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(54) Title: BIOMARKERS FOR ASSESSING EXPOSURE TO IONIZING RADIATION AND ABSORBED DOSE

(57) Abstract: The present invention concerns radiation-responsive biomarkers capable of determining qualitative exposure status (yes/no, i.e., irradiated/non-irradiated) and distinguishing levels of exposure (quantitative absorbed dose determination), and materials and methods to profile the radiation-responsive biomarkers in blood samples to detect radiation exposure and/or to determine the dose of radiation exposure.



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DESCRIPTION

BIOMARKERS FOR ASSESSING EXPOSURE TO IONIZING RADIATION
AND ABSORBED DOSE

GOVERNMENT SUPPORT

This invention was made with government support under U.S. Environmental Protection Agency (EPA) STAR Fellowship Assistance Agreement Number FP-91694801. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The worldwide expansion of nuclear energy as well as ongoing nuclear proliferation in developing, politically unstable countries raises the possibility of large-scale acute radiation exposure. The ability to rapidly triage exposed individuals and assess the extent of exposure is imperative to decrease morbidity and mortality through rationally directed medical intervention.

Significant exposure to ionizing radiation (IR) induces varying degrees of nausea, vomiting, and pancytopenia (1-3). Attempts have been made to exploit these symptoms for estimating dose of exposure. However, these methods lack sensitivity and high inter-individual variability limits their utility. On the molecular level, IR induces DNA double-stranded breaks directly or through the secondary generation of free radicals and reactive oxygen species (4, 5). Improper repair of damaged DNA results in chromosomal aberrations, especially dicentrics, whose frequency has been correlated to radiation dose (6). However, while cytogenetic analysis remains the current "gold standard" of retrospective radiation dose assessment, it is both time- and labor-intensive (1-3) and may not be amenable to mass exposure scenarios.

Previous studies have demonstrated that in addition to DNA damage, exposure of mammalian cells to IR initiates a complex series of signal transduction cascades related to maintaining genomic integrity through damage recognition, repair, induction of cell cycle checkpoints, senescence, and/or initiation of apoptosis. Recent microarray studies have identified transcriptional changes in lymphoid/myeloid cells (7-20), mice (21, 22), primary cells (23-26), ex vivo irradiated whole blood (27, 28) and blood from irradiated patients (22, 29) that could potentially be used for radiation dose assessment. Direct comparison of most array-based analyses remains difficult because these studies were performed using different

array platforms, controls, experimental models, doses of irradiation, and time points. Limiting exclusion criteria to genes identified by at least two independent investigators in more than one model reveals consistent changes in a surprisingly small population of genes including ACTA2, BAX, BBC3, BTG2, CCNG1, CDKN1A, DDB2, FDXR, GADD45A, HSPE1, PCNA, PPM1D, TP53I3, TNF α , and XPC (Table 1B). Furthermore, a dose-dependent induction of GADD45A (30, 31), DDB2 (15, 26, 29, 30), XPC (15, 26) and CDKN1A (8, 15, 26-29, 32) has been observed following irradiation in some contexts.

Published reports of global (microarray) human *in vivo* studies examining changes in gene expression following total body irradiation (TBI) remain limited. Two published studies involving a small number of patients collectively identified subsets of radiation-responsive genes that differed from *in vitro* or *ex vivo* models and emphasize that the effect of irradiation on gene expression profiles may be influenced by the cellular microenvironment within “whole animal” models (22, 29). More importantly, a profile of radiation response genes capable of distinguishing irradiated from non-irradiated blood samples with an accuracy of 90% was identified (22). Taken together, these studies support the hypothesis that there is no single cellular response to radiation and that examining the overall expression profile of multiple biodosimetry genes may be more useful than determining expression of individual genes.

BRIEF SUMMARY OF THE INVENTION

The present invention concerns radiation-responsive biomarkers capable of qualitatively determining exposure status (yes/no, *i.e.*, irradiated/non-irradiated) and distinguishing levels of exposure (quantitative dose determination), and materials and methods to profile the radiation-responsive biomarkers in blood samples to detect radiation exposure and/or to determine the dose of radiation exposure.

The inventors obtained whole blood samples from cancer patients exposed to TBI and used them to identify potential biodosimetry genes. Time- and dose-dependent changes in the expression of these biodosimetry genes were subsequently examined in *in vivo* mouse models. An associative classification algorithm (weighted voting) was then utilized to identify molecular signatures capable of distinguishing irradiated from non-irradiated mice. Multiple linear regression analysis allowed for quantitative dose determination. The results show that gene expression analysis can be used for assessment of radiation exposure and incorporated into current biodosimetry protocols.

Thus, the present invention relates to radiation exposure screening. The genes disclosed herein constitute biomarkers for detection and monitoring of radiation exposure. For example, one or more of the polypeptides disclosed herein, or nucleic acids that encode the polypeptides, can be used to detect, quantify, and monitor radiation exposure. These polypeptides and polynucleotides (referred to herein collectively as “biomarkers”), and agents that bind to them, can be used to detect, quantify, and monitor radiation in mammals, including humans. Biomarkers of the invention include Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

In one aspect, the method of the invention comprises a method of identifying a mammalian subject exposed to ionizing radiation, comprising: detecting the presence of, and/or quantifying the level of, one or more biomarker nucleic acids or proteins in a blood sample or blood derived sample from the subject, wherein the presence of the biomarker, or a level or concentration of the biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject, and wherein the one or more biomarkers comprise or consist of one or more biomarkers among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

The method of the invention can be a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated), a quantitative (dose) assessment of radiation exposure (and, optionally, for two or more time points post-exposure *i.e.*, time-dependent and/or dose-dependent), or both a qualitative and quantitative assessment.

In some embodiments, the method is a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated samples), wherein a level or concentration of the biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject, and wherein a level or concentration of the biomarker below a pre-determined threshold is indicative of a lack of ionizing radiation exposure in the subject. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

In some embodiments, a level or concentration of a biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject within a certain period of time of obtaining a sample from the subject (*e.g.*, within 5 hours to within 48 hours post-exposure; see, for example, Table 4 - Prediction of Exposure in Mice (Non-irradiated vs. Irradiated)). In some embodiments, the one or more biomarkers comprise or consist of one or

two from among Bbc3, Ccng1, and Cdkn1a within 5 hours to within 48 hours post-exposure. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1 within 23 hours post-exposure. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 within 5-12 hours post-exposure. In some embodiments, the one or more
5 biomarkers comprise or consist of Ccng1 and Cdkn1a within 48 hours post-exposure.

In some embodiments, the method is a quantitative (dose) assessment of radiation exposure, in which the actual dose of radiation exposure or a range of exposure is determined (*e.g.*, 0 grays (Gy), 1 Gy, 2 Gy, 3 Gy, 4 Gy, 5 Gy, 6 Gy, 7 Gy, 8 Gy, and so forth). In some
10 embodiments of the quantitative assessment, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1a (see, for example, Table 5 – Predicted Dose based on Multiple Linear Regression Analysis).

In some embodiments, the one or more biomarkers comprise or consist of or one or both biomarkers from among Ccng1 and Tnfrsf10b, wherein a level or concentration of the biomarker(s) above a pre-determined threshold is indicative of ionizing radiation exposure of
15 equal to or greater than 2 Gy in the subject (*e.g.*, within 5 hours, 12 hours, 24 hours, or 48 hours post-exposure), and wherein a level or concentration of the biomarker(s) below a pre-determined threshold is indicative of ionizing radiation exposure of less than 2 Gy in the subject (see, for example, Table 6 – Class Prediction of Dosing Groups in Mice).

Another aspect of the invention is a method of identifying an expression profile in a
20 sample from a mammalian subject that is indicative of the subject's exposure or non-exposure to ionizing radiation, comprising: providing a subject expression profile from the mammalian subject; providing one or more reference profiles, wherein each reference profile is associated with (*i.e.*, representative of) exposure or non-exposure to ionizing radiation, wherein the subject expression profile and each reference profile has a value, wherein each
25 value represents the expression level of one or more biomarkers selected from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b; and identifying the reference profile most similar to the subject expression profile, to thereby identify an expression profile that is indicative of exposure or non-exposure to ionizing radiation. The most similar reference profile can be identified using bioinformatics methods known in the art, such as by weighting a comparison
30 value for each value of each reference profile using a weight value associated with the corresponding biomarker from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the identified reference profile is associated with exposure to ionizing radiation, and the method further comprises treating the mammalian subject for radiation exposure. In some embodiments, the identified reference profile is associated with exposure

to ionizing radiation, and the method further comprises verifying that the mammalian subject is suffering from radiation exposure. In some embodiments, the mammalian subject is human, wherein the identified reference profile is associated with exposure to ionizing radiation, and wherein the method further comprises informing the subject of the exposure or potential exposure to ionizing radiation. In some embodiments, the one or more reference profiles comprise a plurality of reference profiles. Optionally, for quantitative assessment of radiation dose, each reference profile of the plurality of reference profiles can be associated with exposure to a different dose of ionizing radiation, and/or a dose of ionizing radiation exposure after a duration of time. Thus, the method of identifying an expression profile can provide a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated), a quantitative (dose) assessment of radiation exposure (and, optionally, for two or more time points post-exposure, *i.e.*, time-dependent and/or dose-dependent), or both a qualitative and quantitative assessment.

In some embodiments, the method of identifying an expression profile provides a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated samples). In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

In some embodiments, one or more reference profiles are associated with ionizing radiation exposure in the subject within a certain period of time of obtaining a sample from the subject (*e.g.*, within 5 hours to within 48 hours post-exposure; see, for example, Table 4 - Prediction of Exposure in Mice (Non-irradiated vs. Irradiated)). In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a within 5 hours to within 48 hours post-exposure. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1 within 23 hours post-exposure. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 within 5-12 hours post-exposure. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1a within 48 hours post-exposure.

In some embodiments, the method is a quantitative (dose) assessment of radiation exposure, in which the actual dose of radiation exposure or a range of exposure is determined (*e.g.*, 0 grays (Gy), 1 Gy, 2 Gy, 3 Gy, 4 Gy, 5 Gy, 6 Gy, 7 Gy, 8 Gy, and so forth). In some embodiments of the quantitative assessment, the one or more biomarkers comprise or consist

of Ccng1 and Cdkn1a (see, for example, Table 5 – Predicted Dose based on Multiple Linear Regression Analysis).

In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b, wherein a level or concentration of the biomarker(s) above a pre-determined threshold is indicative of ionizing radiation exposure of equal to or greater than 2 Gy in the subject (*e.g.*, within 5 hours, 12 hours, 24 hours, or 48 hours post-exposure), and wherein a level or concentration of the biomarker(s) below a pre-determined threshold is indicative of ionizing radiation exposure of less than 2 Gy in the subject (see, for example, Table 6 – Class Prediction of Dosing Groups in Mice).

