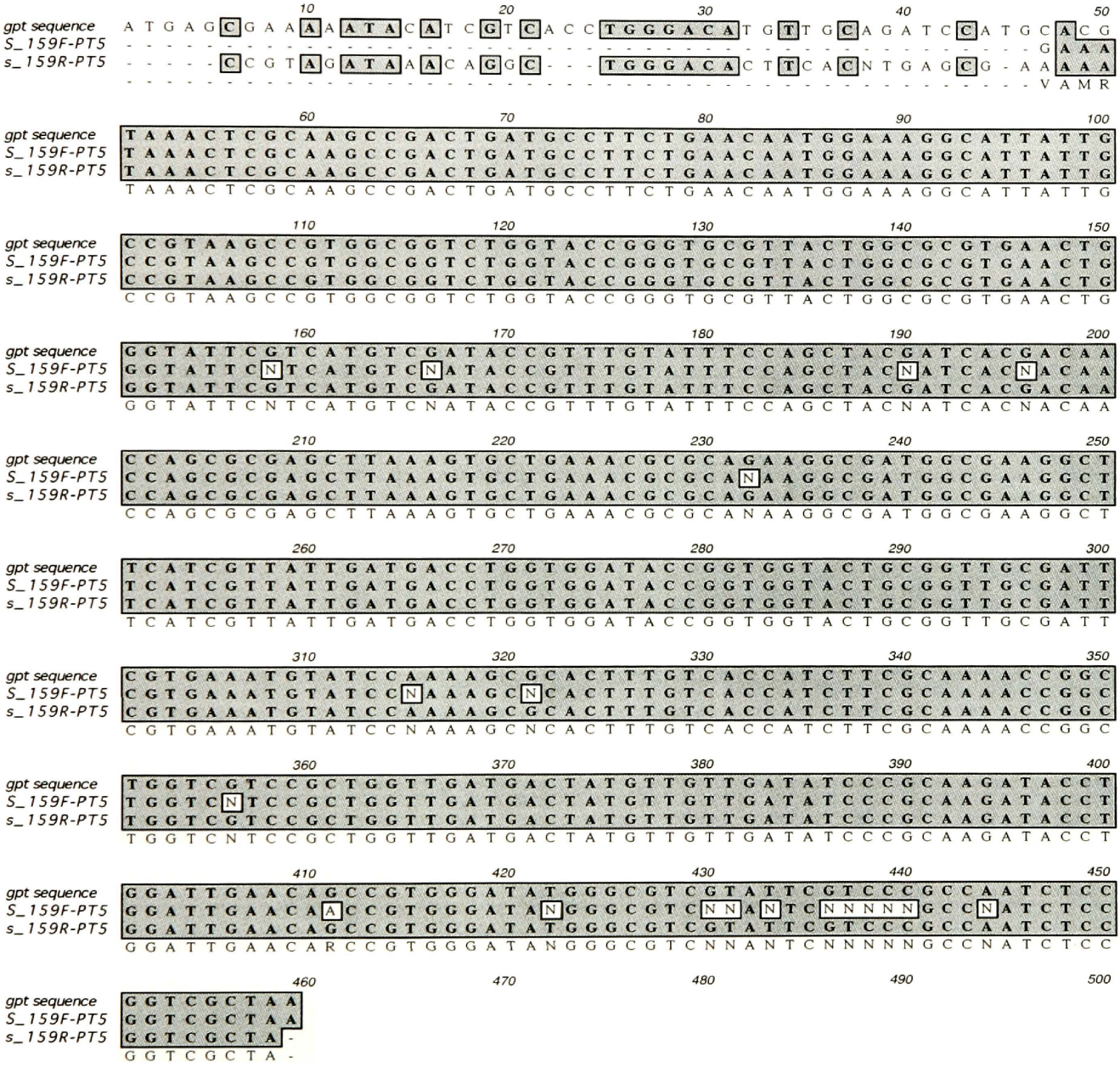
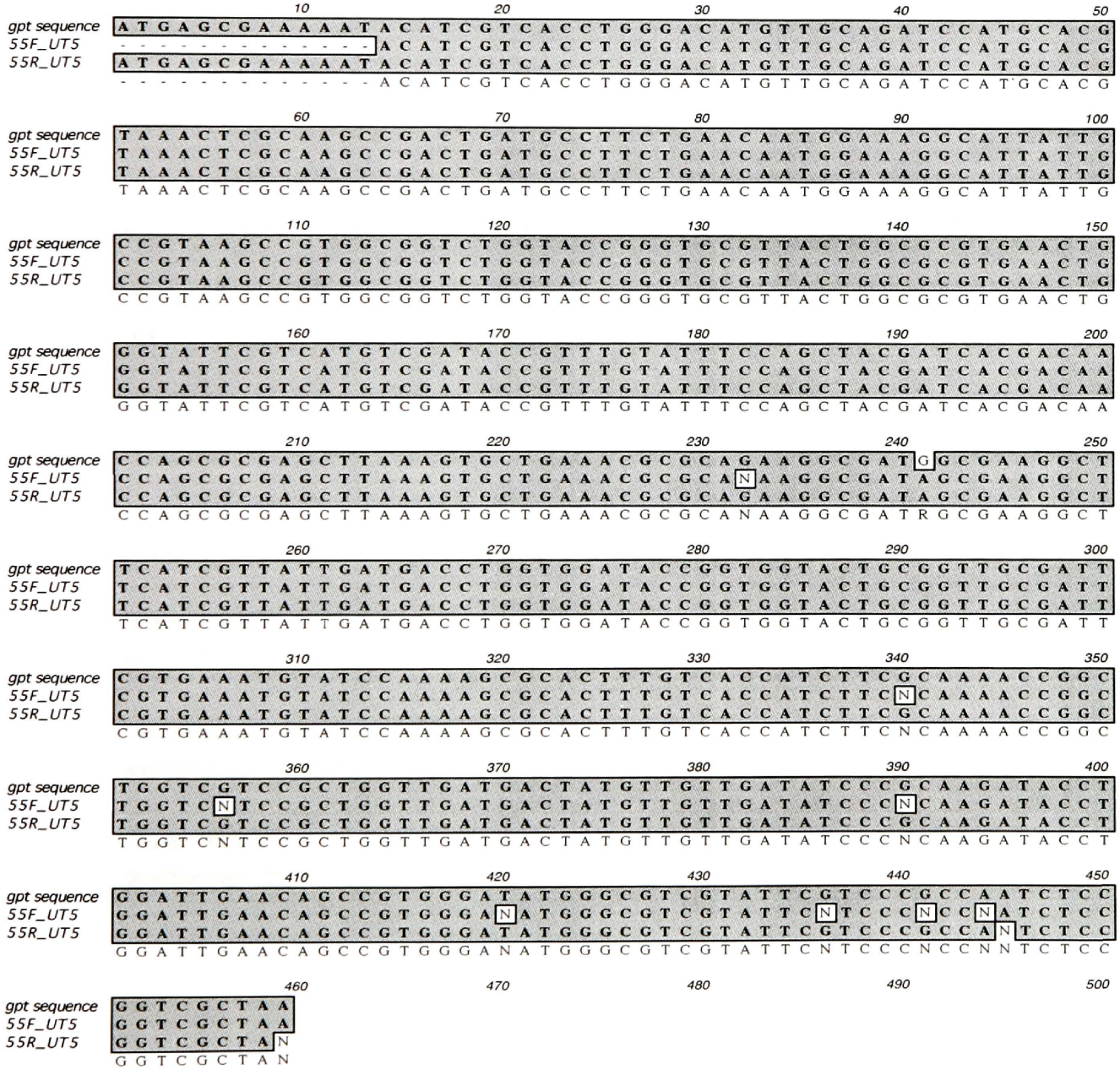
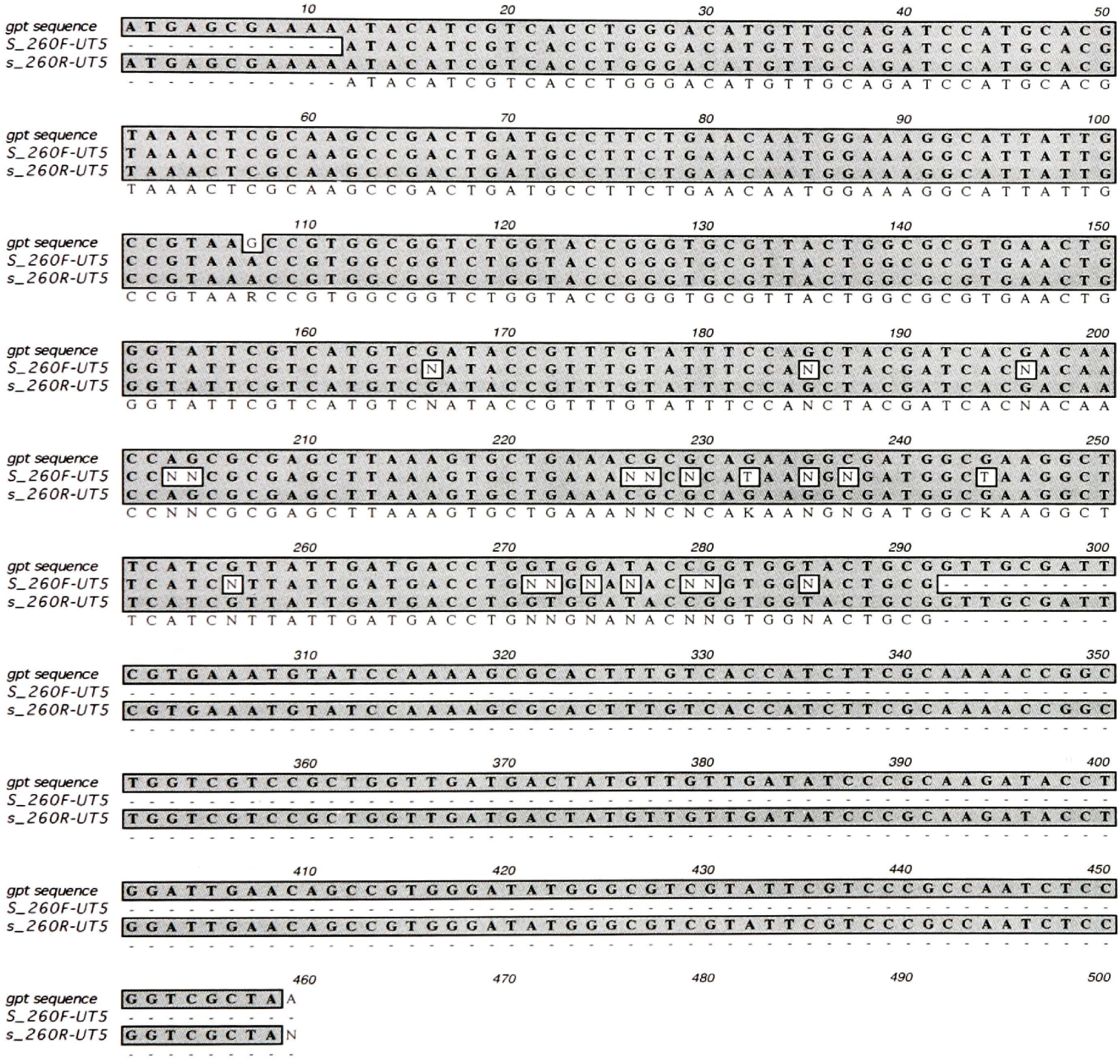


Figure 64. (A-F) Mutations of *gpt* coding sequence from treatment with teak wood dust extract #3

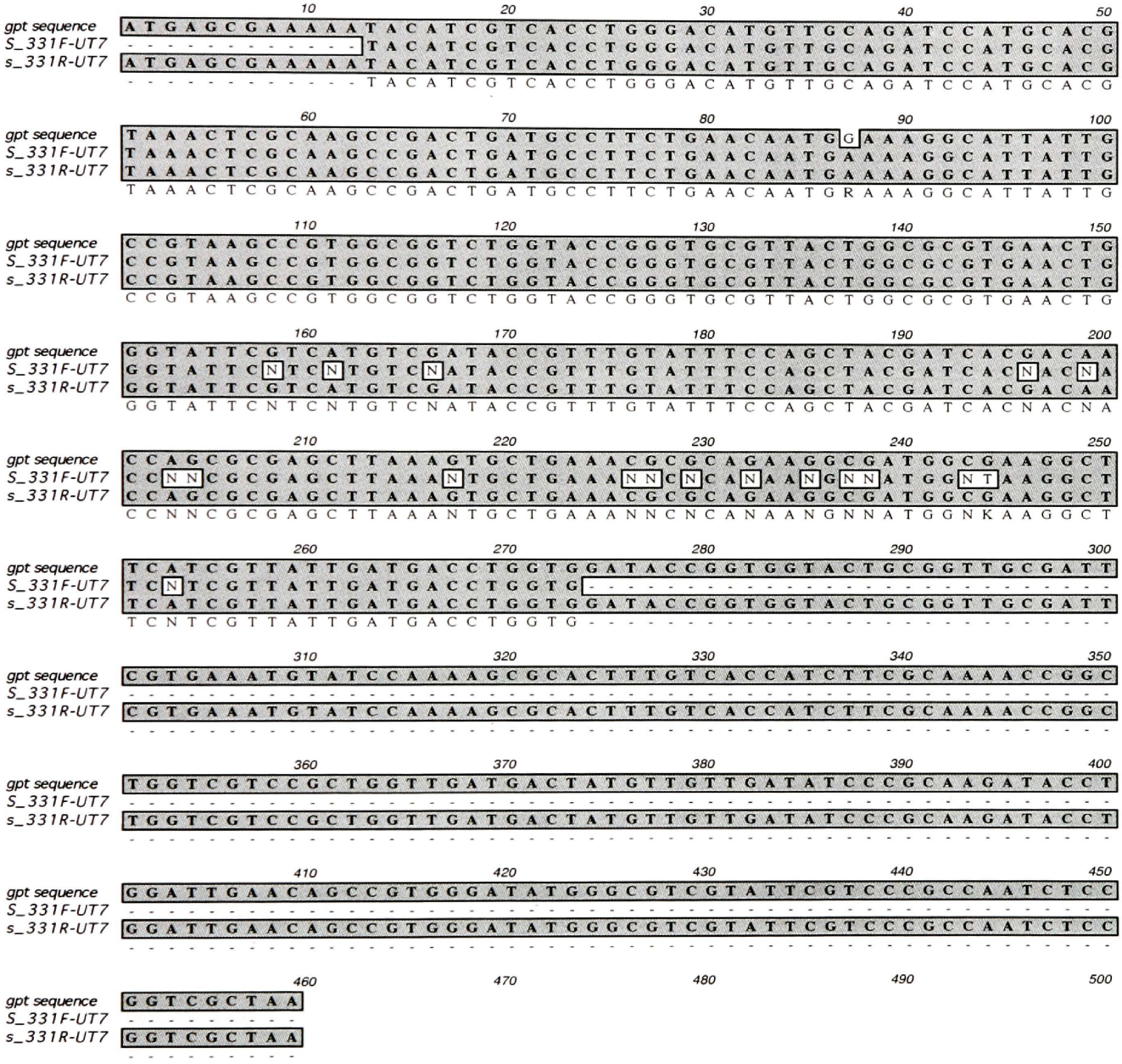
A)



B)



C)

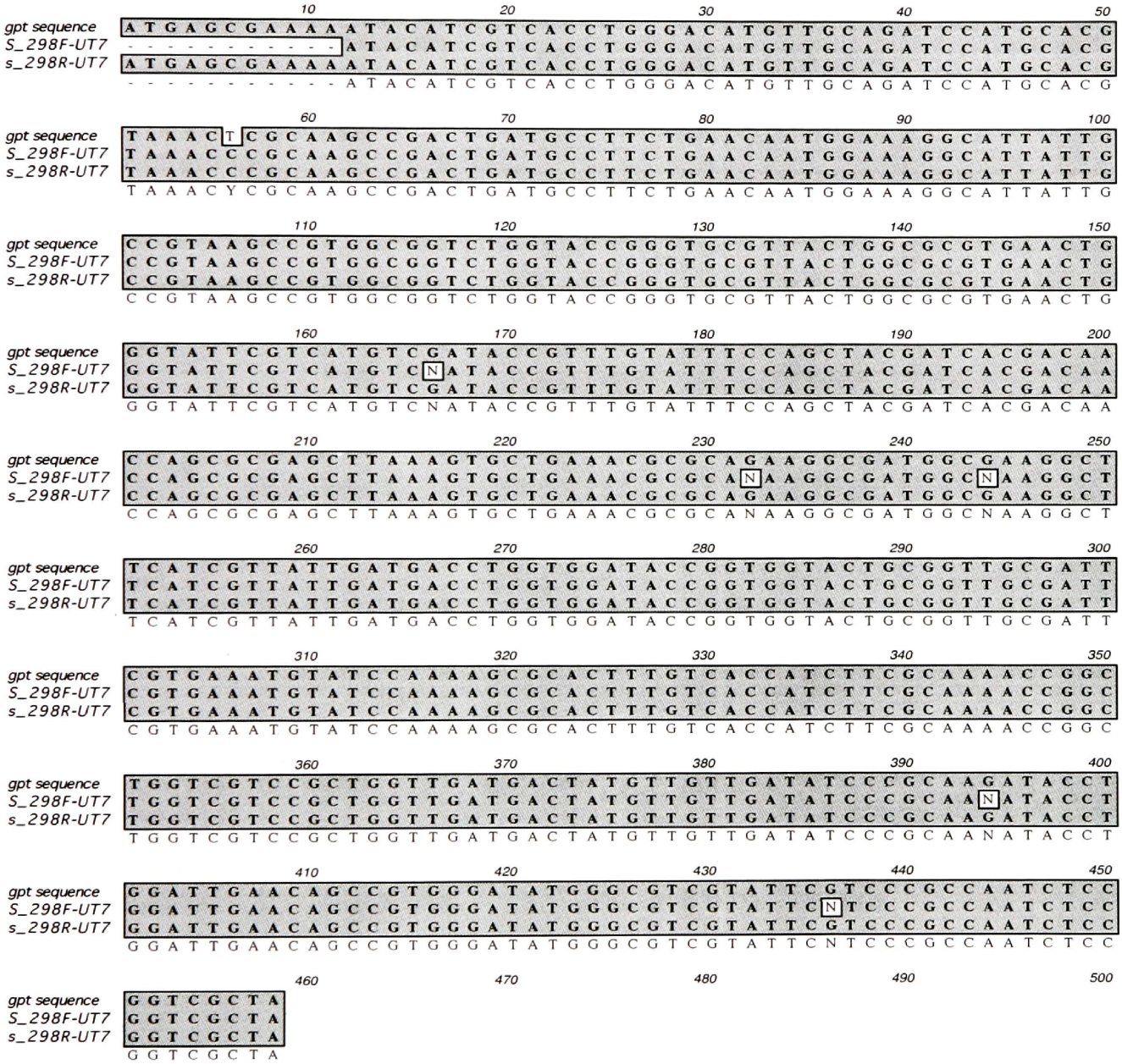

gpt sequence
S_331F-UT7
s_331R-UT7
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
CCGTAAGCCGTGGCGGTCTGGTACCGGGGTGCGTTACTGGCGCGTGAACTG
GGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGATCACGACAA
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCT
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
CGTGAAAATGTATCCAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGC
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATCCCGCAAGATACCT
GGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAATCTCC
GGTCGCTAA

D)


gpt sequence
S_311F-UT7
s_311R-UT7
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
ATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
CCGTAAGCCGTGGCGGTCTGGTACCGGGGTGCGTTACTGGCGCGTGAACTG
GGTATTCGTCATGTCGATACCGTTTGTATTTCCAGCTACGATCACGACAA
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGC
GATGGC
GAAGGCT
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
CGTGAAATGTATCCAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGC
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATCCCGCAAGATACCT
GGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAATCTCC
GGTCGCTAA

E)

F)


gpt sequence
S_298F-UT7
s_298R-UT7
ATGAGCGAAAAATACATCGTCACCTGGGACATGTTGCAGATCCATGCACG
TAAACTCGCAAGCCGACTGATGCCTTCTGAACAATGGAAAGGCATTATTG
CCGTAAGCCGTGGCGGTCTGGTACCGGGTGCGTTACTGGCGCGTGAACTG
GGTATTCGTCATGTCNATACCGTTTGTATTTCCAGCTACGATCACGACAA
CCAGCGCGAGCTTAAAGTGCTGAAACGCGCAGAAGGCGATGGCGAAGGCT
TCATCGTTATTGATGACCTGGTGGATACCGGTGGTACTGCGGTTGCGATT
CGTGAAATGTATCCAAAAGCGCACTTTGTCACCATCTTCGCAAAACCGGC
TGGTCGTCCGCTGGTTGATGACTATGTTGTTGATATCCCGCAAGATACCT
GGATTGAACAGCCGTGGGATATGGGCGTCGTATTCGTCCCGCCAATCTCC
GGTCGCTA

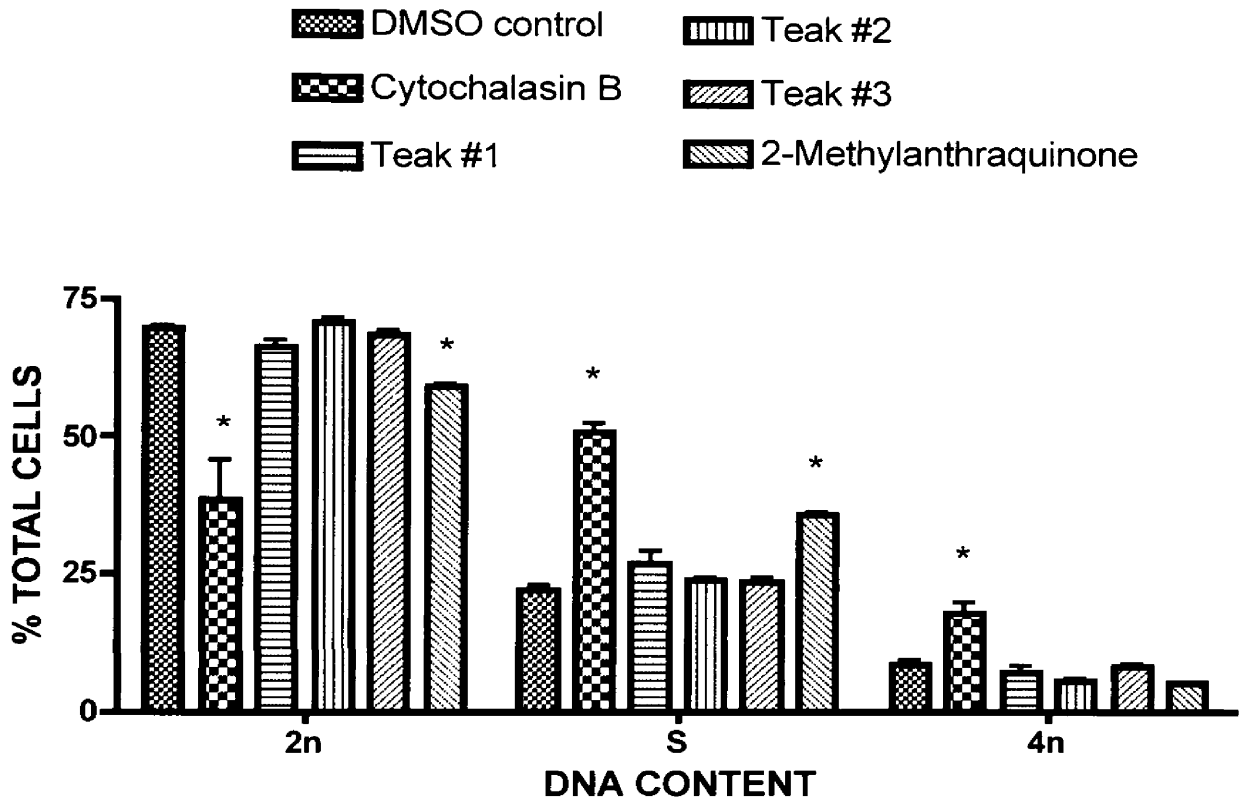
Multinucleate analysis ANOVA table

Table 58. Multinucleate analysis ANOVA table

Table Analyzed				
Data 1				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	5			
F	84.92			
R squared	0.7832			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	107.6			
P value	P<0.0001			
P value summary	***			
Do the variances differ signif. (P < 0.05)	Yes			
ANOVA Table	SS	df	MS	
Treatment (between columns)	712.8	4	178.2	
Residual (within columns)	197.3	94	2.099	
Total	910.1	98		
Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Control vs 3 μM dichromate	-0.7	1.871	P > 0.05	-1.630 to 0.2304
Control vs teak extract 5 μg/mL	-7.933	15	P < 0.01	-9.249 to -6.618
Control vs 2-methylanthraquinone 7 μg/mL	-0.4333	0.9459	P > 0.05	-1.573 to 0.7061
Control vs Cytochalasin-B 1.5 μg/ml	-5.338	11.38	P < 0.01	-6.504 to -4.172

MODFIT cell cycle analysis

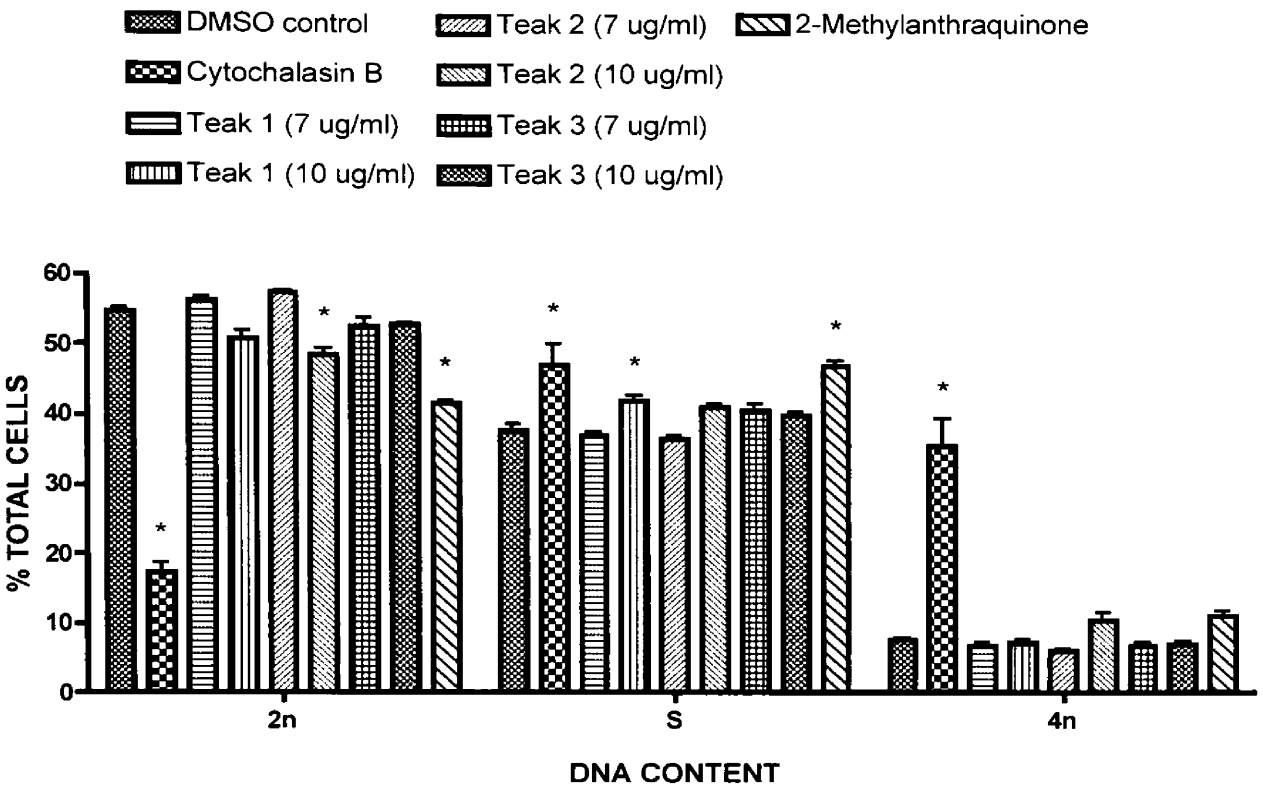
Figure 65. Cell cycle analysis following twenty-four hour recovery period



The x-axis indicates the phases of the cell cycle that are reflected by DNA content.

The y-axis shows the percent of total cells identified in each phase of the cell cycle.

Figure 66. Cell cycle analysis following three-day recovery period



The x-axis indicates the phases of the cell cycle that are reflected by DNA content.

The y-axis shows the percent of total cells identified in each phase of the cell cycle.

ANOVA tables

Table 59. 24-hour recovery experiment ANOVA summary

Two-way ANOVA

Source of Variation	% of total variation	P value
Interaction	14.3	P<0.0001
Treatment	0.14	0.5793
cycle point	84.2	P<0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Treatment	ns	No
cycle point	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	10	4414	441.4	37.96
Treatment	5	44.62	8.924	0.7675
cycle point	2	25990	13000	1118
Residual	36	418.6	11.63	

Number of missing values	0

Bonferroni posttests

DMSO control vs. Cytochalasin B

cycle point	DMSO control	Cytochalasin B	Difference	95% CI of

				diff.
2n	69.49	38.52	-30.97	-39.72 to -22.21
4n	8.33	17.97	9.64	0.8870 to 18.39
S	22.18	50.82	28.64	19.89 to 37.40

cycle point	Difference	T	P value	Summary
2n	-30.97	11.12	P<0.001	***
4n	9.64	3.462	P<0.01	**
S	28.64	10.29	P<0.001	***

DMSO control vs. Teak #1

cycle point	DMSO control	Teak #1	Difference	95% CI of diff.
2n	69.49	66.11	-3.383	-12.14 to 5.370
4n	8.33	7.03	-1.3	-10.05 to 7.453
S	22.18	26.86	4.68	-4.073 to 13.43

cycle point	Difference	T	P value	Summary
2n	-3.383	1.215	P > 0.05	ns
4n	-1.3	0.4669	P > 0.05	ns

S	4.68	1.681	P > 0.05	ns

DMSO control vs. Teak #2

cycle point	DMSO control	Teak #2	Difference	95% CI of diff.
2n	69.49	70.82	1.333	-7.420 to 10.09
4n	8.33	5.337	-2.993	-11.75 to 5.760
S	22.18	23.84	1.663	-7.090 to 10.42

cycle point	Difference	T	P value	Summary
2n	1.333	0.4789	P > 0.05	ns
4n	-2.993	1.075	P > 0.05	ns
S	1.663	0.5974	P > 0.05	ns

DMSO control vs. Teak #3

cycle point	DMSO control	Teak #3	Difference	95% CI of diff.
2n	69.49	68.57	-0.9233	-9.676 to 7.830
4n	8.33	8.097	-0.2333	-8.986 to 8.520
S	22.18	23.33	1.153	-7.600 to 9.906

cycle point	Difference	T	P value	Summary
2n	-0.9233	0.3316	P > 0.05	ns
4n	-0.2333	0.08381	P > 0.05	ns
S	1.153	0.4142	P > 0.05	ns

DMSO control vs. 2-Methylanthraquinone

cycle point	DMSO control	2-Methylanthraquinone	Difference	95% CI of diff.
2n	69.49	59.05	-10.44	-19.19 to -1.684
4n	8.33	5.15	-3.18	-11.93 to 5.573
S	22.18	35.8	13.62	4.864 to 22.37

cycle point	Difference	T	P value	Summary
2n	-10.44	3.749	P<0.01	**
4n	-3.18	1.142	P > 0.05	ns
S	13.62	4.891	P<0.001	***

Table 60. Three-day recovery experiment ANOVA table

Two-way ANOVA

Source of	% of total	P value

Variation	variation	
Interaction	22.61	P<0.0001
Treatment	0	1
cycle point	76.61	P<0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Treatment	ns	No
cycle point	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	16	6184	386.5	97.1
Treatment	8	0.00003948	0.000004935	0.00000124
cycle point	2	20950	10480	2632
Residual	54	214.9	3.98	

Number of missing values	0

Bonferroni posttests

DMSO control vs. Cytochalasin B

cycle point	DMSO control	Cytochalasin B	Difference	95% CI of diff.

2n	54.6	17.32	-37.29	-42.56 to -32.02
4n	7.577	35.55	27.97	22.70 to 33.24
S	37.82	47.14	9.32	4.052 to 14.59

cycle point	Difference	T	P value	Summary
2n	-37.29	22.89	P<0.001	***
4n	27.97	17.17	P<0.001	***
S	9.32	5.721	P<0.001	***

DMSO control vs. Teak#1 (7 ug/ml)

cycle point	DMSO control	Teak #1 7 ug/ml	Difference	95% CI of diff.
2n	54.6	56.2	1.6	-3.668 to 6.868
4n	7.577	6.773	-0.8033	-6.072 to 4.465
S	37.82	37.03	-0.7933	-6.062 to 4.475

cycle point	Difference	T	P value	Summary
2n	1.6	0.9822	P > 0.05	ns
4n	-0.8033	0.4932	P > 0.05	ns
S	-0.7933	0.487	P > 0.05	ns

DMSO control vs. Teak#1 (10 ug/ml)

cycle point	DMSO control	Teak #1 10 ug/ml	Difference	95% CI of diff.
2n	54.6	50.84	-3.76	-9.028 to 1.508
4n	7.577	7.173	-0.4033	-5.672 to 4.865
S	37.82	41.98	4.163	-1.105 to 9.432

cycle point	Difference	T	P value	Summary
2n	-3.76	2.308	P > 0.05	ns
4n	-0.4033	0.2476	P > 0.05	ns
S	4.163	2.556	P < 0.05	*

DMSO control vs. Teak#2 (7 ug/ml)

cycle point	DMSO control	Teak #2 7 ug/ml	Difference	95% CI of diff.
2n	54.6	57.35	2.743	-2.525 to 8.012
4n	7.577	6.013	-1.563	-6.832 to 3.705
S	37.82	36.64	-1.183	-6.452 to 4.085

cycle point	Difference	T	P value	Summary
2n	2.743	1.684	P > 0.05	ns
4n	-1.563	0.9597	P > 0.05	ns
S	-1.183	0.7264	P > 0.05	ns

DMSO control vs. Teak #2 (10 ug/ml)

cycle point	DMSO control	Teak #2 10 ug/ml	Difference	95% CI of diff.
2n	54.6	48.39	-6.21	-11.48 to -0.9415
4n	7.577	10.48	2.907	-2.362 to 8.175
S	37.82	41.12	3.303	-1.965 to 8.572

cycle point	Difference	T	P value	Summary
2n	-6.21	3.812	P<0.01	**
4n	2.907	1.784	P > 0.05	ns
S	3.303	2.028	P > 0.05	ns

DMSO control vs. Teak#3 (7 ug/ml)

cycle point	DMSO control	Teak #3 7 ug/ml	Difference	95% CI of diff.
2n	54.6	52.52	-2.087	-7.355 to 3.182
4n	7.577	6.697	-0.88	-6.148 to

				4.388
				-2.302 to
S	37.82	40.79	2.967	8.235

cycle point	Difference	T	P value	Summary
2n	-2.087	1.281	P > 0.05	ns
4n	-0.88	0.5402	P > 0.05	ns
S	2.967	1.821	P > 0.05	ns

DMSO control vs. Teak#3 (10 ug/ml)

cycle point	DMSO control	Teak #3 10 ug/ml	Difference	95% CI of diff.
				-6.995 to
2n	54.6	52.88	-1.727	3.542
				-5.798 to
4n	7.577	7.047	-0.53	4.738
				-3.008 to
S	37.82	40.08	2.26	7.528

cycle point	Difference	T	P value	Summary
2n	-1.727	1.06	P > 0.05	ns
4n	-0.53	0.3254	P > 0.05	ns
S	2.26	1.387	P > 0.05	ns

DMSO control vs. 2-Methylanthraquinone

cycle point	DMSO	2-	Difference	95% CI of

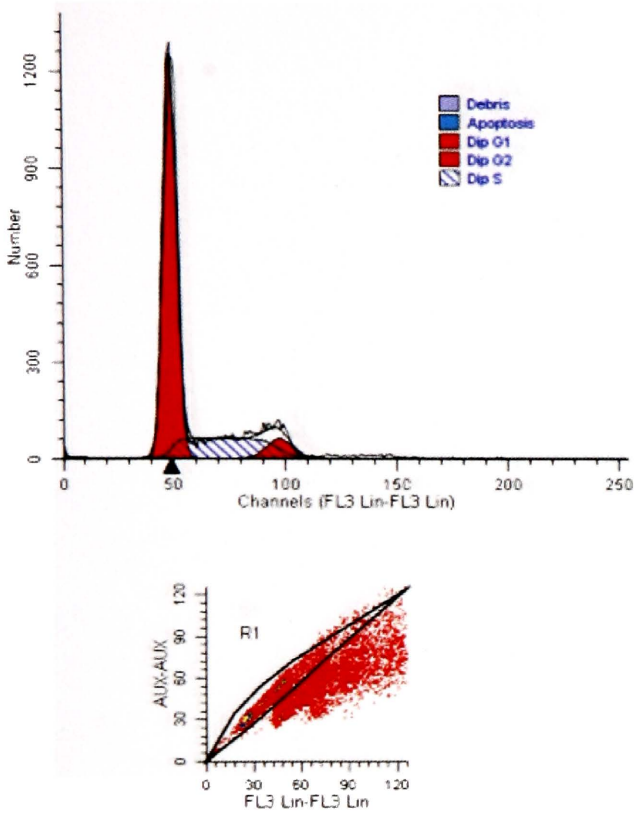
	control	Methylanthraquinone		diff.
2n	54.6	41.66	-12.94	-18.21 to -7.672
4n	7.577	11.13	3.55	-1.718 to 8.818
S	37.82	47.21	9.39	4.122 to 14.66

cycle point	Difference	T	P value	Summary
2n	-12.94	7.944	P<0.001	***
4n	3.55	2.179	P > 0.05	ns
S	9.39	5.764	P<0.001	***

Flow cytometry data from twenty-four hour recovery experiment

Figure 67. DMSO treatment group flow histograms and summaries

A) DMSO treatment sample 1



File analyzed: MW11.19 dna1 00011060 2009-11-19 1128 216.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