In some embodiments of the methods of the invention (*e.g.*, the method of identifying a mammalian subject exposed to ionizing radiation, and the method of identifying an expression profile), radiation exposure is detected by screening for the presence or elevated levels of one or more polypeptides from among the biomarkers Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b, or their encoding polynucleotides, in a blood sample or blood derived sample. Optionally, multiple samples can be obtained from the subject over time and evaluated using the methods of the invention.

Optionally, in the methods of the invention, the quantification (measurement of the level of biomarker polypeptides or encoding nucleic acids) can be used to determine the time or estimated time that the subject was previously exposed to ionizing radiation (*i.e.*, a time-dependent assessment).

Optionally, in the methods of the invention, the subject's sample can be obtained and biomarker nucleic acid and/or protein levels determined by the subject (*i.e.*, as a self-administered test or assay).

Optionally, the methods further comprise verifying that the subject is suffering from the radiation exposure detected (*e.g.*, by assessing the presence of one or more signs or symptoms of radiation exposure, or detecting additional biomarkers of radiation exposure or radiation sickness, or by other confirmatory diagnostic procedures), verifying the amount of exposure, and/or treating the subject for the radiation exposure detected. In addition to a complete physical examination and monitoring clinical signs and symptoms (*e.g.*, vomiting), diagnostic procedures for radiation exposure and/or dosimetry may include, for example, physical dosimetry badges, and laboratory tests such as chromosome aberration cytogenetics and absolute lymphocyte count. Treatments for radiation exposure include, but are not limited to, decontamination, treatment of damaged bone marrow with granulocyte colony-stimulating factor (GC-SF) such as filgrastim (Neupogen) and pegfilgrastim (Neulasta),

transfusions of red blood cells or blood platelets, treatment for internal contamination by specific radionuclides uptake inhibitors or chelating agents such as potassium iodide, Prussian blue, or diethylenetriamine pentaacetic acid (DTPA), supportive treatment for bacterial infections, headache, fever, diarrhea, nausea and vomiting, and dehydration, as well as end-of-life care.

The present invention also relates to kits that may be used for carrying out the methods of the invention. In some embodiments, the kit comprises an array probe (also referred to as a “probe array” or simply an “array”) of nucleic acids attached to a substrate (*e.g.*, solid support), wherein the nucleic acids of the array comprise or consist of one or more oligonucleotides that will hybridize to one or more target nucleic acid molecules of biomarkers from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b (*e.g.*, hybridizing to one or more target nucleic acid sequences encoding the one or more biomarker polypeptides, or hybridizing to a complement thereof). Preferably, the one or more oligonucleotides will hybridize to the nucleic acid molecules under stringent hybridization conditions. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b. In some embodiments, the substrate has no more than 500 oligonucleotides attached to it. In some embodiments, the target nucleic acid sequence bound (hybridized) by each oligonucleotide is unique among a plurality of oligonucleotides attached to the substrate.

Other kits of the invention may comprise primers (*e.g.*, one or more primer pairs) for amplifying target nucleic acid sequences of one or more biomarkers from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

In another aspect, the present invention relates to a device for the rapid detection of one or more of the biomarkers (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) in a blood

sample or blood derived sample. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some
5 embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

Preferably, the device is a lateral flow device. In one embodiment, the device comprises an application zone for receiving a sample of blood; a labeling zone containing a
10 binding agent that binds to a biomarker in the sample; and a detection zone where a biomarker-bound binding agent is retained to give a signal, wherein the signal given for a sample from a subject with a biomarker level lower than a threshold concentration is different from the signal given for a sample from a subject with a biomarker level equal to or greater than a threshold concentration.

In another aspect, the invention relates to a test for radiation biomarkers in blood, similar to currently available in-home diabetic testing equipment, such as blood glucose testing strips that involve collecting blood for the test, most often by pricking the fingertip with a lancet, and squeezing a drop of blood out of the finger. The finger is then pressed against the diabetic testing strip, allowing measurement of blood glucose either with an
15 electronic blood glucose meter or testing strip color chart. Such tests can be used by subjects at home, at a place of employment, in a physicians' office, or at a patient's bedside, *e.g.*, at a health care facility. In one embodiment, the test is a method for detecting and, optionally measuring, one or more radiation biomarkers comprising or consisting of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b in a blood sample or blood derived sample, comprising: (a)
20 obtaining a sample of blood from a subject; (b) contacting the sample with a binding agent that binds to any biomarker in the sample; (c) separating biomarker-bound binding agent; (d) detecting a signal associated with the separated binding agent from (c); and (e) comparing the signal detected in step (d) with a reference signal which corresponds to the signal given by a sample from a subject with a biomarker level equal to a threshold concentration. The test can
25 be a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated), a quantitative (dose) assessment of radiation exposure (and, optionally, for two or more time points post-exposure *i.e.*, time-dependent and/or dose-dependent), or both a qualitative and quantitative assessment. In some embodiments, the test is a qualitative assessment of radiation exposure (distinguishing irradiated versus non-irradiated samples),
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wherein a level or concentration of the biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject, and wherein a level or concentration of the biomarker below a pre-determined threshold is indicative of a lack of ionizing radiation exposure in the subject. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

In some embodiments of the various aspects of the invention (*e.g.*, methods, kits (arrays, primers), devices), the biomarkers correspond to one or more nucleic acid sequences of SEQ ID NOs: 1-25, or polypeptides encoded by such nucleic acids. For example, in some embodiments, Bbc3 is SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7; Ccng1 is SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; Cdkn1a is SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14; Serpine1 is SEQ ID NO: 21 or SEQ ID NO: 22; and/or Tnfrsf10b is SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25.

In the various aspects of the invention (*e.g.*, methods, kits (arrays, primers), devices), the ionizing radiation exposure may occur in potentially any setting in which ionizing radiation exposure is known to occur or there is a risk of occurrence, *e.g.*, medical treatment (*e.g.*, patients receiving total body irradiation), industrial accident (*e.g.*, nuclear power plant or laboratory), environmental exposure, terrorist-driven radiological and nuclear attacks (*e.g.*, warfare).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a patient treatment schema. Patients received fractionated total body irradiation (TBI) prior to bone marrow transplantation. TBI was administered in two daily 2 Gy fractions for 3 consecutive days (12 Gy total). Whole blood for gene expression analysis was collected prior to initiation of TBI and at three time points throughout the treatment regimen.

Figure 2 shows gene expression in healthy volunteers with no treatment (□) (n=9), and in patients at baseline (prior to irradiation) (▤) (n=4), 5 hours (▥) (n=4), 23 hours (▦) (n=4), and 48 hours (▧) (n=4) following the first dose of irradiation. The total body accumulated dose received at 5, 23, and 48 hours was 2 Gy, 4 Gy, and 8 Gy, respectively. Data are presented as mean ± SEM.

Figure 3 shows expression of A) Bbc3, B) Ccng1, C) Cdkn1a, D) Serpine1, and E) Tnfrsf10b in mice at 0 hr (baseline), and 5, 12, 23, and 48 hours following mock (□) (n=14 at each time point) or total body irradiation at doses of 1 (▤), 2 (■), or 6 Gy (▨) (n=8 at each time point for each treated group). Significance was determined using One-way ANOVA followed by Tukey's Multiple Comparison test (*p<0.05, **p<0.01, ***p<0.001). Data are presented as mean ± SEM.

Figure 4 shows a comparison of actual dose vs. predicted dose in the A) training set and B) validation set of mice. Predicted dose was determined based on the multiple linear regression model developed using expression of Ccng1 and Cdkn1a in the training set ($y = -0.7674 + 0.6343 \cdot \text{Ccng1} + 0.0804 \cdot \text{Cdkn1a}$).

Figure 5 shows a flow diagram demonstrating how the materials and methods of the invention can be used to facilitate rapid triage of individuals in need of immediate medical intervention following a radiological event, thereby optimizing the use of available medical resources and to improve treatment and survival. The embodiment shown in Figure 5 utilizes gene expression analysis of radiation biomarkers of the invention to identify and differentiate subjects exposed to radiation from subjects not exposed to radiation, and further determining the absorbed dose of those exposed.

DETAILED DISCLOSURE OF THE INVENTION

Dose assessment following radiological disasters is imperative to decrease mortality through rationally directed medical intervention. The goal of the study described herein was to identify biomarkers capable of qualitative (non-irradiated/irradiated) and/or quantitative (dose) assessment of radiation exposure. Using real-time quantitative PCR, biodosimetry genes were identified in blood samples from cancer patients undergoing total body irradiation. Time (5, 12, 23, 48 hours) and dose (0-8 Gy) dependent changes in gene expression were examined in C57BL/6 mice. A training set was used to derive weighted voting classification algorithms (non-irradiated/irradiated) and continuous regression (dose assessment) models which were tested in a separate validation set of mice. Of eight biodosimetry genes identified in cancer patients (ACTA2, BBC3, CCNG1, CDKN1A, GADD45A, MDK, SERPINE1, TNFRSF10B), expression of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b was significantly (p<0.05) increased in irradiated mice. Ccng1 and Cdkn1a expression segregated irradiated mice from controls with an accuracy, specificity and sensitivity of 96.3, 100.0, 94.4%, respectively, at 48 hours. Multiple linear regression analysis predicted doses for the 0, 1, 2, 4, 6, and 8 Gy treatment groups as 0.0 ± 0.2 , 1.6 ± 1.0 ,

2.9±1.4, 5.1±2.0, 5.3±0.7, and 10.5±5.6 Gy, respectively. Results suggest that gene expression analysis could be incorporated into biodosimetry protocols for qualitative and quantitative assessment of radiation exposure.

The present invention relates to a method for the detection, quantification, and monitoring of radiation exposure, by detecting in a blood sample or blood derived sample from a subject at least one biomarker for radiation exposure identified herein, such as Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. The biomarkers may be detected and, optionally, measured using an agent that detects or binds to the biomarker protein (such as antibodies specifically reactive with the biomarker protein or a portion thereof), or an agent that detects or binds to encoding nucleic acids.

Figure 5 shows a flow diagram demonstrating how the materials and methods of the invention can be used to facilitate rapid triage of individuals in need of immediate medical intervention following a radiological event, thereby optimizing the use of available medical resources and to improve treatment and survival. Potential sources of high-dose radiation include, for example, an accident at a nuclear industrial facility, attack on a nuclear industrial facility, detonation of a small radioactive device, detonation of a conventional explosive device that disperses radioactive material (dirty bomb), and detonation of a standard nuclear weapon. In addition to detection of radiation exposure and dose determination from high-dose radiation from catastrophic events, the materials and methods of the invention can be used to monitor exposure to individuals before, during, and after radiation-dependent medical treatments and diagnostic procedures involving low or intermediate dose radiation or local or focused radiation (*e.g.*, X-ray examinations or other imaging procedures, or radiation therapy for treatment of cancer), and the materials and methods of the invention may be used to detect radiation exposure and determine absorbed dose in these contexts as well. Radiation exposure by patients, as well as medical personnel can be monitored. Radiation exposure can also conceivably occur through the malfunctioning or defect of an implant, resulting in internal contamination, and detection of exposure and dose determination can be carried out in this instance as well.

The present invention concerns materials and methods for detecting a radiation exposure and measuring the dose of radiation exposure received by the subject from which a sample has been obtained. The polypeptides and/or nucleic acid molecules (*e.g.*, DNA or mRNA) encoding the polypeptides disclosed herein (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) can be used as molecular markers for radiation exposure and biodosimetry. The biomarkers of the invention include, but are not limited to, Bbc3, Ccng1, Cdkn1a, Serpine1,

and Tnfrsf10b. Protein and nucleotide sequences of biomarker proteins and nucleic acids encoding them can be found at numerous publicly available sequence databases including GenBank. In one embodiment, the biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from one, two, three, four, or all five of Bbc3, Ccng1, Cdkn1a, Serpin1, and Tnfrsf10b. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

Radiation biomarkers are molecules such as polynucleotides or polypeptides found in the body the presence or absence (*e.g.*, elevation or reduction) of which are associated with radiation exposure and, preferably, radiation dose determination (*e.g.*, dose-dependent elevation or depletion). Measurement or identification of radiation biomarkers can be useful in patient diagnosis or clinical management of radiation exposure. They can be products of the blood cells themselves, or of the body in response to irradiation. As with other radiation biomarkers, the biomarkers described herein can be used for a variety of purposes, such as: screening a healthy population or a high risk population for radiation exposure; making a diagnosis of radiation exposure (such as radiation overdose); determining the prognosis of a subject; and monitoring the course in a subject in remission or while receiving surgery, radiation, chemotherapy, or other treatment. Thus, levels of these biomarkers in the blood can be used to detect and/or monitor the occurrence and extent of radiation exposure throughout a course of treatment, for example, and can be used in predicting therapeutic and prognostic outcome. In addition to monitoring an individual's or population's exposure to radiation and the radiation dose received during acute or chronic radiation treatment, the radiation biomarkers can be used to monitor an individual or population's exposure following a known radiation exposure or suspected radiation exposure.

One aspect of the invention concerns a method for identifying a mammalian subject exposed to radiation, comprising detecting the presence of and/or quantifying the level of at least one biomarker selected from among Bbc3, Ccng1, Cdkn1a, Serpin1, and Tnfrsf10b in a blood sample or blood derived sample from the subject, wherein the presence of the biomarker, or a level (*e.g.*, concentration) of the biomarker above a pre-determined threshold is indicative of radiation exposure in the subject. In some embodiments, the one or more

biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some
5 embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

In one embodiment of a method of the invention, the detecting comprises: (a) contacting a blood sample or blood derived with a binding agent (or binding agents) that binds the biomarker protein (or biomarker proteins) to form a complex (or complexes); and
10 (b) detecting the complex(es); and optionally correlating the detected complex(es) to the amount of biomarker protein(s) in the sample, wherein the presence of one or more biomarkers, or the presence of elevated levels biomarker protein(s), is indicative of ionizing radiation exposure. In a specific embodiment, the binding agent is an aptamer or peptide or antibody. In a specific embodiment, the binding agent for detecting of step (b) further
15 comprises a label linked or incorporated onto the agent. In one embodiment, the detecting comprises using ELISA-based immunoenzymatic detection. In another embodiment, the detecting comprises using Western blotting or radioimmunoassays (RIA).

In another embodiment, the biomarker protein (or proteins) is detected using electrophoretic, chromatographic, or spectroscopy methods, or a combination thereof. In one
20 embodiment, proteins can be identified using 2-D gel electrophoresis of a sample followed by digestion of the separated proteins and mass spectrometry of peptides. In another embodiment, proteins or peptides can be identified by amino acid sequencing all or a portion of the molecule. In a specific embodiment, the mass spectrometry is tandem mass spectrometry (MS/MS). In a further embodiment, the mass spectrometry is carried out using
25 matrix-assisted laser desorption/ionization (MALDI), such as MALDI-TOF. In a still further embodiment, proteins or peptides are identified using liquid chromatography (LC) methods, such as high pressure LC (HPLC).

Antibodies against numerous biomarker proteins of the invention are commercially available. Antibodies can also be readily prepared using standard and routine procedures
30 known in the art.

Optionally, the methods of the invention further comprise detecting and/or quantifying one or more additional biomarkers of radiation exposure in the same blood sample or blood derived sample, or a different blood sample or blood derived sample, or a same or different biological sample (*e.g.*, tissue such as a biopsy), obtained from the same

subject, before, during, or after said detecting of the biomarker(s) of the invention is carried out on the sample. In this way, one or more biomarkers of the invention can be used as part of a panel of biomarkers utilized in surveillance protocols for detecting radiation exposure and, optionally, radiation dose. For example, a panel of 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more markers could be utilized.

In some embodiments, the detecting is performed at two or more time points or intervals. For example, the detecting can be performed at various time points or intervals as part of monitoring of the subject before, during, or after radiation treatment (*e.g.*, for treatment of cancer) or some other radiation exposure.

Optionally, the methods of the invention further comprise comparing the level of one or more radiation biomarkers in a blood sample, or blood derived sample, with the level of the radiation biomarker present in a normal control sample, wherein a higher level of biomarker in the test sample as compared to the level in the normal control sample is indicative of the presence of radiation exposure.

In some embodiments, the subject exhibits no signs or symptoms of radiation sickness or radiation poisoning at the time the detecting of the radiation biomarker(s) is carried out. In other embodiments, the subject exhibits one or more signs or symptoms of radiation exposure. Signs and symptoms of radiation sickness include, but are not limited to, signs and symptoms resulting from an acute absorbed dose of about 1 to 2 Gy, *i.e.*, mild radiation sickness (*e.g.*, nausea and vomiting within 24 to 48 hours, headache, fatigue, weakness); signs and symptoms resulting from an acute absorbed dose of about 2 to 3.5 Gy, *i.e.*, moderate radiation sickness (*e.g.*, nausea and vomiting within 12 to 24 hours, fever, hair loss, infections, vomiting blood, bloody stool, poor wound healing, any signs and symptoms associated with a lower absorbed dose); signs and symptoms resulting from an absorbed dose of about 3.5 to 5.5 Gy, *i.e.*, severe radiation sickness (*e.g.*, nausea and vomiting less than one hour after exposure to radiation, diarrhea, high fever, any signs and symptoms associated with a lower absorbed dose); and signs and symptoms resulting from an absorbed dose greater than about 5.5 to 8 Gy, *i.e.*, very severe radiation sickness (*e.g.*, nausea and vomiting less than 30 minutes after exposure to radiation, dizziness, disorientation, low blood pressure (hypotension), and any signs and symptoms associated with a lower absorbed dose).

The subject invention also concerns methods for prognostic evaluation of a subject having, or suspected of having, radiation exposure, comprising: a) determining the level of one or more radiation biomarkers of the present invention from among Bbc3, Ccng1, Cdkn1a, Serpin1, and Tnfrsf10b in a blood sample or blood derived sample obtained from the

subject; b) comparing the level determined in step (a) to a level or range of the one or more radiation biomarkers known to be present in a blood sample or blood derived sample obtained from a normal subject not exposed to radiation; and c) determining the prognosis of the subject based on the comparison of step (b), wherein a high level of the one or more radiation biomarkers in step (a) indicates a higher absorbed dose of radiation and, therefore, a poorer prognosis. In one embodiment, the radiation biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

The terms “detecting” or “detect” include assaying or otherwise establishing the presence or absence of the target radiation biomarker (one or more encoding nucleic acid sequences or gene products (polypeptides) of the invention (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b), subunits thereof, or combinations of agent bound targets, and the like, or assaying for, interrogating, ascertaining, establishing, or otherwise determining one or more factual characteristics of radiation sickness or poisoning. The term encompasses diagnostic, prognostic, and monitoring applications for one or more of the radiation biomarkers of the invention (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) and, optionally, in combination with other radiation biomarkers. The term encompasses quantitative, semi-quantitative, and qualitative detection methodologies. In embodiments of the invention involving detection of one or more polypeptides (as opposed to nucleic acid molecules encoding the polypeptides), the detection method may be, for example, an ELISA-based method. Preferably, in the various embodiments of the invention, the detection method provides an output (*i.e.*, readout or signal) with information concerning the presence, absence, or amount of the radiation biomarker(s) in a blood sample, or blood derived sample, from a subject. For example, the output may be qualitative (*e.g.*, “positive” or “negative”; or “yes” or “no”; or “irradiated” or “non-irradiated”), or quantitative (*e.g.*, a concentration such as nanograms per milliliter, or gray unit (Gy)), and/or unit of time post-exposure (*e.g.*, 5 or 6 hours post-radiation exposure, 12 hours post-radiation exposure, 23 or 24 hours post-radiation exposure, 48 hours post-radiation exposure).

In one embodiment, the invention relates to a method for detecting radiation exposure in a subject by quantitating one or more radiation biomarker polypeptides of the invention (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b), or their encoding nucleic acids (DNA or RNA), in a blood sample or blood derived sample from the subject, comprising (a) contacting
5 (reacting) the sample with an antibody specific for the radiation biomarker polypeptide(s) which is directly or indirectly labeled with a detectable substance; and (b) detecting the detectable substance. In one embodiment, the radiation biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the one or more biomarkers
10 comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers
15 from among Ccng1 and Tnfrsf10b.

In one aspect, the invention relates to a method for diagnosing and/or monitoring radiation exposure in a subject by quantitating one or more radiation biomarker polypeptides of the invention in a blood sample or blood derived sample from the subject, comprising (a)
20 reacting the sample with an antibody or antibodies specific for the biomarker or biomarkers which are directly or indirectly labeled with a detectable substance; and (b) detecting the detectable substance. In one embodiment, the biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the one or more biomarkers comprise or consist of one or
25 two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

Some embodiments of the methods of the invention involve (a) contacting a blood
30 sample, or blood derived sample, from a subject with an antibody or antibodies specific for the biomarker or biomarker polypeptides of the invention which are directly or indirectly labeled with an enzyme; (b) adding a substrate for the enzyme wherein the substrate is selected so that the substrate, or a reaction product of the enzyme and substrate, forms fluorescent complexes; (c) quantitating the biomarker(s) in the sample by measuring

fluorescence of the fluorescent complexes; and (d) comparing the quantitated levels to that of a standard. In one embodiment, the biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b. In some embodiments, the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a. In some embodiments, the one or more biomarkers comprise or consist of Bbc3 and Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1. In some embodiments, the one or more biomarkers comprise or consist of Ccng1 and Cdkn1. In some embodiments, the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

A specific embodiment of the invention comprises:

A method for detecting radiation exposure, comprising (a) incubating a blood sample or blood derived sample with a first antibody specific for at least one radiation biomarker polypeptide of the invention which is directly or indirectly labeled with a detectable substance, and a second antibody specific for the biomarker polypeptide which is immobilized;

(b) separating the first antibody from the second antibody to provide a first antibody phase and a second antibody phase;

(c) detecting the detectable substance in the first or second antibody phase thereby quantitating the biomarker in the sample; and

(d) comparing the quantitated biomarker with a standard.