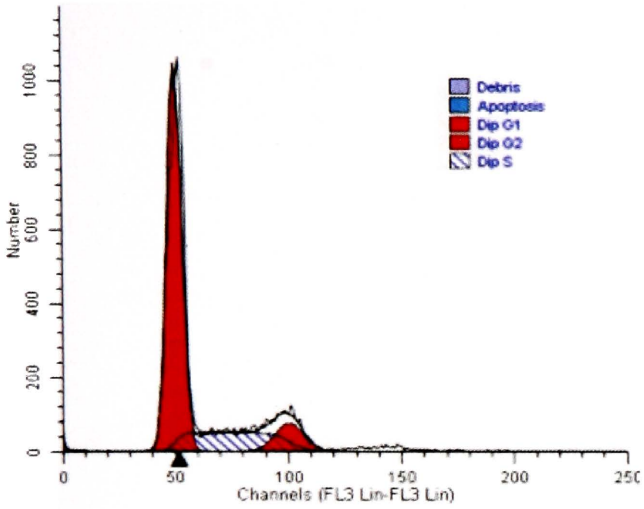
Diploid: 100.00 %
Dip G1: 70.44 % at 48.68
Dip G2: 6.77 % at 97.35
Dip S: 22.79 % G2/G1: 2.00
%CV: 5.95

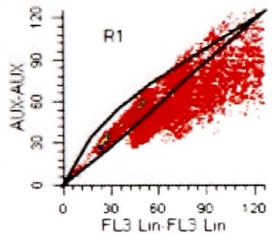
Total S-Phase: 22.79 %
Total B.A.D.: 0.06 % no aggs

Apoptosis: 0.11 % Mean: 1.45

Debris: 0.80 %
Aggregates: 0.00 %
Modeled events: 13207
All cycle events: 13087
Cycle events per channel: 263
RCS: 3.855

B) DMSO treatment sample 2





File analyzed: MW11.19 dna2 00011061 2009-11-19 1132 217.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

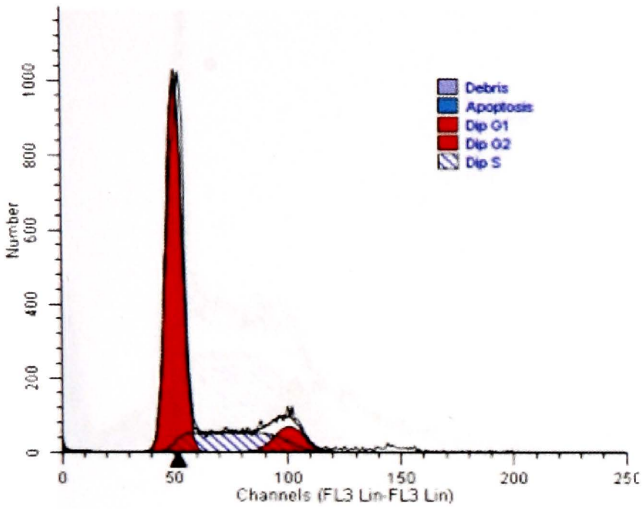
Diploid: 100.00 %
Dip G1: 68.62 % at 50.28
Dip G2: 10.09 % at 100.55
Dip S: 21.29 % G2/G1: 2.00
%CV: 6.34

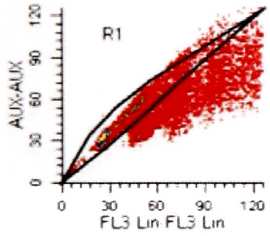
Total S-Phase: 21.29 %
Total B.A.D.: 0.07 % no aggs

Apoptosis: 0.02 % Mean: 6.10

Debris: 1.05 %
Aggregates: 0.00 %
Modeled events: 12101
All cycle events: 11972
Cycle events per channel: 233
RCS: 4.325

C) DMSO treatment sample 3





File analyzed: MW11.19 dna3 00011062
2009-11-19 1135 218.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 69.02 % at 50.46
Dip G2: 9.23 % at 100.93
Dip S: 21.75 % G2/G1: 2.00
%CV: 6.54

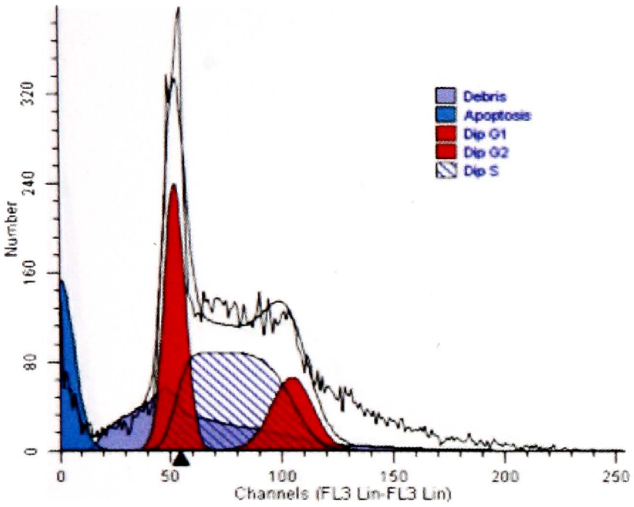
Total S-Phase: 21.75 %
Total B.A.D.: 0.04 % no aggs

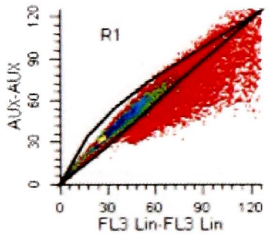
Apoptosis: 0.37 % Mean: 0.17

Debris: 0.46 %
Aggregates: 0.00 %
Modeled events: 12274
All cycle events: 12173
Cycle events per channel: 237
RCS: 4.533

Figure 68. Cytochalasin-B treatment group flow histograms and summaries

A) Cytochalasin-B treatment sample 1





File analyzed: MW11.19 dna4 00011063
2009-11-19 1137 219.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

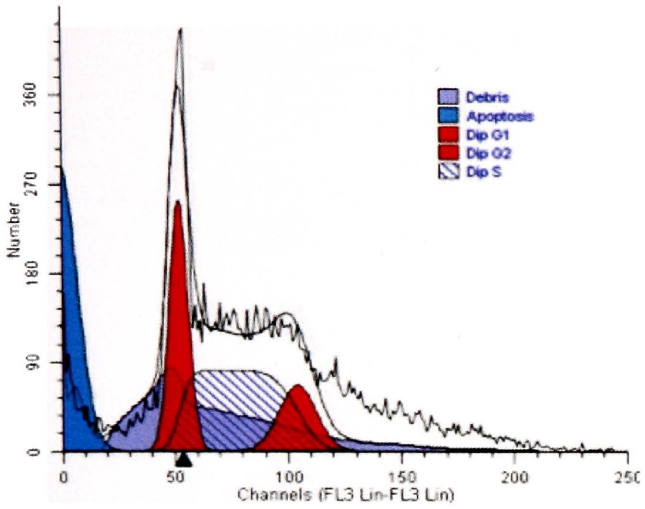
Diploid: 100.00 %
Dip G1: 31.26 % at 52.34
Dip G2: 17.13 % at 104.67
Dip S: 51.60 % G2/G1: 2.00
%CV: 8.84

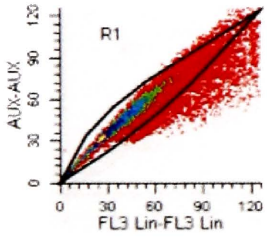
Total S-Phase: 51.60 %
Total B.A.D.: 16.35 % no aggs

Apoptosis: 13.57 % Mean: 1.39

Debris: 17.95 %
Aggregates: 0.00 %
Modeled events: 12639
All cycle events: 8963
Cycle events per channel: 168
RCS: 7.725

B) Cytochalasin-B treatment sample 2





File analyzed: MW11.19 dna5 00011064
2009-11-19 1139 220.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

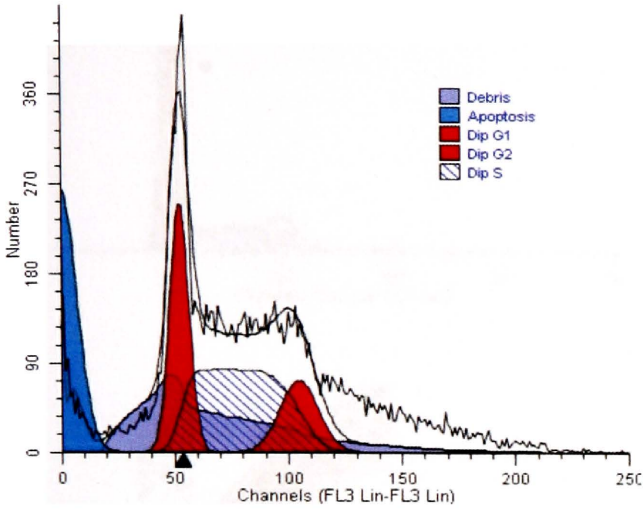
Diploid: 100.00 %
Dip G1: 31.72 % at 52.14
Dip G2: 16.69 % at 104.28
Dip S: 51.60 % G2/G1: 2.00
%CV: 7.83

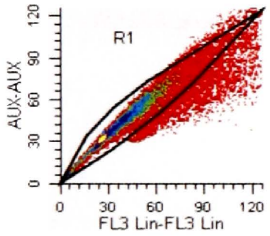
Total S-Phase: 51.60 %
Total B.A.D.: 24.72 % no aggs

Apoptosis: 25.41 % Mean: 0.11

Debris: 20.05 %
Aggregates: 0.00 %
Modeled events: 13792
All cycle events: 8225
Cycle events per channel: 155
RCS: 10.801

C) Cytochalasin-B treament sample 3





File analyzed: MW11.19 dna6 00011065
2009-11-19 1142 221.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 30.98 % at 52.42
Dip G2: 18.00 % at 104.83
Dip S: 51.02 % G2/G1: 2.00
%CV: 8.04

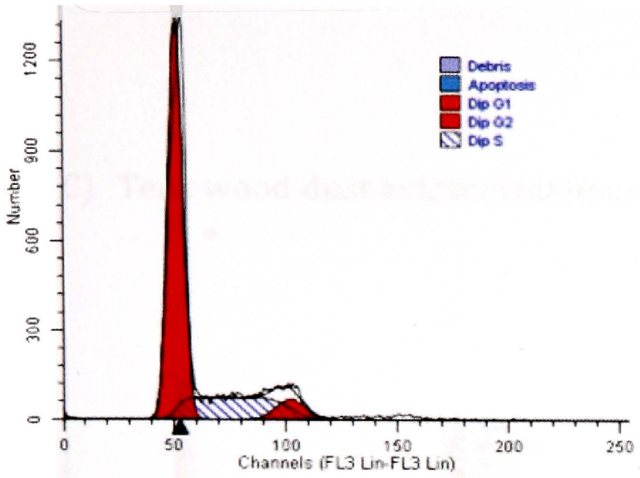
Total S-Phase: 51.02 %
Total B.A.D.: 23.09 % no aggs

Apoptosis: 22.94 % Mean: 0.33

Debris: 19.20 %
Aggregates: 0.00 %
Modeled events: 13783
All cycle events: 8583
Cycle events per channel: 161
RCS: 10.218

Figure 69. Teak wood dust extract 1 treatment group flow histograms and summaries

A) Teak wood dust extract treatment sample 1

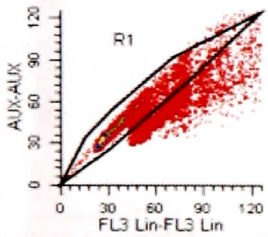


2009-11-19 1145 222.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 68.95 % at 51.08
Dip G2: 7.08 % at 102.16
Dip S: 23.97 % G2/G1: 2.00
%CV: 6.26

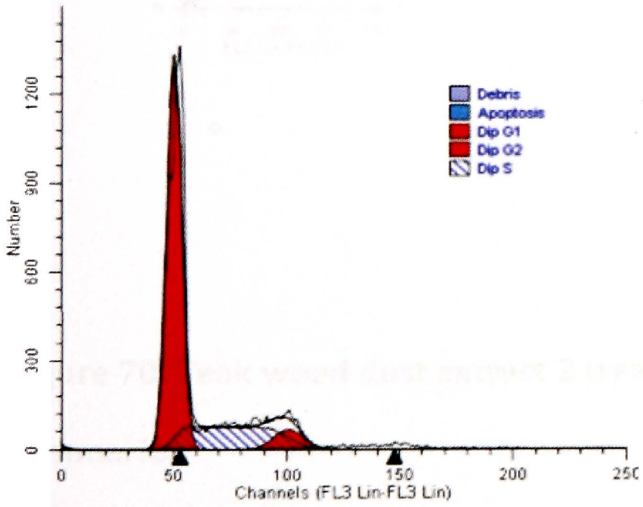
Total S-Phase: 23.97 %
Total B.A.D.: 0.04 % no aggs



Apoptosis: 0.02 % Mean: 0.16

Debris: 0.56 %
Aggregates: 0.00 %
Modeled events: 15654
All cycle events: 15563
Cycle events per channel: 299
RCS: 5.953

B) Teak wood dust extract treatment sample 2

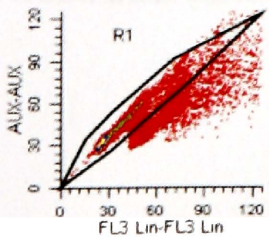


File analyzed: MW11.19 dna8 00011067
2009-11-19 1147 223.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 68.25 % at 50.49
Dip G2: 6.88 % at 100.98
Dip S: 24.87 % G2/G1: 2.00
%CV: 6.37

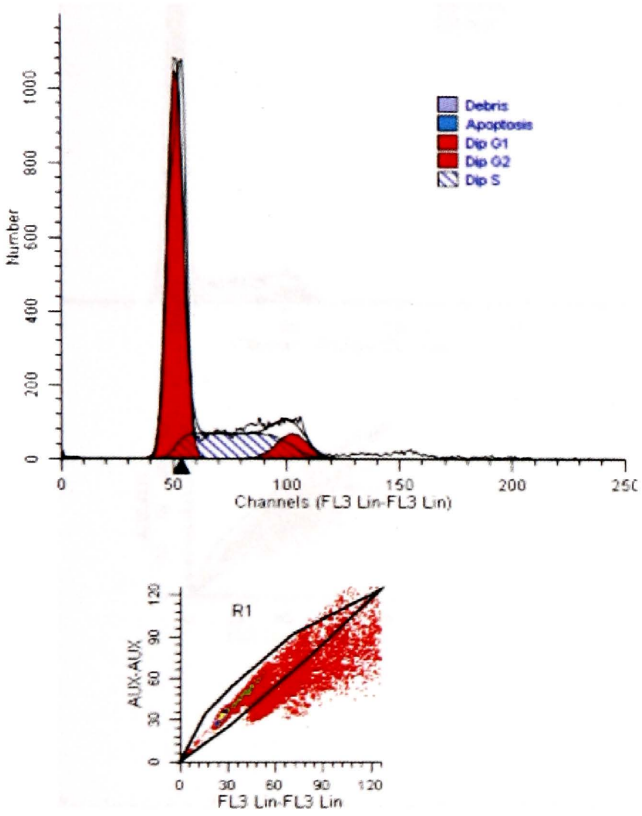
Total S-Phase: 24.87 %
Total B.A.D.: 0.02 % no aggs



Apoptosis: 0.06 % Mean: 4.05

Debris: 0.42 %
Aggregates: 0.00 %
Modeled events: 15565
All cycle events: 15492
Cycle events per channel: 301
RCS: 6.942

C) Teak wood dust extract treatment sample 3



File analyzed: MW11.19 dna9 00011068
2009-11-19 1149 224.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 65.93 % at 51.37
Dip G2: 8.40 % at 102.73
Dip S: 25.67 % G2/G1: 2.00
%CV: 6.61

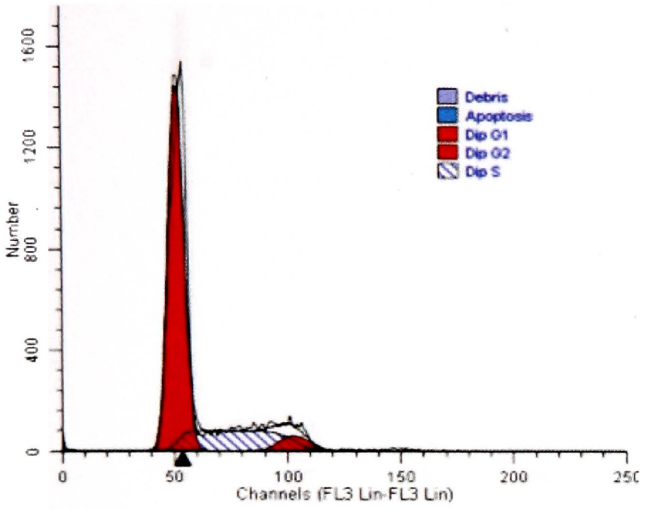
Total S-Phase: 25.67 %
Total B.A.D.: 0.02 % no aggs

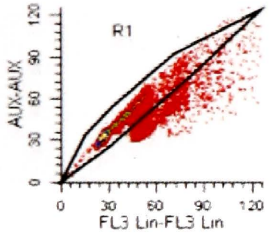
Apoptosis: 0.16 % Mean: 1.45

Debris: 0.37 %
Aggregates: 0.00 %
Modeled events: 13749
All cycle events: 13676
Cycle events per channel: 261
RCS: 6.518

Figure 70. Teak wood dust extract 2 treatment group flow histograms and summaries

A) Teak wood dust extract 2 treatment sample 1





File analyzed: MW11.19 dna10 00011069 2009-11-19 1152 225.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

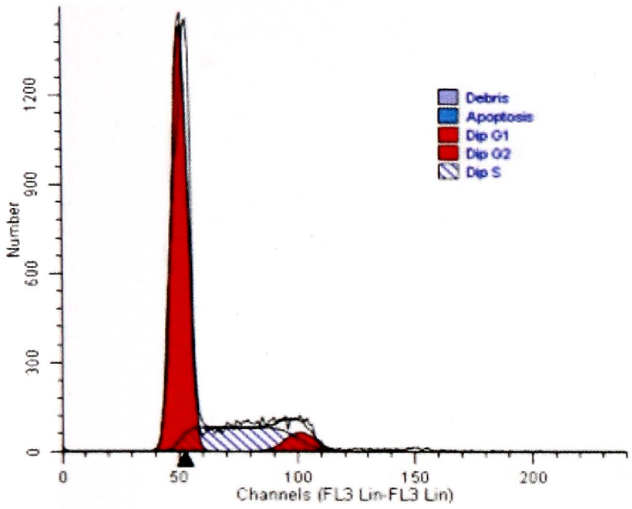
Diploid: 100.00 %
Dip G1: 70.33 % at 51.42
Dip G2: 5.81 % at 102.84
Dip S: 23.86 % G2/G1: 2.00
%CV: 6.42

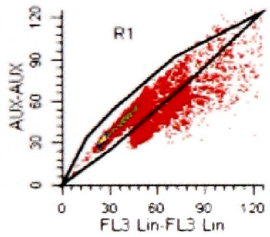
Total S-Phase: 23.86 %
Total B.A.D.: 0.01 % no aggs

Apoptosis: 0.10 % Mean: 1.45

Debris: 0.47 %
Aggregates: 0.00 %
Modeled events: 17348
All cycle events: 17249
Cycle events per channel: 329
RCS: 6.090

B) Teak wood dust extract 2 treatment sample 2





File analyzed: MW11.19 dna11 00011070 2009-11-19 1155 226.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

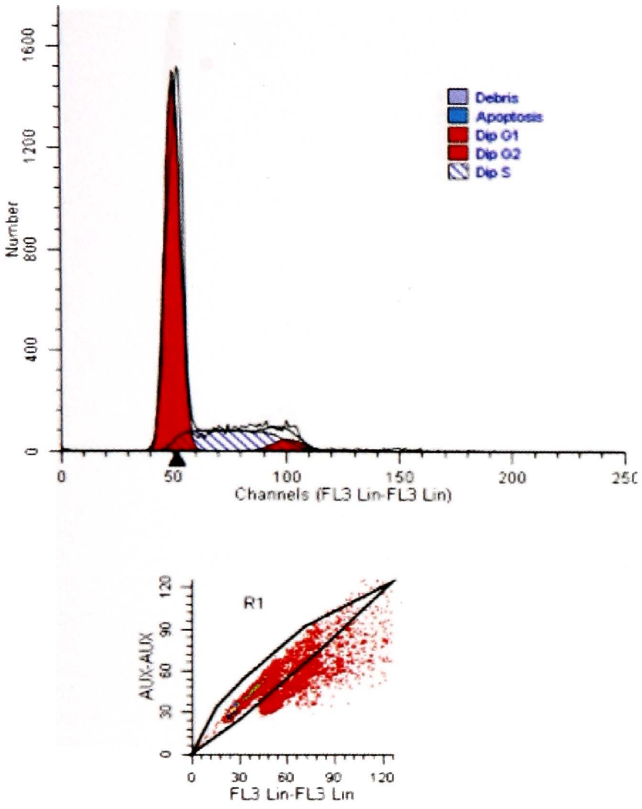
Diploid: 100.00 %
Dip G1: 69.34 % at 50.63
Dip G2: 6.12 % at 101.27
Dip S: 24.53 % G2/G1: 2.00
%CV: 6.27

Total S-Phase: 24.53 %
Total B.A.D.: 0.02 % no aggs

Apoptosis: 0.00 % Mean: 11.29

Debris: 0.27 %
Aggregates: 0.00 %
Modeled events: 16701
All cycle events: 16657
Cycle events per channel: 323
RCS: 6.683

D) Teak wood dust extract 2 treatment sample 3



File analyzed: MW11.19 dna12 00011071 2009-11-19 1157 227.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 71.91 % at 50.40
Dip G2: 4.62 % at 100.80
Dip S: 23.47 % G2/G1: 2.00
%CV: 6.42

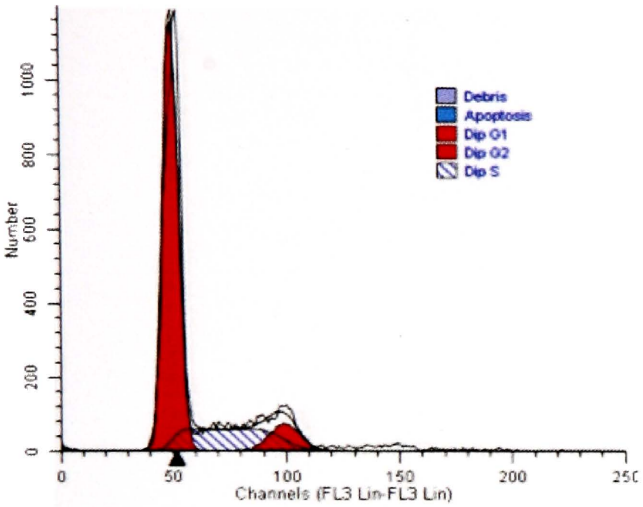
Total S-Phase: 23.47 %
Total B.A.D.: 0.02 % no aggs

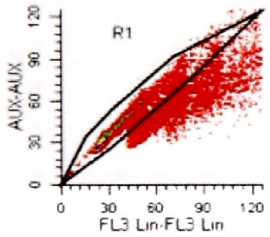
Apoptosis: 0.05 % Mean: 2.92

Debris: 0.27 %
Aggregates: 0.00 %
Modeled events: 16745
All cycle events: 16691
Cycle events per channel: 325
RCS: 6.657

Figure 71. Teak wood dust extract 3 treatment group flow histograms and summaries

A) Teak wood dust extract 3 treatment sample1





File analyzed: MW11.19 dna13 00011072
2009-11-19 1159 228.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

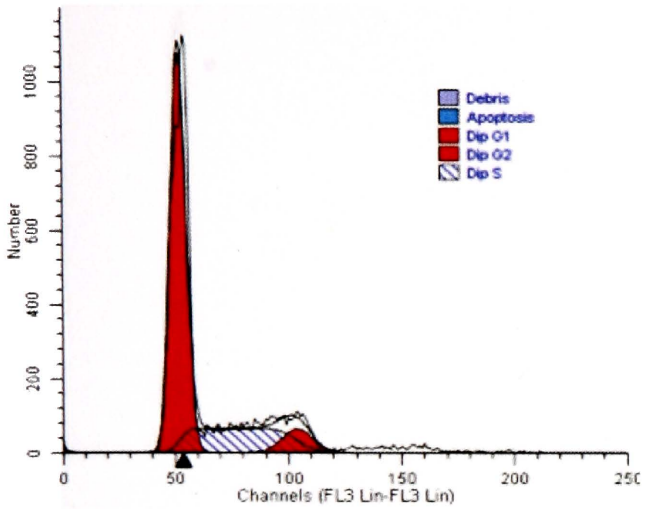
Diploid: 100.00 %
Dip G1: 69.92 % at 49.68
Dip G2: 8.72 % at 99.37
Dip S: 21.36 % G2/G1: 2.00
%CV: 6.64

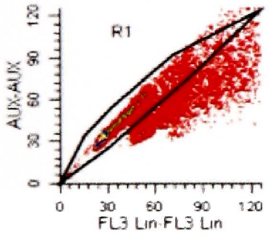
Total S-Phase: 21.36 %
Total B.A.D.: 0.05 % no aggs

Apoptosis: 0.05 % Mean: 2.72

Debris: 0.60 %
Aggregates: 0.00 %
Modeled events: 13867
All cycle events: 13776
Cycle events per channel: 272
RCS: 6.203

B) Teak wood dust extract 3 treatment sample 2





File analyzed: MW11.19 dna14 00011073
2009-11-19 1203 229.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

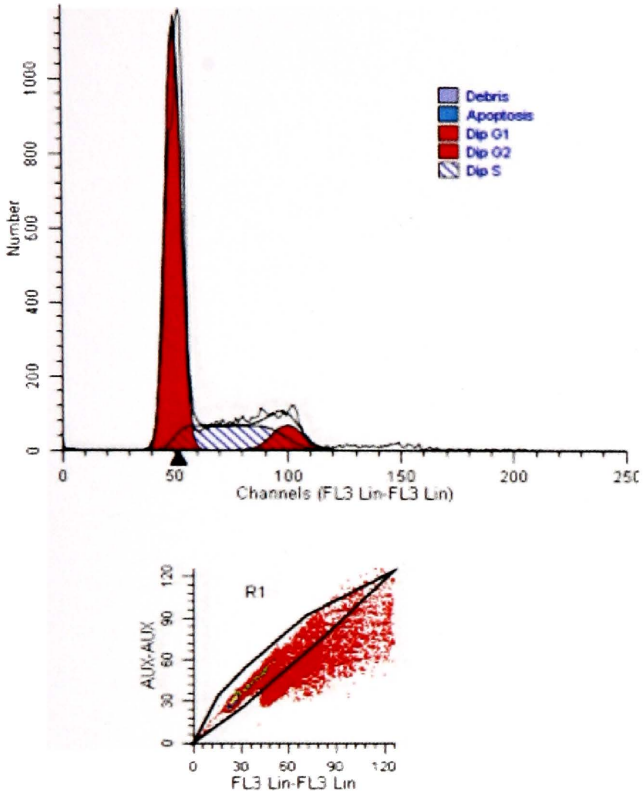
Diploid: 100.00 %
Dip G1: 66.83 % at 51.98
Dip G2: 7.82 % at 103.97
Dip S: 25.35 % G2/G1: 2.00
%CV: 6.37

Total S-Phase: 25.35 %
Total B.A.D.: 0.05 % no aggs

Apoptosis: 0.00 % Mean: 1.02

Debris: 0.57 %
Aggregates: 0.00 %
Modeled events: 13517
All cycle events: 13439
Cycle events per channel: 254
RCS: 7.009

C) Teak wood dust extract 3 treatment sample 3



File analyzed: MW11.19 dna15 00011074
2009-11-19 1205 230.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 68.47 % at 49.97
Dip G2: 8.05 % at 99.94
Dip S: 23.47 % G2/G1: 2.00
%CV: 6.75

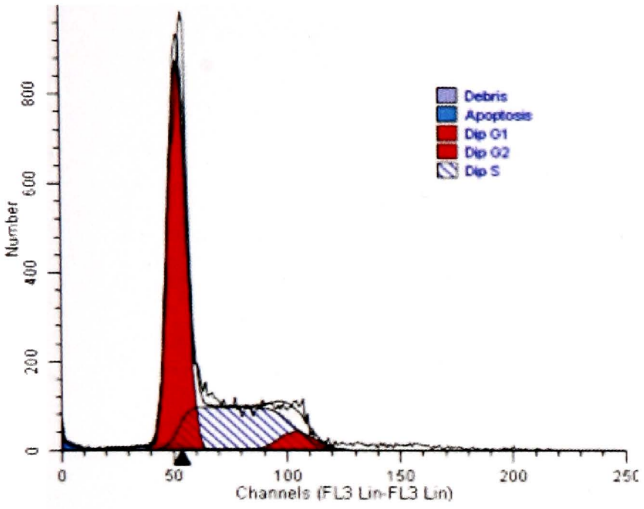
Total S-Phase: 23.47 %
Total B.A.D.: 0.04 % no aggs

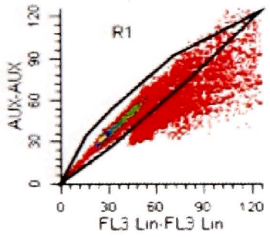
Apoptosis: 0.01 % Mean: 4.22

Debris: 0.43 %
Aggregates: 0.00 %
Modeled events: 14174
All cycle events: 14111
Cycle events per channel: 277
RCS: 6.702

Figure 72. 2-methylanthraquinone treatment group flow histograms and summaries

A) 2-methylanthraquinone treatment sample1





File analyzed: MW11.19 dna16 00011075
2009-11-19 1208 231.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

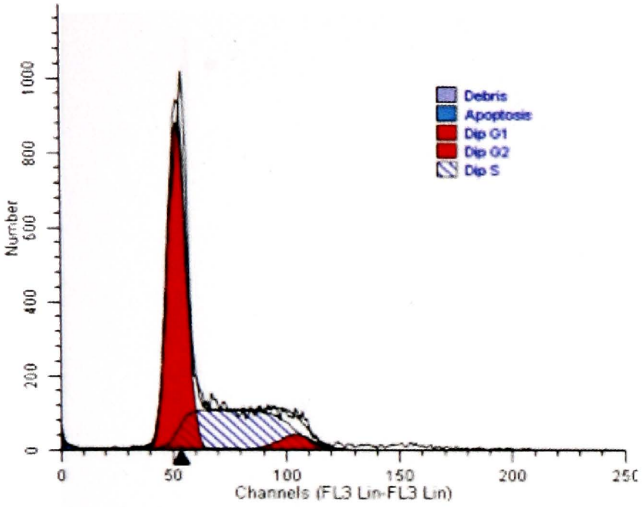
Diploid: 100.00 %
Dip G1: 58.92 % at 52.12
Dip G2: 5.63 % at 104.24
Dip S: 35.46 % G2/G1: 2.00
%CV: 7.36

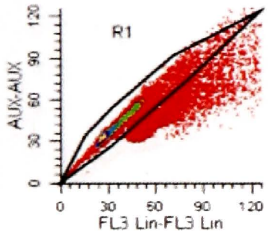
Total S-Phase: 35.46 %
Total B.A.D.: 1.37 % no aggs

Apoptosis: 1.24 % Mean: 1.82

Debris: 3.01 %
Aggregates: 0.00 %
Modeled events: 14954
All cycle events: 14323
Cycle events per channel: 270
RCS: 4.797

B) 2-methylanthraquinone treatment sample 2





File analyzed: MW11.19 dna17 00011076
2009-11-19 1210 232.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

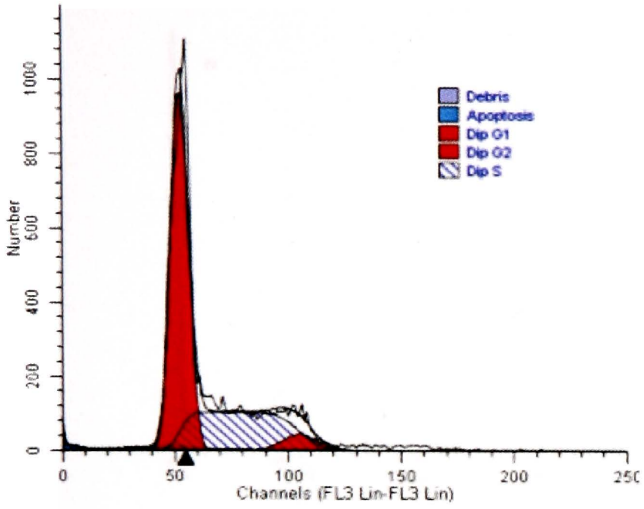
Diploid: 100.00 %
Dip G1: 58.10 % at 52.14
Dip G2: 5.15 % at 104.29
Dip S: 36.75 % G2/G1: 2.00
%CV: 7.47

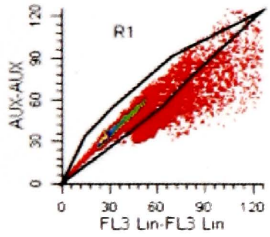
Total S-Phase: 36.75 %
Total B.A.D.: 1.00 % no aggs

Apoptosis: 0.51 % Mean: 3.76

Debris: 2.74 %
Aggregates: 0.00 %
Modeled events: 15383
All cycle events: 14886
Cycle events per channel: 280
RCS: 5.246

C) 2-methylanthraquinone treatment sample 3





File analyzed: MW11.19 dna18 00011077
2009-11-19 1212 233.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 59.64 % at 52.56
Dip G2: 5.35 % at 105.11
Dip S: 35.01 % G2/G1: 2.00
%CV: 7.22

Total S-Phase: 35.01 %
Total B.A.D.: 1.09 % no aggs

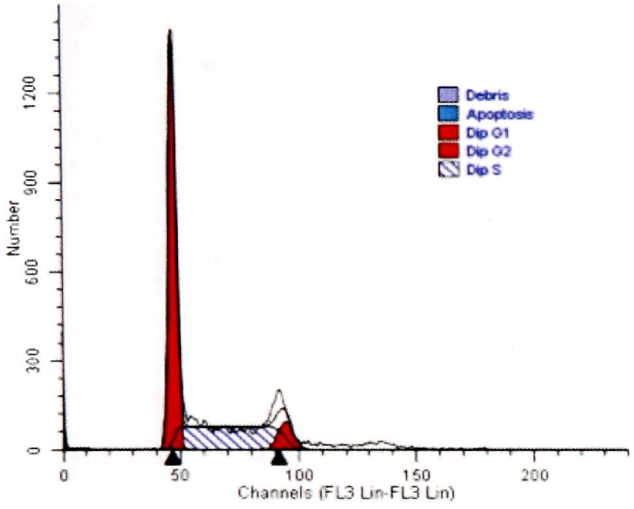
Apoptosis: 0.93 % Mean: 3.36

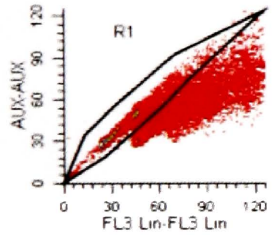
Debris: 2.50 %
Aggregates: 0.00 %
Modeled events: 16064
All cycle events: 15517
Cycle events per channel: 290
RCS: 4.448

Flow cytometry data from three day recovery experiment

Figure 73. DMSO treatment group flow histograms and summaries

A) DMSO treatment sample 1





File analyzed: MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

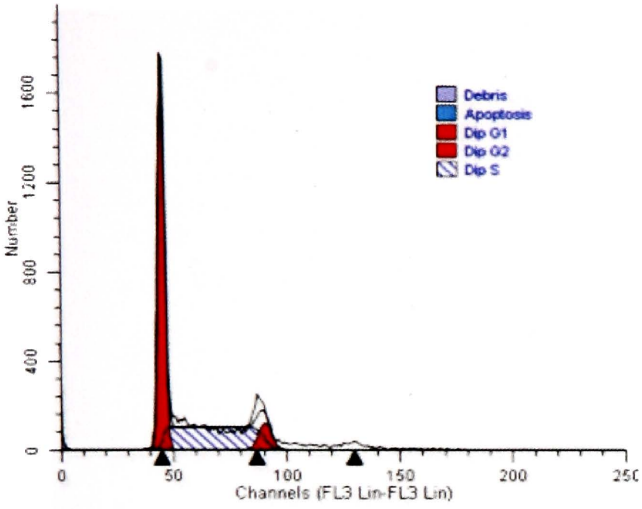
Diploid: 100.00 %
Dip G1: 55.64 % at 47.36
Dip G2: 7.41 % at 94.73
Dip S: 36.95 % G2/G1: 2.00
%CV: 3.25

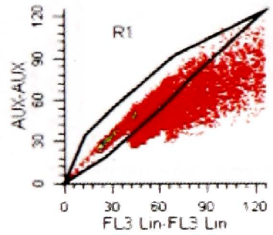
Total S-Phase: 36.95 %
Total B.A.D.: 0.13 % no aggs

Apoptosis: 1.33 % Mean: 0.53

Debris: 1.14 %
Aggregates: 0.00 %
Modeled events: 10154
All cycle events: 9904
Cycle events per channel: 205
RCS: 5.594

B) DMSO treatment sample 2





File analyzed: MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

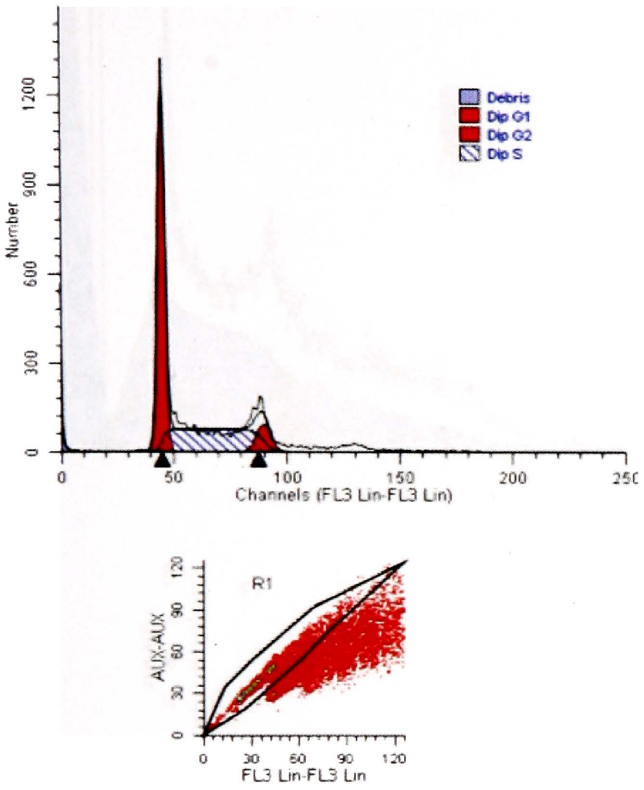
Diploid: 100.00 %
Dip G1: 53.55 % at 45.30
Dip G2: 7.20 % at 90.60
Dip S: 39.25 % G2/G1: 2.00
%CV: 3.06

Total S-Phase: 39.25 %
Total B.A.D.: 0.47 % no aggs

Apoptosis: 0.00 % Mean: 3.48

Debris: 1.70 %
Aggregates: 0.00 %
Modeled events: 11961
All cycle events: 11758
Cycle events per channel: 254
RCS: 6.738

C) DMSO treatment sample 3



File analyzed: MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 54.62 % at 44.98
Dip G2: 8.12 % at 89.96
Dip S: 37.26 % G2/G1: 2.00
%CV: 3.45

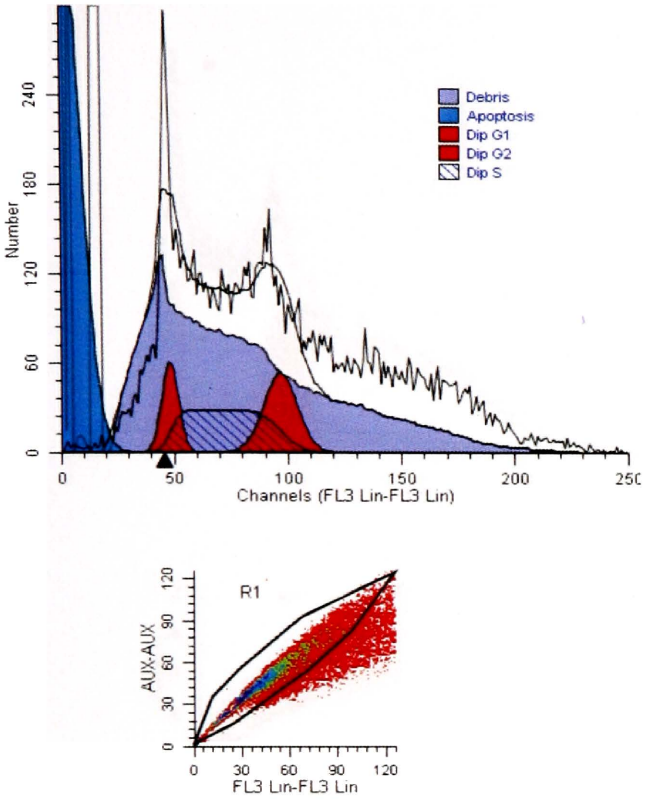
Total S-Phase: 37.26 %
Total B.A.D.: 0.62 % no aggs

Apoptosis: % Mean:

Debris: 3.04 %
Aggregates: 0.00 %
Modeled events: 9302
All cycle events: 9019
Cycle events per channel: 196
RCS: 5.550

Figure 74. Cytochalasin B treatment group flow histograms and summaries

A) Cytochalsain-B treatment sample 1



File analyzed: MW1.11 cb1 00011428 2010-01-12 1138 544.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

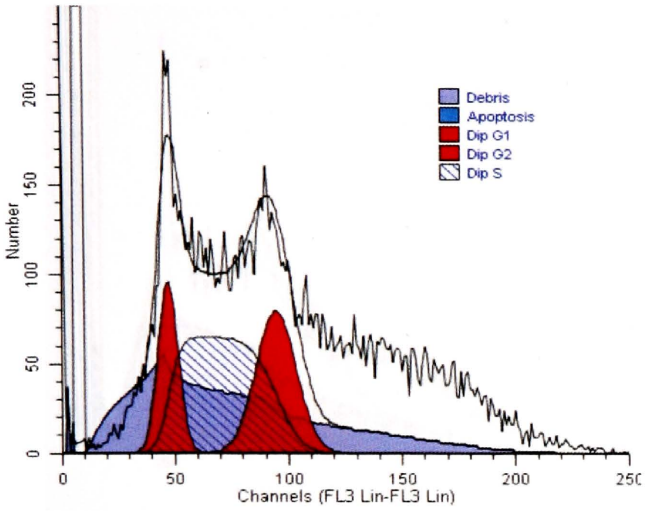
Diploid: 100.00 %
Dip G1: 19.00 % at 48.44
Dip G2: 33.44 % at 96.89
Dip S: 47.57 % G2/G1: 2.00
%CV: 7.50

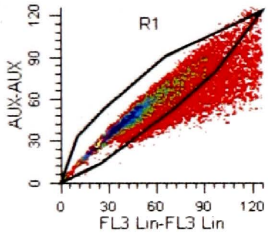
Total S-Phase: 47.57 %
Total B.A.D.: 65.69 % no aggs

Apoptosis: 60.69 % Mean: 0.63

Debris: 41.28 %
Aggregates: 0.00 %
Modeled events: 12535
All cycle events: 2893
Cycle events per channel: 59
RCS: 10.546

B) Cytochalsain-B treatment sample 2





File analyzed: MW1.11 cb2 00011429 2010-01-12 1209 545.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

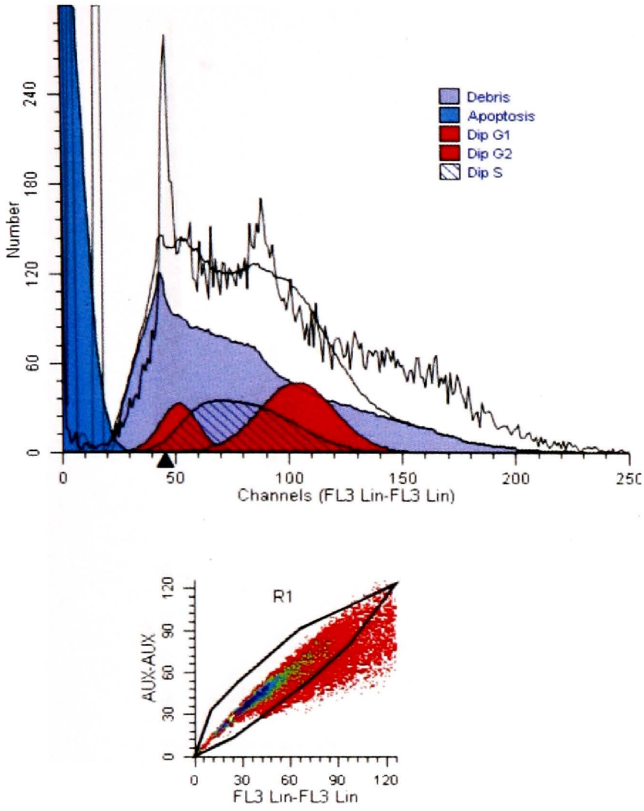
Diploid: 100.00 %
Dip G1: 18.09 % at 47.37
Dip G2: 29.86 % at 94.74
Dip S: 52.05 % G2/G1: 2.00
%CV: 9.25

Total S-Phase: 52.05 %
Total B.A.D.: 28.66 % no aggs

Apoptosis: 0.29 % Mean: 4.97

Debris: 40.03 %
Aggregates: 0.00 %
Modeled events: 9846
All cycle events: 5887
Cycle events per channel: 122
RCS: 15.779

C) Cytochalsain-B treatment sample 3



File analyzed: MW1.11 cb3 00011430 2010-01-12 1213 546.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 14.86 % at 51.92
Dip G2: 43.34 % at 103.84
Dip S: 41.80 % G2/G1: 2.00
%CV: 15.66

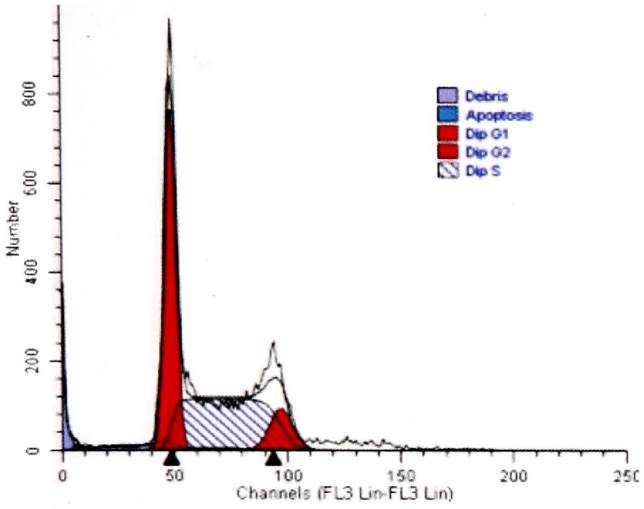
Total S-Phase: 41.80 %
Total B.A.D.: 56.93 % no aggs

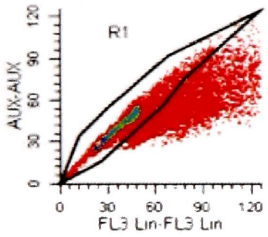
Apoptosis: 50.69 % Mean: 0.98

Debris: 33.36 %
Aggregates: 0.00 %
Modeled events: 13579
All cycle events: 4462
Cycle events per channel: 84
RCS: 10.632

Figure 75. 2-methylanthraquinone treatment group flow histograms and summaries

A) 2-methylanthraquinone treatment sample 1





File analyzed: MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

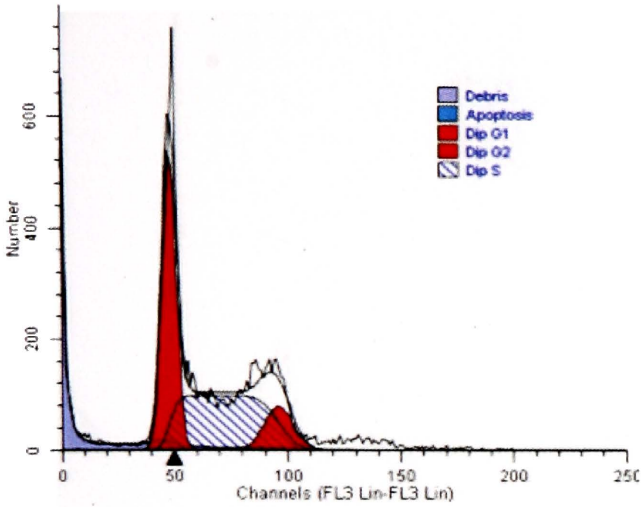
Diploid: 100.00 %
Dip G1: 42.39 % at 48.63
Dip G2: 10.16 % at 97.26
Dip S: 47.45 % G2/G1: 2.00
%CV: 5.18

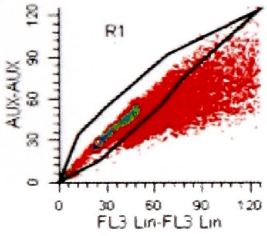
Total S-Phase: 47.45 %
Total B.A.D.: 2.74 % no aggs

Apoptosis: 0.27 % Mean: 6.51

Debris: 10.51 %
Aggregates: 0.00 %
Modeled events: 13000
All cycle events: 11602
Cycle events per channel: 234
RCS: 5.465

B) 2-methylanthraquinone treatment sample 2





File analyzed: MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

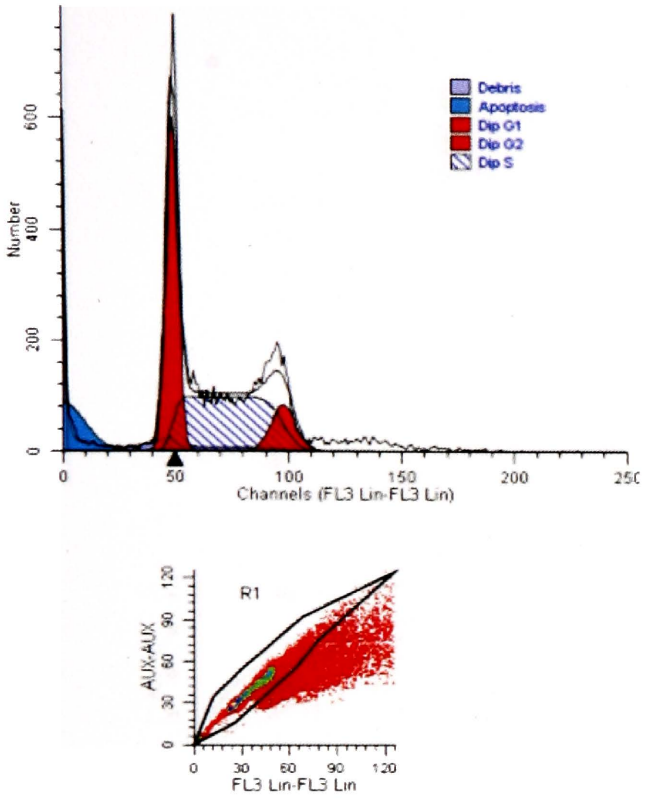
Diploid: 100.00 %
Dip G1: 41.76 % at 48.08
Dip G2: 12.05 % at 96.17
Dip S: 46.19 % G2/G1: 2.00
%CV: 6.52

Total S-Phase: 46.19 %
Total B.A.D.: 3.41 % no aggs

Apoptosis: 0.05 % Mean: 7.16

Debris: 17.13 %
Aggregates: 0.00 %
Modeled events: 12288
All cycle events: 10177
Cycle events per channel: 207
RCS: 5.258

C) 2-methylanthraquinone treatment sample 3



File analyzed: MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 40.84 % at 48.93
Dip G2: 11.17 % at 97.86
Dip S: 47.99 % G2/G1: 2.00
%CV: 5.52

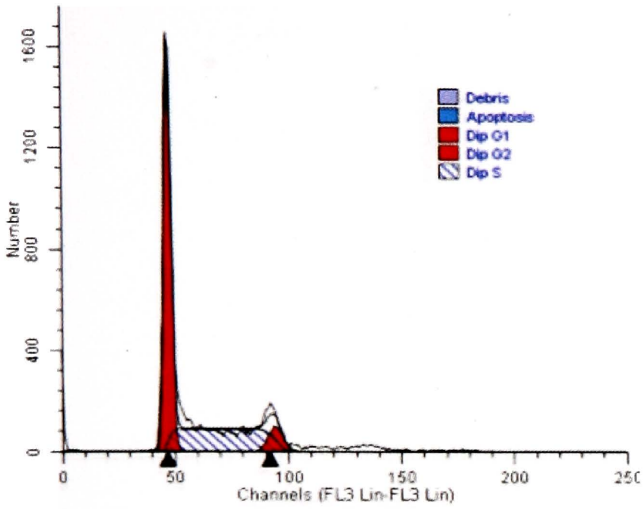
Total S-Phase: 47.99 %
Total B.A.D.: 4.23 % no aggs

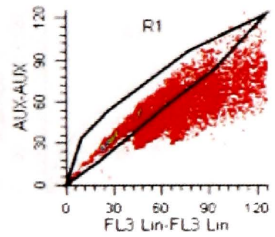
Apoptosis: 10.42 % Mean: 0.19

Debris: 6.81 %
Aggregates: 0.00 %
Modeled events: 12021
All cycle events: 10035
Cycle events per channel: 201
RCS: 5.542

Figure 76. Teak wood extract #1 (7 μg/ml treatment group) flow histograms and summaries

A) Teak wood extract #1 treatment sample 1





File analyzed: MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

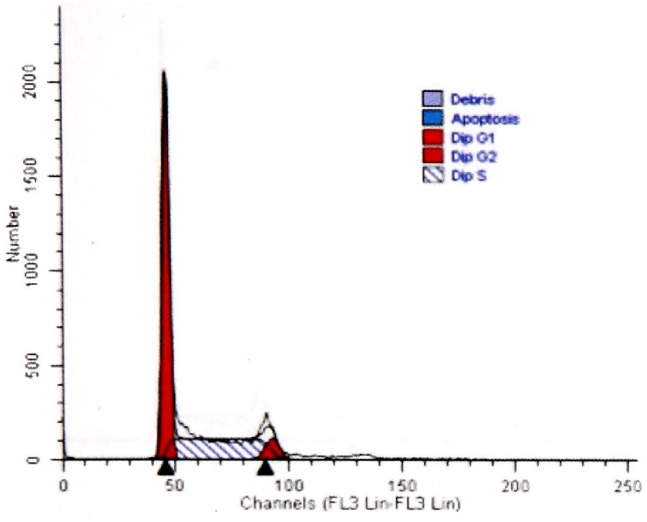
Diploid: 100.00 %
Dip G1: 56.92 % at 47.21
Dip G2: 6.75 % at 94.42
Dip S: 36.33 % G2/G1: 2.00
%CV: 3.37

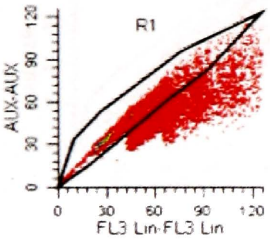
Total S-Phase: 36.33 %
Total B.A.D.: 0.47 % no aggs

Apoptosis: 0.16 % Mean: 5.21

Debris: 1.10 %
Aggregates: 0.00 %
Modeled events: 11705
All cycle events: 11558
Cycle events per channel: 240
RCS: 6.081

B) Teak wood extract #1 treatment sample 2





File analyzed: MW1.11 bt7.2 00011432
2010-01-12 1223 548.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

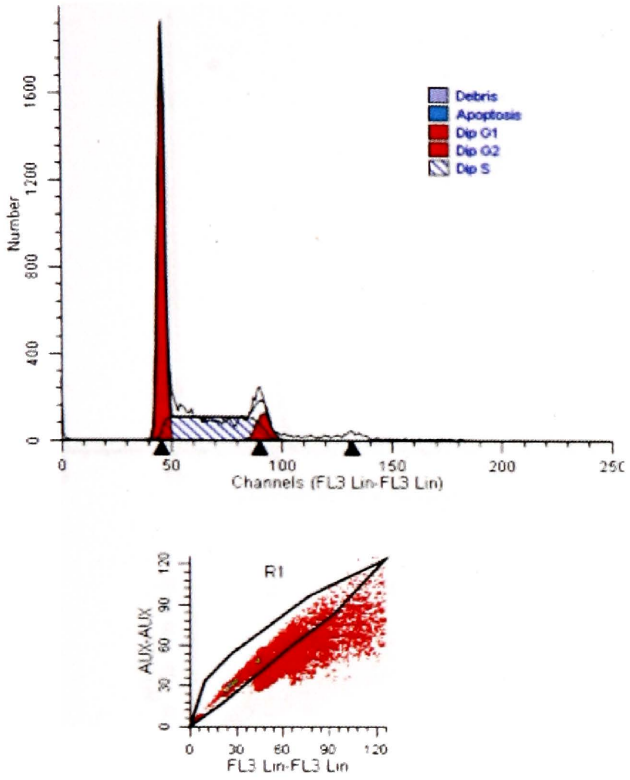
Diploid: 100.00 %
Dip G1: 56.37 % at 46.32
Dip G2: 6.18 % at 92.65
Dip S: 37.46 % G2/G1: 2.00
%CV: 3.18

Total S-Phase: 37.46 %
Total B.A.D.: 0.44 % no aggs

Apoptosis: 0.03 % Mean: 4.60

Debris: 1.13 %
Aggregates: 0.00 %
Modeled events: 13809
All cycle events: 13648
Cycle events per channel: 288
RCS: 6.186

C) Teak wood extract #1 treatment sample 3



File analyzed: MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 55.32 % at 45.94
Dip G2: 7.39 % at 91.87
Dip S: 37.29 % G2/G1: 2.00
%CV: 3.29

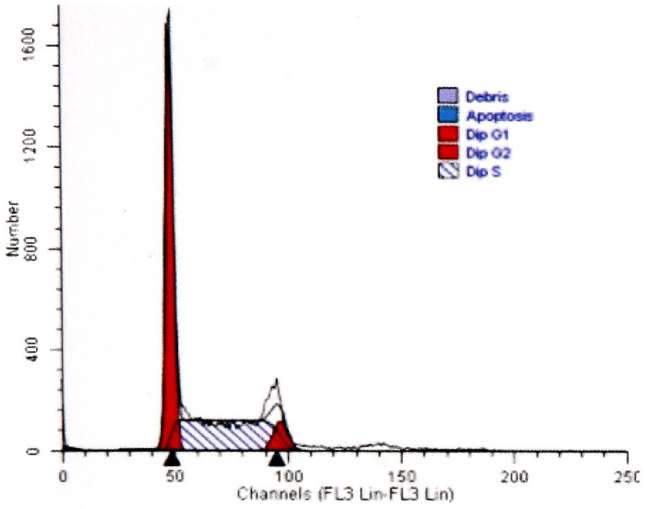
Total S-Phase: 37.29 %
Total B.A.D.: 0.20 % no aggs

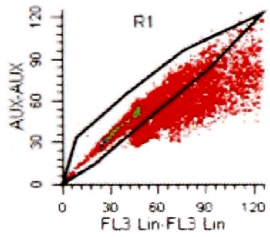
Apoptosis: 0.03 % Mean: 4.49

Debris: 0.73 %
Aggregates: 0.00 %
Modeled events: 13014
All cycle events: 12916
Cycle events per channel: 275
RCS: 7.167

Figure 77. Teak wood dust extract #1 (10 μg/ml treatment group) flow histograms and summaries

A) Teak wood dust extract #1 treatment sample 1





File analyzed: MW1.11 bt10.1 00011434
2010-01-12 1231 550.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

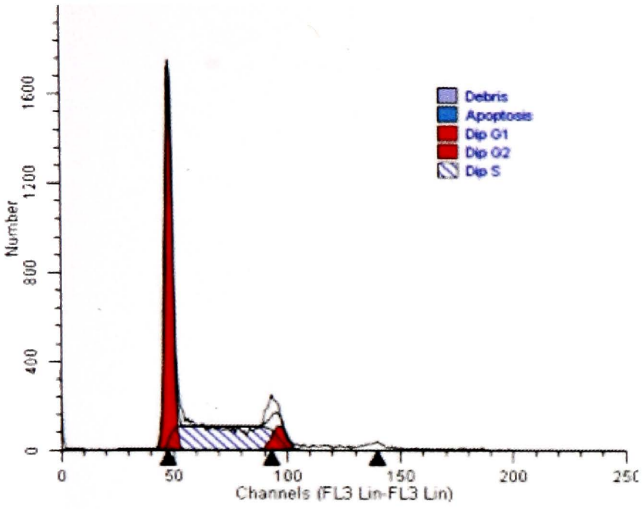
Diploid: 100.00 %
Dip G1: 50.49 % at 48.36
Dip G2: 6.89 % at 96.72
Dip S: 42.62 % G2/G1: 2.00
%CV: 3.26

Total S-Phase: 42.62 %
Total B.A.D.: 0.56 % no aggs

Apoptosis: 0.73 % Mean: 2.22

Debris: 1.15 %
Aggregates: 0.00 %
Modeled events: 13571
All cycle events: 13317
Cycle events per channel: 270
RCS: 6.620

B) Teak wood dust extract #1 treatment sample 2

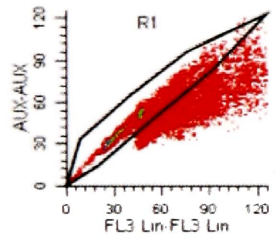


File analyzed: MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 52.69 % at 48.25
Dip G2: 6.73 % at 96.50
Dip S: 40.58 % G2/G1: 2.00
%CV: 3.17

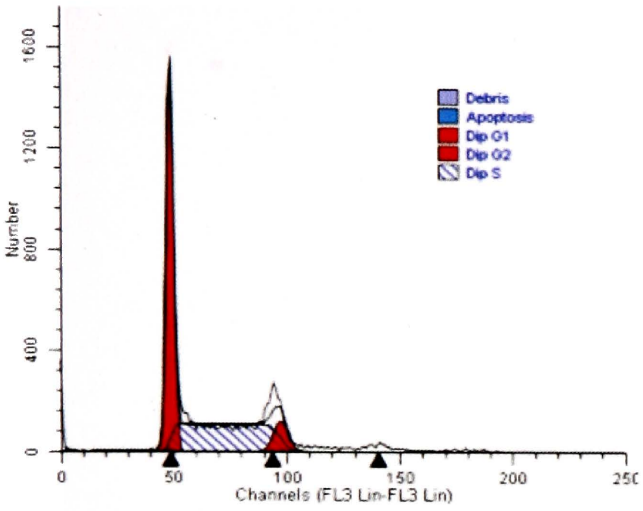
Total S-Phase: 40.58 %
Total B.A.D.: 0.63 % no aggs

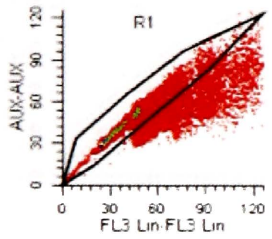


Apoptosis: 0.35 % Mean: 6.88

Debris: 1.45 %
Aggregates: 0.00 %
Modeled events: 12762
All cycle events: 12532
Cycle events per channel: 254
RCS: 6.958

C) Teak wood dust extract #1 treatment sample 3





File analyzed: MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 49.35 % at 48.68
Dip G2: 7.90 % at 97.36
Dip S: 42.75 % G2/G1: 2.00
%CV: 3.30

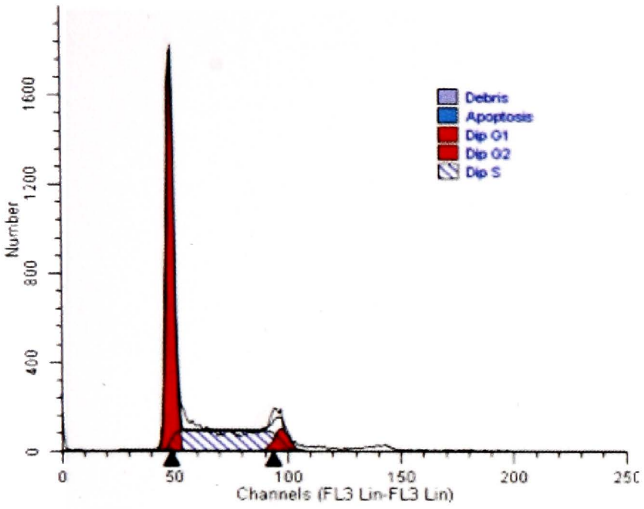
Total S-Phase: 42.75 %
Total B.A.D.: 0.76 % no aggs

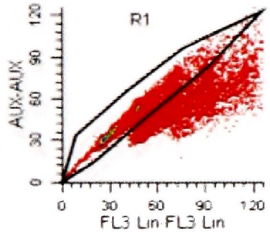
Apoptosis: 0.00 % Mean: 7.34

Debris: 2.24 %
Aggregates: 0.00 %
Modeled events: 12479
All cycle events: 12199
Cycle events per channel: 246
RCS: 7.003

Figure 78. Teak wood dust extract #2 (7 μg/ml treatment group) flow histograms and summaries

A) Teak wood dust extract #2 treatment sample 1





File analyzed: MW1.11 pt7.1 00011437
2010-01-12 1245 553.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

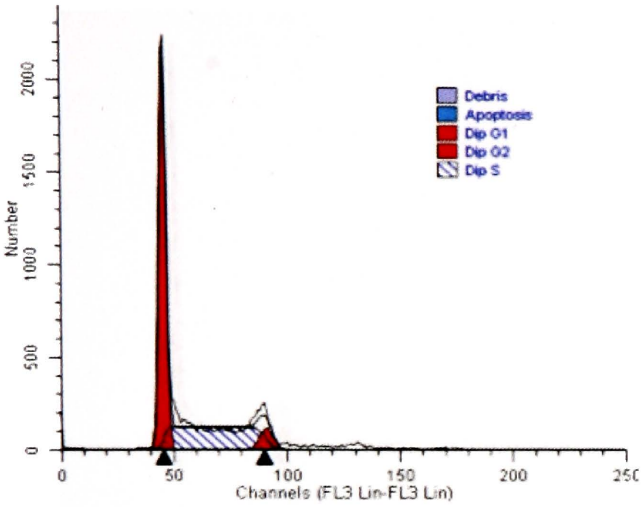
Diploid: 100.00 %
Dip G1: 57.56 % at 48.65
Dip G2: 6.39 % at 97.31
Dip S: 36.04 % G2/G1: 2.00
%CV: 3.29

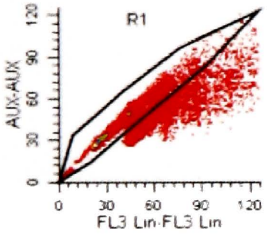
Total S-Phase: 36.04 %
Total B.A.D.: 0.54 % no aggs

Apoptosis: 0.09 % Mean: 7.75

Debris: 1.45 %
Aggregates: 0.00 %
Modeled events: 12796
All cycle events: 12599
Cycle events per channel: 254
RCS: 5.953

B) Teak wood dust extract #2 treatment sample 2





File analyzed: MW1.11 pt7.2 00011438 2010-01-12 1248 554.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

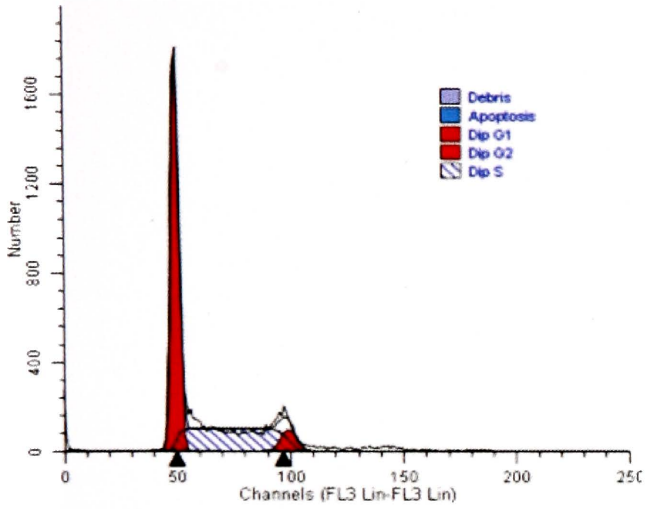
Diploid: 100.00 %
Dip G1: 56.73 % at 45.49
Dip G2: 5.69 % at 90.99
Dip S: 37.58 % G2/G1: 2.00
%CV: 3.19

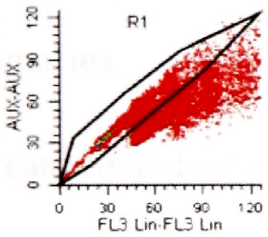
Total S-Phase: 37.58 %
Total B.A.D.: 0.47 % no aggs

Apoptosis: 0.49 % Mean: 5.73

Debris: 0.89 %
Aggregates: 0.00 %
Modeled events: 14727
All cycle events: 14524
Cycle events per channel: 312
RCS: 6.744

C) Teak wood dust extract #2 treatment sample 3





File analyzed: MW1.11 pt7.3 00011439 2010-01-12 1251 555.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 57.75 % at 49.60
Dip G2: 5.96 % at 99.19
Dip S: 36.29 % G2/G1: 2.00
%CV: 3.44

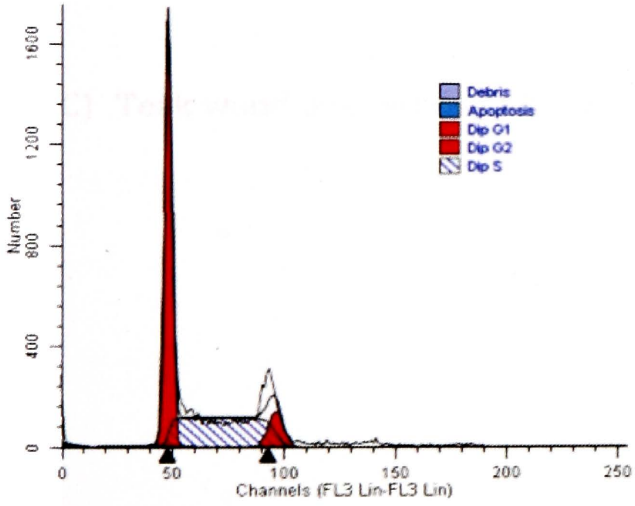
Total S-Phase: 36.29 %
Total B.A.D.: 0.20 % no aggs

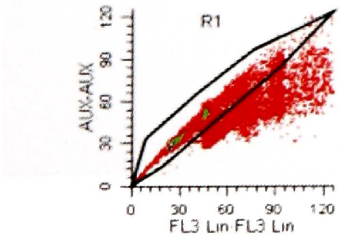
Apoptosis: 0.00 % Mean: 5.02

Debris: 0.99 %
Aggregates: 0.00 %
Modeled events: 13648
All cycle events: 13513
Cycle events per channel: 267
RCS: 5.321

Figure 79. Teak wood dust extract #2 (10 μg/ml treatment group) flow histograms and summaries

A) Teak wood dust extract #2 treatment sample 1





File analyzed: MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

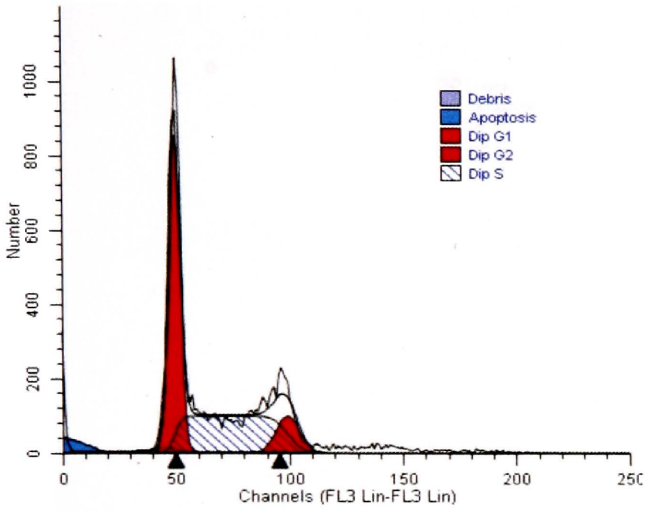
Diploid: 100.00 %
Dip G1: 50.61 % at 48.14
Dip G2: 8.59 % at 96.29
Dip S: 40.80 % G2/G1: 2.00
%CV: 3.42

Total S-Phase: 40.80 %
Total B.A.D.: 1.06 % no aggs

Apoptosis: 1.05 % Mean: 2.57

Debris: 2.02 %
Aggregates: 0.00 %
Modeled events: 13955
All cycle events: 13528
Cycle events per channel: 275
RCS: 6.054

B) Teak wood dust extract #2 treatment sample 2

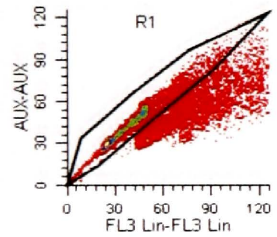


File analyzed: MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 47.19 % at 49.60
Dip G2: 10.70 % at 99.20
Dip S: 42.11 % G2/G1: 2.00
%CV: 5.07

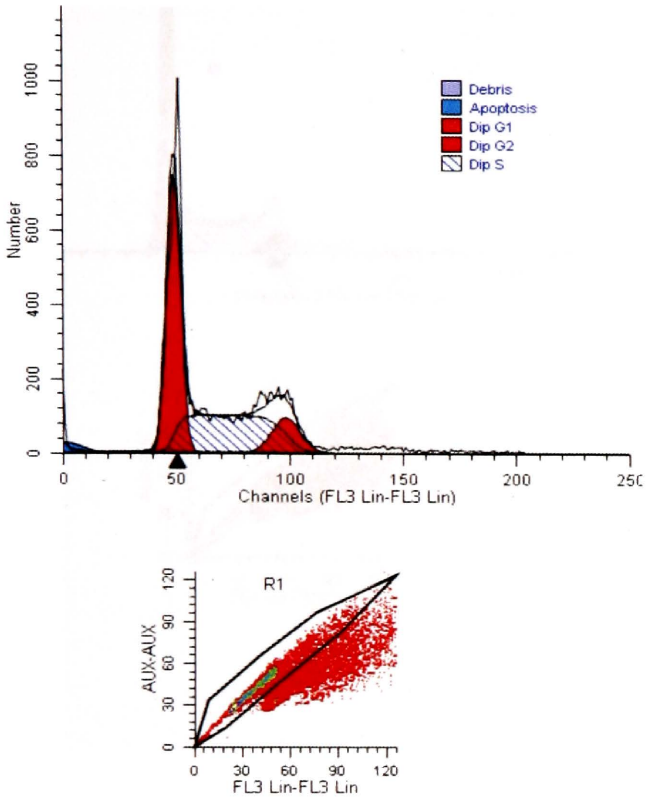
Total S-Phase: 42.11 %
Total B.A.D.: 1.90 % no aggs



Apoptosis: 4.14 % Mean: 0.80

Debris: 0.84 %
Aggregates: 0.00 %
Modeled events: 12248
All cycle events: 11643
Cycle events per channel: 230
RCS: 6.295

C) Teak wood dust extract #2 treatment sample 3



File analyzed: MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 47.38 % at 49.20
Dip G2: 12.16 % at 98.41
Dip S: 40.46 % G2/G1: 2.00
%CV: 6.26

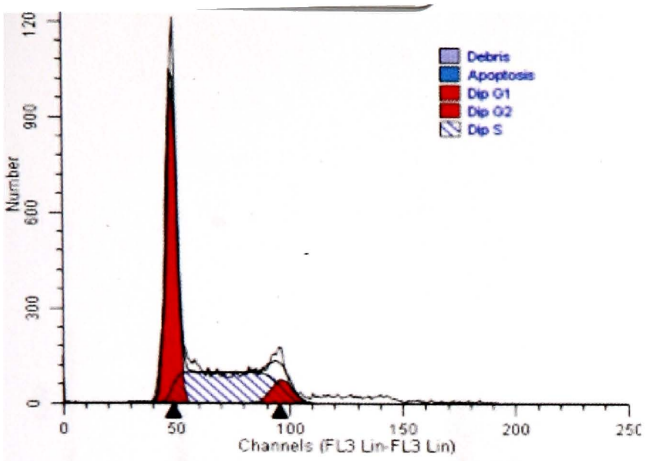
Total S-Phase: 40.46 %
Total B.A.D.: 1.32 % no aggs

Apoptosis: 2.48 % Mean: 1.37

Debris: 1.33 %
Aggregates: 0.00 %
Modeled events: 12758
All cycle events: 12276
Cycle events per channel: 245
RCS: 6.018

Figure 80. Teak wood dust extract #3 (7 μg/ml treatment group) flow histograms and summaries