A standard used in a method of the invention may correspond to biomarker levels obtained for samples from healthy control subjects, from subjects with benign disease, subjects with a known extent of radiation exposure, or from other samples of the subject, or other reference samples. Increased levels of a radiation biomarker as compared to the standard may be indicative of radiation exposure. In one embodiment, the biomarker is one or more polypeptides, and/or nucleic acids encoding the polypeptides, selected from Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

The invention also contemplates using the methods, devices, and kits described herein in conjunction with one or more additional radiation biomarkers. Therefore, the invention contemplates a method for analyzing a blood sample or blood derived sample, for the presence of a radiation biomarker of the invention (a polypeptide or encoding nucleic acid molecule selected from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) and analyzing the same sample, or another sample from the same subject, for other markers that are specific indicators of radiation exposure and/or radiation sickness or poisoning. The one

or more additional biomarkers may be detected before, during, and/or after detection of the one or more radiation biomarkers of the invention is carried out. The methods, devices, and kits described herein may be modified by including agents to detect the additional markers, or nucleic acids encoding the markers.

5 In one embodiment, the one or more additional biomarker in the methods, devices, and kits described herein is one or more additional diagnostic and/or prognostic biomarkers for radiation exposure. In one embodiment, the additional radiation biomarker is one or more selected from Table 1B herein. In another embodiment, the additional radiation biomarker is one or more disclosed in WO 2009/145830 (Chute *et al.*), which is incorporated herein by
10 reference in its entirety.

As indicated above, the present invention provides a method for detecting and/or quantifying radiation exposure in a subject by detecting and/or measuring a radiation biomarker of the invention in a blood sample or blood derived sample from the subject. In one embodiment, the method comprises contacting the sample with an antibody specific for
15 the biomarker polypeptide which is directly or indirectly labeled with a detectable substance, and detecting the detectable substance.

The methods, devices, and kits of the invention can be used for the detection of either an over-abundance or an under-abundance of one or more radiation biomarkers relative to a non-exposure state, or relative to a known exposure state, or the presence of a modified (*e.g.*,
20 less than full length) radiation biomarker which correlates with a disorder state (*e.g.*, radiation sickness or poisoning), or a progression toward a disorder state.

The methods, devices, and kits of the invention can be used in the detection and, optionally, determination of the absorbed radiation dose when the subject is symptomatic or asymptomatic of radiation exposure, and for monitoring and evaluating the prognosis of
25 radiation sickness or poisoning progression and mortality. Depending upon the particular radiation biomarker of the invention, increased levels or decreased levels of detected biomarker blood sample or blood derived sample compared to a standard (*e.g.*, levels for no or normal radiation exposure, levels for benign disorders, or other reference levels) may be indicative of extent of radiation exposure (dose and/or duration) and radiation sickness or
30 poisoning progression and mortality.

The terms “sample”, “biological sample”, and the like refer to a type of material known to or suspected of expressing or containing a radiation biomarker, such as a blood sample or a sample derived from blood that has undergone separation, purification, or preparative steps. The test sample can be used directly as obtained from the source or

following a pretreatment to modify the character of the sample. The sample can be derived from any biological source, such as tissues or extracts, including cells and physiological fluids, such as, for example, whole blood, plasma, serum, peritoneal fluid, ascites, and the like. The sample can be obtained from mammals, most preferably humans. The sample can be pretreated by any method and/or can be prepared in any convenient medium that does not interfere with the assay. The sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, applying one or more protease inhibitors to samples, and the like. Sample treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, the addition of reagents, and the like.

The presence and quantity of a radiation biomarker of the invention or nucleic acids (DNA or RNA) encoding the polypeptides may be detected in human or non-human mammalian blood samples or blood derived samples.

In several embodiments of the invention, the method described herein is adapted for determining the dose of radiation exposure by quantitating a biomarker of the invention in blood samples or blood derived samples from a subject. Preferably, the amount of biomarker quantitated in a sample from a subject being tested is compared to levels quantitated for another sample or an earlier sample from the subject, or levels quantitated for a control sample. Levels for control samples from healthy subjects may be established by prospective and/or retrospective statistical studies. Healthy subjects who have no clinically evident radiation exposure, or disease or abnormalities, may be selected for statistical studies. Diagnosis may be made by a finding of statistically different levels of one or more biomarkers compared to a control sample or previous levels quantitated for the same subject.

The radiation biomarkers of the invention include all homologs, naturally occurring allelic variants, isoforms and precursors of the human or non-human mammalian molecules. In general, naturally occurring allelic variants of human biomarkers will share significant sequence homology (70-90%) to other sequences. Allelic variants may contain conservative amino acid substitutions or will contain a substitution of an amino acid from a corresponding position in a homologue. Preferably, the homologs or variants of the biomarkers selected for detection will be appropriately matched to the species of the mammalian subject (species) from which the sample is obtained (human sequences within a human subject).

In some embodiments of the various aspects of the invention (*e.g.*, methods, kits (arrays, primers), devices), the biomarkers correspond to one or more nucleic acid sequences of SEQ ID NOs: 1-25, or polypeptides encoded by such nucleic acids. For example, in some embodiments, Bbc3 is SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7;

Ccng1 is SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; Cdkn1a is SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14; Serpin1 is SEQ ID NO: 21 or SEQ ID NO: 22; and/or Tnfrsf10b is SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25.

The terms “subject”, “individual”, and “patient” are used interchangeably herein to refer to a mammal, which may be exposed to ionizing radiation. The subject may be male or female. The subject may be any age (adult, child, *etc.*). The term includes dogs, cats, and horses. The term also includes primates such as apes, chimps, monkeys, and humans.

Agents that are capable of detecting radiation biomarkers of the invention in the samples of subjects include those that interact or bind with the polypeptide or the nucleic acid molecule (*e.g.*, nucleic acid encoding the polypeptide). Examples of such agents (also referred to herein as binding agents) include, but are not limited to, antibodies or fragments thereof that bind the polypeptide, polypeptide binding partners, and nucleic acid molecules that hybridize to the nucleic acid molecules encoding the polypeptides. Preferably, the binding agent is labeled with a detectable substance (*e.g.*, a detectable moiety). The binding agent may itself function as a label.

Biomarker Antibodies

Antibodies specific for radiation biomarkers of the invention that are used in the methods, devices, and kits of the invention may be obtained from scientific or commercial sources. Alternatively, the isolated native polypeptides or recombinant polypeptides may be utilized to prepare antibodies, monoclonal or polyclonal antibodies, and immunologically active fragments (*e.g.*, a Fab or (Fab)₂ fragment), an antibody heavy chain, an antibody light chain, humanized antibodies, a genetically engineered single chain F_v molecule (Ladne *et al.*, U.S. Patent No. 4,946,778), or a chimeric antibody, for example, an antibody which contains the binding specificity of a murine antibody, but in which the remaining portions are of human origin. Antibodies, including monoclonal and polyclonal antibodies, fragments and chimeras, may be prepared using methods known to those skilled in the art. Preferably, antibodies used in the methods of the invention are reactive against biomarkers of the invention if they bind with a K_a of greater than or equal to 10⁷ M. In a sandwich immunoassay of the invention, mouse polyclonal antibodies and rabbit polyclonal antibodies can be utilized, for example.

In order to produce monoclonal antibodies, a host mammal is inoculated with a protein or peptide representing a radiation biomarker of the invention and then boosted. Spleens are collected from inoculated mammals a few days after the final boost. Cell

suspensions from the spleens are fused with a tumor cell in accordance with the general method described by Kohler and Milstein (*Nature*, 1975, 256:495-497). In order to be useful, a peptide fragment must contain sufficient amino acid residues to define the epitope of the biomarker molecule being detected.

5 If the fragment is too short to be immunogenic, it may be conjugated to a carrier molecule. Some suitable carrier molecules include keyhole limpet hemocyanin and bovine serum albumin. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule. The peptide fragments may be synthesized by methods known in the art. Some
10 suitable methods are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984).

Purification of the antibodies or fragments can be accomplished by a variety of methods known to those skilled in the art including, precipitation by ammonium sulfate or sodium sulfate followed by dialysis against saline, ion exchange chromatography, affinity or
15 immunoaffinity chromatography as well as gel filtration, zone electrophoresis, *etc.* (Goding in, *Monoclonal Antibodies: Principles and Practice*, 2d ed., pp. 104-126, Orlando, Fla., Academic Press). It is preferable to use purified antibodies or purified fragments of the antibodies having at least a portion of a biomarker binding region, including such as Fv, F(ab')₂, Fab fragments (Harlow and Lane, 1988, *Antibody* Cold Spring Harbor) for the
20 detection of the biomarker(s) in the blood samples or blood derived samples of subjects (*e.g.*, subjects suspected of radiation exposure or at risk of radiation exposure).

For use in detection, dose determination, and/or monitoring of radiation exposure, the purified antibodies can be covalently attached, either directly or via linker, to a compound which serves as a reporter group to permit detection of the presence of the biomarker. A
25 variety of different types of substances can serve as the reporter group, including but not limited to enzymes, dyes, radioactive metal and non-metal isotopes, fluorogenic compounds, fluorescent compounds, *etc.* Methods for preparation of antibody conjugates of the antibodies (or fragments thereof) of the invention useful for detection, monitoring are described in U.S. Patent Nos. 4,671,958; 4,741,900 and 4,867,973.

30 In one aspect of the invention, preferred binding epitopes may be identified from a known biomarker gene sequence and its encoded amino acid sequence and used to generate antibodies to the biomarker with high binding affinity. Also, identification of binding epitopes on the biomarker polypeptide can be used in the design and construction of preferred antibodies. For example, a DNA encoding a preferred epitope on a biomarker polypeptide

may be recombinantly expressed and used to select an antibody which binds selectively to that epitope. The selected antibodies then are exposed to the sample under conditions sufficient to allow specific binding of the antibody to the specific binding epitope on the biomarker and the amount of complex formed then detected. Specific antibody methodologies are well understood and described in the literature. A more detailed description of their preparation can be found, for example, in Practical Immunology, Butt, W. R., ed., Marcel Dekker, New York, 1984.