A) Teak wood dust extract #3 treatment sample 1

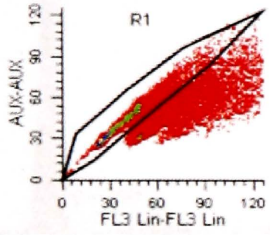


2010-01-12 1302 559.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 51.15 % at 48.30
Dip G2: 7.54 % at 96.59
Dip S: 41.31 % G2/G1: 2.00
%CV: 4.77

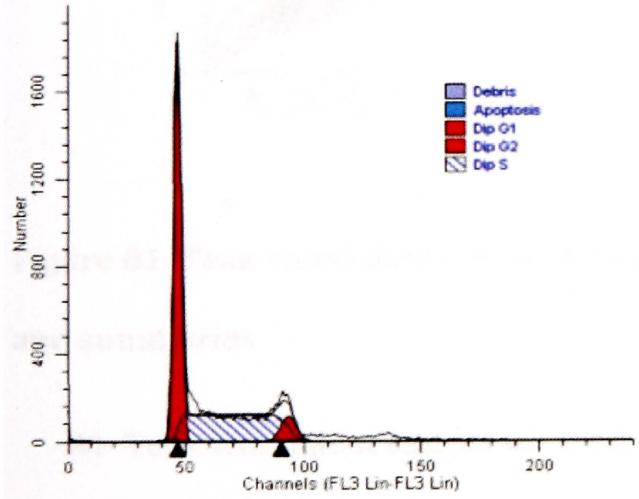
Total S-Phase: 41.31 %
Total B.A.D.: 0.42 % no aggs



Apoptosis: 0.04 % Mean: 6.13

Debris: 0.88 %
Aggregates: 0.00 %
Modeled events: 11637
All cycle events: 11530
Cycle events per channel: 234
RCS: 6.984

B) Teak wood dust extract #3 treatment sample 2

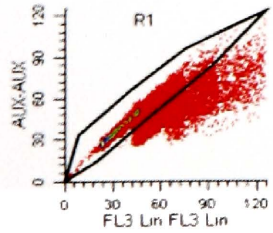


File analyzed: MW1.11 ut7.2 00011444
2010-01-12 1305 560.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 51.61 % at 46.85
Dip G2: 6.56 % at 93.69
Dip S: 41.83 % G2/G1: 2.00
%CV: 3.32

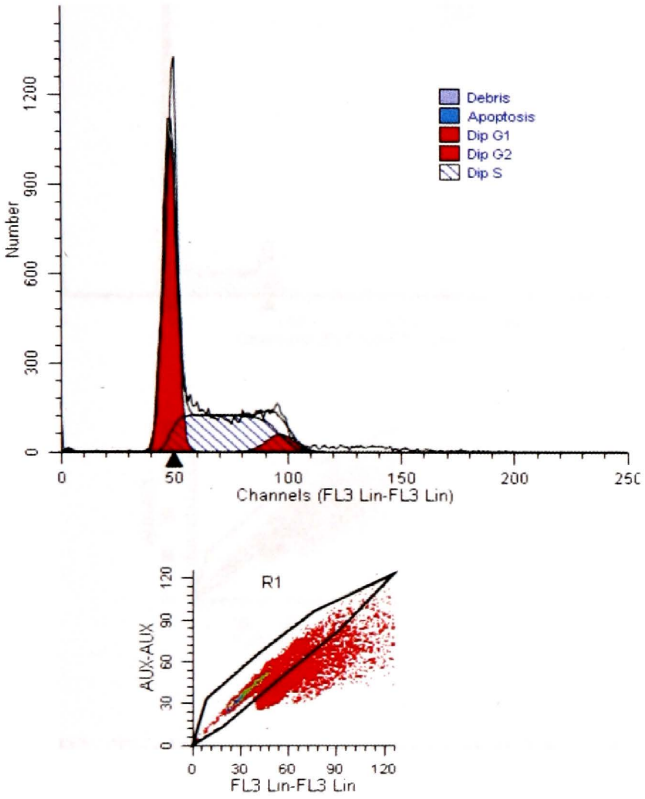
Total S-Phase: 41.83 %
Total B.A.D.: 0.42 % no aggs



Apoptosis: 0.58 % Mean: 0.61

Debris: 0.46 %
Aggregates: 0.00 %
Modeled events: 13738
All cycle events: 13596
Cycle events per channel: 284
RCS: 6.666

C) Teak wood dust extract #3 treatment sample 3



File analyzed: MW1.11 ut7.3 00011445
2010-01-12 1315 561.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 54.79 % at 48.31
Dip G2: 5.99 % at 96.63
Dip S: 39.22 % G2/G1: 2.00
%CV: 6.25

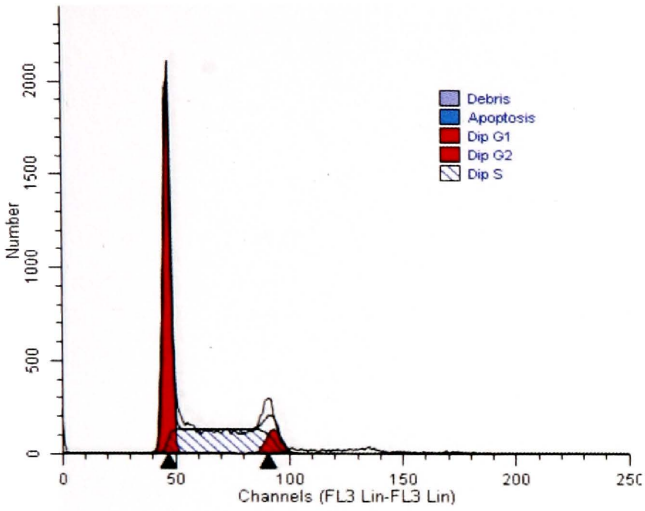
Total S-Phase: 39.22 %
Total B.A.D.: 0.07 % no aggs

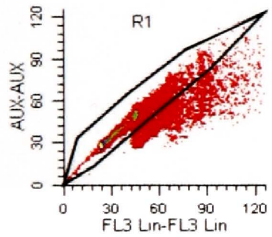
Apoptosis: 0.35 % Mean: 3.15

Debris: 0.26 %
Aggregates: 0.00 %
Modeled events: 14975
All cycle events: 14884
Cycle events per channel: 302
RCS: 5.961

Figure 81. Teak wood dust extract #3 (10 μg/ml treatment group) flow histograms and summaries

A) Teak wood dust extract #3 treatment sample 1





File analyzed: MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

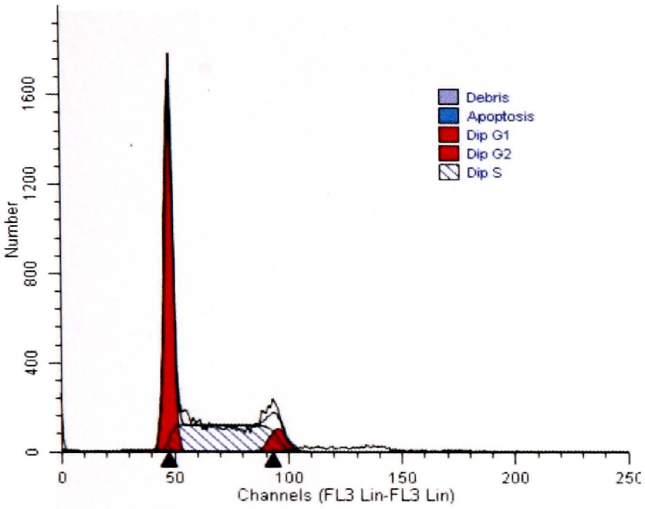
Diploid: 100.00 %
Dip G1: 53.34 % at 46.54
Dip G2: 6.75 % at 93.07
Dip S: 39.92 % G2/G1: 2.00
%CV: 3.34

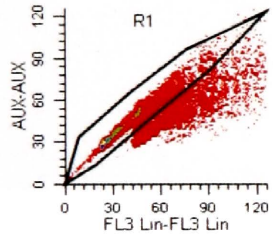
Total S-Phase: 39.92 %
Total B.A.D.: 0.25 % no aggs

Apoptosis: 0.03 % Mean: 7.55

Debris: 0.73 %
Aggregates: 0.00 %
Modeled events: 15184
All cycle events: 15068
Cycle events per channel: 317
RCS: 6.440

B) Teak wood dust extract #3 treatment sample 2





File analyzed: MW1.11 ut10.2 00011447
2010-01-12 1319 563.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

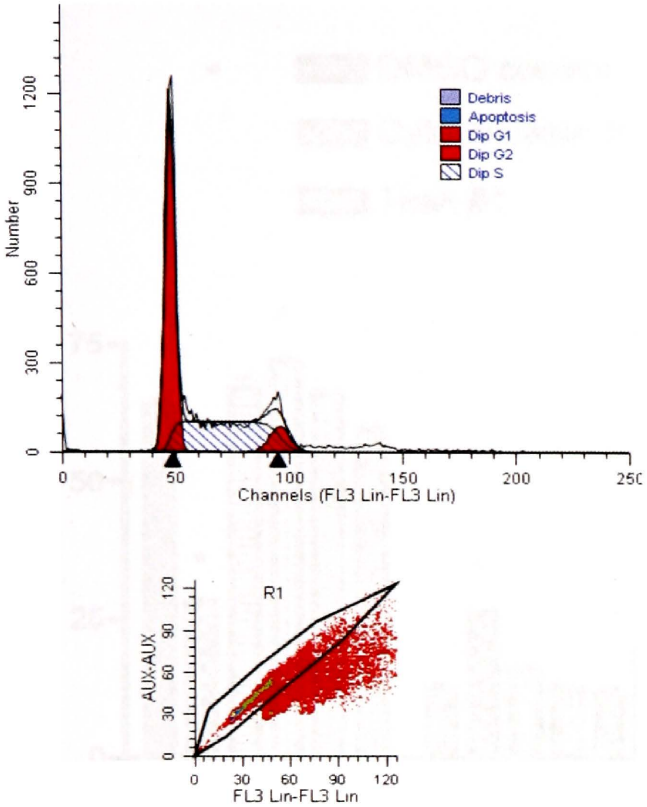
Diploid: 100.00 %
Dip G1: 52.53 % at 47.70
Dip G2: 6.67 % at 95.40
Dip S: 40.80 % G2/G1: 2.00
%CV: 3.73

Total S-Phase: 40.80 %
Total B.A.D.: 0.50 % no aggs

Apoptosis: 0.38 % Mean: 2.25

Debris: 1.09 %
Aggregates: 0.00 %
Modeled events: 14159
All cycle events: 13952
Cycle events per channel: 286
RCS: 6.331

C) Teak wood dust extract #3 treatment sample 3



File analyzed: MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD
Date analyzed: 12-Jan-2010
Model: 1Dn0A_DSD
Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 52.76 % at 47.96
Dip G2: 7.72 % at 95.91
Dip S: 39.52 % G2/G1: 2.00
%CV: 4.54

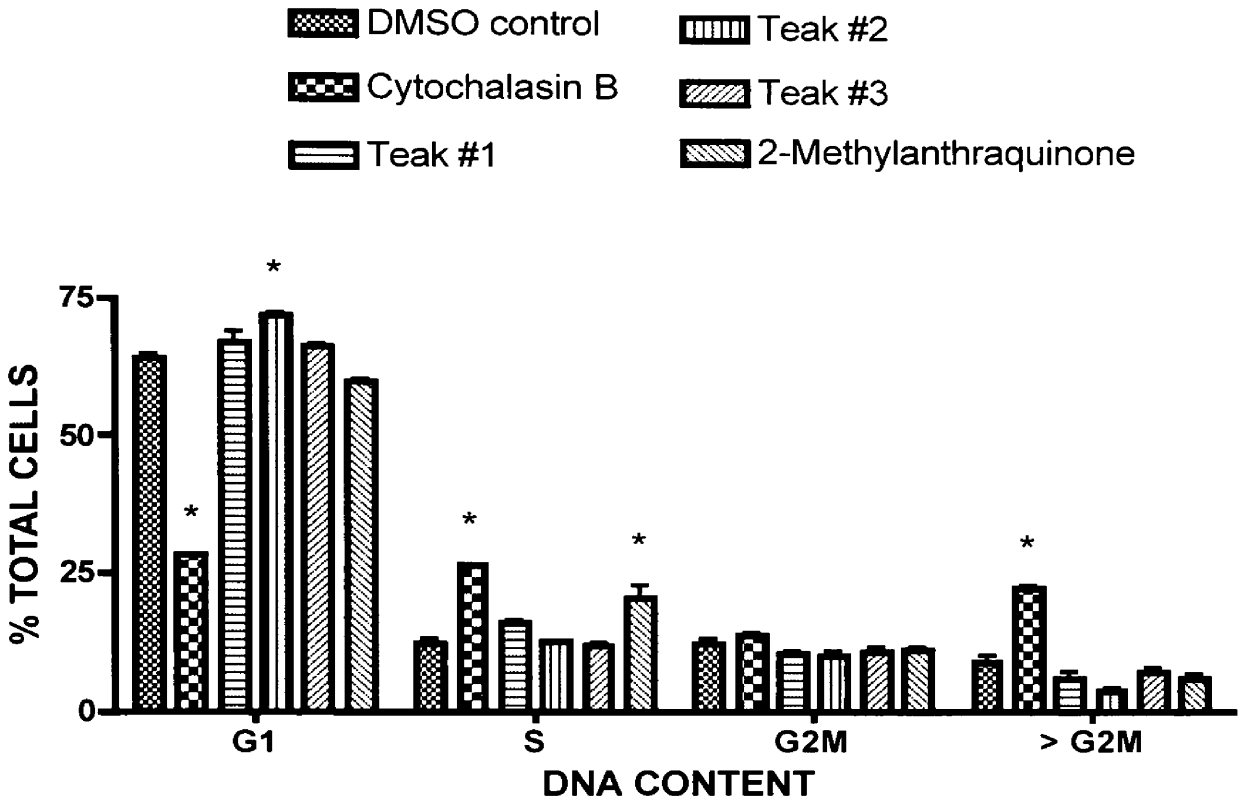
Total S-Phase: 39.52 %
Total B.A.D.: 0.27 % no aggs

Apoptosis: 0.13 % Mean: 5.87

Debris: 0.85 %
Aggregates: 0.00 %
Modeled events: 12296
All cycle events: 12177
Cycle events per channel: 249
RCS: 5.985

Manual cell cycle analysis

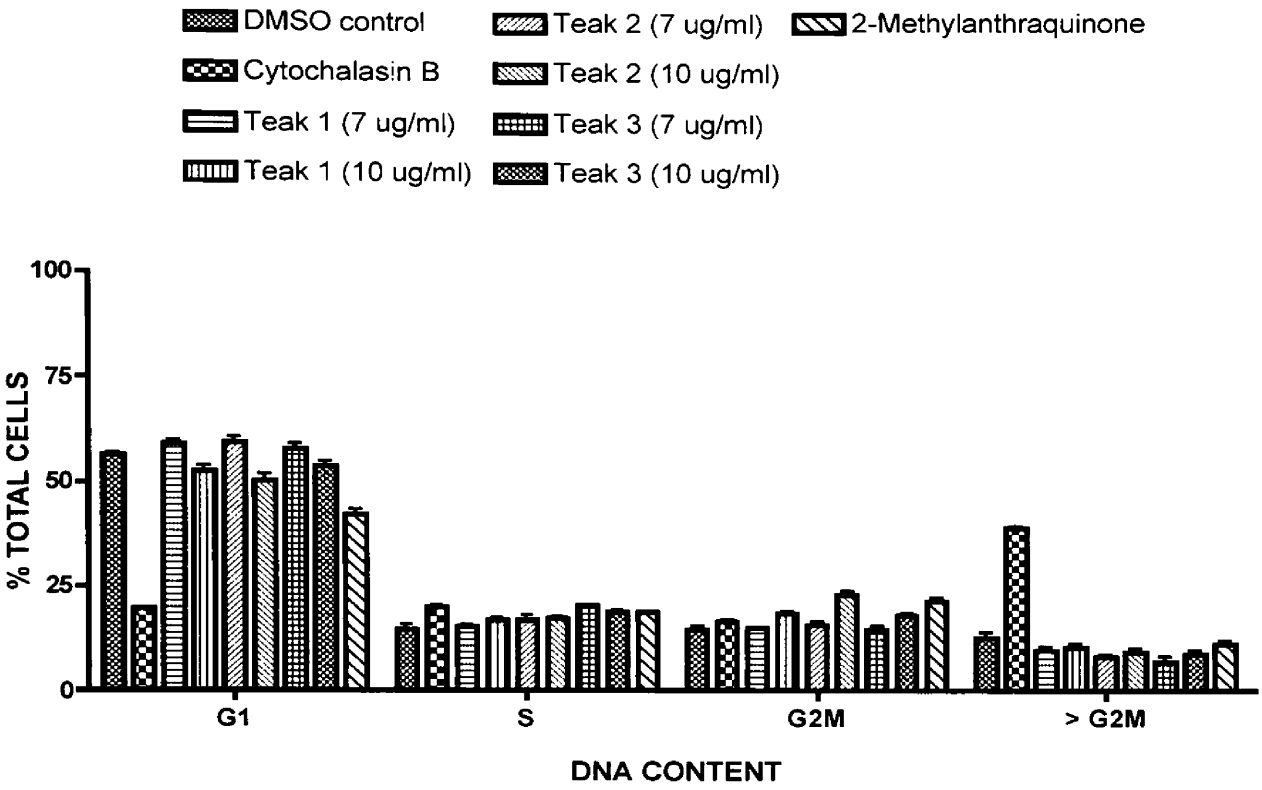
Figure 82. Cell cycle analysis following twenty-four hour recovery period



The x-axis indicates the phases of the cell cycle that are reflected by DNA content.

The y-axis shows the percent of total cells identified in each phase of the cell cycle.

Figure 83. Cell cycle analysis following three-day recovery period



The x-axis indicates the phases of the cell cycle that are reflected by DNA content.

The y-axis shows the percent of total cells identified in each phase of the cell cycle.

ANOVA tables

Table 61. Dichromate and teak extract treatment ANOVA table

Parameter	Value	dichromate	teak extract
Table Analyzed			

Data 1

One-way analysis of variance

P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	3
F	166.7
R squared	0.8327

Bartlett's test for equal variances

Bartlett's statistic (corrected)	87.06
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	500.3	2	250.1
Residual (within columns)	100.5	67	1.5
Total	600.8	69	

Dunnett's Multiple	Mean	q	P value	95% CI of

Comparison Test	Diff.			diff
Control vs 3 mM			P >	-1.417 to
dichromate	-0.7	2.213	0.05	0.01677
Control vs teak 5			P <	-8.947 to
mg/ml	-7.933	17.74	0.01	-6.920

Table 62. 24-hour recovery experiment ANOVA summary

Two-way ANOVA

Source of Variation	% of total variation	P value
Interaction	13.81	P<0.0001
Treatment	0.09	0.0068
cycle point	85.85	P<0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Treatment	**	Yes
cycle point	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	15	4931	328.7	178.1
Treatment	5	33.9	6.78	3.674
cycle point	3	30650	10220	5535
Residual	48	88.58	1.846	

Number of missing values	0

Bonferroni posttests

DMSO control vs. Cytochalasin B

cycle point	DMSO control	Cytochalasin B	Difference	95% CI of diff.
G1	64.03	28.32	-35.71	-39.25 to -32.17
S	12.14	26.4	14.26	10.72 to 17.80
G2M	12.32	13.68	1.367	-2.173 to 4.906
> G2M	8.783	22.27	13.49	9.950 to 17.03

cycle point	Difference	t	P value	Summary
G1	-35.71	32.19	P<0.001	***
S	14.26	12.86	P<0.001	***
G2M	1.367	1.232	P > 0.05	ns
> G2M	13.49	12.16	P<0.001	***

DMSO control vs. Burmese teak

cycle point	DMSO control	Burmese teak	Difference	95% CI of diff.
G1	64.03	67.14	3.113	-0.4262 to 6.653
S	12.14	15.86	3.72	0.1804 to 7.260
G2M	12.32	10.27	-2.05	-5.590 to 1.490
> G2M	8.783	6.047	-2.737	-6.276 to 0.8029

cycle point	Difference	t	P value	Summary
G1	3.113	2.807	P < 0.05	*
S	3.72	3.354	P<0.01	**
G2M	-2.05	1.848	P > 0.05	ns
> G2M	-2.737	2.467	P > 0.05	ns

DMSO control vs. Plantation Teak

cycle point	DMSO control	Plantation Teak	Difference	95% CI of diff.
G1	64.03	71.8	7.77	4.230 to 11.31
S	12.14	12.44	0.3067	-3.233 to 3.846
G2M	12.32	10.12	-2.193	-5.733 to 1.346
> G2M	8.783	3.553	-5.23	-8.770 to -1.690

cycle point	Difference	t	P value	Summary
G1	7.77	7.005	P<0.001	***
S	0.3067	0.2765	P > 0.05	ns
G2M	-2.193	1.977	P > 0.05	ns

> G2M	-5.23	4.715	P<0.001	***

DMSO control vs. Unknown teak

cycle point	DMSO control	Unknown teak	Difference	95% CI of diff.
G1	64.03	66.15	2.12	-1.420 to 5.660
S	12.14	12.03	-0.11	-3.650 to 3.430
G2M	12.32	10.92	-1.397	-4.936 to 2.143
> G2M	8.783	7.077	-1.707	-5.246 to 1.833

cycle point	Difference	t	P value	Summary
G1	2.12	1.911	P > 0.05	ns
S	-0.11	0.09917	P > 0.05	ns
G2M	-1.397	1.259	P > 0.05	ns
> G2M	-1.707	1.539	P > 0.05	ns

DMSO control vs. 2-Methylanthraquinone

cycle point	DMSO control	2-Methylanthraquinone	Difference	95% CI of diff.
G1	64.03	59.7	-4.327	-7.866 to -0.7871
S	12.14	20.67	8.533	4.994 to 12.07
G2M	12.32	10.99	-1.33	-4.870 to 2.210
> G2M	8.783	5.963	-2.82	-6.360 to 0.7196

cycle point	Difference	t	P value	Summary
G1	-4.327	3.901	P<0.01	**
S	8.533	7.693	P<0.001	***
G2M	-1.33	1.199	P > 0.05	ns
> G2M	-2.82	2.542	P > 0.05	ns

Table 63. Three-day recovery experiment ANOVA table

Two-way ANOVA

Source of Variation	% of total variation	P value
Interaction	23.75	P<0.0001
Treatment	0.11	0.0282
cycle point	75.73	P<0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Treatment	*	Yes
cycle point	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	21	6134	292.1	174.4
Treatment	7	28.53	4.076	2.434
cycle point	3	19560	6520	3894
Residual	64	107.2	1.675	
Number of missing values	12			

Bonferroni posttests

DMSO control vs. Cytochalasin B

cycle point	DMSO control	Cytochalasin B	Difference	95% CI of diff.
G1	56.18	19.47	-36.71	-40.20 to -33.22
S	14.81	20	5.187	1.695 to 8.678
G2M	14.64	16.52	1.88	-1.611 to 5.371
> G2M	12.66	39.06	26.4	22.91 to 29.89

cycle point	Difference	t	P value	Summary
G1	-36.71	34.74	P<0.001	***
S	5.187	4.909	P<0.001	***
G2M	1.88	1.779	P > 0.05	ns
> G2M	26.4	24.99	P<0.001	***

DMSO control vs. Burmese teak 7 ug/ml

cycle point	DMSO control	Burmese teak 7 ug/ml	Difference	95% CI of diff.
G1	56.18	59.07	2.897	-0.5945 to 6.388
S	14.81	15.48	0.67	-2.821 to 4.161
G2M	14.64	14.88	0.2367	-3.255 to

> G2M	12.66	9.787	-2.873	3.728 to -6.365 to 0.6179

cycle point	Difference	t	P value	Summary
G1	2.897	2.742	P < 0.05	*
S	0.67	0.6341	P > 0.05	ns
G2M	0.2367	0.224	P > 0.05	ns
> G2M	-2.873	2.719	P < 0.05	*

DMSO control vs. Burmese teak 10 ug/ml

cycle point	DMSO control	Burmese teak 10 ug/ml	Difference	95% CI of diff.
G1	56.18	52.51	-3.663	-7.155 to -0.1721
S	14.81	17.13	2.32	-1.171 to 5.811
G2M	14.64	18.35	3.703	0.2121 to 7.195
> G2M	12.66	10.58	-2.08	-5.571 to 1.411

cycle point	Difference	t	P value	Summary
G1	-3.663	3.467	P<0.01	**
S	2.32	2.196	P > 0.05	ns
G2M	3.703	3.505	P<0.01	**
> G2M	-2.08	1.969	P > 0.05	ns

DMSO control vs. Plantation Teak 7 ug/ml

cycle point	DMSO control	Plantation Teak 7 ug/ml	Difference	95% CI of diff.
G1	56.18	59.38	3.207	-0.2845 to 6.698
S	14.81	16.97	2.157	-1.335 to 5.648
G2M	14.64	15.7	1.057	-2.435 to 4.548
> G2M	12.66	8.233	-4.427	-7.918 to -0.9355

cycle point	Difference	t	P value	Summary
G1	3.207	3.035	P < 0.05	*
S	2.157	2.041	P > 0.05	ns
G2M	1.057	1	P > 0.05	ns
> G2M	-4.427	4.19	P<0.001	***

DMSO control vs. Plantation Teak 10 ug/ml

cycle point	DMSO control	Plantation Teak 10 ug/ml	Difference	95% CI of diff.
G1	56.18	50.08	-6.097	-9.588 to -2.605

S	14.81	17.47	2.657	-0.8345 to 6.148
G2M	14.64	23.08	8.433	4.942 to 11.92
> G2M	12.66	9.21	-3.45	-6.941 to 0.04121

cycle point	Difference	t	P value	Summary
G1	-6.097	5.77	P<0.001	***
S	2.657	2.514	P > 0.05	ns
G2M	8.433	7.982	P<0.001	***
> G2M	-3.45	3.265	P<0.01	**

DMSO control vs. Unknown Teak 10 ug/ml

cycle point	DMSO control	Unknown Teak 10 ug/ml	Difference	95% CI of diff.
G1	56.18	53.81	-2.367	-5.858 to 1.125
S	14.81	18.74	3.923	0.4321 to 7.415
G2M	14.64	18.21	3.567	0.07546 to 7.058
> G2M	12.66	8.793	-3.867	-7.358 to -0.3755

cycle point	Difference	t	P value	Summary
G1	-2.367	2.24	P > 0.05	ns
S	3.923	3.713	P<0.01	**
G2M	3.567	3.376	P<0.01	**
> G2M	-3.867	3.66	P<0.01	**

DMSO control vs. 2-Methylanthraquinone

cycle point	DMSO control	2-Methylanthraquinone	Difference	95% CI of diff.
G1	56.18	42.19	-13.99	-17.48 to -10.50
S	14.81	18.76	3.943	0.4521 to 7.435
G2M	14.64	21.71	7.063	3.572 to 10.55
> G2M	12.66	11.28	-1.377	-4.868 to 2.115

cycle point	Difference	t	P value	Summary
G1	-13.99	13.24	P<0.001	***
S	3.943	3.732	P<0.01	**
G2M	7.063	6.685	P<0.001	***
> G2M	-1.377	1.303	P > 0.05	ns

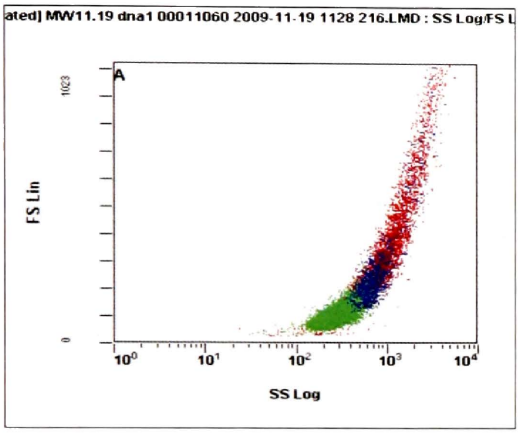
Flow cytometry data from twenty-four hour recovery experiment

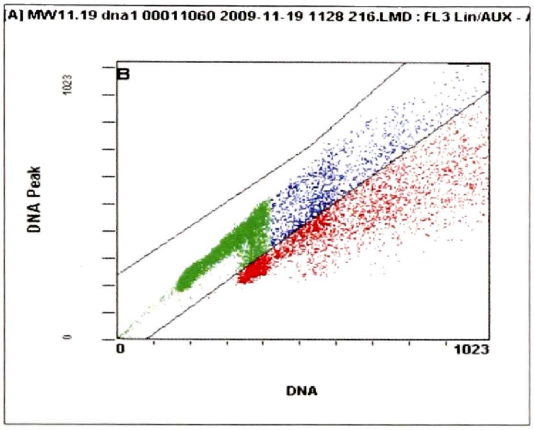
Figure 84. DMSO treatment group flow histograms and summaries

A) DMSO treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:26:00
Settings File: MW DNA.PRO, 19-Nov-2009, 11:27:14
Listmode File: MW11.19 dna1 00011060 2009-11-19 1128 216.LMD

Run Date: 19-Nov-09, 11:2
Sample ID: MW11.19 d
User ID:
Acquisition Time/Events: 98.3s / 20000 (PROTOC
Tube ID: NoF



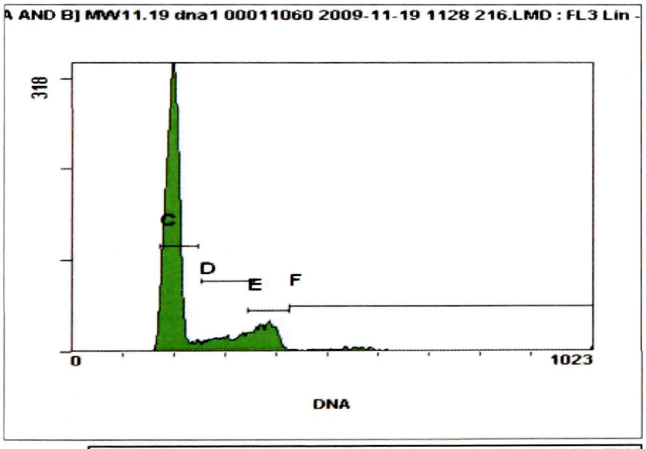


(F1)[Ungated] MW11.19 dna1 00011060 2009-11-19 1128 216.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	561	179
A	20000	100.00	100.00	561	179

(F1)[A] MW11.19 dna1 00011060 2009-11-19 1128 216.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	382	365
B	15073	75.36	75.36	272	309



(F1)MW11.19 dna1 00011060 2009-11-19 1128 216.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna1 00011060 2009-11-19 1128 216.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna1

MW DNA

(F1)[A AND B] MW11.19 dna1 00011060 2009-11-19 1128 216.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15073	75.36	100.00	272	###
C	9875	49.38	65.51	197	###
D	1808	9.04	11.99	310	###
E	1939	9.70	12.86	379	###
F	1393	6.96	9.24	655	###

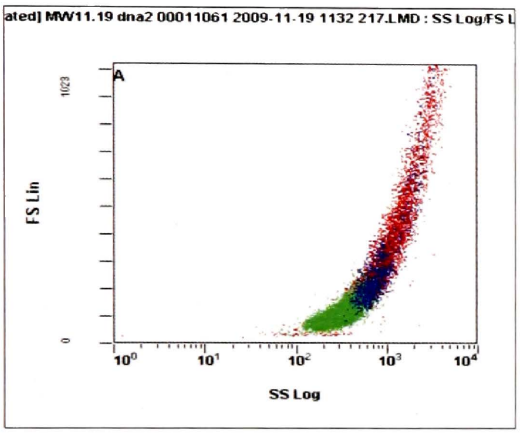
(F1)[Ungated] MW11.19 dna1 00011060 2009-11-19 1128 216.LMD :

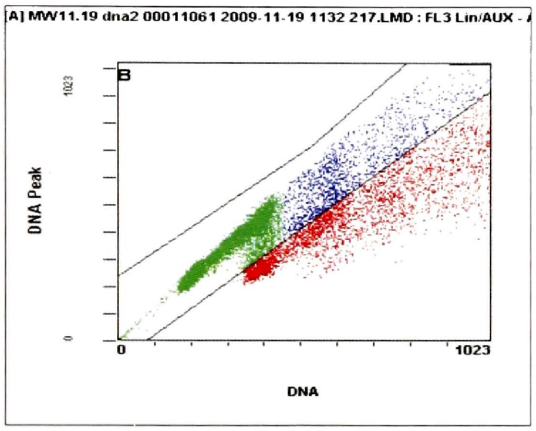
Color	Name	% Gated	% Total	Number	Cells/μL
	F	6.96	6.96	1393	ERROR
	B	75.36	75.36	15073	ERROR
	A	100.00	100.00	20000	ERROR

B) DMSO treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:26:34
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna2 00011061 2009-11-19 1132 217.LMD

Run Date: 19-Nov-09, 11:2
Sample ID: MW11.19 d
User ID:
Acquisition Time/Events: 36.3s / 20000 (PROTOC
Tube ID: NoF



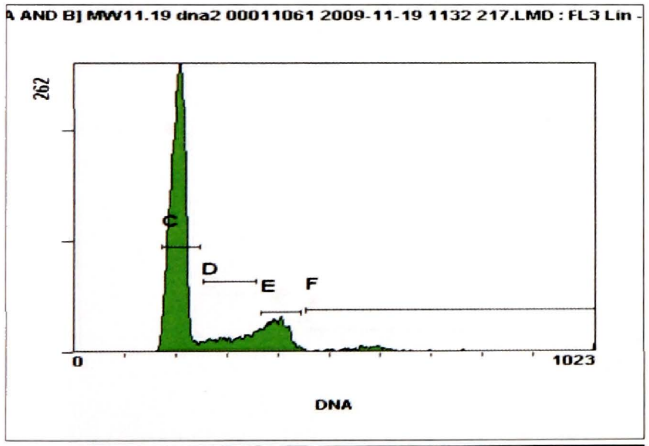


(F1)[Ungated] MW11.19 dna2 00011061 2009-11-19 1132 217.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	689	206
A	20000	100.00	100.00	689	206

(F1)[A] MW11.19 dna2 00011061 2009-11-19 1132 217.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	431	413
B	14284	71.42	71.42	298	333



(F1)MW11.19 dna2 00011061 2009-11-19 1132 217.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna2 00011061 2009-11-19 1132 217.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna2

MW DNA

(F1)[A AND B] MW11.19 dna2 00011061 2009-11-19 1132 217.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14284	71.42	100.00	298	###
C	8924	44.62	62.48	203	###
D	1511	7.55	10.58	308	###
E	1857	9.29	13.00	398	###
F	1591	7.96	11.14	713	###

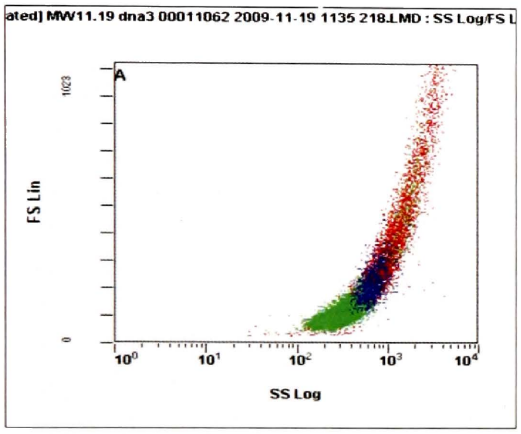
(F1)[Ungated] MW11.19 dna2 00011061 2009-11-19 1132 217.LMD :

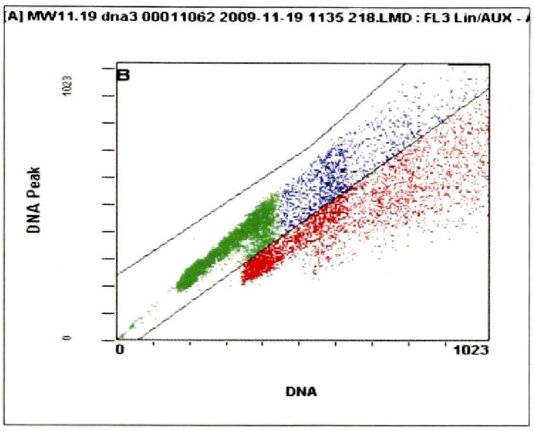
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.96	7.96	1591	ERROR
	B	71.42	71.42	14284	ERROR
	A	100.00	100.00	20000	ERROR

C) DMSO treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:34:36
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna3 00011062 2009-11-19 1135 218.LMD

Run Date: 19-Nov-09, 11:3
Sample ID: MW11.19 c
User ID:
Acquisition Time/Events: 56.4s / 20000 (PROTOC
Tube ID: NoF



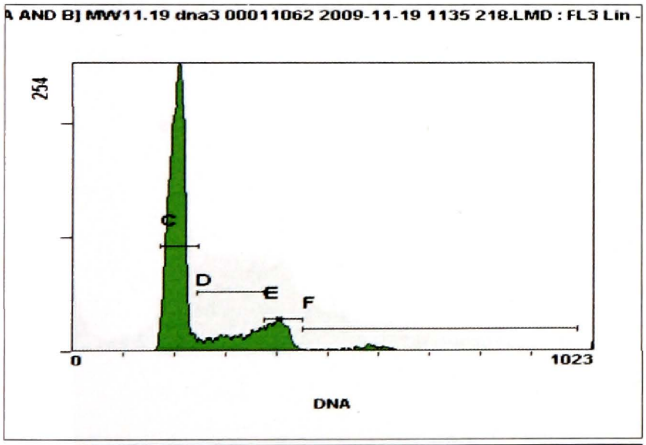


(F1)[Ungated] MW11.19 dna3 00011062 2009-11-19 1135 218.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	672	199
A	20000	100.00	100.00	672	199

(F1)[A] MW11.19 dna3 00011062 2009-11-19 1135 218.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	428	413
B	14202	71.01	71.01	293	333



(F1)MW11.19 dna3 00011062 2009-11-19 1135 218.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna3 00011062 2009-11-19 1135 218.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna3

MW DNA

(F1)[A AND B] MW11.19 dna3 00011062 2009-11-19 1135 218.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14202	71.01	100.00	293	###
C	9103	45.52	64.10	204	###
D	1966	9.83	13.84	314	###
E	1575	7.88	11.09	403	###
F	1171	5.86	8.25	622	###

(F1)[Ungated] MW11.19 dna3 00011062 2009-11-19 1135 218.LMD :

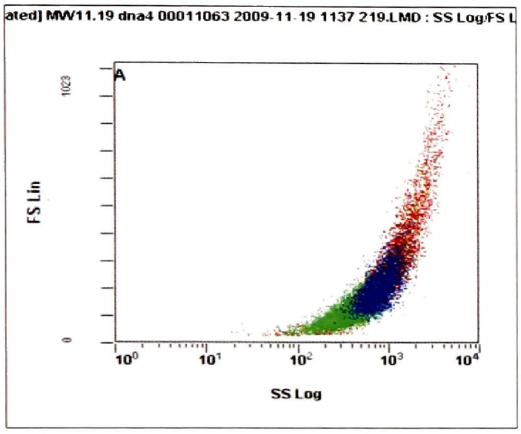
Color	Name	% Gated	% Total	Number	Cells/μL
	F	5.86	5.86	1171	ERROR
	B	71.01	71.01	14202	ERROR
	A	100.00	100.00	20000	ERROR

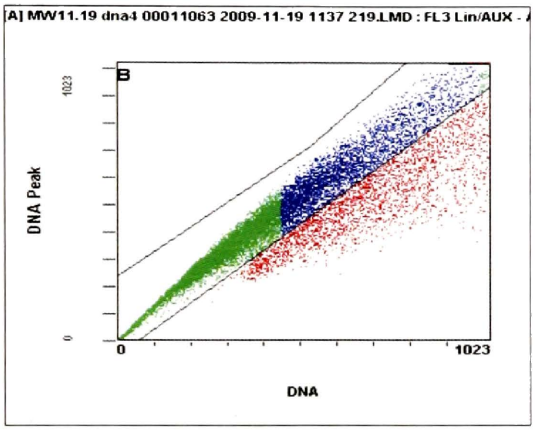
Figure 85. Cytochalasin-B treatment group flow histograms and summaries

A) Cytochalasin-B treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:35:25
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna4 00011063 2009-11-19 1137 219.LMD

Run Date: 19-Nov-09, 11:3(
Sample ID: MW11.19 d
User ID:
Acquisition Time/Events: 61.4s / 20000 (PROTOC
Tube ID: NoF



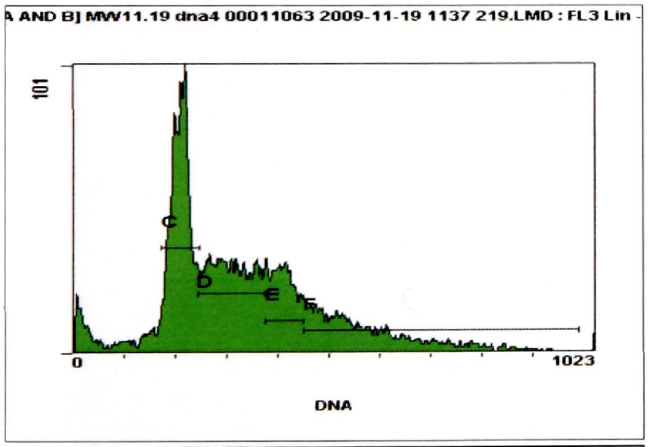


(F1)[Ungated] MW11.19 dna4 00011063 2009-11-19 1137 219.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	714	180
A	20000	100.00	100.00	714	180

(F1)[A] MW11.19 dna4 00011063 2009-11-19 1137 219.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	446	439
B	15814	79.07	79.07	358	389



(F1)MW11.19 dna4 00011063 2009-11-19 1137 219.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna4 00011063 2009-11-19 1137 219.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna4

MW DNA

(F1)[A AND B] MW11.19 dna4 00011063 2009-11-19 1137 219.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15814	79.07	100.00	358	###
C	4419	22.09	27.94	210	###
D	4168	20.84	26.36	307	###
E	2210	11.05	13.97	408	###
F	3643	18.22	23.04	609	###

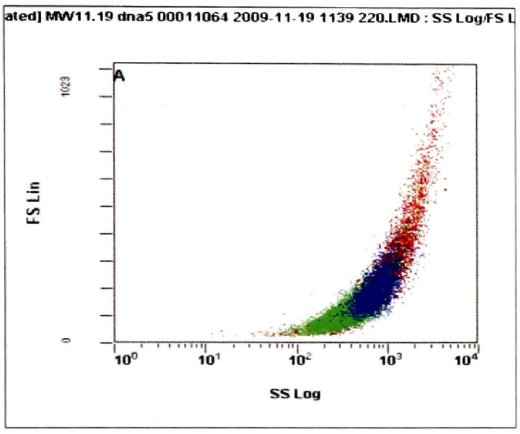
(F1)[Ungated] MW11.19 dna4 00011063 2009-11-19 1137 219.LMD :

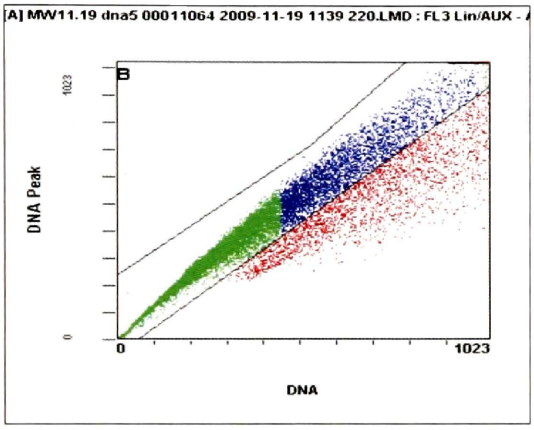
Color	Name	% Gated	% Total	Number	Cells/µL
	F	18.22	18.22	3643	ERROR
	B	79.07	79.07	15814	ERROR
	A	100.00	100.00	20000	ERROR

B) Cytochalasin-B treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:35:54
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna5 00011064 2009-11-19 1139 220.LMD

Run Date: 19-Nov-09, 11:3
Sample ID: MW11.19 d
User ID:
Acquisition Time/Events: 46.1s / 20000 (PROTOC
Tube ID: NoF



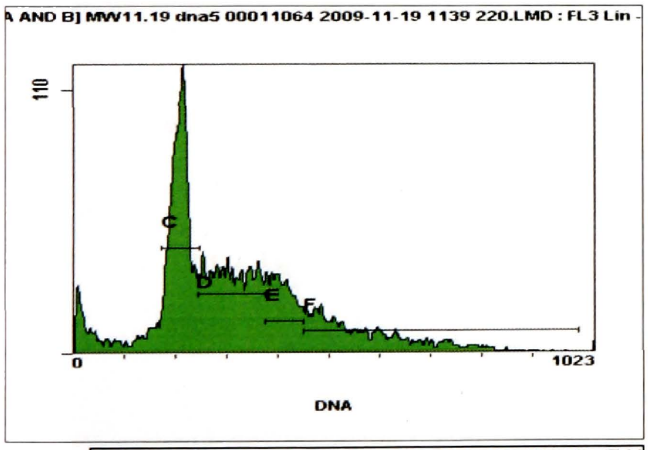


(F1)[Ungated] MW11.19 dna5 00011064 2009-11-19 1139 220.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	665	172
A	20000	100.00	100.00	665	172

(F1)[A] MW11.19 dna5 00011064 2009-11-19 1139 220.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	429	426
B	16071	80.36	80.36	347	378



(F1)MW11.19 dna5 00011064 2009-11-19 1139 220.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna5 00011064 2009-11-19 1139 220.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna5

MW DNA

(F1)[A AND B] MW11.19 dna5 00011064 2009-11-19 1139 220.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	16071	80.36	100.00	347	###
C	4575	22.88	28.47	209	###
D	4239	21.20	26.38	307	###
E	2097	10.48	13.05	408	###
F	3545	17.73	22.06	609	###

(F1)[Ungated] MW11.19 dna5 00011064 2009-11-19 1139 220.LMD

Color	Name	% Gated	% Total	Number	Cells/μL
	F	17.73	17.73	3545	ERROR
	B	80.36	80.36	16071	ERROR
	A	100.00	100.00	20000	ERROR

C) Cytochalasin-B treament sample 3

Institution: Tulane Center for Gene Therapy

Protocol: DNA.PRO

Listmode Replay: New Protocol

Analysis Date: 19-Jul-2010, 13:36:21

Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18

Listmode File: MW11.19 dna6 00011065 2009-11-19 1142 221.LMD

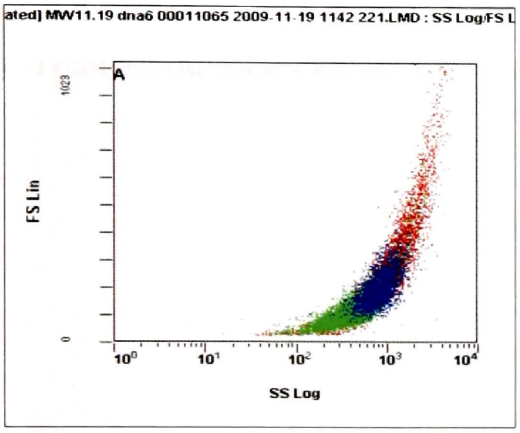
Run Date: 19-Nov-09, 11:4

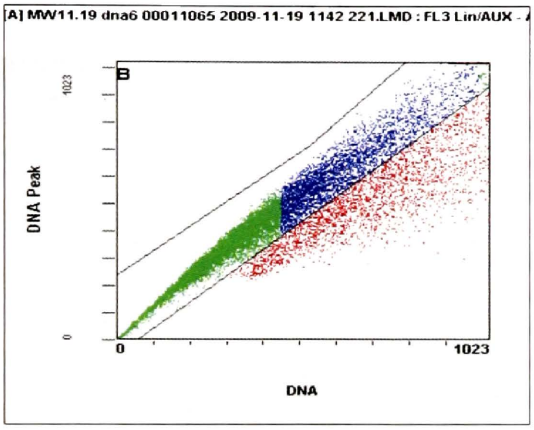
Sample ID: MW11.19 d

User ID:

Acquisition Time/Events: 63.3s / 20000 (PROTOC

Tube ID: NoF



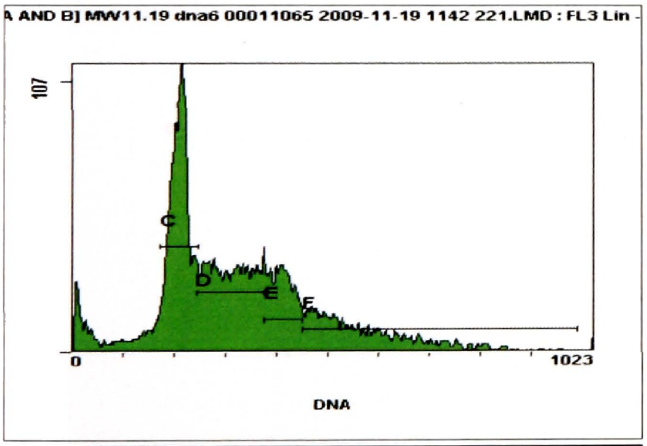


(F1)[Ungated] MW11.19 dna6 00011065 2009-11-19 1142 221.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	695	172
A	20000	100.00	100.00	695	172

(F1)[A] MW11.19 dna6 00011065 2009-11-19 1142 221.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	433	429
B	16037	80.19	80.19	351	382



(F1)MW11.19 dna6 00011065 2009-11-19 1142 221.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna6 00011065 2009-11-19 1142 221.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna6

MW DNA

(F1)[A AND B] MW11.19 dna6 00011065 2009-11-19 1142 221.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	16037	80.19	100.00	351	###
C	4579	22.90	28.55	210	###
D	4243	21.22	26.46	307	###
E	2250	11.25	14.03	408	###
F	3483	17.41	21.72	609	###

(F1)[Ungated] MW11.19 dna6 00011065 2009-11-19 1142 221.LMD :

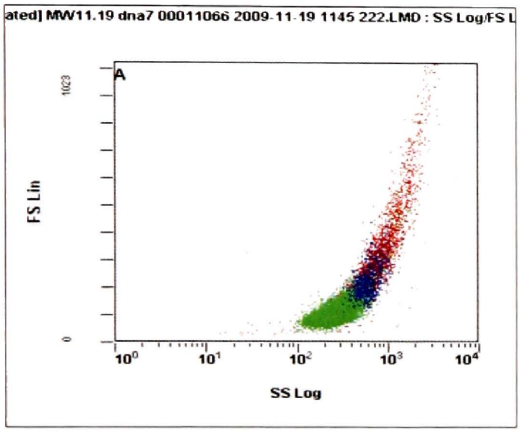
Color	Name	% Gated	% Total	Number	Cells/µL
	F	17.41	17.41	3483	ERROR
	B	80.19	80.19	16037	ERROR
	A	100.00	100.00	20000	ERROR

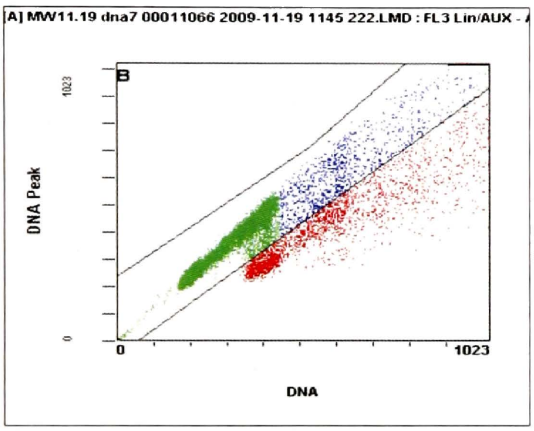
Figure 86. Teak wood dust extract 1 treatment group flow histograms and summaries

E) Teak wood dust extract treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:38:31
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna7 00011066 2009-11-19 1145 222.LMD

Run Date: 19-Nov-09, 11:4
Sample ID: MW11.19 c
User ID:
Acquisition Time/Events: 34.9s / 20000 (PROTOC
Tube ID: NoF



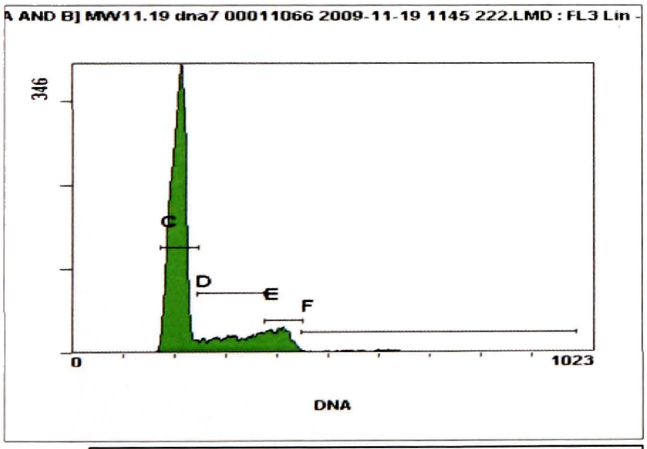


(F1)[Ungated] MW11.19 dna7 00011066 2009-11-19 1145 222.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	368	140
A	20000	100.00	100.00	368	140

(F1)[A] MW11.19 dna7 00011066 2009-11-19 1145 222.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	323	342
B	17185	85.92	85.92	266	313



(F1)MW11.19 dna7 00011066 2009-11-19 1145 222.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna7 00011066 2009-11-19 1145 222.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna7

MW DNA

(F1)[A AND B] MW11.19 dna7 00011066 2009-11-19 1145 222.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	17185	85.92	100.00	266	###
C	11891	59.45	69.19	206	###
D	2610	13.05	15.19	312	###
E	1750	8.75	10.18	404	###
F	893	4.46	5.20	621	###

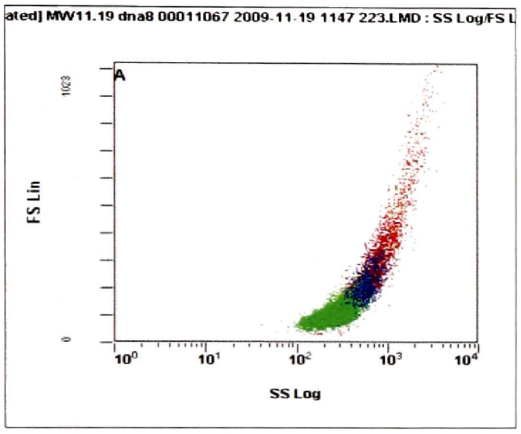
(F1)[Ungated] MW11.19 dna7 00011066 2009-11-19 1145 222.LMD :

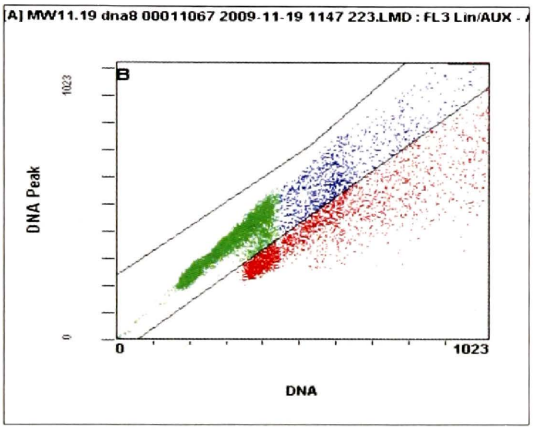
Color	Name	% Gated	% Total	Number	Cells/μL
	F	4.46	4.46	893	ERROR
	B	85.92	85.92	17185	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:37:55
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna8 00011067 2009-11-19 1147 223.LMD

Run Date: 19-Nov-09, 11:4
Sample ID: MW11.19 d
User ID:
Acquisition Time/Events: 21.6s / 20000 (PROTOC
Tube ID: NoF



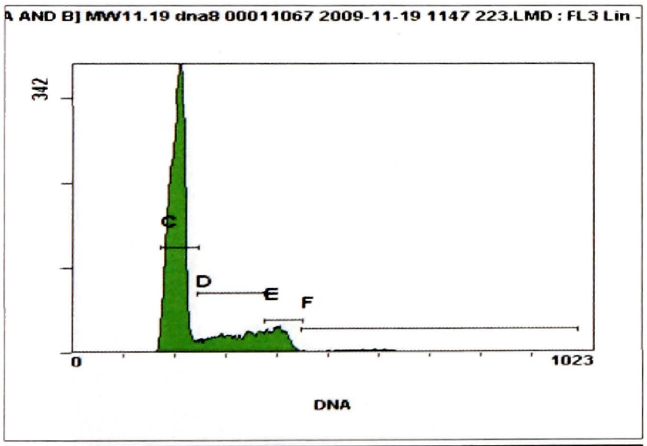


(F1)[Ungated] MW11.19 dna8 00011067 2009-11-19 1147 223.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	367	139
A	20000	100.00	100.00	367	139

(F1)[A] MW11.19 dna8 00011067 2009-11-19 1147 223.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	319	338
B	17189	85.94	85.94	265	310



(F1)MW11.19 dna8 00011067 2009-11-19 1147 223.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna8 00011067 2009-11-19 1147 223.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna8

MW DNA

(F1)[A AND B] MW11.19 dna8 00011067 2009-11-19 1147 223.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	17189	85.94	100.00	265	###
C	11778	58.89	68.52	204	###
D	2794	13.97	16.25	313	###
E	1613	8.06	9.38	402	###
F	916	4.58	5.33	611	###

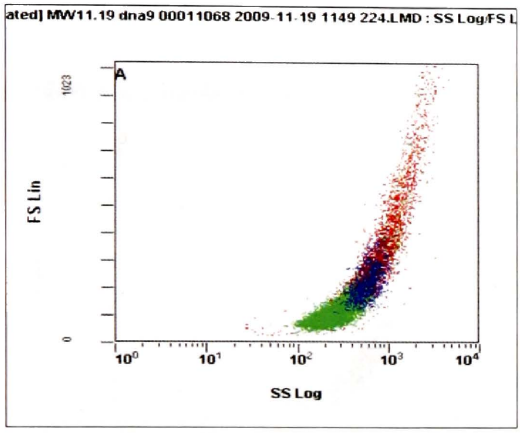
(F1)[Ungated] MW11.19 dna8 00011067 2009-11-19 1147 223.LMD :

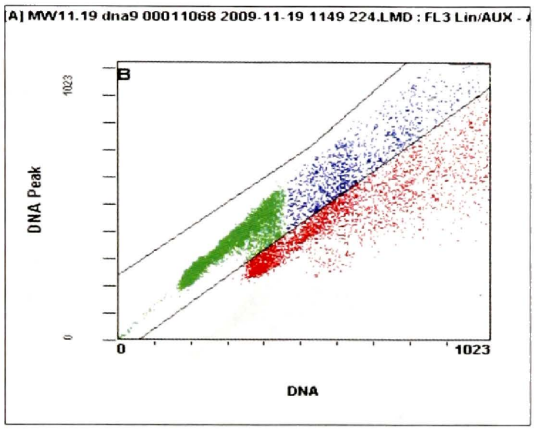
Color	Name	% Gated	% Total	Number	Cells/μL
	F	4.58	4.58	916	ERROR
	B	85.94	85.94	17189	ERROR
	A	100.00	100.00	20000	ERROR

G) Teak wood dust extract treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:39:11
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna9 00011068 2009-11-19 1149 224.LMD

Run Date: 19-Nov-09, 11:4
Sample ID: MW11.19 c
User ID:
Acquisition Time/Events: 55.6s / 20000 (PROTOC
Tube ID: NoF



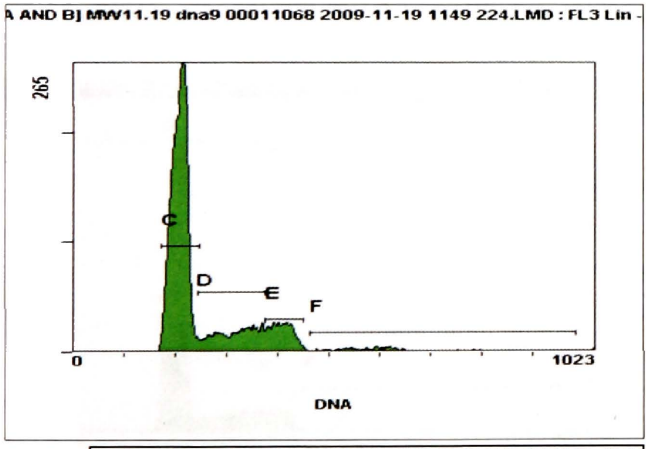


(F1)[Ungated] MW11.19 dna9 00011068 2009-11-19 1149 224.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	429	163
A	20000	100.00	100.00	429	163

(F1)[A] MW11.19 dna9 00011068 2009-11-19 1149 224.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	384	386
B	15698	78.49	78.49	289	334



(F1)MW11.19 dna9 00011068 2009-11-19 1149 224.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna9 00011068 2009-11-19 1149 224.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna9

MW DNA

(F1)[A AND B] MW11.19 dna9 00011068 2009-11-19 1149 224.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15698	78.49	100.00	289	###
C	10003	50.02	63.72	207	###
D	2532	12.66	16.13	314	###
E	1765	8.82	11.24	407	###
F	1194	5.97	7.61	643	###

(F1)[Ungated] MW11.19 dna9 00011068 2009-11-19 1149 224.LMD :

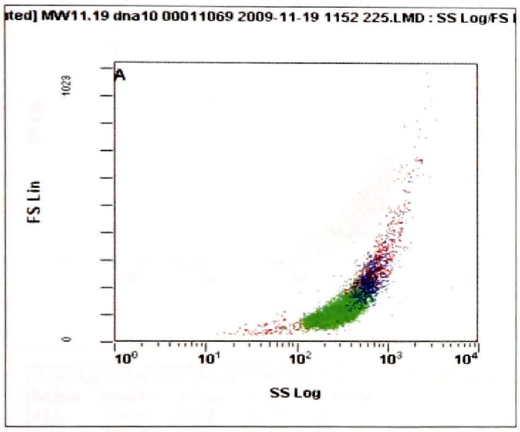
Color	Name	% Gated	% Total	Number	Cells/μL
	F	5.97	5.97	1194	ERROR
	B	78.49	78.49	15698	ERROR
	A	100.00	100.00	20000	ERROR

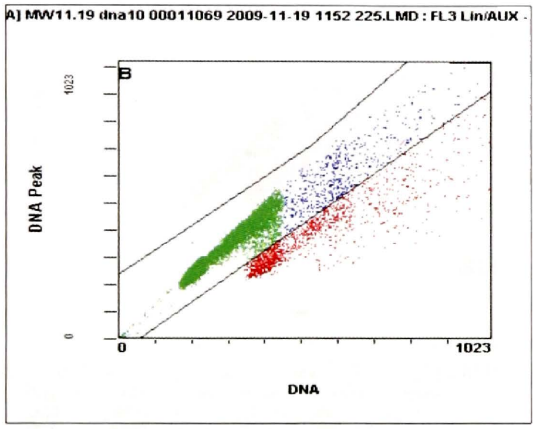
Figure 87. Teak wood dust extract 2 treatment group flow histograms and summaries

A) Teak wood dust extract treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:27:27
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna10 00011069 2009-11-19 1152 225.LMD

Run Date: 19-Nov-09, 11:5
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 21.6s / 20000 (PROTOC
Tube ID: NoF



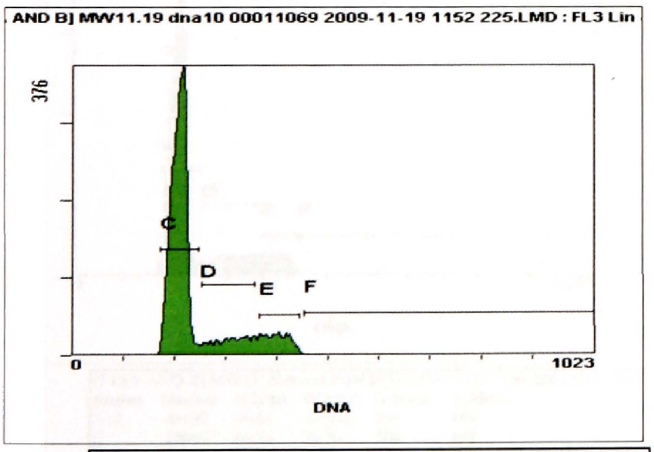


(F1)[Ungated] MW11.19 dna10 00011069 2009-11-19 1152 225.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	314	115
A	20000	100.00	100.00	314	115

(F1)[A] MW11.19 dna10 00011069 2009-11-19 1152 225.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	274	306
B	18589	92.94	92.94	254	300



(F1)MW11.19 dna10 00011069 2009-11-19 1152 225.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna10 00011069 2009-11-19 1152 225.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna10

MW DNA

(F1)[A AND B] MW11.19 dna10 00011069 2009-11-19 1152 225.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	18589	92.94	100.00	254	###
C	13408	67.04	72.13	207	###
D	2242	11.21	12.06	308	###
E	2053	10.27	11.04	400	###
F	534	2.67	2.87	632	###

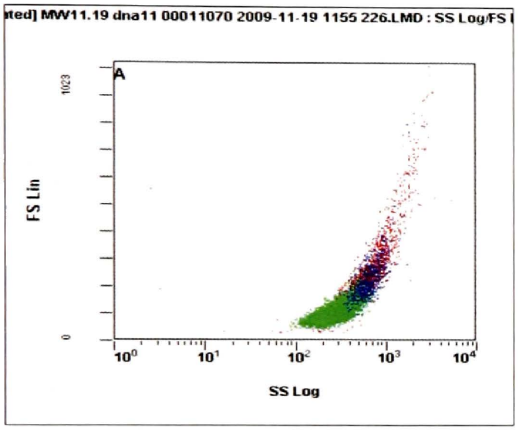
(F1)[Ungated] MW11.19 dna10 00011069 2009-11-19 1152 225.LMD

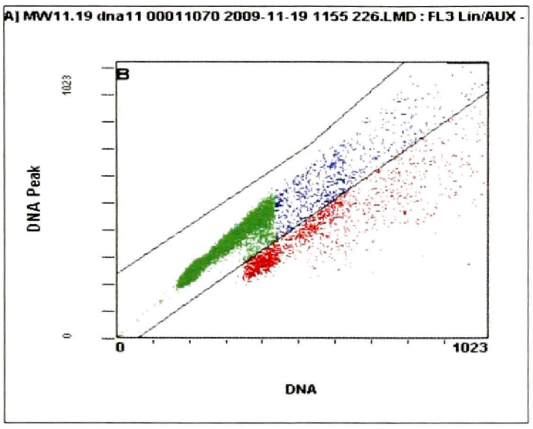
Color	Name	% Gated	% Total	Number	Cells/μL
	F	2.67	2.67	534	ERROR
	B	92.94	92.94	18589	ERROR
	A	100.00	100.00	20000	ERROR

B) Teak wood dust extract treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:27:56
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna11 00011070 2009-11-19 1155 226.LMD

Run Date: 19-Nov-09, 11:5
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 19.9s / 20000 (PROTOC
Tube ID: NoF



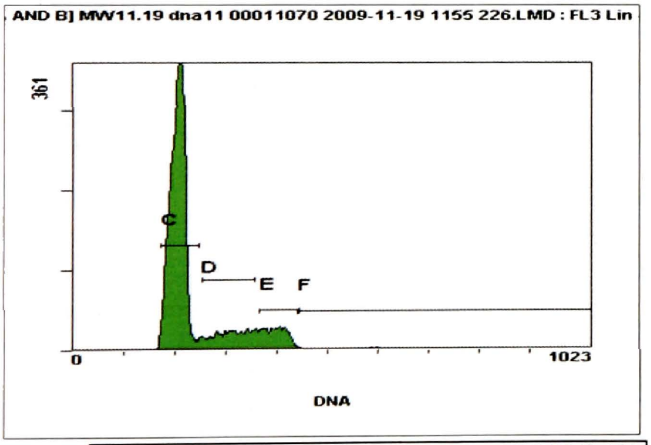


(F1)[Ungated] MW11.19 dna11 00011070 2009-11-19 1155 226.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	329	120
A	20000	100.00	100.00	329	120

(F1)[A] MW11.19 dna11 00011070 2009-11-19 1155 226.LMD : FL3 Lin/Al

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	286	314
B	18197	90.98	90.98	256	301



(F1)MW11.19 dna11 00011070 2009-11-19 1155 226.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna11 00011070 2009-11-19 1155 226.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna11

MW DNA

(F1)[A AND B] MW11.19 dna11 00011070 2009-11-19 1155 226.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	18197	90.98	100.00	256	###
C	12866	64.33	70.70	204	###
D	2329	11.65	12.80	309	###
E	1886	9.43	10.36	398	###
F	759	3.79	4.17	617	###

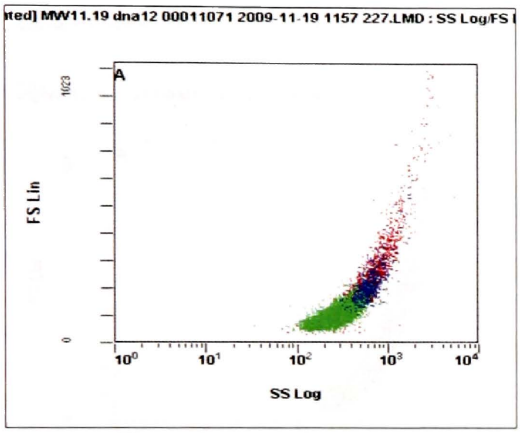
(F1)[Ungated] MW11.19 dna11 00011070 2009-11-19 1155 226.LMD

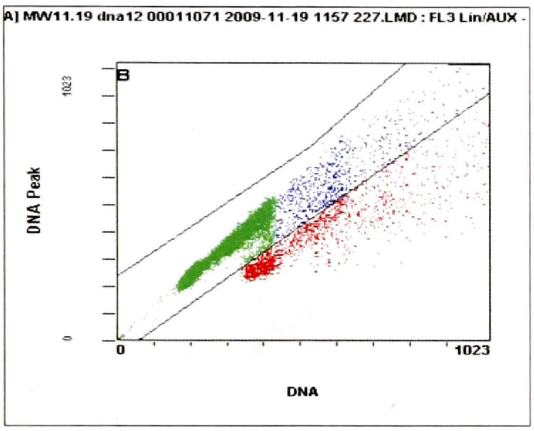
Color	Name	% Gated	% Total	Number	Cells/μL
	F	3.79	3.79	759	ERROR
	B	90.98	90.98	18197	ERROR
	A	100.00	100.00	20000	ERROR

C) Teak wood dust extract treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:28:18
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna12 00011071 2009-11-19 1157 227.LMD

Run Date: 19-Nov-09, 11:5
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 17.8s / 20000 (PROTOC
Tube ID: NoF



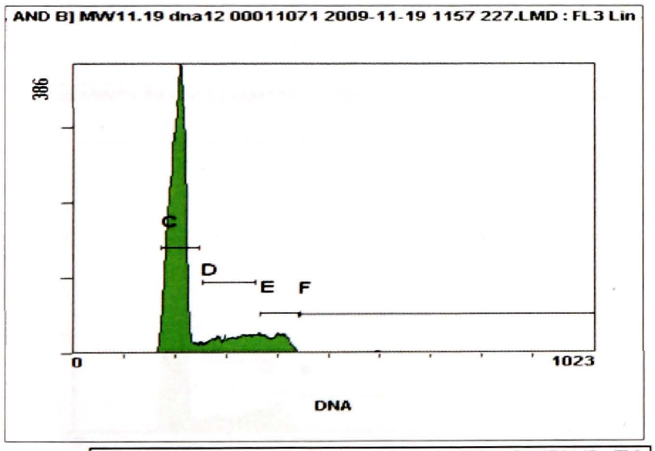


(F1)[Ungated] MW11.19 dna12 00011071 2009-11-19 1157 227.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	323	114
A	20000	100.00	100.00	323	114

(F1)[A] MW11.19 dna12 00011071 2009-11-19 1157 227.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	279	306
B	18271	91.36	91.36	250	295



(F1)MW11.19 dna12 00011071 2009-11-19 1157 227.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna12 00011071 2009-11-19 1157 227.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna12

MW DNA

(F1)[A AND B] MW11.19 dna12 00011071 2009-11-19 1157 227.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	18271	91.36	100.00	250	###
C	13260	66.30	72.57	203	###
D	2278	11.39	12.47	309	###
E	1639	8.20	8.97	397	###
F	662	3.31	3.62	621	###

(F1)[Ungated] MW11.19 dna12 00011071 2009-11-19 1157 227.LMD

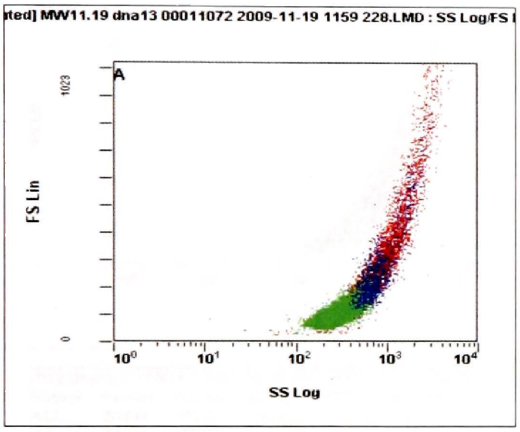
Color	Name	% Gated	% Total	Number	Cells/µL
	F	3.31	3.31	662	ERROR
	B	91.36	91.36	18271	ERROR
	A	100.00	100.00	20000	ERROR

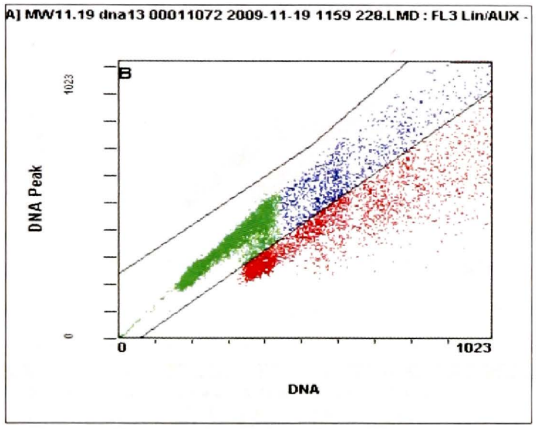
Figure 88. Teak wood dust extract 3 treatment group flow histograms and summaries

D) Teak wood dust extract 3 treatment sample1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:28:49
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna13 00011072 2009-11-19 1159 228.LMD

Run Date: 19-Nov-09, 11:5
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 30.8s / 20000 (PROTOC
Tube ID: NoF



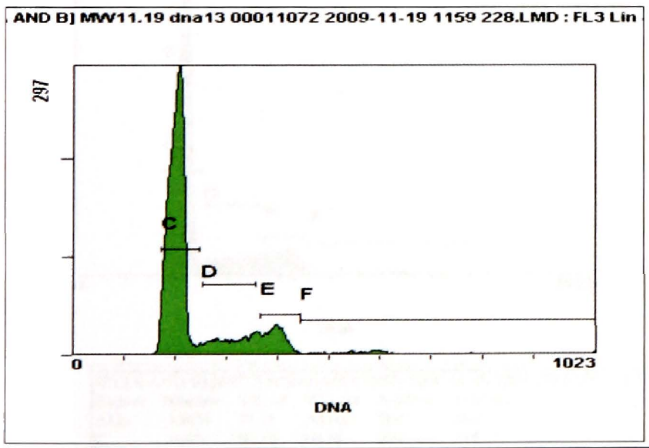


(F1)[Ungated] MW11.19 dna13 00011072 2009-11-19 1159 228.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	512	162
A	20000	100.00	100.00	512	162

(F1)[A] MW11.19 dna13 00011072 2009-11-19 1159 228.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	366	362
B	15723	78.61	78.61	273	312



(F1)MW11.19 dna13 00011072 2009-11-19 1159 228.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna13 00011072 2009-11-19 1159 228.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna13

MW DNA

(F1)[A AND B] MW11.19 dna13 00011072 2009-11-19 1159 228.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15723	78.61	100.00	273	###
C	10499	52.49	66.77	201	###
D	1833	9.16	11.66	308	###
E	1658	8.29	10.55	396	###
F	1244	6.22	7.91	683	###

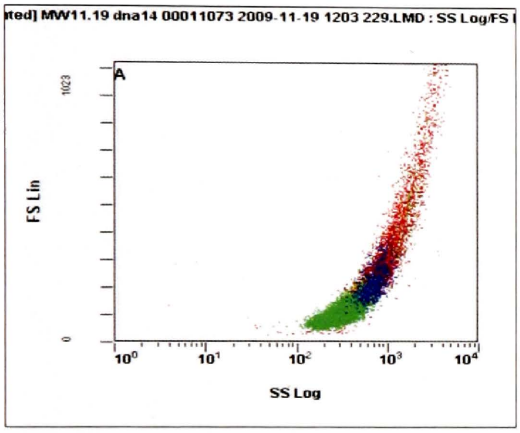
(F1)[Ungated] MW11.19 dna13 00011072 2009-11-19 1159 228.LMD