The present invention also contemplates the detection of radiation biomarker antibodies. Thus, detection of antibodies to the radiation biomarkers of the invention in blood samples or blood derived samples of a subject may enable the detection of radiation exposure and dose determination, and is also contemplated within the scope of the invention.

Protein Binding Assays

Antibodies specifically reactive with the radiation biomarkers disclosed or their derivatives, such as enzyme conjugates or labeled derivatives, may be used to detect radiation biomarkers in various biological samples, for example they may be used in any known immunoassays which rely on the binding interaction between an antigenic determinant of a protein and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassay (*e.g.*, ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, and histochemical tests.

An antibody specific for a biomarker of the invention can be labeled with a detectable substance and localized in biological samples such as blood based upon the presence of the detectable substance. Examples of detectable substances include, but are not limited to, the following radioisotopes (*e.g.*, ^3H , ^{14}C , ^{35}S , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), luminescent labels such as luminol; enzymatic labels (*e.g.*, horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase), biotinyl groups (which can be detected by marked avidin, *e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against the biomarker. By way of

example, if the antibody having specificity against a biomarker is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labeled with a detectable substance.

Methods for conjugating or labeling the antibodies discussed above may be readily accomplished by one of ordinary skill in the art. (See, for example, Imman, *Methods In Enzymology*, Vol. 34, Affinity Techniques, Enzyme Purification: Part B, Jakoby and Wichek (eds.), Academic Press, New York, p. 30, 1974; and Wilchek and Bayer, "The Avidin-Biotin Complex in Bioanalytical Applications," *Anal. Biochem.*, 1988, 171:1-32, regarding methods for conjugating or labeling the antibodies with an enzyme or ligand binding partner).

Time-resolved fluorometry may be used to detect a signal. For example, the method described in Christopoulos T.K. and Diamandis E.P., *Anal. Chem.*, 1992:64:342-346 may be used with a conventional time-resolved fluorometer.

Therefore, in accordance with an embodiment of the invention, a method is provided wherein an antibody to a radiation biomarker of the invention is labeled with an enzyme, a substrate for the enzyme is added wherein the substrate is selected so that the substrate, or a reaction product of the enzyme and substrate, forms fluorescent complexes with a lanthanide metal. A lanthanide metal is added and the biomarker is quantitated in the sample by measuring fluorescence of the fluorescent complexes. The antibodies specific for the biomarkers may be directly or indirectly labeled with an enzyme. Enzymes are selected based on the ability of a substrate of the enzyme, or a reaction product of the enzyme and substrate, to complex with lanthanide metals such as europium and terbium. Examples of suitable enzymes include alkaline phosphatase and beta-galactosidase. Preferably, the enzyme is alkaline phosphatase. The biomarker antibodies may also be indirectly labeled with an enzyme. For example, the antibodies may be conjugated to one partner of a ligand binding pair, and the enzyme may be coupled to the other partner of the ligand binding pair. Representative examples include avidin-biotin, and riboflavin-riboflavin binding protein. Preferably the antibodies are biotinylated, and the enzyme is coupled to streptavidin.

In an embodiment of the method, antibody bound to a radiation biomarker of the invention in a sample is detected by adding a substrate for the enzyme. The substrate is selected so that in the presence of a lanthanide metal (*e.g.*, europium, terbium, samarium, and dysprosium, preferably europium and terbium), the substrate or a reaction product of the enzyme and substrate, forms a fluorescent complex with the lanthanide metal. Examples of enzymes and substrates for enzymes that provide such fluorescent complexes are described in U.S. Patent No. 5,312,922 to Diamandis. By way of example, when the antibody is directly or indirectly labeled with alkaline phosphatase, the substrate employed in the method may be

4-methylumbelliferyl phosphate, or 5-fluoropsalicyl phosphate. The fluorescence intensity of the complexes is typically measured using a time-resolved fluorometer, *e.g.*, a CyberFluor 615 Immoanalyzer (Nordion International, Kanata Ontario).

The sample, antibody specific for the radiation biomarker, or the biomarker itself, may be immobilized on a carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, liposomes, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, *etc.* The carrier may be in the shape of, for example, a tube, test plate, well, beads, disc, sphere, *etc.* The immobilized antibody may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

In accordance with an embodiment, the present invention provides a mode for determining the presence and, optionally, the amount of radiation biomarker in an appropriate sample such as blood by measuring the biomarker(s) by immunoassay. It will be evident to a skilled artisan that a variety of immunoassay methods can be used to measure the biomarkers of the invention. In general, a biomarker immunoassay method may be competitive or noncompetitive. Competitive methods typically employ an immobilized or immobilizable antibody to the biomarker (anti-biomarker such as anti-Serpine1) and a labeled form of the biomarker (such as labeled Serpine1). Sample biomarker and labeled biomarker compete for binding to anti-biomarker. After separation of the resulting labeled biomarker that has become bound to anti-biomarker (bound fraction) from that which has remained unbound (unbound fraction), the amount of the label in either bound or unbound fraction is measured and may be correlated with the amount of biomarker in the biological sample in any conventional manner, *e.g.*, by comparison to a standard curve.

Preferably, a noncompetitive method is used for the determination of one or more radiation biomarkers of the invention, with the most common method being the “sandwich” method. In this assay, two anti-biomarker antibodies, such as two anti-serpine1 antibodies, are employed. One of the anti-biomarker antibodies is directly or indirectly labeled (also referred to as the “detection antibody”) and the other is immobilized or immobilizable (also referred to as the “capture antibody”). The capture and detection antibodies can be contacted simultaneously or sequentially with the biological sample. Sequential methods can be accomplished by incubating the capture antibody with the sample, and adding the detection antibody at a predetermined time thereafter (sometimes referred to as the “forward” method);

or the detection antibody can be incubated with the sample first and then the capture antibody added (sometimes referred to as the “reverse” method). After the necessary incubation(s) have occurred, to complete the assay, the capture antibody is separated from the liquid test mixture, and the label is measured in at least a portion of the separated capture antibody phase or the remainder of the liquid test mixture. Generally, it is measured in the capture antibody phase since it comprises the biomarker bound by (“sandwiched” between) the capture and detection antibodies.

In a typical two-site immunometric assay for a biomarker, one or both of the capture and detection antibodies are polyclonal antibodies. The label used in the detection antibody can be selected from any of those known conventionally in the art. As with other embodiments of the protein detection assay, the label can be an enzyme or a chemiluminescent moiety, for example, or a radioactive isotope, a fluorophore, a detectable ligand (*e.g.*, detectable by a secondary binding by a labeled binding partner for the ligand), and the like. Preferably, the antibody is labeled with an enzyme that is detected by adding a substrate that is selected so that a reaction product of the enzyme and substrate forms fluorescent complexes. The capture antibody is selected so that it provides a mode for being separated from the remainder of the test mixture. Accordingly, the capture antibody can be introduced to the assay in an already immobilized or insoluble form, or can be in an immobilizable form, that is, a form which enables immobilization to be accomplished subsequent to introduction of the capture antibody to the assay. An immobilized capture antibody can comprise an antibody covalently or noncovalently attached to a solid phase such as a magnetic particle, a latex particle, a microtiter multi-well plate, a bead, a cuvette, or other reaction vessel. An example of an immobilizable capture antibody is an antibody that has been chemically modified with a ligand moiety, *e.g.*, a hapten, biotin, or the like, and that can be subsequently immobilized by contact with an immobilized form of a binding partner for the ligand, *e.g.*, an antibody, avidin, or the like. In an embodiment, the capture antibody can be immobilized using a species specific antibody for the capture antibody that is bound to the solid phase.

A particular sandwich immunoassay method of the invention employs two antibodies reactive against a biomarker of the invention, a second antibody having specificity against an antibody reactive against the biomarker labeled with an enzymatic label, and a fluorogenic substrate for the enzyme. In an embodiment, the enzyme is alkaline phosphatase (ALP) and the substrate is 5-fluorosalicyl phosphate. ALP cleaves phosphate out of the fluorogenic substrate, 5-fluorosalicyl phosphate, to produce 5-fluorosalicylic acid (FSA). 5-

Fluorosalicyclic acid can then form a highly fluorescent ternary complex of the form FSA-Tb(3+)-EDTA, which can be quantified by measuring the Tb³⁺ fluorescence in a time-resolved mode. Fluorescence intensity is typically measured using a time-resolved fluorometry as described herein.

5 The above-described immunoassay methods and formats are intended to be exemplary and are not limiting since, in general, it will be understood that any immunoassay method or format can be used in the present invention.

The protein detection methods, devices, and kits of the invention can utilize nanowire sensor technology (Zhen *et al.*, *Nature Biotechnology*, 2005, 23(10):1294-1301; Lieber *et al.*,
10 *Anal. Chem.*, 2006, 78(13):4260-4269, which are incorporated herein by reference) or microcantilever technology (Lee *et al.*, *Biosens. Bioelectron.*, 2005, 20(10):2157-2162; Wee *et al.*, *Biosens. Bioelectron.*, 2005, 20(10):1932-1938; Campbell and Mutharasan, *Biosens. Bioelectron.*, 2005, 21(3):462-473; Campbell and Mutharasan, *Biosens. Bioelectron.*, 2005, 21(4):597-607; Hwang *et al.*, *Lab Chip*, 2004, 4(6):547-552; Mukhopadhyay *et al.*, *Nano.*
15 *Lett.*, 2005, 5(12):2835-2388, which are incorporated herein by reference) for detection of one or more biomarkers of the invention in samples. In addition, Huang *et al.* describe a prostate specific antigen immunoassay on a commercially available surface plasmon resonance biosensor (*Biosens. Bioelectron.*, 2005, 21(3):483-490, which is incorporated herein by reference) which may be adapted for detection of one or more biomarkers of the
20 invention. High-sensitivity miniaturized immunoassays may also be utilized for detection of the biomarkers (Cesaro-Tadic *et al.*, *Lab Chip*, 2004, 4(6):563-569; Zimmerman *et al.*, *Biomed. Microdevices*, 2005, 7(2):99-110, which are incorporated herein by reference).

Nucleic Acids

25 Nucleic acids including naturally occurring nucleic acids, oligonucleotides, antisense oligonucleotides, and synthetic oligonucleotides that hybridize to target nucleic acids within biomarker genes or transcripts of the invention (*e.g.*, encoding biomarker polypeptides of the invention), are useful as agents to detect the presence of radiation biomarkers of the invention in blood samples or blood derived samples of subjects, preferably in blood samples or blood
30 derived samples from those exposed to radiation or at risk for radiation exposure. The present invention contemplates the use of nucleic acid sequences corresponding to the coding sequence of radiation biomarkers of the invention and to the complementary sequence thereof, as well as sequences complementary to the biomarker transcript sequences occurring further upstream or downstream from the coding sequence (*e.g.*, sequences contained in, or

extending into, the 5' and 3' untranslated regions) for use as agents for detecting the expression of radiation biomarkers of the invention in samples of subjects.