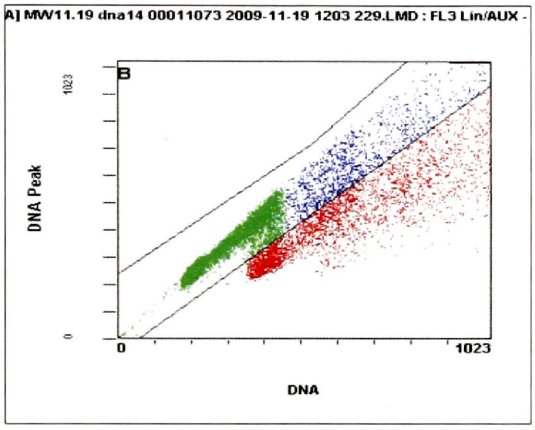
Color	Name	% Gated	% Total	Number	Cells/µL
	F	6.22	6.22	1244	ERROR
	B	78.61	78.61	15723	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood dust extract 3 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:29:42
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna14 00011073 2009-11-19 1203 229.LMD

Run Date: 19-Nov-09, 12:0
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 85.0s / 20000 (PROTOC
Tube ID: NoF



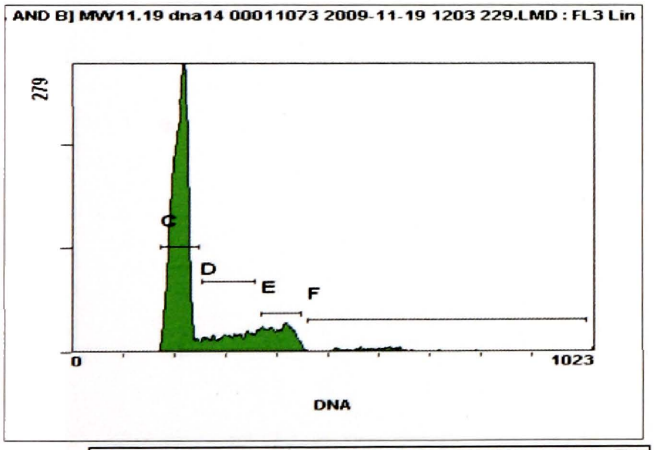


(F1)[Ungated] MW11.19 dna14 00011073 2009-11-19 1203 229.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	531	166
A	20000	100.00	100.00	531	166

(F1)[A] MW11.19 dna14 00011073 2009-11-19 1203 229.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	390	386
B	15434	77.17	77.17	289	331



(F1)MW11.19 dna14 00011073 2009-11-19 1203 229.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna14 00011073 2009-11-19 1203 229.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna14

MW DNA

(F1)[A AND B] MW11.19 dna14 00011073 2009-11-19 1203 229.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15434	77.17	100.00	289	###
C	10021	50.10	64.93	209	###
D	1817	9.09	11.77	307	###
E	1860	9.30	12.05	405	###
F	1138	5.69	7.37	645	###

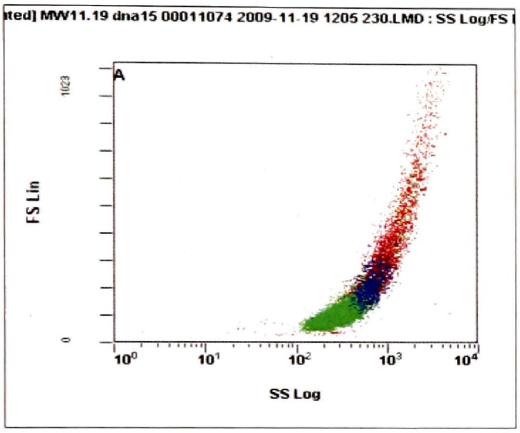
(F1)[Ungated] MW11.19 dna14 00011073 2009-11-19 1203 229.LMD

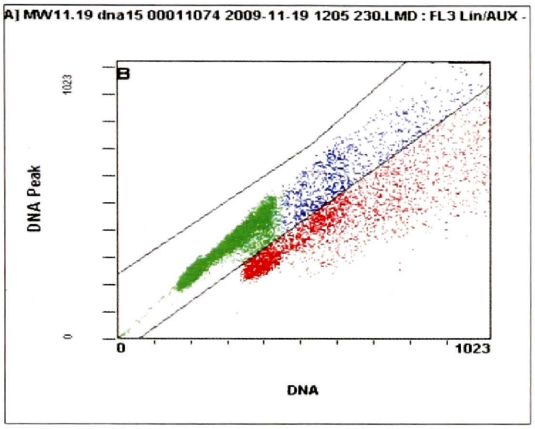
Color	Name	% Gated	% Total	Number	Cells/µL
	F	5.69	5.69	1138	ERROR
	B	77.17	77.17	15434	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract 3 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:30:19
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna15 00011074 2009-11-19 1205 230.LMD

Run Date: 19-Nov-09, 12:0
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 30.3s / 20000 (PROTOC
Tube ID: NoF



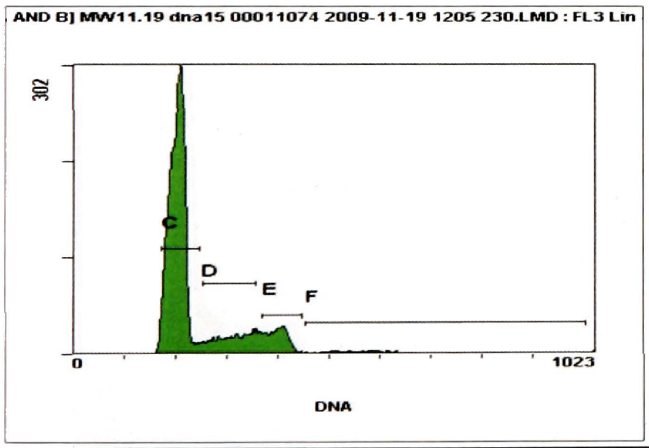


(F1)[Ungated] MW11.19 dna15 00011074 2009-11-19 1205 230.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	485	154
A	20000	100.00	100.00	485	154

(F1)[A] MW11.19 dna15 00011074 2009-11-19 1205 230.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	359	359
B	15971	79.86	79.86	271	311



(F1)MW11.19 dna15 00011074 2009-11-19 1205 230.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna15 00011074 2009-11-19 1205 230.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna15

MW DNA

(F1)[A AND B] MW11.19 dna15 00011074 2009-11-19 1205 230.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15971	79.86	100.00	271	###
C	10661	53.31	66.75	202	###
D	2020	10.10	12.65	309	###
E	1622	8.11	10.16	399	###
F	950	4.75	5.95	626	###

(F1)[Ungated] MW11.19 dna15 00011074 2009-11-19 1205 230.LMD

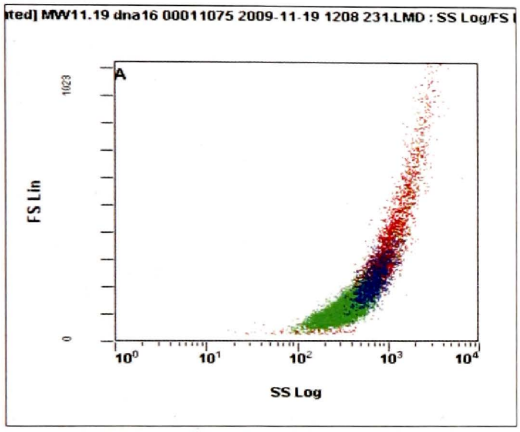
Color	Name	% Gated	% Total	Number	Cells/µL
	F	4.75	4.75	950	ERROR
	B	79.86	79.86	15971	ERROR
	A	100.00	100.00	20000	ERROR

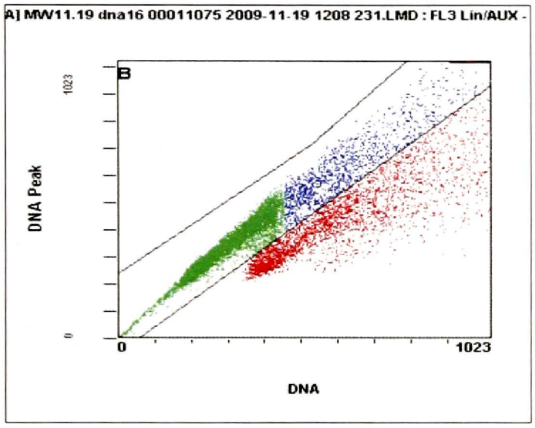
Figure 89. 2-methylanthraquinone treatment group flow histograms and summaries

D) 2-methylanthraquinone treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:30:50
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna16 00011075 2009-11-19 1208 231.LMD

Run Date: 19-Nov-09, 12:0
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 53.4s / 20000 (PROTOC
Tube ID: NoF



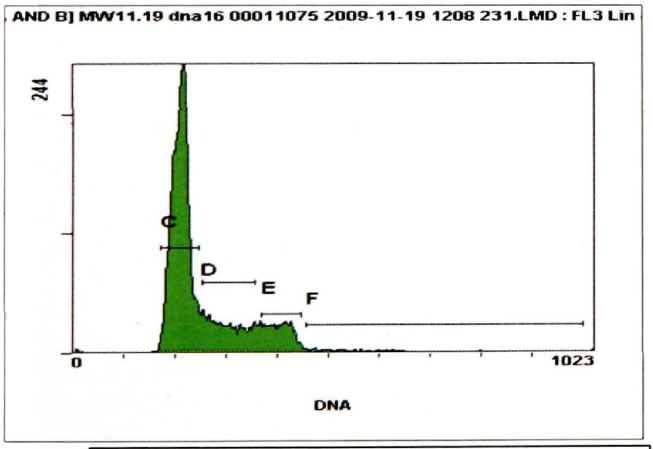


(F1)[Ungated] MW11.19 dna16 00011075 2009-11-19 1208 231.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	486	161
A	20000	100.00	100.00	486	161

(F1)[A] MW11.19 dna16 00011075 2009-11-19 1208 231.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	360	363
B	16228	81.14	81.14	283	324



(F1)MW11.19 dna16 00011075 2009-11-19 1208 231.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna16 00011075 2009-11-19 1208 231.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna16

MW DNA

(F1)[A AND B] MW11.19 dna16 00011075 2009-11-19 1208 231.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	16228	81.14	100.00	283	###
C	9614	48.07	59.24	210	###
D	2694	13.47	16.60	301	###
E	1874	9.37	11.55	404	###
F	1081	5.41	6.66	624	###

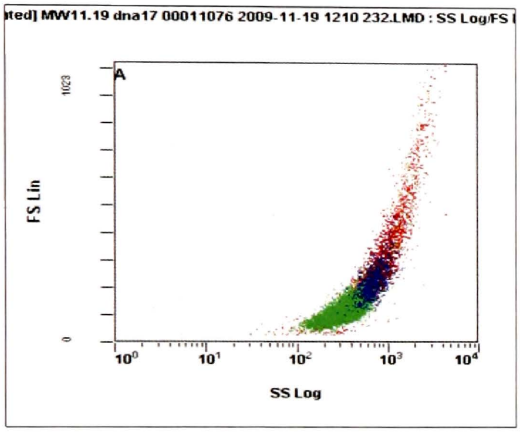
(F1)[Ungated] MW11.19 dna16 00011075 2009-11-19 1208 231.LMD

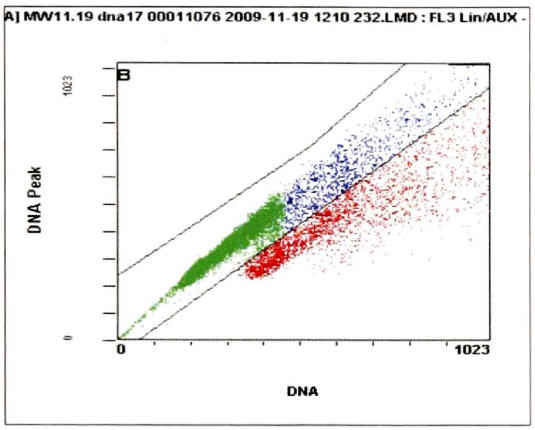
Color	Name	% Gated	% Total	Number	Cells/µL
	F	5.41	5.41	1081	ERROR
	B	81.14	81.14	16228	ERROR
	A	100.00	100.00	20000	ERROR

E) 2-methylanthraquinone treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:31:34
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna17 00011076 2009-11-19 1210 232.LMD

Run Date: 19-Nov-09, 12:0
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 41.6s / 20000 (PROTOC
Tube ID: NoF



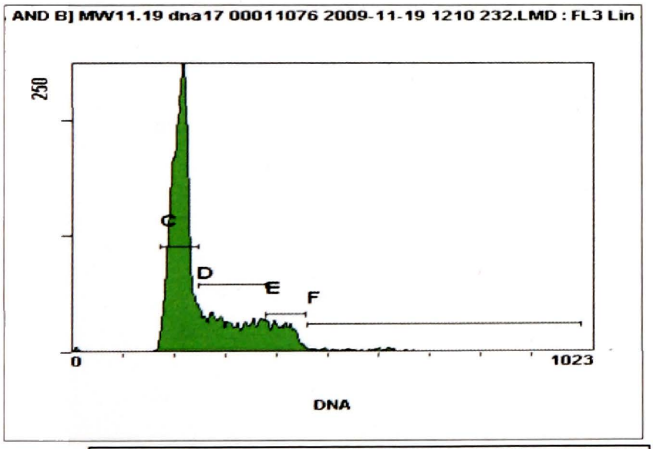


(F1)[Ungated] MW11.19 dna17 00011076 2009-11-19 1210 232.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	455	152
A	20000	100.00	100.00	455	152

(F1)[A] MW11.19 dna17 00011076 2009-11-19 1210 232.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	347	354
B	16617	83.08	83.08	281	322



(F1)MW11.19 dna17 00011076 2009-11-19 1210 232.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna17 00011076 2009-11-19 1210 232.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna17

MW DNA

(F1)[A AND B] MW11.19 dna17 00011076 2009-11-19 1210 232.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	16617	83.08	100.00	281	###
C	9823	49.12	59.11	210	###
D	3850	19.25	23.17	308	###
E	1688	8.44	10.16	411	###
F	1028	5.14	6.19	626	###

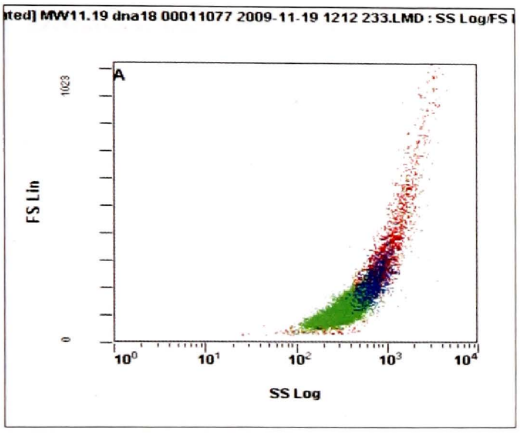
(F1)[Ungated] MW11.19 dna17 00011076 2009-11-19 1210 232.LMD

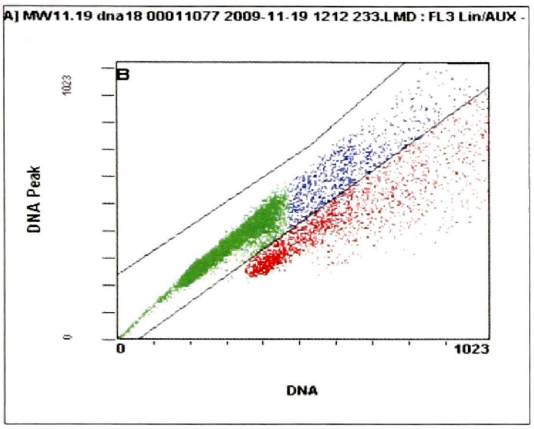
Color	Name	% Gated	% Total	Number	Cells/μL
	F	5.14	5.14	1028	ERROR
	B	83.08	83.08	16617	ERROR
	A	100.00	100.00	20000	ERROR

F) 2-methylanthraquinone treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:32:17
Settings File: MW DNA.PRO, 19-Nov-2009, 11:28:18
Listmode File: MW11.19 dna18 00011077 2009-11-19 1212 233.LMD

Run Date: 19-Nov-09, 12:1
Sample ID: MW11.19 dr
User ID:
Acquisition Time/Events: 36.4s / 20000 (PROTOC
Tube ID: NoF



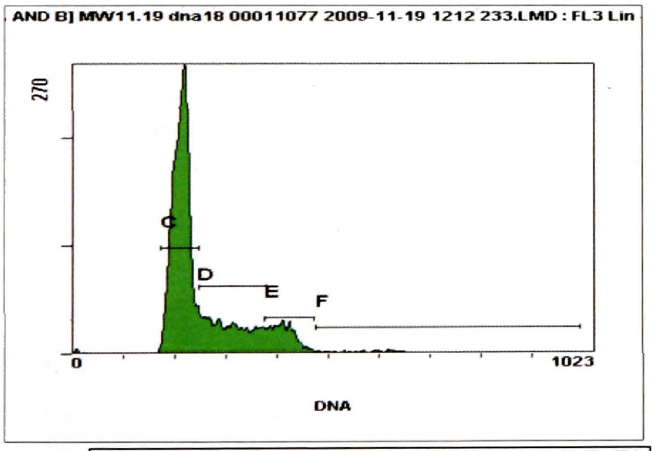


(F1)[Ungated] MW11.19 dna18 00011077 2009-11-19 1212 233.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	434	142
A	20000	100.00	100.00	434	142

(F1)[A] MW11.19 dna18 00011077 2009-11-19 1212 233.LMD : FL3 Lin/A

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	331	344
B	17182	85.91	85.91	277	318



(F1)MW11.19 dna18 00011077 2009-11-19 1212 233.LMD : FCS Information
Cytomics FC 500
19-Nov-09
MW11.19 dna18 00011077 2009-11-19 1212 233.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW11.19 dna18

MW DNA

(F1)[A AND B] MW11.19 dna18 00011077 2009-11-19 1212 233.LMD : FL3

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	17182	85.91	100.00	277	###
C	10439	52.20	60.76	211	###
D	3822	19.11	22.24	306	###
E	1933	9.66	11.25	412	###
F	866	4.33	5.04	634	###

(F1)[Ungated] MW11.19 dna18 00011077 2009-11-19 1212 233.LMD

Color	Name	% Gated	% Total	Number	Cells/µL
	F	4.33	4.33	866	ERROR
	B	85.91	85.91	17182	ERROR
	A	100.00	100.00	20000	ERROR

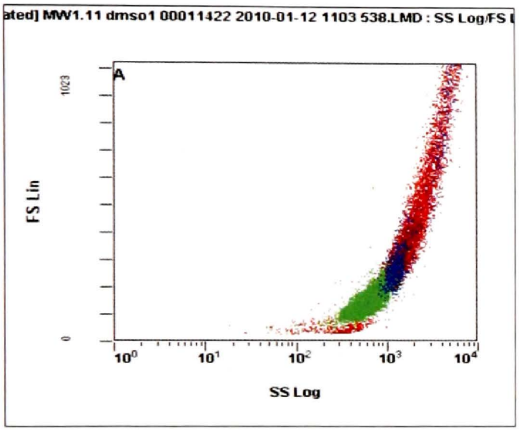
Flow cytometry data from three day recovery experiment

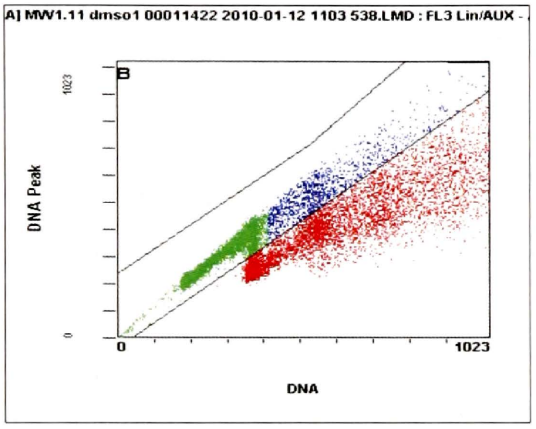
Figure 90. DMSO treatment group flow histograms and summaries

D) DMSO treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:03:50
Settings File: MW DNA.PRO, 12-Jan-2010, 10:57:57
Listmode File: MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD

Run Date: 12-Jan-10, 10:5
Sample ID: MW1.11 dn
User ID:
Acquisition Time/Events: 91.2s / 20000 (PROTOC
Tube ID: NoF



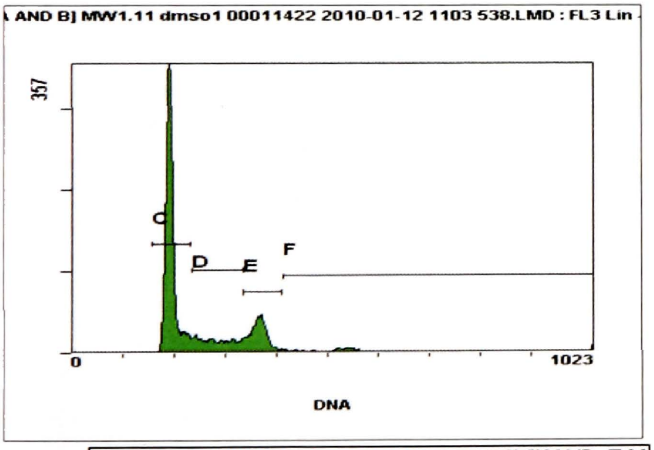


(F1)[Ungated] MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.46e+003	289
A	20000	100.00	100.00	1.46e+003	289

(F1)[A] MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	465	401
B	11626	58.13	58.13	291	311



(F1)MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 dmso1

MW DNA

(F1)[A AND B] MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	11626	58.13	100.00	291	###
C	6489	32.45	55.81	193	###
D	1724	8.62	14.83	281	###
E	1900	9.50	16.34	365	###
F	1409	7.04	12.12	668	###

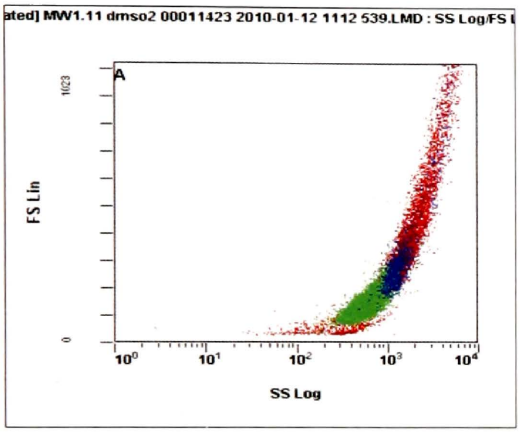
(F1)[Ungated] MW1.11 dmso1 00011422 2010-01-12 1103 538.LMD

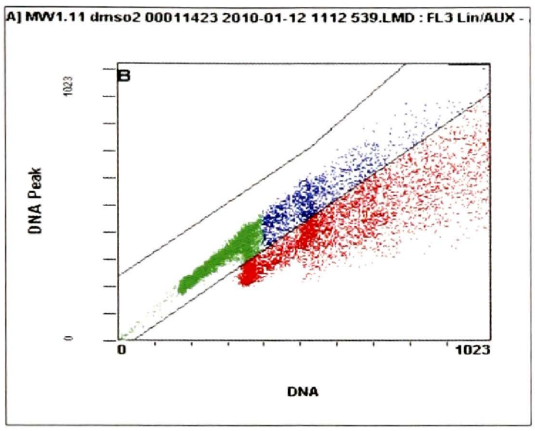
Color	Name	% Gated	% Total	Number	Cells/μL
	F	7.04	7.04	1409	ERROR
	B	58.13	58.13	11626	ERROR
	A	100.00	100.00	20000	ERROR

E) DMSO treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:06:32
Settings File: MW DNA.PRO, 12-Jan-2010, 10:57:57
Listmode File: MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD

Run Date: 12-Jan-10, 11:0
Sample ID: MW1.11 dn
User ID:
Acquisition Time/Events: 55.1s / 20000 (PROTOC
Tube ID: NoF



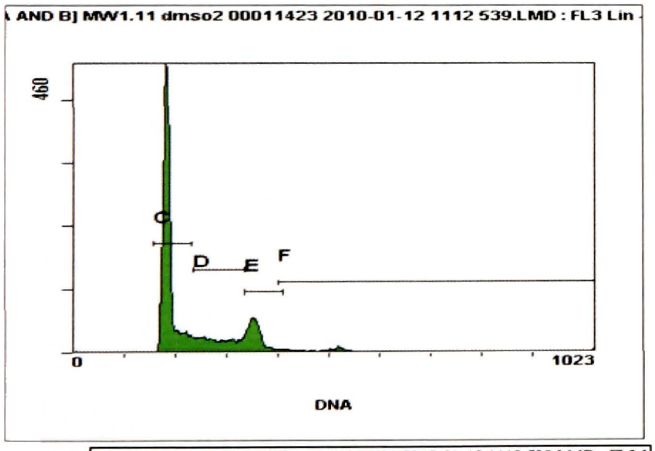


(F1)[Ungated] MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.18e+003	243
A	20000	100.00	100.00	1.18e+003	243

(F1)[A] MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	394	357
B	13624	68.12	68.12	272	296



(F1)MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 dmso2

MW DNA

(F1)[A AND B] MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13624	68.12	100.00	272	###
C	7804	39.02	57.28	186	###
D	2286	11.43	16.78	281	###
E	1982	9.91	14.55	357	###
F	1492	7.46	10.95	611	###

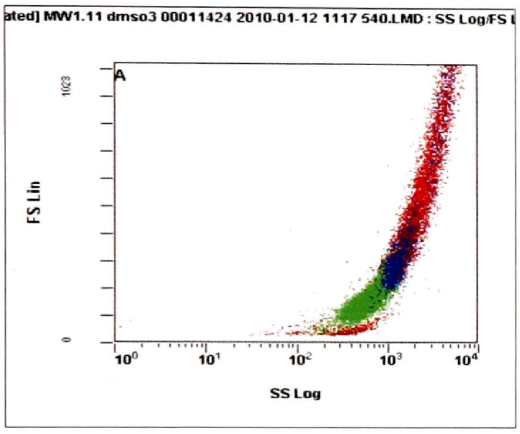
(F1)[Ungated] MW1.11 dmso2 00011423 2010-01-12 1112 539.LMD

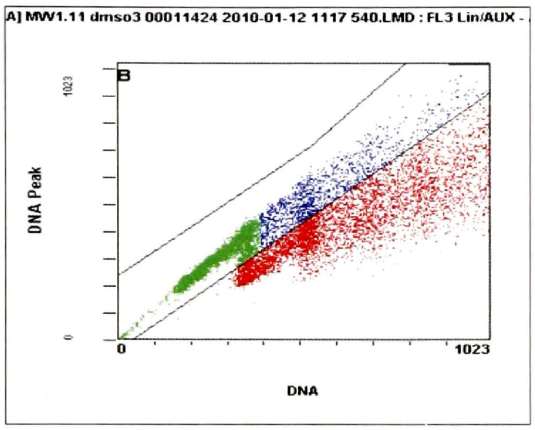
Color	Name	% Gated	% Total	Number	Cells/μL
	F	7.46	7.46	1492	ERROR
	B	68.12	68.12	13624	ERROR
	A	100.00	100.00	20000	ERROR

F) DMSO treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:07:22
Settings File: MW DNA.PRO, 12-Jan-2010, 11:13:14
Listmode File: MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD

Run Date: 12-Jan-10, 11:1:
Sample ID: MW1.11 dn
User ID:
Acquisition Time/Events: 103.6s / 20000 (PROTOC
Tube ID: NoF



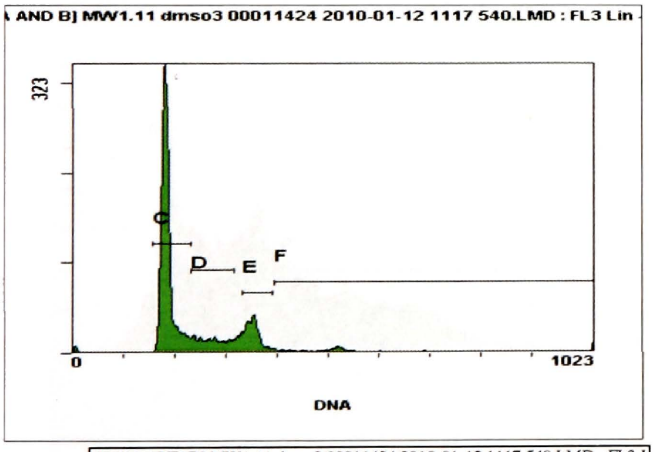


(F1)[Ungated] MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.72e+003	337
A	20000	100.00	100.00	1.72e+003	337

(F1)[A] MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	485	427
B	11017	55.09	55.09	293	316



(F1)MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 dmso3

MW DNA

(F1)[A AND B] MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	11017	55.09	100.00	293	###
C	6108	30.54	55.44	185	###
D	1414	7.07	12.83	272	###
E	1437	7.18	13.04	352	###
F	1643	8.21	14.91	680	###

(F1)[Ungated] MW1.11 dmso3 00011424 2010-01-12 1117 540.LMD

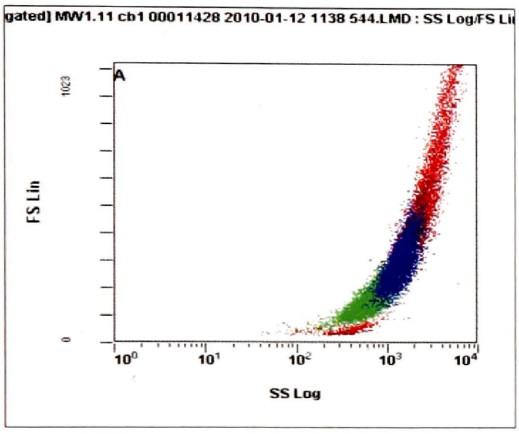
Color	Name	% Gated	% Total	Number	Cells/μL
	F	8.21	8.21	1643	ERROR
	B	55.09	55.09	11017	ERROR
	A	100.00	100.00	20000	ERROR

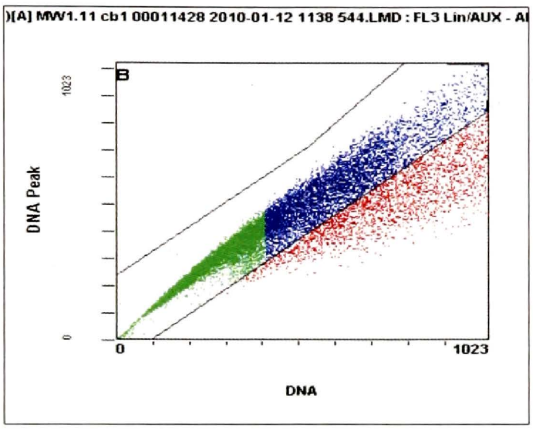
Figure 91. Cytochalasin B treatment group flow histograms and summaries

D) Cytochalsain-B treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:01:09
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 cb1 00011428 2010-01-12 1138 544.LMD

Run Date: 12-Jan-10, 11:3
Sample ID: MW1.11
User ID:
Acquisition Time/Events: 190.9s / 20000 (PROTOC
Tube ID: NoF



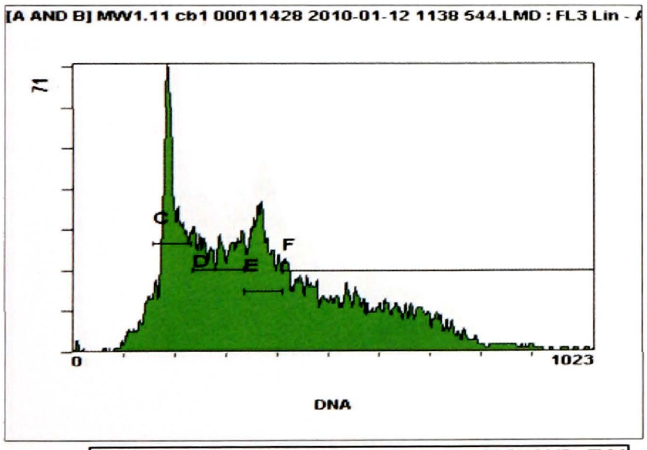


(F1)[Ungated] MW1.11 cb1 00011428 2010-01-12 1138 544.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.61e+003	315
A	20000	100.00	100.00	1.61e+003	315

(F1)[A] MW1.11 cb1 00011428 2010-01-12 1138 544.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	527	478
B	14082	70.41	70.41	397	399



(F1)MW1.11 cb1 00011428 2010-01-12 1138 544.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 cb1 00011428 2010-01-12 1138 544.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 cb1

MW DNA

(F1)[A AND B] MW1.11 cb1 00011428 2010-01-12 1138 544.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14082	70.41	100.00	397	###
C	2770	13.85	19.67	194	###
D	2802	14.01	19.90	283	###
E	2259	11.30	16.04	369	###
F	5581	27.91	39.63	601	###

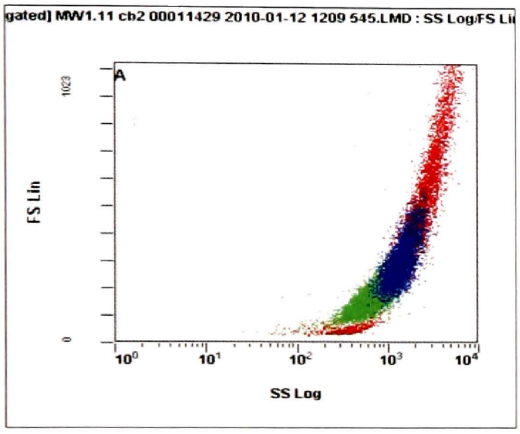
(F1)[Ungated] MW1.11 cb1 00011428 2010-01-12 1138 544.LMD : L

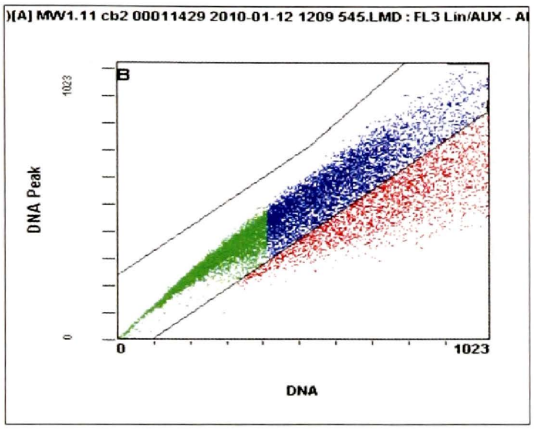
Color	Name	% Gated	% Total	Number	Cells/μL
	F	27.91	27.91	5581	ERROR
	B	70.41	70.41	14082	ERROR
	A	100.00	100.00	20000	ERROR

E) Cytochalsain-B treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:01:46
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 cb2 00011429 2010-01-12 1209 545.LMD

Run Date: 12-Jan-10, 11:4
Sample ID: MW1.11
User ID:
Acquisition Time/Events: 208.1s / 20000 (PROTOC
Tube ID: NoF



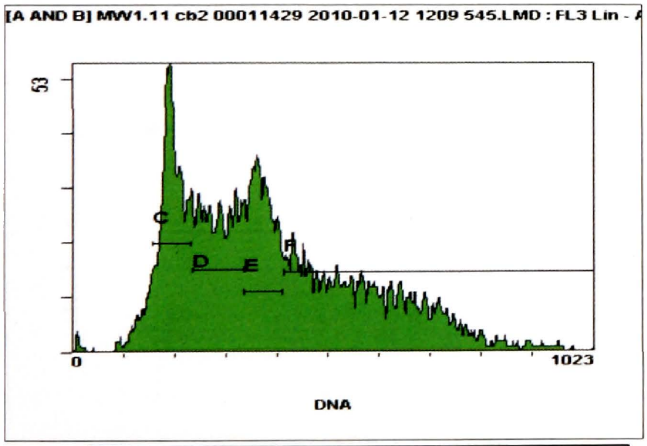


(F1)[Ungated] MW1.11 cb2 00011429 2010-01-12 1209 545.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.61e+003	323
A	20000	100.00	100.00	1.61e+003	323

(F1)[A] MW1.11 cb2 00011429 2010-01-12 1209 545.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	528	477
B	13691	68.45	68.45	400	403



(F1)MW1.11 cb2 00011429 2010-01-12 1209 545.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 cb2 00011429 2010-01-12 1209 545.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 cb2

MW DNA

(F1)[A AND B] MW1.11 cb2 00011429 2010-01-12 1209 545.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13691	68.45	100.00	400	###
C	2587	12.94	18.90	195	###
D	2696	13.48	19.69	284	###
E	2317	11.59	16.92	369	###
F	5386	26.93	39.34	605	###

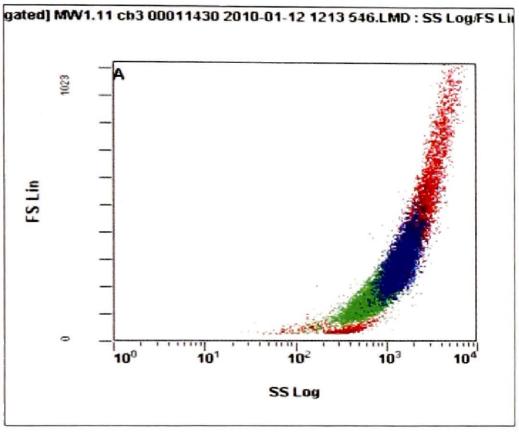
(F1)[Ungated] MW1.11 cb2 00011429 2010-01-12 1209 545.LMD : L

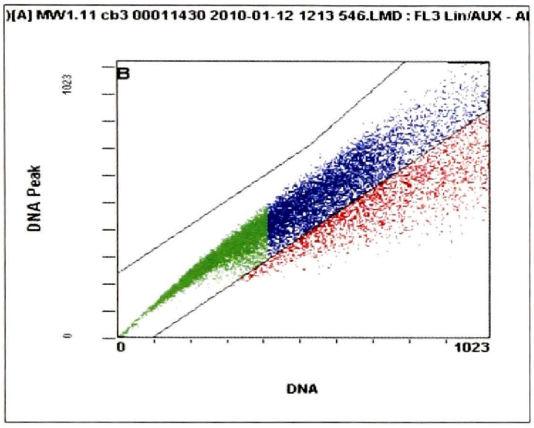
Color	Name	% Gated	% Total	Number	Cells/μL
	F	26.93	26.93	5386	ERROR
	B	68.45	68.45	13691	ERROR
	A	100.00	100.00	20000	ERROR

F) Cytochalsain-B treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:02:19
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 cb3 00011430 2010-01-12 1213 546.LMD

Run Date: 12-Jan-10, 12:1
Sample ID: MW1.11
User ID:
Acquisition Time/Events: 106.1s / 20000 (PROTOC
Tube ID: NoF



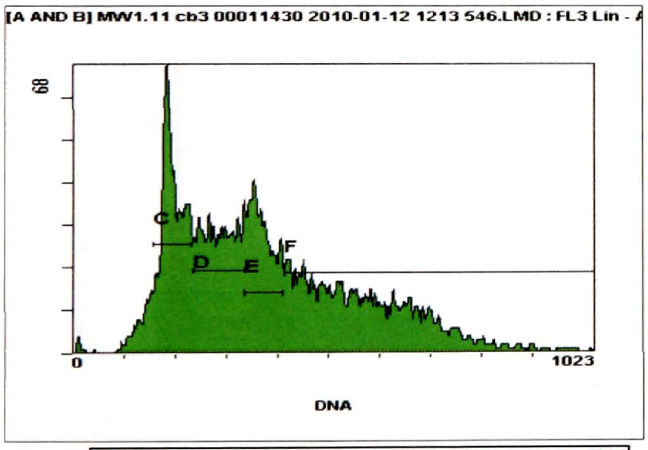


(F1)[Ungated] MW1.11 cb3 00011430 2010-01-12 1213 546.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.46e+003	283
A	20000	100.00	100.00	1.46e+003	283

(F1)[A] MW1.11 cb3 00011430 2010-01-12 1213 546.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	492	449
B	14887	74.44	74.44	392	394



(F1)MW1.11 cb3 00011430 2010-01-12 1213 546.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 cb3 00011430 2010-01-12 1213 546.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 cb3

MW DNA

(F1)[A AND B] MW1.11 cb3 00011430 2010-01-12 1213 546.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14887	74.44	100.00	392	###
C	2954	14.77	19.84	194	###
D	3039	15.20	20.41	284	###
E	2473	12.37	16.61	368	###
F	5688	28.44	38.21	597	###

(F1)[Ungated] MW1.11 cb3 00011430 2010-01-12 1213 546.LMD : L

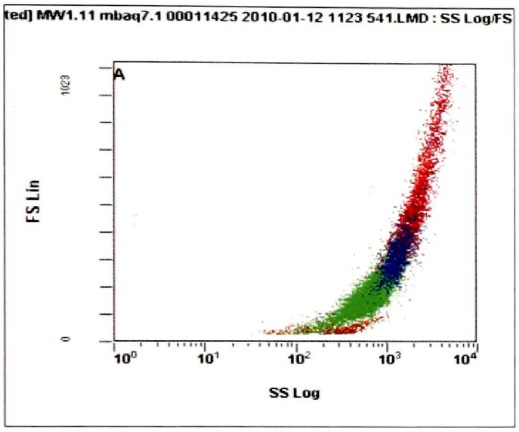
Color	Name	% Gated	% Total	Number	Cells/μL
	F	28.44	28.44	5688	ERROR
	B	74.44	74.44	14887	ERROR
	A	100.00	100.00	20000	ERROR

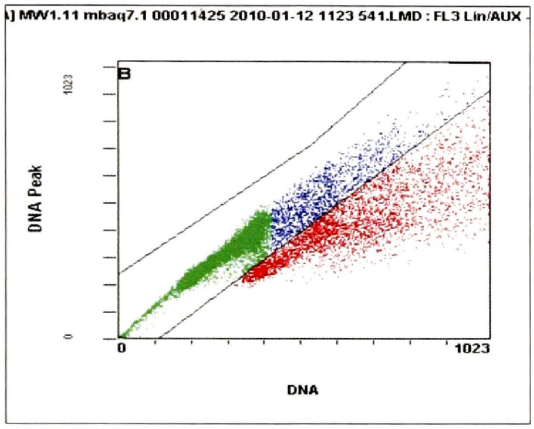
Figure 92. 2-methylanthraquinone treatment group flow histograms and summaries

D) 2-methylanthraquinone treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:08:45
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD

Run Date: 12-Jan-10, 11:1
Sample ID: MW1.11 mba
User ID:
Acquisition Time/Events: 167.9s / 20000 (PROTOC
Tube ID: NoF



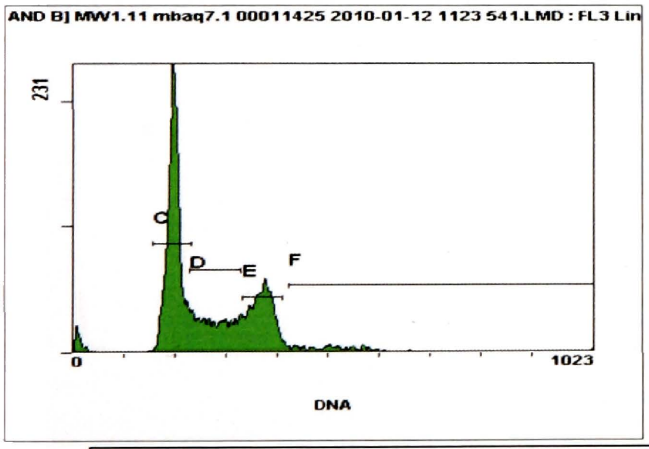


(F1)[Ungated] MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.09e+003	255
A	20000	100.00	100.00	1.09e+003	255

(F1)[A] MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	386	345
B	14402	72.01	72.01	287	305



(F1)MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 mbaq7.1

MW DNA

(F1)[A AND B] MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14402	72.01	100.00	287	###
C	6386	31.93	44.34	197	###
D	2749	13.74	19.09	276	###
E	3170	15.85	22.01	369	###
F	1458	7.29	10.12	605	###

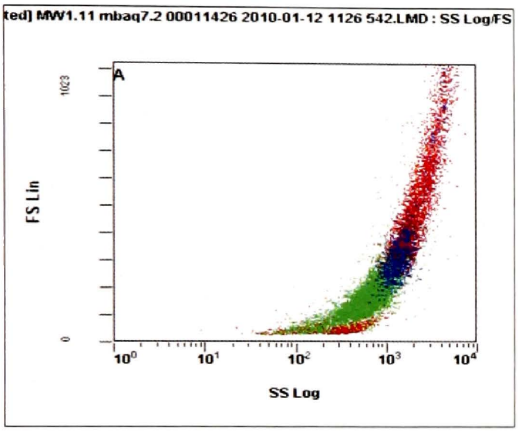
(F1)[Ungated] MW1.11 mbaq7.1 00011425 2010-01-12 1123 541.LMI

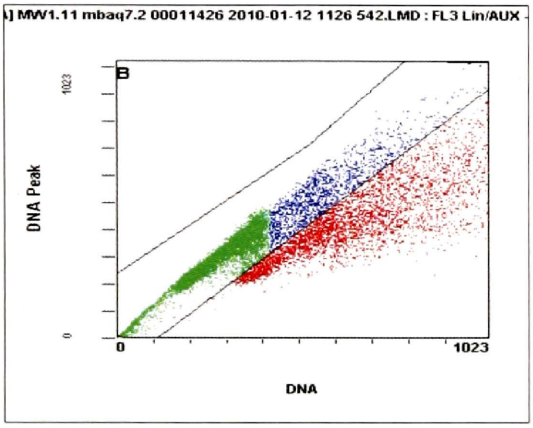
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.29	7.29	1458	ERROR
	B	72.01	72.01	14402	ERROR
	A	100.00	100.00	20000	ERROR

E) 2-methylanthraquinone treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:09:59
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD

Run Date: 12-Jan-10, 11:2·
Sample ID: MW1.11 mba
User ID:
Acquisition Time/Events: 73.6s / 20000 (PROTOC
Tube ID: NoF



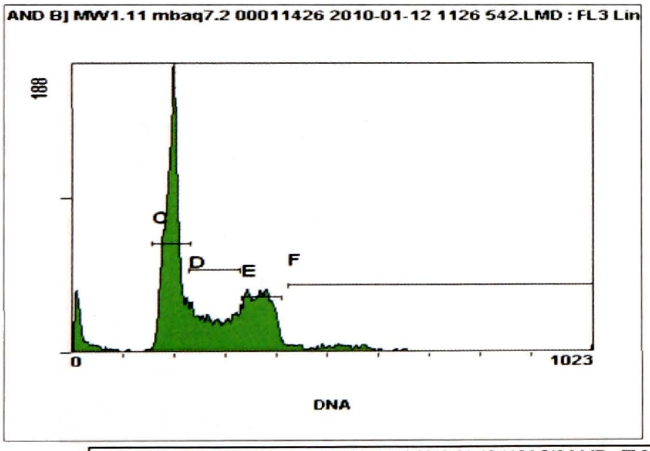


(F1)[Ungated] MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.23e+003	265
A	20000	100.00	100.00	1.23e+003	265

(F1)[A] MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	399	351
B	13397	66.98	66.98	285	299



(F1)MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 mbaq7.2

MW DNA

(F1)[A AND B] MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMD : FL3 I

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13397	66.98	100.00	285	###
C	5530	27.65	41.28	195	###
D	2460	12.30	18.36	276	###
E	2787	13.94	20.80	366	###
F	1522	7.61	11.36	629	###

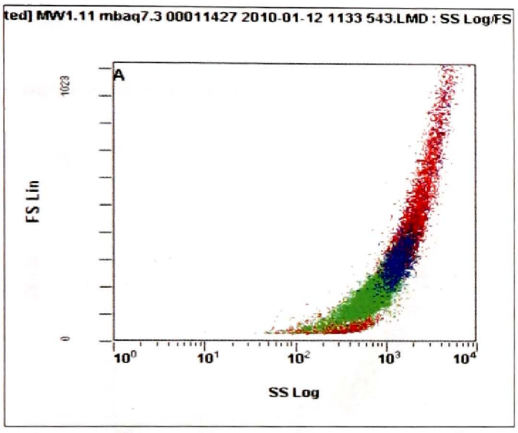
(F1)[Ungated] MW1.11 mbaq7.2 00011426 2010-01-12 1126 542.LMI

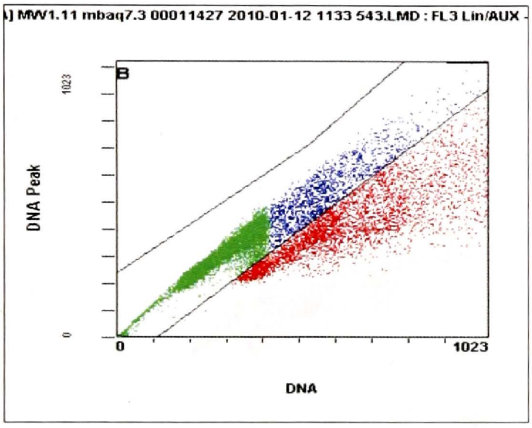
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.61	7.61	1522	ERROR
	B	66.98	66.98	13397	ERROR
	A	100.00	100.00	20000	ERROR

F) 2-methylanthraquinone treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:10:24
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD

Run Date: 12-Jan-10, 11:2
Sample ID: MW1.11 mba
User ID:
Acquisition Time/Events: 155.0s / 20000 (PROTOC
Tube ID: NoF



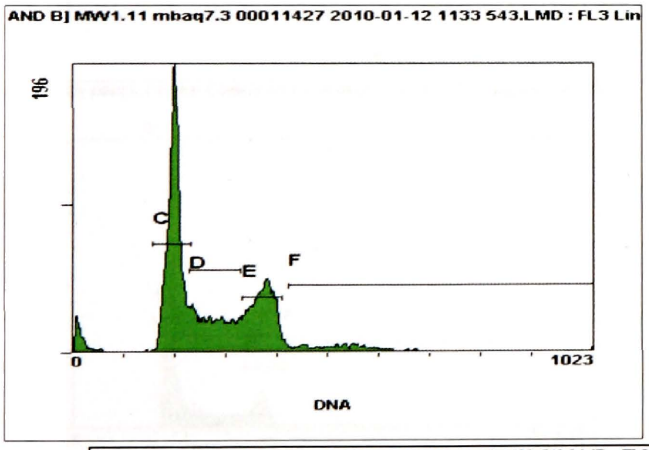


(F1)[Ungated] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.24e+003	261
A	20000	100.00	100.00	1.24e+003	261

(F1)[A] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	405	355
B	13188	65.94	65.94	297	311



(F1)MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 mbaq7.3

MW DNA

(F1)[A AND B] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13188	65.94	100.00	297	###
C	5400	27.00	40.95	198	###
D	2482	12.41	18.82	275	###
E	2942	14.71	22.31	370	###
F	1631	8.15	12.37	617	###

(F1)[Ungated] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMI

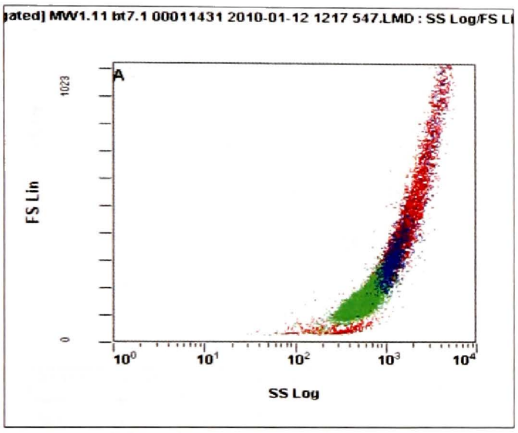
Color	Name	% Gated	% Total	Number	Cells/μL
	F	8.15	8.15	1631	ERROR
	B	65.94	65.94	13188	ERROR
	A	100.00	100.00	20000	ERROR

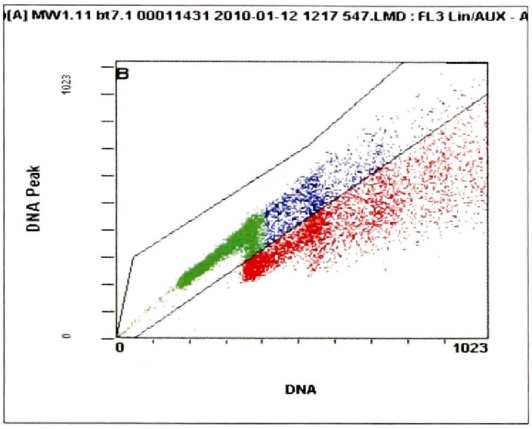
Figure 93. Teak wood extract #1 (7 μg/ml treatment group) flow histograms and summaries

D) Teak wood extract #1 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:58:41
Settings File: MW DNA.PRO, 12-Jan-2010, 12:14:51
Listmode File: MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD

Run Date: 12-Jan-10, 12:1
Sample ID: MW1.11 b
User ID:
Acquisition Time/Events: 105.0s / 20000 (PROTOC
Tube ID: NoF



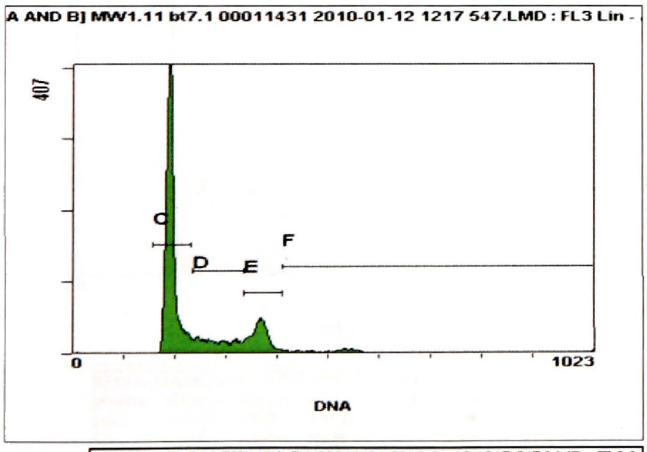


(F1)[Ungated] MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.15e+003	267
A	20000	100.00	100.00	1.15e+003	267

(F1)[A] MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	404	368
B	13500	67.50	67.50	279	305



(F1)MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt7.1

MW DNA

(F1)[A AND B] MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13500	67.50	100.00	279	###
C	7853	39.27	58.17	192	###
D	2003	10.02	14.84	281	###
E	2063	10.32	15.28	366	###
F	1458	7.29	10.80	633	###

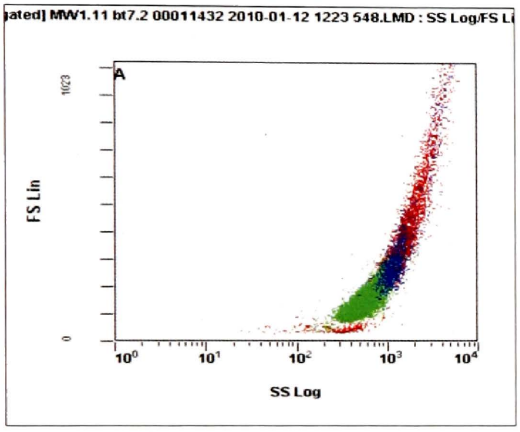
(F1)[Ungated] MW1.11 bt7.1 00011431 2010-01-12 1217 547.LMD :

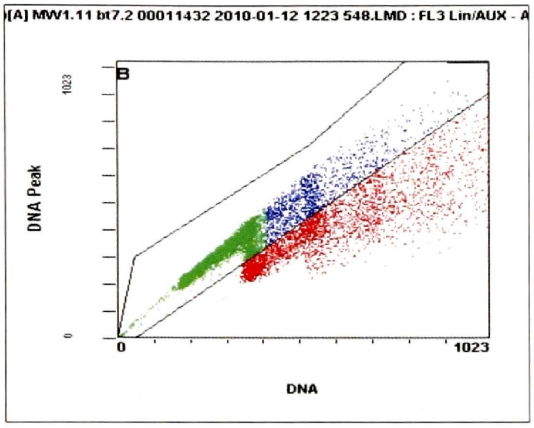
Color	Name	% Gated	% Total	Number	Cells/μL
	F	7.29	7.29	1458	ERROR
	B	67.50	67.50	13500	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood extract #1 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:59:16
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD

Run Date: 12-Jan-10, 12:2
Sample ID: MW1.11 b
User ID:
Acquisition Time/Events: 57.2s / 20000 (PROTOC
Tube ID: NoF



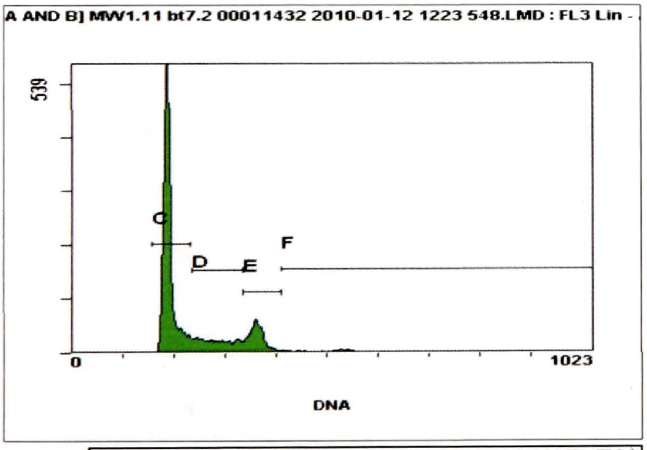


(F1)[Ungated] MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	888	212
A	20000	100.00	100.00	888	212

(F1)[A] MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	344	328
B	15545	77.72	77.72	263	290



(F1)MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt7.2

MW DNA

(F1)[A AND B] MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15545	77.72	100.00	263	###
C	9409	47.05	60.53	190	###
D	2481	12.40	15.96	281	###
E	2229	11.15	14.34	362	###
F	1307	6.54	8.41	598	###

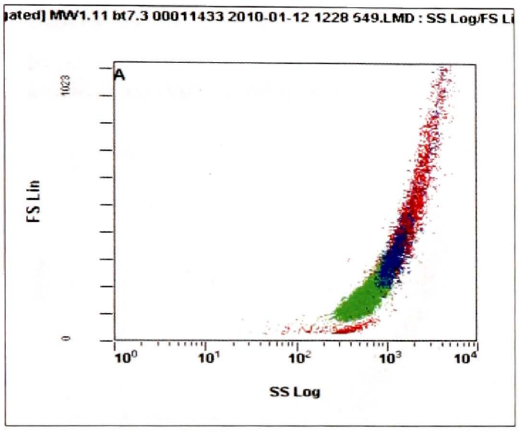
(F1)[Ungated] MW1.11 bt7.2 00011432 2010-01-12 1223 548.LMD :

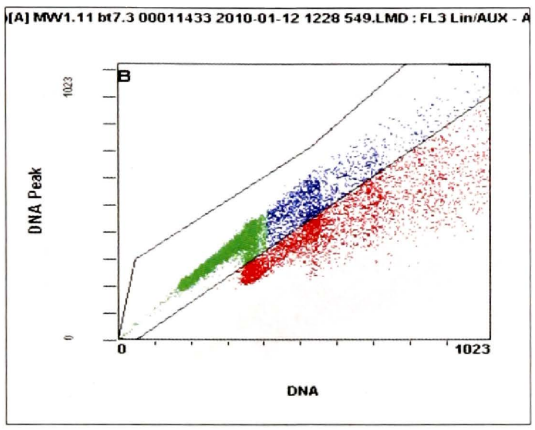
Color	Name	% Gated	% Total	Number	Cells/μL
	F	6.54	6.54	1307	ERROR
	B	77.72	77.72	15545	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood extract #1 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:59:44
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD

Run Date: 12-Jan-10, 12:2·
Sample ID: MW1.11 b
User ID:
Acquisition Time/Events: 55.6s / 20000 (PROTOC
Tube ID: NoF



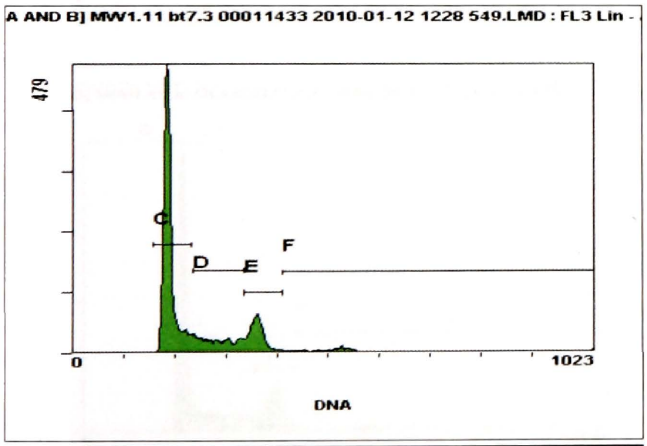


(F1)[Ungated] MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	971	244
A	20000	100.00	100.00	971	244

(F1)[A] MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	363	342
B	15049	75.25	75.25	270	297



(F1)MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt7.3

MW DNA

(F1)[A AND B] MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15049	75.25	100.00	270	###
C	8807	44.03	58.52	188	###
D	2355	11.78	15.65	281	###
E	2261	11.31	15.02	361	###
F	1528	7.64	10.15	603	###

(F1)[Ungated] MW1.11 bt7.3 00011433 2010-01-12 1228 549.LMD :

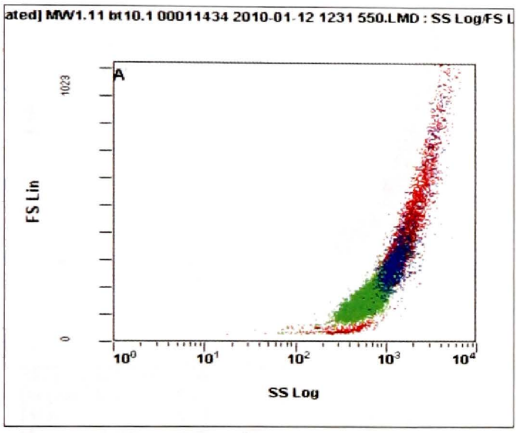
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.64	7.64	1528	ERROR
	B	75.25	75.25	15049	ERROR
	A	100.00	100.00	20000	ERROR

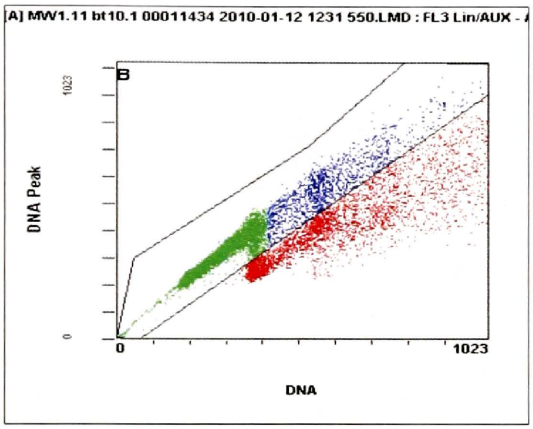
Figure 94. Teak wood dust extract #1 (10 µg/ml treatment group) flow histograms and summaries

D) Teak wood dust extract #1 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:56:22
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD

Run Date: 12-Jan-10, 12:2
Sample ID: MW1.11 bt
User ID:
Acquisition Time/Events: 84.9s / 20000 (PROTOC
Tube ID: NoF



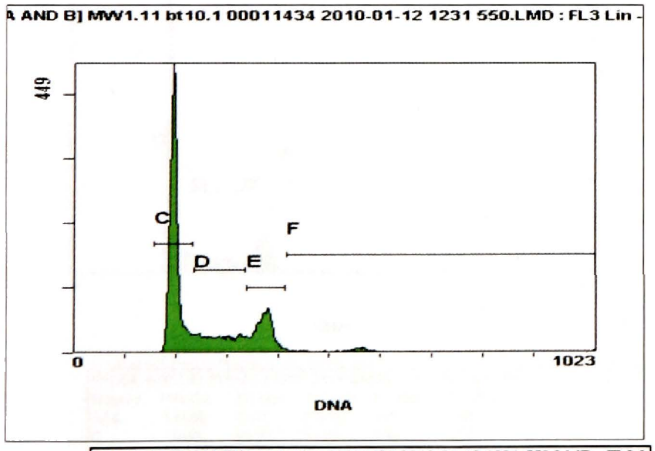


(F1)[Ungated] MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	954	219
A	20000	100.00	100.00	954	219

(F1)[A] MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	369	348
B	15329	76.64	76.64	285	313



(F1)MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt10.1

MW DNA

(F1)[A AND B] MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15329	76.64	100.00	285	###
C	8068	40.34	52.63	197	###
D	2702	13.51	17.63	283	###
E	2871	14.36	18.73	371	###
F	1464	7.32	9.55	627	###

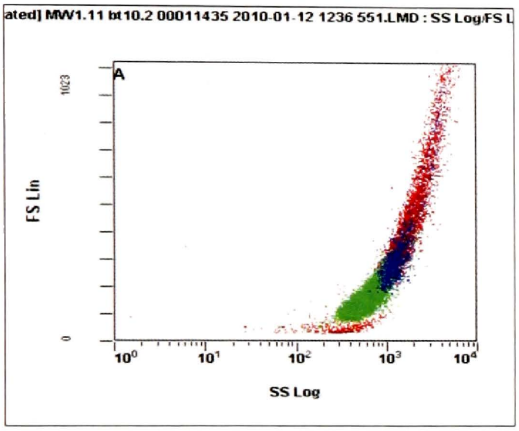
(F1)[Ungated] MW1.11 bt10.1 00011434 2010-01-12 1231 550.LMD

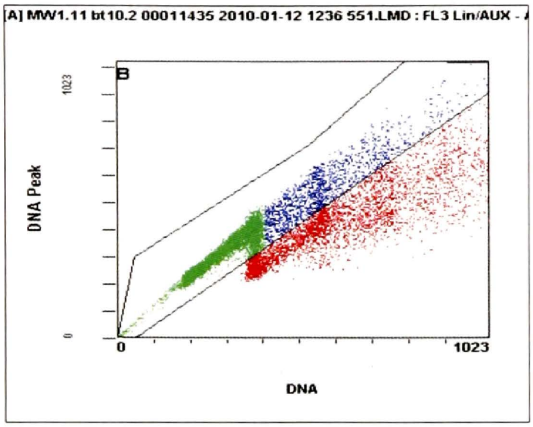
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.32	7.32	1464	ERROR
	B	76.64	76.64	15329	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood dust extract #1 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:57:07
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD

Run Date: 12-Jan-10, 12:3
Sample ID: MW1.11 bt
User ID:
Acquisition Time/Events: 86.9s / 20000 (PROTOC
Tube ID: NoF



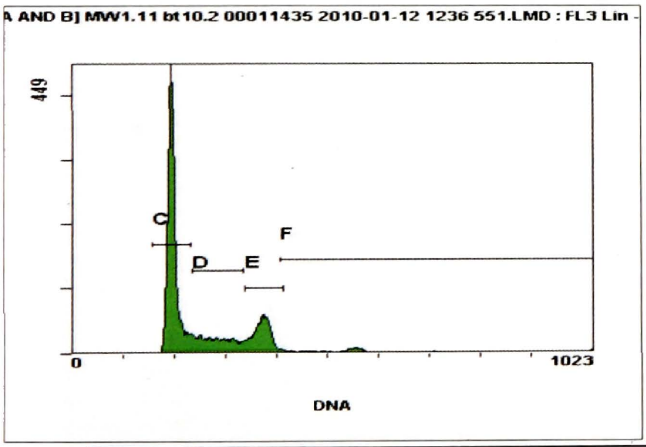


(F1)[Ungated] MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.04e+003	241
A	20000	100.00	100.00	1.04e+003	241

(F1)[A] MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	386	359
B	14602	73.01	73.01	286	313



(F1)MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD
Tulane Center for Gene Therapy
*
L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt10.2

MW DNA

(F1)[A AND B] MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14602	73.01	100.00	286	###
C	7915	39.58	54.20	196	###
D	2421	12.11	16.58	281	###
E	2502	12.51	17.13	370	###
F	1604	8.02	10.98	621	###

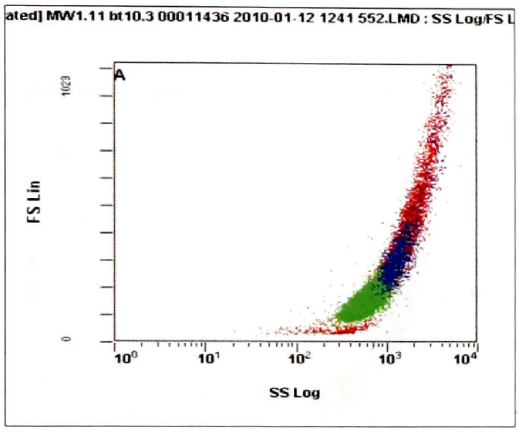
(F1)[Ungated] MW1.11 bt10.2 00011435 2010-01-12 1236 551.LMD

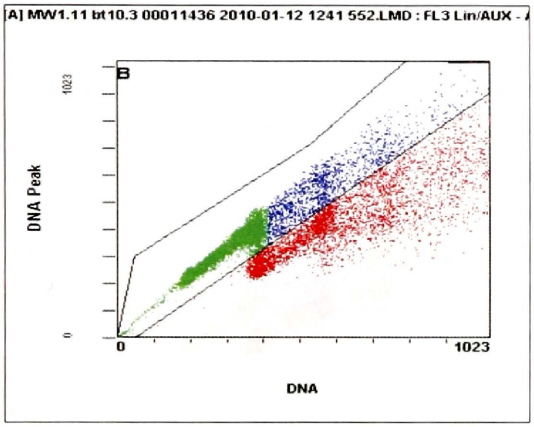
Color	Name	% Gated	% Total	Number	Cells/µL
	F	8.02	8.02	1604	ERROR
	B	73.01	73.01	14602	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract #1 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 12:57:41
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD

Run Date: 12-Jan-10, 12:3
Sample ID: MW1.11 bt
User ID:
Acquisition Time/Events: 117.0s / 20000 (PROTOC
Tube ID: NoF



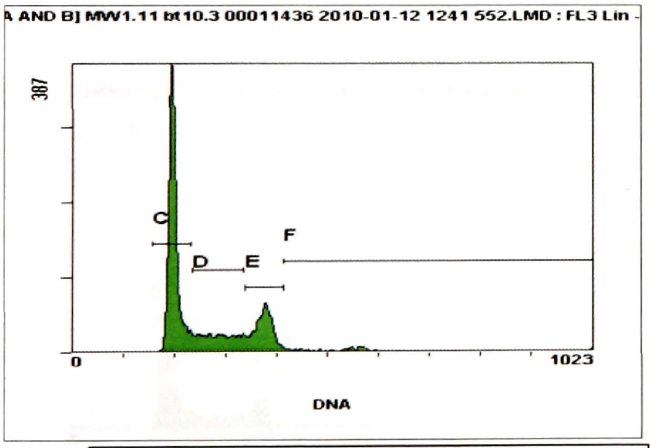


(F1)[Ungated] MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.07e+003	248
A	20000	100.00	100.00	1.07e+003	248

(F1)[A] MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	397	367
B	14278	71.39	71.39	294	321



(F1)MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 bt10.3

MW DNA

(F1)[A AND B] MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14278	71.39	100.00	294	###
C	7241	36.20	50.71	198	###
D	2455	12.28	17.19	283	###
E	2738	13.69	19.18	373	###
F	1600	8.00	11.21	630	###

(F1)[Ungated] MW1.11 bt10.3 00011436 2010-01-12 1241 552.LMD

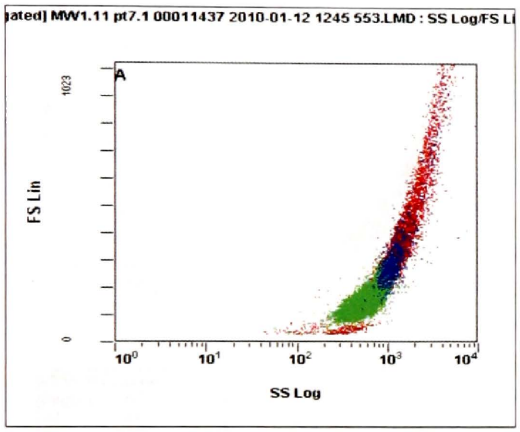
Color	Name	% Gated	% Total	Number	Cells/µL
	F	8.00	8.00	1600	ERROR
	B	71.39	71.39	14278	ERROR
	A	100.00	100.00	20000	ERROR

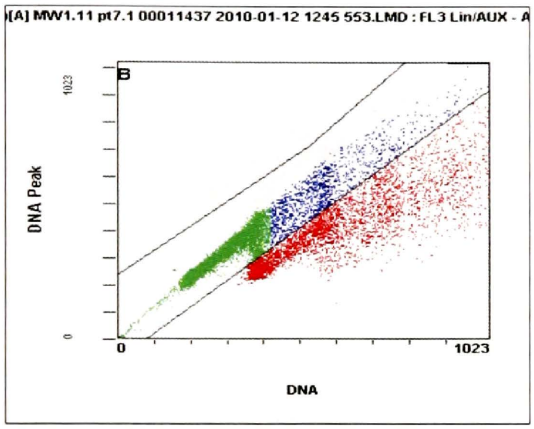
Figure 95. Teak wood dust extract #2 (7 µg/ml treatment group) flow histograms and summaries

D) Teak wood dust extract #2 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:13:59
Settings File: MW DNA.PRO, 12-Jan-2010, 12:20:45
Listmode File: MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD

Run Date: 12-Jan-10, 12:4
Sample ID: MW1.11 p
User ID:
Acquisition Time/Events: 138.6s / 20000 (PROTOC
Tube ID: NoF



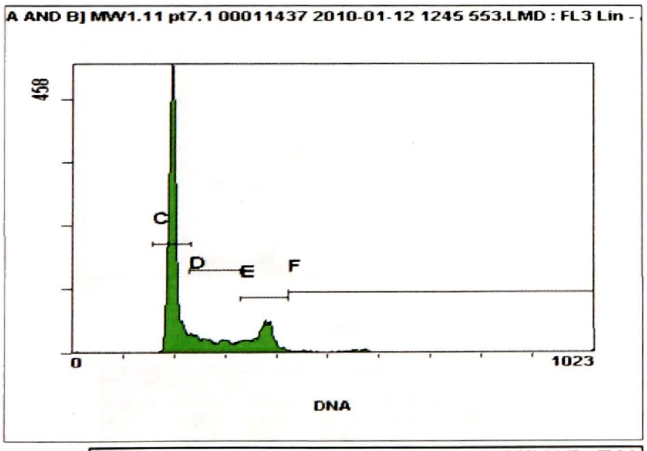


(F1)[Ungated] MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD : SS Log/FS Li

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	971	237
A	20000	100.00	100.00	971	237

(F1)[A] MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	379	350
B	14473	72.36	72.36	276	302



(F1)MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 pt7.1

MW DNA

(F1)[A AND B] MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14473	72.36	100.00	276	###
C	8410	42.05	58.11	197	###
D	2280	11.40	15.75	273	###
E	2508	12.54	17.33	371	###
F	1315	6.58	9.09	615	###

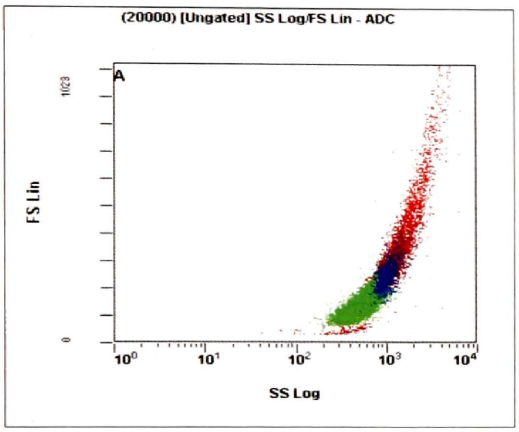
(F1)[Ungated] MW1.11 pt7.1 00011437 2010-01-12 1245 553.LMD :