The preferred oligonucleotides for detecting the presence of radiation biomarkers of the invention in samples are those that are complementary to at least part of the cDNA sequence encoding the biomarker. These complementary sequences are also known in the art as "antisense" sequences. These oligonucleotides may be oligoribonucleotides or oligodeoxyribonucleotides. In addition, oligonucleotides may be natural oligomers composed of the biologically significant nucleotides, *i.e.*, A (adenine), dA (deoxyadenine), G (guanine), dG (deoxyguanine), C (cytosine), dC (deoxycytosine), T (thymine) and U (uracil), or modified oligonucleotide species, substituting, for example, a methyl group or a sulfur atom for a phosphate oxygen in the inter-nucleotide phosphodiester linkage. Additionally, these nucleotides themselves, and/or the ribose moieties may be modified.

The oligonucleotides may be synthesized chemically, using any of the known chemical oligonucleotide synthesis methods well described in the art. For example, the oligonucleotides can be prepared by using any of the commercially available, automated nucleic acid synthesizers. Alternatively, the oligonucleotides may be created by standard recombinant DNA techniques, for example, inducing transcription of the noncoding strand. The DNA sequence encoding the biomarker may be inverted in a recombinant DNA system, *e.g.*, inserted in reverse orientation downstream of a suitable promoter, such that the noncoding strand now is transcribed.

Although any length oligonucleotide may be utilized to hybridize to a target nucleic acid within biomarker genes or transcripts of the invention (*e.g.*, to a nucleic acid encoding a biomarker polypeptide), oligonucleotides typically within the range of 8-100 nucleotides are preferred. Most preferable oligonucleotides for use in detecting biomarkers in blood samples or blood derived samples are those within the range of 15-50 nucleotides.

In some embodiments, the substrate (*e.g.*, solid support) of the array of the invention has no more than 500 oligonucleotides attached to it. In some embodiments, the substrate has no more than 100 oligonucleotides attached to it. In some embodiments, the substrate has no more than 50 oligonucleotides attached to it. In some embodiments, the substrate has no more than 20 oligonucleotides attached to it. In some embodiments, the substrate has no more than 10 oligonucleotides attached to it. In some embodiments, the substrate has no more than 5 oligonucleotides attached to it. In some embodiments, the substrate has no more than 4 oligonucleotides attached to it. In some embodiments, the substrate has no more than 3 oligonucleotides attached to it. In some embodiments, the substrate has no more than 2

oligonucleotides attached to it. In some embodiments, the substrate has no more than 1 oligonucleotide attached to it.

When referring to hybridization of one nucleic to another, "low stringency conditions" means in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42 °C., followed by washing in 1X SSPE, 0.2% SDS, at 50 °C.; "moderate stringency conditions" means in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42 °C., followed by washing in 0.2X SSPE, 0.2% SDS, at 65 °C.; and "high stringency conditions" means in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42 °C., followed by washing in 0.1X SSPE, and 0.1% SDS at 65 °C. The phrase "stringent hybridization conditions" means low, moderate, or high stringency conditions.

The oligonucleotide selected for hybridizing to the biomarker nucleic acid molecule, whether synthesized chemically or by recombinant DNA technology, can be isolated and purified using standard techniques and then preferably labeled (*e.g.*, with ³⁵S or ³²P) using standard labeling protocols. Oligonucleotides can be attached or immobilized to a suitable solid support using methods known in the art.

The present invention also contemplates the use of oligonucleotide pairs (*e.g.*, primers) in polymerize chain reactions (PCR) to detect the expression of the biomarker in biological samples. The oligonucleotide pairs include a forward primer and a reverse primer.

The presence of biomarkers in a sample from a subject may be determined by nucleic acid hybridization, such as but not limited to Northern blot analysis, dot blotting, Southern blot analysis, fluorescence in situ hybridization (FISH), and PCR. Chromatography, preferably HPLC, and other known assays may also be used to determine messenger RNA levels of biomarkers in a sample.

Nucleic acid molecules encoding a biomarker of the present invention can be found in the biological fluids inside a biomarker-positive cell that is present in a biological sample under investigation, *e.g.*, blood. Nucleic acids encoding biomarkers can also be found directly (*i.e.*, cell-free) in the fluid or biological sample, *e.g.*, blood.

In one aspect, the present invention contemplates the use of nucleic acids as agents for detecting radiation biomarkers of the invention in blood samples or blood derived samples of subjects, wherein the nucleic acids are labeled. The nucleic agents may be labeled with a radioactive label, a fluorescent label, an enzyme, a chemiluminescent tag, a colorimetric tag or other labels or tags that are discussed above or that are known in the art.

In another aspect, the present invention contemplates the use of Northern blot analysis to detect the presence of biomarker mRNA in a blood sample or blood derived

sample. The first step of the analysis involves separating a sample containing biomarker nucleic acid by gel electrophoresis. The dispersed nucleic acids are then transferred to a nitrocellulose filter or another filter. Subsequently, the labeled oligonucleotide is exposed to the filter under suitable hybridizing conditions, *e.g.*, 50% formamide, 5 x SSPE, 2 x Denhardt's solution, 0.1% SDS at 42° C, as described in *Molecular Cloning: A Laboratory Manual*, Maniatis *et al.* (1982, CSH Laboratory). Other useful procedures known in the art include solution hybridization, dot and slot RNA hybridization, and probe based microarrays. Measuring the radioactivity of hybridized fragments, using standard procedures known in the art quantitates the amount of biomarker nucleic acid present in the sample of a subject.

Dot blotting involves applying samples containing the nucleic acid of interest to a membrane. The nucleic acid can be denatured before or after application to the membrane. The membrane is incubated with a labeled probe. Dot blot procedures are well known to the skilled artisan and are described more fully in U.S. Patent Nos. 4,582,789 and 4,617,261, the disclosures of which are incorporated herein by reference.

Polymerase chain reaction (PCR) is a process for amplifying one or more target nucleic acid sequences present in a nucleic acid sample using primers and agents for polymerization and then detecting the amplified sequence. The extension product of one primer when hybridized to the other becomes a template for the production of the desired specific nucleic acid sequence, and vice versa, and the process is repeated as often as is necessary to produce the desired amount of the sequence. The skilled artisan to detect the presence of desired sequence (U.S. Patent No. 4,683,195) routinely uses polymerase chain reaction.

A specific example of PCR that is routinely performed by the skilled artisan to detect desired sequences is reverse transcript PCR (RT-PCR; Saiki *et al.*, *Science*, 1985, 230:1350; Scharf *et al.*, *Science*, 1986, 233:1076). RT-PCR involves isolating total RNA from biological fluid, denaturing the RNA in the presence of primers that recognize the desired nucleic acid sequence, using the primers to generate a cDNA copy of the RNA by reverse transcription, amplifying the cDNA by PCR using specific primers, and detecting the amplified cDNA by electrophoresis or other methods known to the skilled artisan.

In a preferred embodiment, the methods of detecting biomarker nucleic acid in blood samples or blood derived samples of subjects include Northern blot analysis, dot blotting, Southern blot analysis, FISH, and PCR.

Devices

The methods of the invention can be carried out on a solid support. The solid supports used may be those which are conventional for the purpose of assaying an analyte in a biological sample, and are typically constructed of materials such as cellulose, polysaccharide such as Sephadex, and the like, and may be partially surrounded by a housing for protection and/or handling of the solid support. The solid support can be rigid, semi-rigid, flexible, elastic (having shape-memory), *etc.*, depending upon the desired application. Radiation biomarkers of the invention can be detected in a sample *in vivo* or *in vitro* (*ex vivo*). When, according to an embodiment of the invention, the amount of biomarker in a sample is to be determined without removing the sample from the body (*i.e.*, *in vivo*, such as with an indwelling catheter or probe), the support should be one which is harmless to the subject and may be in any form convenient for insertion into an appropriate part of the body. For example, the support may be a probe made of polytetrafluoroethylene, polystyrene or other rigid non-harmful plastic material and having a size and shape to enable it to be introduced into a subject. The selection of an appropriate inert support is within the competence of those skilled in the art, as are its dimensions for the intended purpose.

A contacting step in an assay (method) of the invention can involve contacting, combining, or mixing the biological sample and the solid support, such as a reaction vessel, microvessel, tube, microtube, well, multi-well plate, or other solid support. In an embodiment of the invention, the solid support to be contacted with the biological sample (*e.g.*, blood) has an absorbent pad or membrane for lateral flow of the liquid medium to be assayed, such as those available from Millipore Corp. (Bedford, MA), including but not limited to Hi-Flow Plus™ membranes and membrane cards, and SureWick™ pad materials.

The diagnostic device useful in carrying out the methods of the invention can be constructed in any form adapted for the intended use. Thus, in one embodiment, the device of the invention can be constructed as a disposable or reusable test strip or stick to be contacted with a blood sample or blood derived sample for which the presence of the biomarker or biomarker level is to be determined. In another embodiment, the device can be constructed using art recognized micro-scale manufacturing techniques to produce needle-like embodiments capable of being implanted or injected into an anatomical site, such as a vein or artery, for indwelling diagnostic applications. In other embodiments, devices intended for repeated laboratory use can be constructed in the form of an elongated probe or catheter, for sampling of blood.

In preferred embodiments, the devices of the invention comprise a solid support (such as a strip or dipstick), with a surface that functions as a lateral flow matrix defining a flow path for a biological sample such as blood.

Immunochromatographic assays, also known as lateral flow test strips or simply strip tests, for detecting various analytes of interest, have been known for some time, and may be used for detection of radiation biomarkers of the invention. The benefits of lateral flow tests include a user-friendly format, rapid results, long-term stability over a wide range of climates, and relatively low cost to manufacture. These features make lateral flow tests ideal for applications involving home testing, rapid point of care testing, and testing in the field for various analytes. The principle behind the test is straightforward. Essentially, any ligand that can be bound to a visually detectable solid support, such as dyed microspheres, can be tested for, qualitatively, and in many cases even semi-quantitatively. For example, a one-step lateral flow immunostrip for the detection of free and total prostate specific antigen in serum is described in Fernandez-Sanchez *et al. (J. Immuno. Methods*, 2005, 307(1-2):1-12, which is incorporated herein by reference) and may be adapted for detection of biomarkers of the invention in a biological sample such as blood.