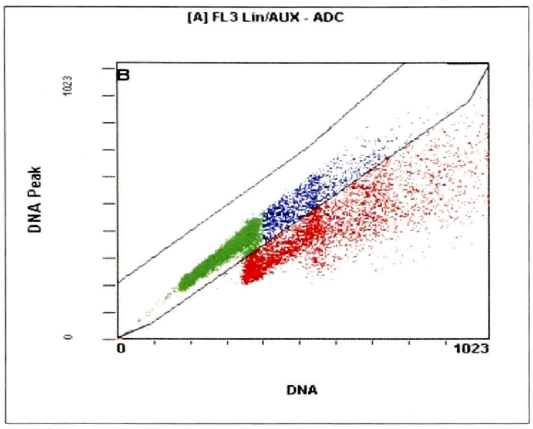
Color	Name	% Gated	% Total	Number	Cells/μL
	F	6.58	6.58	1315	ERROR
	B	72.36	72.36	14473	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood dust extract #2 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 22-Jul-2010, 14:41:20
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut7.2 00011444 2010-01-12 1305 560.LMD

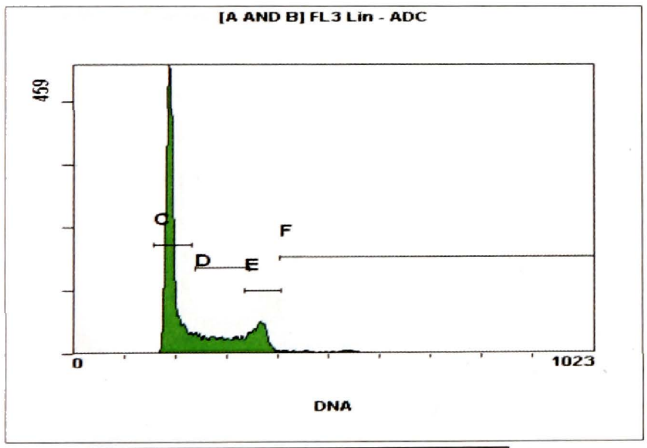
Run Date: 12-Jan-10, 13:0
Sample ID: MW1.11 u
User ID:
Acquisition Time/Events: 70.3s / 20000 (PROTOC
Tube ID: NoF





(20000) [Ungated] SS Log/FS Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	776	190
A	20000	100.00	100.00	776	190

[A] FL3 Lin/AUX					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	348	325
B	14831	74.16	74.16	257	285



FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut7.2 00011444 2010-01-12 1305 560.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut7.2

MW DNA

[A AND B] FL3 Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14831	74.16	100.00	257	###
C	8725	43.63	58.83	192	###
D	3017	15.09	20.34	290	###
E	2191	10.96	14.77	362	###
F	1070	5.35	7.21	522	###

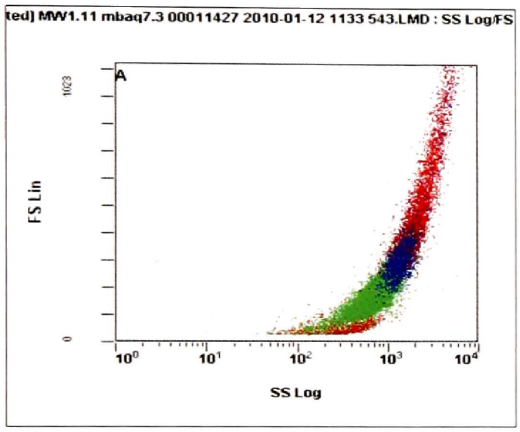
[Ungated] Legend

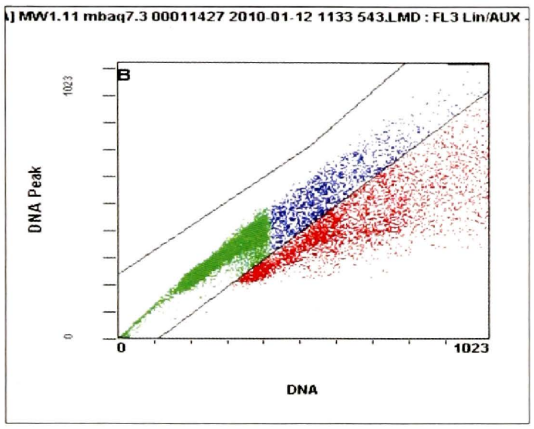
Color	Name	% Gated	% Total	Number	Cells/µL
	F	5.35	5.35	1070	ERROR
	B	74.16	74.16	14831	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract #2 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:10:24
Settings File: MW DNA.PRO, 12-Jan-2010, 11:19:13
Listmode File: MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD

Run Date: 12-Jan-10, 11:2
Sample ID: MW1.11 mba
User ID:
Acquisition Time/Events: 155.0s / 20000 (PROTOC
Tube ID: NoF



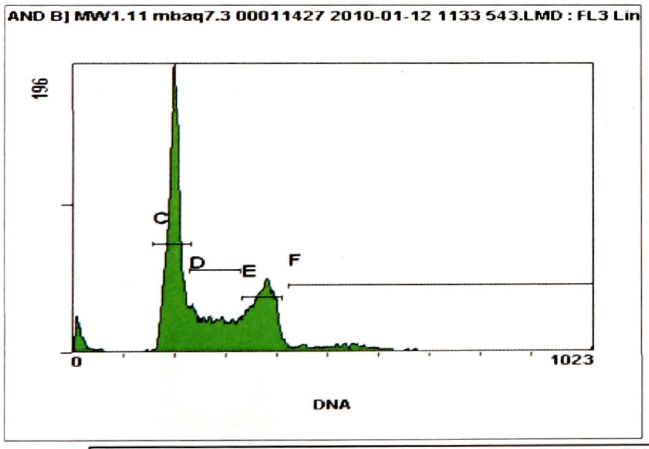


(F1)[Ungated] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : SS Log/FS

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.24e+003	261
A	20000	100.00	100.00	1.24e+003	261

(F1)[A] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	405	355
B	13188	65.94	65.94	297	311



(F1)MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 mbaq7.3

MW DNA

(F1)[A AND B] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13188	65.94	100.00	297	###
C	5400	27.00	40.95	198	###
D	2482	12.41	18.82	275	###
E	2942	14.71	22.31	370	###
F	1631	8.15	12.37	617	###

(F1)[Ungated] MW1.11 mbaq7.3 00011427 2010-01-12 1133 543.LMI

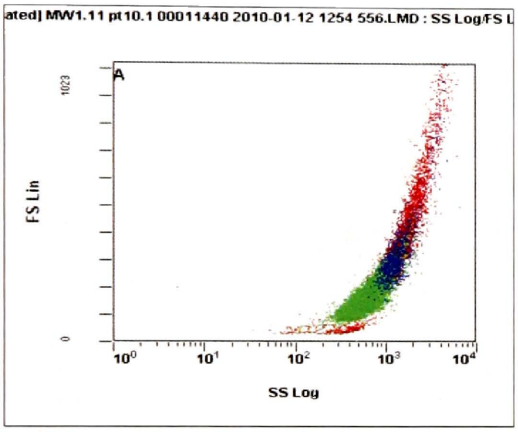
Color	Name	% Gated	% Total	Number	Cells/μL
	F	8.15	8.15	1631	ERROR
	B	65.94	65.94	13188	ERROR
	A	100.00	100.00	20000	ERROR

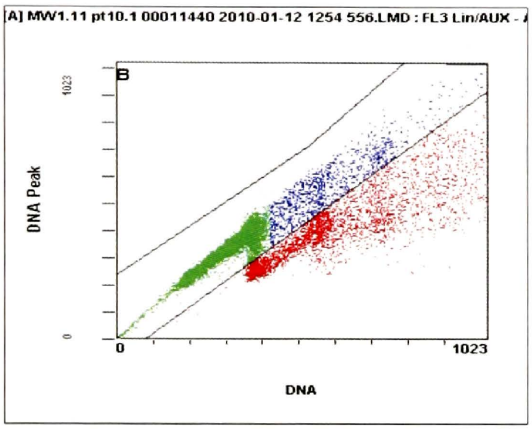
Figure 96. Teak wood dust extract #2 (10 μg/ml treatment group) flow histograms and summaries

D) Teak wood dust extract #2 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:12:39
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD

Run Date: 12-Jan-10, 12:5
Sample ID: MW1.11 pt
User ID:
Acquisition Time/Events: 98.5s / 20000 (PROTOC
Tube ID: NoF



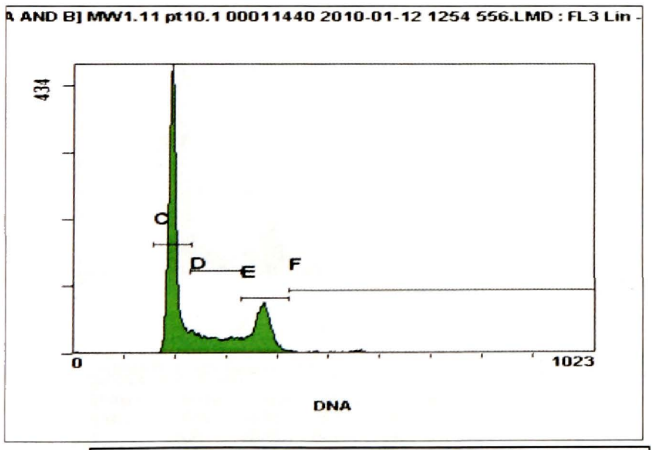


(F1)[Ungated] MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	930	223
A	20000	100.00	100.00	930	223

(F1)[A] MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	356	337
B	15563	77.81	77.81	276	304



(F1)MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 pt10.1

MW DNA

(F1)[A AND B] MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15563	77.81	100.00	276	###
C	8284	41.42	53.23	196	###
D	2690	13.45	17.28	275	###
E	3411	17.06	21.92	368	###
F	1170	5.85	7.52	613	###

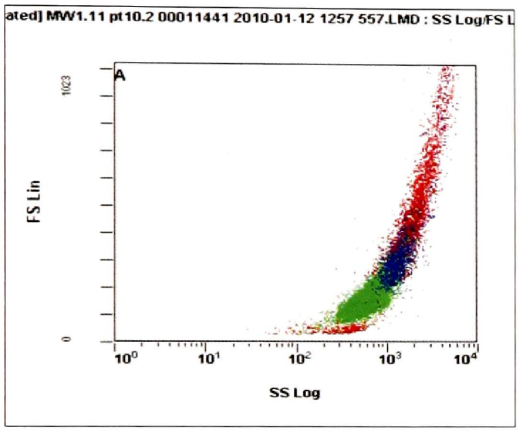
(F1)[Ungated] MW1.11 pt10.1 00011440 2010-01-12 1254 556.LMD :

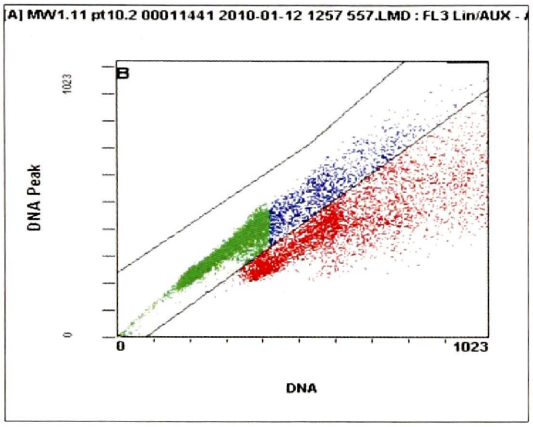
Color	Name	% Gated	% Total	Number	Cells/µL
	F	5.85	5.85	1170	ERROR
	B	77.81	77.81	15563	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood dust extract #2 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:12:14
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD

Run Date: 12-Jan-10, 12:5
Sample ID: MW1.11 pt
User ID:
Acquisition Time/Events: 111.4s / 20000 (PROTOC
Tube ID: NoF



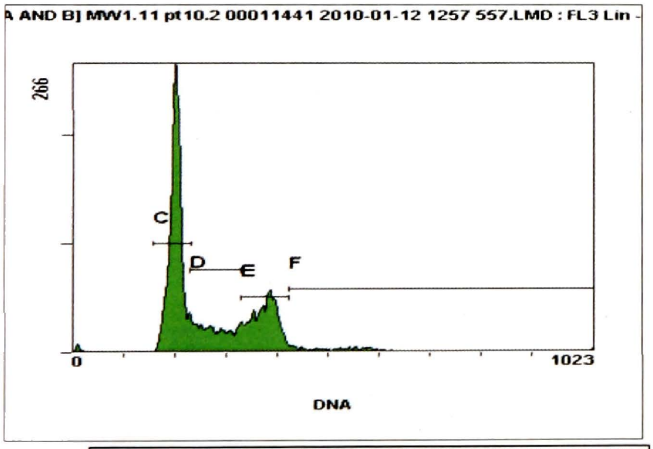


(F1)[Ungated] MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.11e+003	252
A	20000	100.00	100.00	1.11e+003	252

(F1)[A] MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	405	358
B	13906	69.53	69.53	294	315



(F1)MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 pt10.2

MW DNA

(F1)[A AND B] MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13906	69.53	100.00	294	###
C	6689	33.45	48.10	200	###
D	2407	12.04	17.31	274	###
E	3372	16.86	24.25	373	###
F	1400	7.00	10.07	618	###

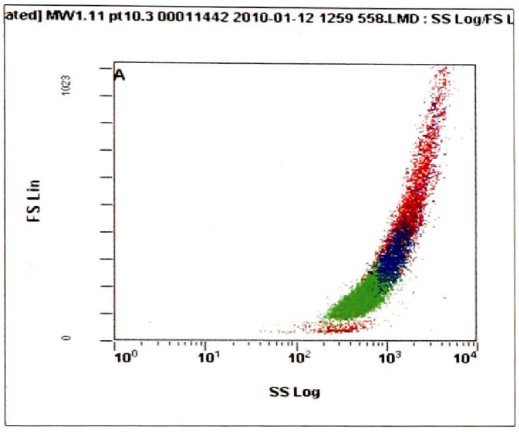
(F1)[Ungated] MW1.11 pt10.2 00011441 2010-01-12 1257 557.LMD

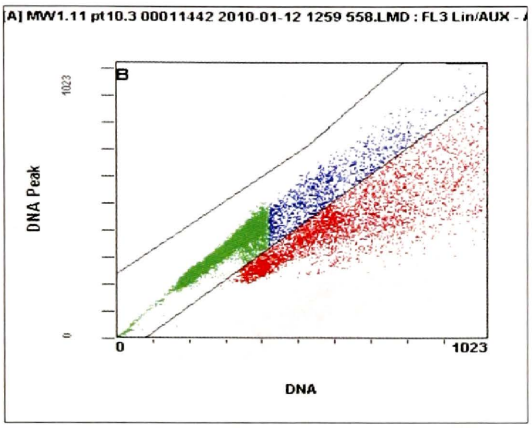
Color	Name	% Gated	% Total	Number	Cells/μL
	F	7.00	7.00	1400	ERROR
	B	69.53	69.53	13906	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract #2 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:13:04
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD

Run Date: 12-Jan-10, 12:5
Sample ID: MW1.11 pt
User ID:
Acquisition Time/Events: 18.5s / 20000 (PROTOC
Tube ID: NoF



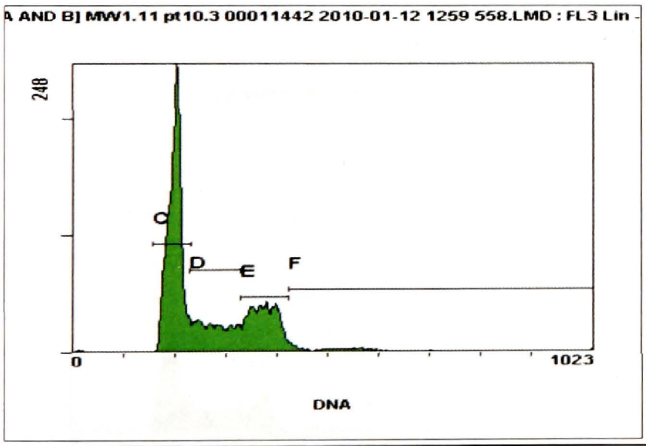


(F1)[Ungated] MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	1.06e+003	266
A	20000	100.00	100.00	1.06e+003	266

(F1)[A] MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	407	369
B	14229	71.14	71.14	293	317



(F1)MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 pt10.3

MW DNA

(F1)[A AND B] MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14229	71.14	100.00	293	###
C	6960	34.80	48.91	199	###
D	2536	12.68	17.82	276	###
E	3281	16.41	23.06	372	###
F	1429	7.14	10.04	628	###

(F1)[Ungated] MW1.11 pt10.3 00011442 2010-01-12 1259 558.LMD :

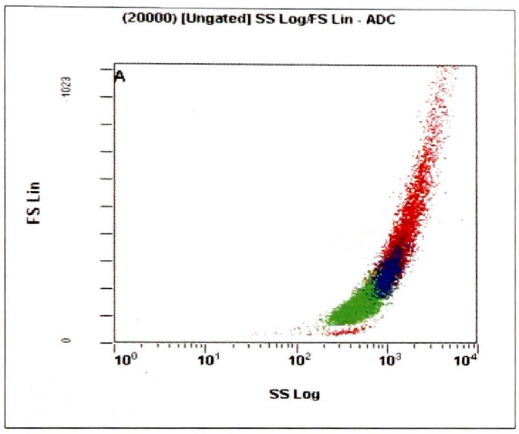
Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.14	7.14	1429	ERROR
	B	71.14	71.14	14229	ERROR
	A	100.00	100.00	20000	ERROR

Figure 97. Teak wood dust extract #3 (7 µg/ml treatment group) flow histograms and summaries

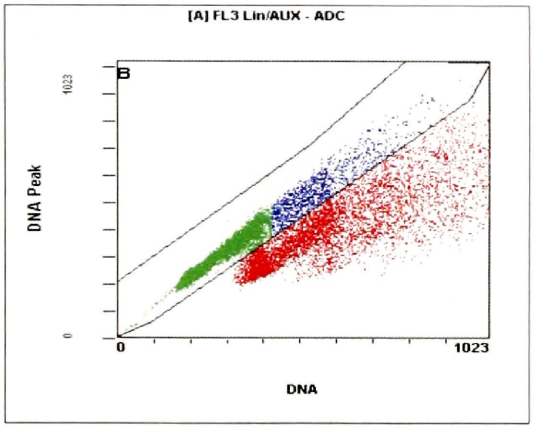
D) Teak wood dust extract #3 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 22-Jul-2010, 14:40:29
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut7.1 00011443 2010-01-12 1302 559.LMD

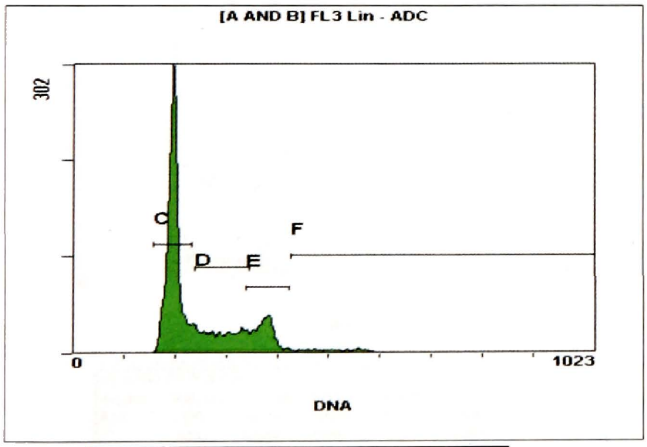
Run Date: 12-Jan-10, 13:0
Sample ID: MW1.11 u
User ID:
Acquisition Time/Events: 86.3s / 20000 (PROTOC
Tube ID: NoF



(20000) [Ungated] SS Log/FS Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	905	226
A	20000	100.00	100.00	905	226



[A] FL3 Lin/AUX					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	408	357
B	12865	64.33	64.33	272	299



FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut7.1 00011443 2010-01-12 1302 559.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut7.1

MW DNA

[A AND B] FL3 Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	12865	64.33	100.00	272	###
C	7128	35.64	55.41	195	###
D	2585	12.93	20.09	290	###
E	2065	10.32	16.05	372	###
F	1104	5.52	8.58	556	###

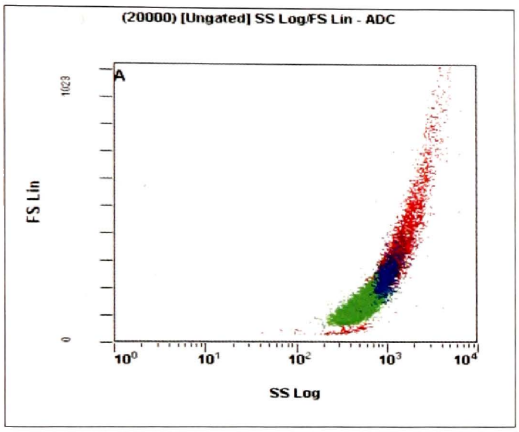
[Ungated] Legend

Color	Name	% Gated	% Total	Number	Cells/µL
	F	5.52	5.52	1104	ERROR
	B	64.33	64.33	12865	ERROR
	A	100.00	100.00	20000	ERROR

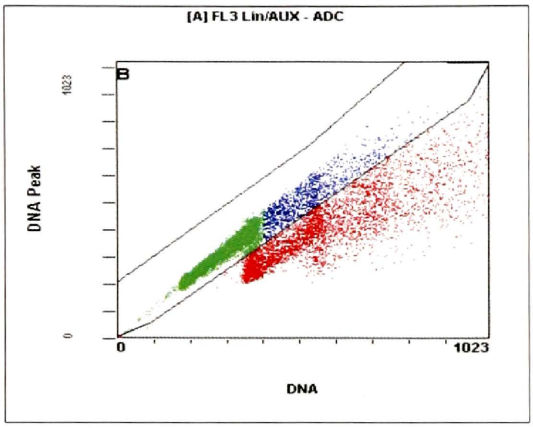
E) Teak wood dust extract #3 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 22-Jul-2010, 14:41:20
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut7.2 00011444 2010-01-12 1305 560.LMD

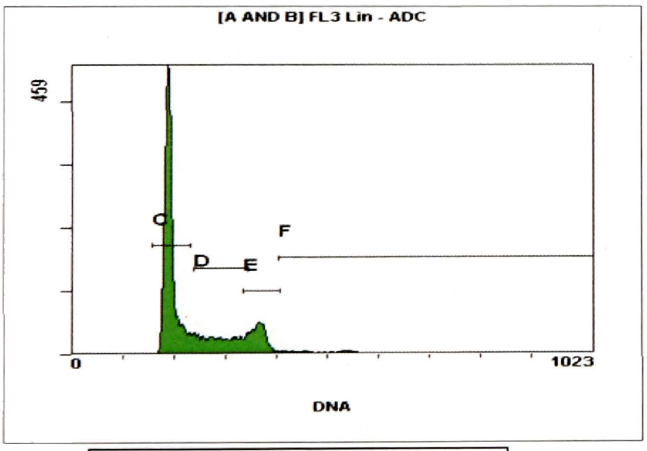
Run Date: 12-Jan-10, 13:0
Sample ID: MW1.11 u
User ID:
Acquisition Time/Events: 70.3s / 20000 (PROTOC
Tube ID: NoF



(20000) [Ungated] SS Log/FS Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	776	190
A	20000	100.00	100.00	776	190



[A] FL3 Lin/AUX					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	348	325
B	14831	74.16	74.16	257	285



FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut7.2 00011444 2010-01-12 1305 560.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut7.2

MW DNA

[A AND B] FL3 Lin					
Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	14831	74.16	100.00	257	###
C	8725	43.63	58.83	192	###
D	3017	15.09	20.34	290	###
E	2191	10.96	14.77	362	###
F	1070	5.35	7.21	522	###

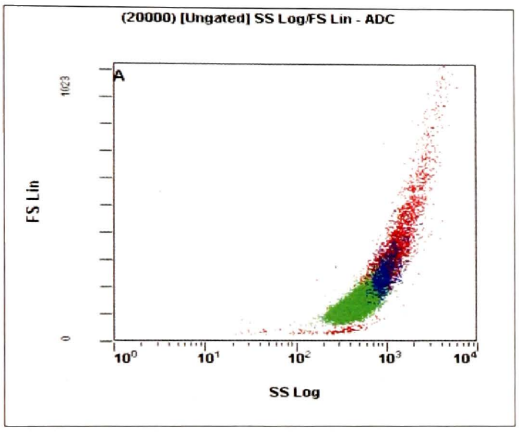
[Ungated] Legend

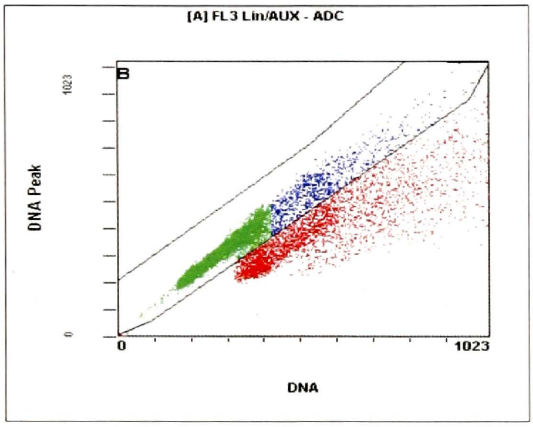
Color	Name	% Gated	% Total	Number	Cells/μL
	F	5.35	5.35	1070	ERROR
	B	74.16	74.16	14831	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract #3 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 22-Jul-2010, 14:42:06
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut7.3 00011445 2010-01-12 1315 561.LMD

Run Date: 12-Jan-10, 13:1
Sample ID: MW1.11 L
User ID:
Acquisition Time/Events: 42.6s / 20000 (PROTOC
Tube ID: NoF



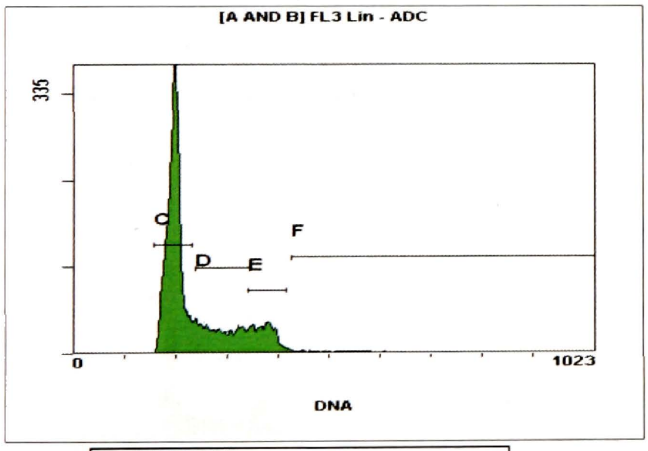


(20000) [Ungated] SS Log/FS Lin

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	649	176
A	20000	100.00	100.00	649	176

[A] FL3 Lin/AUX

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	325	320
B	15978	79.89	79.89	258	291



FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut7.3 00011445 2010-01-12 1315 561.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut7.3

MW DNA

[A AND B] FL3 Lin

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15978	79.89	100.00	258	###
C	9553	47.77	59.79	195	###
D	3280	16.40	20.53	289	###
E	2076	10.38	12.99	372	###
F	914	4.57	5.72	561	###

[Ungated] Legend

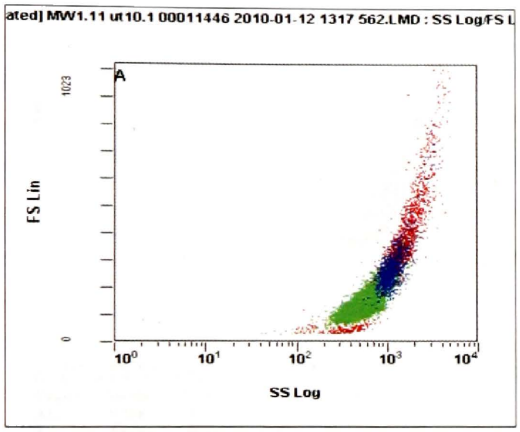
Color	Name	% Gated	% Total	Number	Cells/µL
	F	4.57	4.57	914	ERROR
	B	79.89	79.89	15978	ERROR
	A	100.00	100.00	20000	ERROR

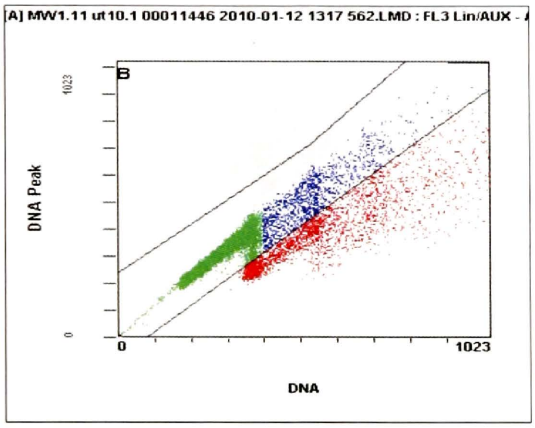
Figure 98. Teak wood dust extract #3 (10 µg/ml treatment group) flow histograms and summaries

D) Teak wood dust extract #3 treatment sample 1

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:16:47
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD

Run Date: 12-Jan-10, 13:1(
Sample ID: MW1.11 ut
User ID:
Acquisition Time/Events: 43.2s / 20000 (PROTOC
Tube ID: NoF



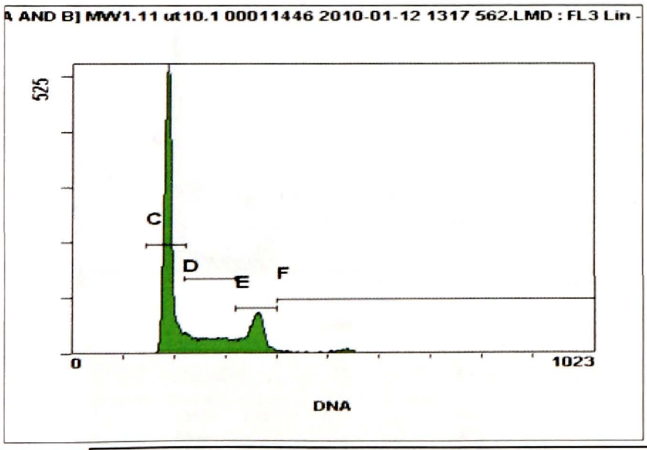


(F1)[Ungated] MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	710	172
A	20000	100.00	100.00	710	172

(F1)[A] MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	309	307
B	16842	84.21	84.21	261	290



(F1)MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut10.1

MW DNA

(F1)[A AND B] MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	16842	84.21	100.00	261	###
C	9402	47.01	55.82	189	###
D	3171	15.86	18.83	266	###
E	3125	15.63	18.55	356	###
F	1300	6.50	7.72	551	###

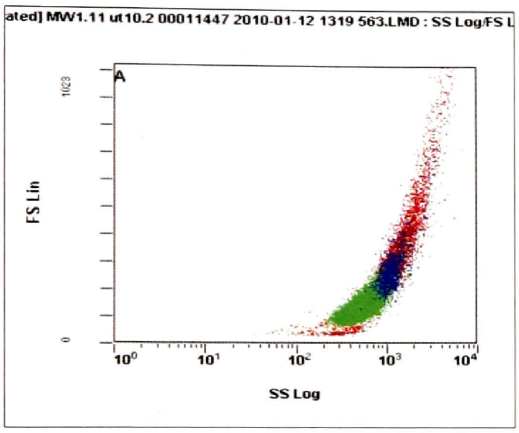
(F1)[Ungated] MW1.11 ut10.1 00011446 2010-01-12 1317 562.LMD :

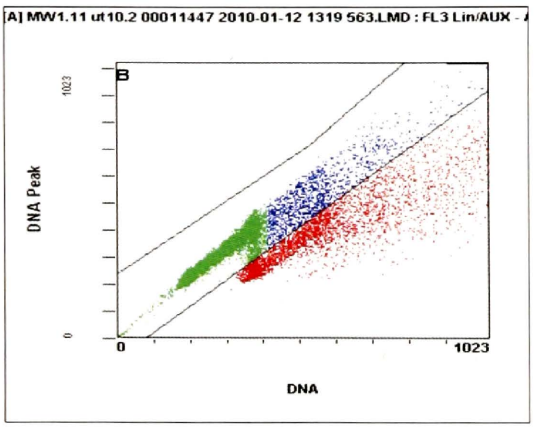
Color	Name	% Gated	% Total	Number	Cells/μL
	F	6.50	6.50	1300	ERROR
	B	84.21	84.21	16842	ERROR
	A	100.00	100.00	20000	ERROR

E) Teak wood dust extract #3 treatment sample 2

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:17:30
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD

Run Date: 12-Jan-10, 13:1
Sample ID: MW1.11 ut
User ID:
Acquisition Time/Events: 66.8s / 20000 (PROTOC
Tube ID: NoF



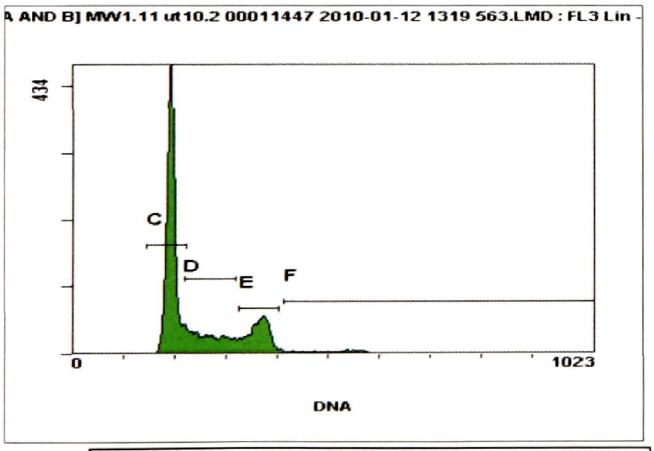


(F1)[Ungated] MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	773	186
A	20000	100.00	100.00	773	186

(F1)[A] MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	340	326
B	15777	78.89	78.89	271	300



(F1)MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut10.2

MW DNA

(F1)[A AND B] MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	15777	78.89	100.00	271	###
C	8440	42.20	53.50	193	###
D	3088	15.44	19.57	264	###
E	2874	14.37	18.22	363	###
F	1340	6.70	8.49	579	###

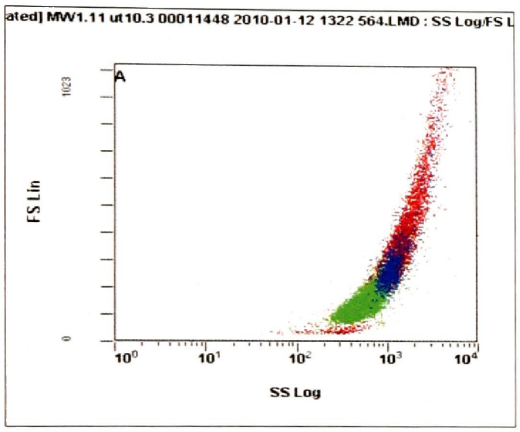
(F1)[Ungated] MW1.11 ut10.2 00011447 2010-01-12 1319 563.LMD :

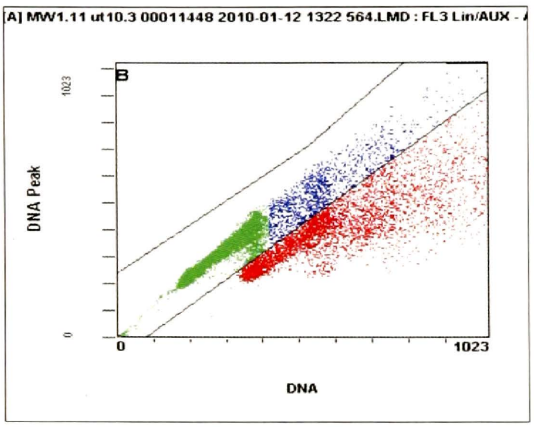
Color	Name	% Gated	% Total	Number	Cells/µL
	F	6.70	6.70	1340	ERROR
	B	78.89	78.89	15777	ERROR
	A	100.00	100.00	20000	ERROR

F) Teak wood dust extract #3 treatment sample 3

Institution: Tulane Center for Gene Therapy
Protocol: DNA.PRO
Listmode Replay: New Protocol
Analysis Date: 19-Jul-2010, 13:18:12
Settings File: MW DNA.PRO, 12-Jan-2010, 12:52:31
Listmode File: MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD

Run Date: 12-Jan-10, 13:2(
Sample ID: MW1.11 ut
User ID:
Acquisition Time/Events: 75.7s / 20000 (PROTOC
Tube ID: NoF



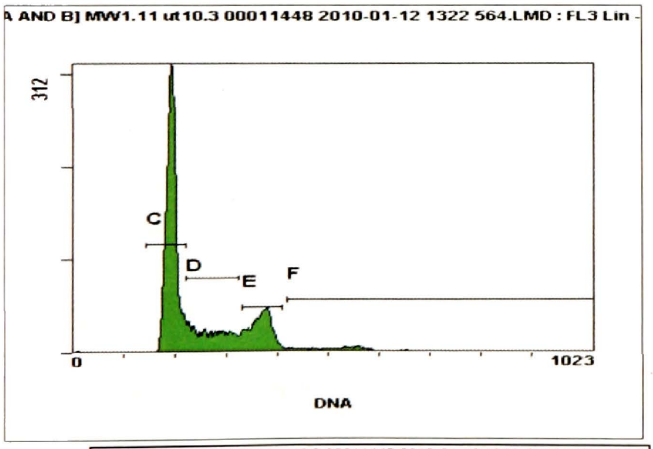


(F1)[Ungated] MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD : SS Log/FS L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	916	224
A	20000	100.00	100.00	916	224

(F1)[A] MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD : FL3 Lin/AU

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	20000	100.00	100.00	390	352
B	13950	69.75	69.75	281	309



(F1)MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD : FCS Information
Cytomics FC 500
12-Jan-10
MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD
Tulane Center for Gene Therapy

L
Alan
FL3 Lin
SS Log
FS Lin
FL1 Log
FL1 Lin
MW1.11 ut10.3

MW DNA

(F1)[A AND B] MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD : FL3 L

Region	Number	%Total	%Gated	X-Mean	Y-Mean
ALL	13950	69.75	100.00	281	###
C	7270	36.35	52.11	193	###
D	2484	12.42	17.81	271	###
E	2491	12.46	17.86	367	###
F	1419	7.09	10.17	606	###

(F1)[Ungated] MW1.11 ut10.3 00011448 2010-01-12 1322 564.LMD :

Color	Name	% Gated	% Total	Number	Cells/µL
	F	7.09	7.09	1419	ERROR
	B	69.75	69.75	13950	ERROR
	A	100.00	100.00	20000	ERROR