Some of the more common immunochromatographic assays currently on the market are tests for pregnancy (as an over-the-counter (OTC) test kit), Strep throat, and Chlamydia. Many new tests for well-known antigens have been recently developed using the immunochromatographic assay method. For instance, the antigen for the most common cause of community acquired pneumonia has been known since 1917, but a simple assay was developed only recently, and this was done using this simple test strip method (Murdoch, D.R. *et al. J Clin Microbiol*, 2001, 39:3495-3498). Human immunodeficiency virus (HIV) has been detected rapidly in pooled blood using a similar assay (Soroka, S.D. *et al. J Clin Virol*, 2003, 27:90-96). A nitrocellulose membrane card has also been used to diagnose schistosomiasis by detecting the movement and binding of nanoparticles of carbon (van Dam, G.J. *et al. J Clin Microbiol*, 2004, 42:5458-5461).

The two common approaches to the immunochromatographic assay are the non-competitive (or direct) and competitive (or competitive inhibition) reaction schemes (TechNote #303, Rev. #001, 1999, Bangs Laboratories, Inc., Fishers, IN). The direct (double antibody sandwich) format is typically used when testing for larger analytes with multiple antigenic sites such as luteinizing hormone (LH), human chorionic gonadotropin (hCG), and HIV. In this instance, less than an excess of sample analyte is desired, so that some of the microspheres will not be captured at the capture line, and will continue to flow toward the

second line of immobilized antibodies, the control zone. This control line uses species-specific anti-immunoglobulin antibodies, specific for the conjugate antibodies on the microspheres. Free antigen, if present, is introduced onto the device by adding sample (blood, *etc.*) onto a sample addition pad. Free antigen then binds to antibody-microsphere complexes. Antibody 1, specific for epitope 1 of sample antigen, is coupled to dye microspheres and dried onto the device. When sample is added, microsphere-antibody complex is rehydrated and carried to a capture zone and control lines by liquid. Antibody 2, specific for a second antigenic site (epitope 2) of sample antigen, is dried onto a membrane at the capture line. Antibody 3, a species-specific, anti-immunoglobulin antibody that will react with antibody 1, is dried onto the membrane at the control line. If antigen is present in the sample (*i.e.*, a positive test), it will bind by its two antigenic sites, to both antibody 1 (conjugated to microspheres) and antibody 2 (dried onto membrane at the capture line). Antibody 1-coated microspheres are bound by antibody 3 at the control line, whether antigen is present or not. If antigen is not present in the sample (a negative test), microspheres pass the capture line without being trapped, but are caught by the control line.

The competitive reaction scheme is typically used when testing for small molecules with single antigenic determinants, which cannot bond to two antibodies simultaneously. As with double antibody sandwich assay, free antigen, if present is introduced onto the device by adding sample onto a sample pad. Free antigen present in the sample binds to an antibody-microsphere complex. Antibody 1 is specific for sample antigen and couple to dyed microspheres. An antigen-carrier molecule (typically BSA) conjugate is dried onto a membrane at the capture line. Antibody 2 (Ab2) is dried onto the membrane at the control line, and is a species-specific anti-immunoglobulin that will capture the reagent particles and confirm that the test is complete. If antigen is present in the sample (a positive test), antibody on microspheres (Ab1) is already saturated with antigen from sample and, therefore, antigen conjugate bound at the capture line does not bind to it. Any microspheres not caught by the antigen carrier molecule can be caught by Ab2 on the control line. If antigen is not present in the sample (a negative test), antibody-coated dyed microspheres are allowed to be captured by antigen conjugate bound at the capture line.

Normally, the membranes used to hold the antibodies in place on these devices are made of primary hydrophobic materials, such as nitrocellulose. Both the microspheres used as the solid phase supports and the conjugate antibodies are hydrophobic, and their interaction with the membrane allows them to be effectively dried onto the membrane.

Samples and/or biomarker-specific binding agents may be arrayed on the solid support, or multiple supports can be utilized, for multiplex detection or analysis. "Arraying" refers to the act of organizing or arranging members of a library (*e.g.*, an array of different samples or an array of devices that target the same target molecules or different target molecules), or other collection, into a logical or physical array. Thus, an "array" refers to a physical or logical arrangement of, *e.g.*, biological samples. A physical array can be any "spatial format" or physically gridded format" in which physical manifestations of corresponding library members are arranged in an ordered manner, lending itself to combinatorial screening. For example, samples corresponding to individual or pooled members of a sample library can be arranged in a series of numbered rows and columns, *e.g.*, on a multi-well plate. Similarly, binding agents can be plated or otherwise deposited in microtitered, *e.g.*, 96-well, 384-well, or-1536 well, plates (or trays). Optionally, biomarker-specific binding agents may be immobilized on the solid support.

Detection of biomarkers of the invention and other radiation biomarkers, and other assays that are to be carried out on samples, can be carried out simultaneously or sequentially with the detection of other target molecules, and may be carried out in an automated fashion, in a high-throughput format.

The biomarker-specific binding agents can be deposited but "free" (non-immobilized) in the conjugate zone, and be immobilized in the capture zone of a solid support. The biomarker-specific binding agents may be immobilized by non-specific adsorption onto the support or by covalent bonding to the support, for example. Techniques for immobilizing binding agents on supports are known in the art and are described for example in U.S. Patent Nos. 4,399,217, 4,381,291, 4,357,311, 4,343,312 and 4,260,678, which are incorporated herein by reference. Such techniques can be used to immobilize the binding agents in the invention. When the solid support is polytetrafluoroethylene, it is possible to couple hormone antibodies onto the support by activating the support using sodium and ammonia to aminate it and covalently bonding the antibody to the activated support by means of a carbodiimide reaction (yon Klitzing, Schultek, Strasburger, Fricke and Wood in "Radioimmunoassay and Related Procedures in Medicine 1982", International Atomic Energy Agency, Vienna (1982), pages 57-62.).

The diagnostic device of the invention can utilize lateral flow strip (LFS) technology, which has been applied to a number of other rapid strip assay systems, such as over-the-counter early pregnancy test strips based on antibodies to human chorionic gonadotropin (hCG). As with many other diagnostic devices, the device utilizes a binding agent to bind the

target molecule (biomarker of the invention). The device has an application zone for receiving a biological sample such as blood, a labeling zone containing label which binds to biomarker in the sample, and a detection zone where biomarker label is retained.

Binding agent retained in the detection zone gives a signal, and the signal differs depending on whether biomarker levels in the biological sample are lower than, equal to, or greater than a given threshold concentration.

A sample from a subject having a biomarker level equal to or greater than the given reference biomarker concentration can be referred to as a “threshold level”, “threshold amount”, or “threshold sample”. The application zone in the device is suitable for receiving the biological sample to be assayed. It is typically formed from absorbent material such as blotting paper. The labeling zone contains binding agent that binds to any biomarker in the sample. In one embodiment, the binding agent is an antibody (*e.g.*, monoclonal antibody, polyclonal antibody, antibody fragment). For ease of detection, the binding agent is preferably in association with a label that provides a signal that is visible to the naked eye, *e.g.*, it is tagged with a fluorescent tag or a colored tag such as conjugated colloidal gold, which is visible as a pink color.

The detection zone retains biomarker to which the binding agent has bound. This will typically be achieved using an immobilized binding agent such as an immobilized antibody. Where the binding agent in the labeling zone and the detection zone are both antibodies, they will typically recognize different epitopes on the target molecule (biomarker protein). This allows the formation of a “sandwich” comprising antibody-biomarker-antibody.

The detection zone is downstream of the application zone, with the labelling zone typically located between the two. A sample will thus migrate from the application zone into the labeling zone, where any biomarker in the sample binds to the label. Biomarker-binding agent complexes continue to migrate into the detection zone together with excess binding agent. When the biomarker-binding agent complex encounters the capture reagent, the complex is retained whilst the sample and excess binding agent continue to migrate. As biomarker levels in the sample increase, the amount of binding agent (in the form of biomarker-binding agent complex) retained in the detection zone increases proportionally.

In preferred embodiments, the device of the invention has the ability to distinguish between samples according to the threshold concentration. This can be achieved in various ways.

One type of device includes a reference zone that includes a signal of fixed intensity against which the amount of binding agent retained in the detection zone can be compared--

when the signal in the detection zone equals the signal in the reference zone, the sample is a threshold sample; when the signal in the detection zone is less intense than the reference zone, the sample contains less biomarker than a threshold sample; when the signal in the detection zone is more intense than the reference zone, the sample contains more biomarker than a threshold sample.

A suitable reference zone can be prepared and calibrated without difficulty. For this type of device, the binding agent will generally be present in excess to biomarker in the sample, and the reference zone may be upstream or, preferably, downstream of the detection zone. The signal in the reference zone will be of the same type as the signal in the detection zone, *i.e.*, they will typically both be visible to the naked eye, *e.g.*, they will use the same tag. A preferred reference zone in a device of this type comprises immobilized protein (*e.g.*, bovine serum albumin) which is tagged with colloidal gold.

In another device of the invention, the reference zone is downstream of the detection zone and includes a reagent which captures binding agent (*e.g.*, an immobilized anti-binding agent antibody). Binding agent that flows through the device is not present in excess, but is at a concentration such that 50% of it is bound by a sample having biomarker at the threshold concentration. In a threshold sample, therefore, 50% of the binding agent will be retained in the detection zone and 50% in the reference zone. If the biomarker level in the sample is greater than in a threshold sample, less than 50% of the binding agent will reach the reference zone and the detection zone will give a more intense signal than the reference zone; conversely, if the biomarker level in the sample is less than in a threshold sample, less than 50% of the binding agent will be retained in the detection zone and the reference zone will give a more intense signal than the detection zone.

In another device of the invention which operates according to similar principles, the reference zone is downstream of the detection zone and includes a limiting amount of a reagent which captures binding agent (*e.g.*, an immobilized anti-binding agent antibody). The reagent is present at a level such that it retains the same amount of label which would bind to the detection zone for a threshold sample, with excess label continuing to migrate beyond the reference zone.

In these three types of device, therefore, a comparison between the detection zone and the reference zone is used to compare the sample with the threshold concentration. The detection:reference binding ratio can preferably be determined by eye. Close juxtaposition of the detection and reference zones is preferred in order to facilitate visual comparison of the signal intensities in the two zones.

In a fourth type of device, no reference zone is needed, but the detection zone is configured such that it gives an essentially on/off response, *e.g.*, no signal is given below the threshold concentration but, at or above the threshold, signal is given.

In a fifth type of device, no reference zone is needed, but an external reference is used which corresponds to the threshold concentration. This can take various forms, *e.g.*, a printed card against which the signal in the detection zone can be compared, or a machine reader which compares an absolute value measured in the detection zone (*e.g.*, a calorimetric signal) against a reference value stored in the machine.

In some embodiments of the invention, the device includes a control zone downstream of the detection zone. This will generally be used to capture excess binding agent that passes through the detection and/or reference zones (*e.g.*, using immobilized anti-binding agent antibody). When binding agent is retained at the control zone, this confirms that mobilization of the binding agent and migration through the device have both occurred. It will be appreciated that this function may be achieved by the reference zone.

In a preferred embodiment, the detection, reference and control zones are preferably formed on a nitrocellulose support.

Migration from the application zone to the detection zone will generally be assisted by a wick downstream of the detection zone to aid capillary movement. This wick is typically formed from absorbent material such as blotting or chromatography paper.

The device of the invention can be produced simply and cheaply, conveniently in the form of a dipstick. Furthermore, it can be used very easily, for instance by the home user. The invention thus provides a device which can be used as a screen for radiation exposure.

Kits for detecting ionizing radiation exposure, determination of absorbed dose, and time of exposure

The present invention also concerns kits comprising the required elements for detecting radiation exposure in a subject and, optionally, determining the absorbed dose of radiation, and the time of exposure. In one embodiment, the kits comprise one or more containers for collecting a blood sample or blood derived sample from a subject and an agent for detecting the presence of radiation biomarker polypeptides or nucleic acids encoding the polypeptides in the fluid. The radiation biomarker components of the kits can be packaged either in aqueous medium or in lyophilized form.

The methods of the invention can be carried out using a diagnostic kit for qualitatively or quantitatively detecting one or more radiation biomarkers of the invention in a sample such

as blood. By way of example, the kit can contain binding agents (*e.g.*, antibodies) specific for biomarkers of the invention, antibodies labeled with a detectable label or substance that can bind to the binding agents. If the detectable substance is an enzyme, then the kit can comprise a substrate for the enzyme. The kit can also contain a solid support (such as microtiter multi-well plates, nitrocellulose, *etc.*), standards, assay diluent, wash buffer, adhesive plate covers, and/or instructions for carrying out a method of the invention using the kit. In one embodiment, the kit includes one or more protease inhibitors (*e.g.*, a protease inhibitor cocktail) and/or nuclease inhibitors to be applied to the biological sample to be assayed (such as blood).

Kits for detecting radiation exposure determining absorbed dose containing one or more agents that detect the biomarker polypeptides, such as but not limited to biomarker antibodies, fragments thereof, or biomarker binding partners, can be prepared. The agent(s) can be packaged with a container for collecting the biological sample from a patient. When the antibodies or binding partner are used in the kits in the form of conjugates in which a label is attached, such as a radioactive metal ion or a moiety, the components of such conjugates can be supplied either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit.

Kits containing one or more agents that detect biomarker nucleic acids, such as but not limited to the full length biomarker nucleic acid, biomarker oligonucleotides, and pairs of biomarker primers can also be prepared. The agent(s) can be packaged with a container for collecting biological samples from a patient. The nucleic acid can be in the labeled form or unlabeled form.

Other components of the kit may include, but are not limited to, means for collecting biological samples (*e.g.*, lancets, needles), means for labeling the detecting agent (binding agent), means for immobilizing the biomarker protein or biomarker nucleic acid in the biological sample (*e.g.*, support membranes, nitrocellulose, *etc.*), means for applying the biological sample to an immobilizing support means for binding the agent to the biomarker in the biological sample of a subject, a second antibody, a means for isolating total RNA or mRNA from a biological fluid of a subject, means for performing gel electrophoresis, means for generating cDNA from isolated total RNA or mRNA, means for performing hybridization assays, and means for performing PCR, *etc.*

Gene specific primers and methods of using same are described in U.S. Patent No. 5,994,076. In addition to the specific primers designed to selectively amplify the biomarker

nucleic acids, the kits of the invention can also include reagents such as dNTPs (labeled or unlabeled) and/or rNTPs, buffers, enzymes, *etc.*

As used herein, the term “ELISA” includes an enzyme-linked immunoabsorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen (*e.g.*, biomarker of the invention) or antibody present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites *et al.*, 1982, published by Lange Medical Publications of Los Altos, Calif. and in U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043, the disclosures of which are herein incorporated by reference. ELISA is an assay that can be used to quantitate the amount of antigen, proteins, or other molecules of interest in a sample. In particular, ELISA can be carried out by attaching on a solid support (*e.g.*, polyvinylchloride) an antibody specific for an antigen or protein of interest. Cell extract or other biological sample of interest such as blood can be added for formation of an antibody-antigen complex, and the extra, unbound sample is washed away. An enzyme-linked antibody, specific for a different site on the antigen is added. The support is washed to remove the unbound enzyme-linked second antibody. The enzyme-linked antibody can include, but is not limited to, alkaline phosphatase. The enzyme on the second antibody can convert an added colorless substrate into a colored product or can convert a non-fluorescent substrate into a fluorescent product. The ELISA-based assay method provided herein can be conducted in a single chamber or on an array of chambers and can be adapted for automated processes.

In these exemplary embodiments, the antibodies can be labeled with pairs of FRET dyes, bioluminescence resonance energy transfer (BRET) protein, fluorescent dye-quencher dye combinations, beta gal complementation assays protein fragments. The antibodies may participate in FRET, BRET, fluorescence quenching or beta-gal complementation to generate fluorescence, colorimetric or enhanced chemiluminescence (ECL) signals, for example.

These methods are routinely employed in the detection of antigen-specific antibody responses, and are well described in general immunology text books such as Immunology by Ivan Roitt, Jonathan Brostoff and David Male (London: Mosby, c1998. 5th ed. and Immunobiology: Immune System in Health and Disease/Charles A. Janeway and Paul Travers. Oxford: Blackwell Sci. Pub., 1994), the contents of which are herein incorporated by reference.

Definitions

A “sample” (biological sample) can be any composition of matter of interest from a human or non-human subject, in any physical state (*e.g.*, solid, liquid, semi-solid, vapor) and of any complexity. The sample can be any composition reasonably suspecting of containing one or more biomarkers of the invention that can be analyzed by the methods, devices, and kits of the invention. Preferably, the sample is a fluid (biological fluid such as blood). Samples can include human or animal samples. The sample may be contained within a test tube, culture vessel, multi-well plate, or any other container or supporting substrate. The sample can be, for example, a cell culture, human or animal tissue. Fluid homogenates of cellular tissues are biological fluids that may contain biomarkers for detection by the invention. Preferably, the sample is a blood sample or blood derived sample.

The “complexity” of a sample refers to the relative number of different molecular species that are present in the sample.

The terms “body fluid” and “bodily fluid”, as used herein, refer to a composition obtained from a human or animal subject. Bodily fluids include, but are not limited to, whole blood, blood plasma, serum, urine, bladder wash, bladder barbotage specimen, tears, semen, saliva, sputum, exhaled breath, nasal secretions, pharyngeal exudates, bronchoalveolar lavage, tracheal aspirations, interstitial fluid, lymph fluid, meningeal fluid, amniotic fluid, glandular fluid, feces, perspiration, mucous, vaginal or urethral secretion, cerebrospinal fluid, and transdermal exudate. Bodily fluid also includes experimentally separated fractions of all of the preceding solutions or mixtures containing homogenized solid material, such as feces, tissues, and biopsy samples.

The term “*ex vivo*,” as used herein, refers to an environment outside of a subject. Accordingly, a sample of bodily fluid collected from a subject is an *ex vivo* sample of bodily fluid as contemplated by the subject invention. In-dwelling embodiments of the method and device of the invention obtain samples *in vivo*.

As used herein, the term “conjugate” refers to a compound comprising two or more molecules bound together, optionally through a linking group, to form a single structure. The binding can be made by a direct connection (*e.g.*, a chemical bond) between the molecules or by use of a linking group.

As used herein, the terms solid “support”, “substrate”, and “surface” refer to a solid phase which is a porous or non-porous water insoluble material that can have any of a number of shapes, such as strip, rod, particle, beads, or multi-welled plate. In some embodiments, the support has a fixed organizational support matrix that preferably functions

as an organization matrix, such as a microtiter tray. Solid support materials include, but are not limited to, cellulose, polysaccharide such as Sephadex, glass, polyacryloylmorpholide, silica, controlled pore glass (CPG), polystyrene, polystyrene/latex, polyethylene such as ultra high molecular weight polyethylene (UPE), polyamide, polyvinylidene fluoride (PVDF),
5 polytetrafluoroethylene (PTFE; TEFLON), carboxyl modified Teflon, nylon, nitrocellulose, and metals and alloys such as gold, platinum and palladium. The solid support can be biological, non-biological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, pads, cards, strips, dipsticks, test strips, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.*, depending upon the
10 particular application. Preferably, the solid support is planar in shape, to facilitate contact with a biological sample such as whole blood, plasma, serum, urine, peritoneal fluid, or ascites fluid. Other suitable solid support materials will be readily apparent to those of skill in the art. The solid support can be a membrane, with or without a backing (*e.g.*, polystyrene or polyester card backing), such as those available from Millipore Corp. (Bedford, MA), *e.g.*,
15 Hi-Flow™ Plus membrane cards. The surface of the solid support may contain reactive groups, such as carboxyl, amino, hydroxyl, thiol, or the like for the attachment of nucleic acids, proteins, *etc.* Surfaces on the solid support will sometimes, though not always, be composed of the same material as the support. Thus, the surface can be composed of any of a wide variety of materials, such as polymers, plastics, resins, polysaccharides, silica or silica-
20 based materials, carbon, metals, inorganic glasses, membranes, or any of the aforementioned support materials (*e.g.*, as a layer or coating).

As used herein, the terms “label” and “tag” refer to substances that may confer a detectable signal, and include, but are not limited to, enzymes such as alkaline phosphatase, glucose-6-phosphate dehydrogenase, and horseradish peroxidase, ribozyme, a substrate for a
25 replicase such as QB replicase, promoters, dyes, fluorescers, such as fluorescein, isothiocyanate, rhodamine compounds, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine, chemiluminescers such as isoluminol, sensitizers, coenzymes, enzyme substrates, radiolabels, particles such as latex or carbon particles, liposomes, cells, *etc.*, which may be further labeled with a dye, catalyst or other detectable
30 group.

As used herein, the term “receptor” and “receptor protein” are used herein to indicate a biologically active proteinaceous molecule that specifically binds to (or with) other molecules such as biomarkers of the invention.

As used herein, the term “ligand” refers to a molecule that contains a structural portion that is bound by specific interaction with a particular receptor protein.

As used herein, the term “antibody” refers to immunoglobulin molecules and immunologically active portions (fragments) of immunoglobulin molecules, *i.e.*, molecules that contain an antibody combining site or paratope. The term is inclusive of monoclonal antibodies and polyclonal antibodies.

As used here, the terms “monoclonal antibody” or “monoclonal antibody composition” refer to an antibody molecule that contains only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody composition thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody composition is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) only one type of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. Such antibodies were first described by Kohler and Milstein, *Nature*, 1975, 256:495-497, the disclosure of which is herein incorporated by reference. An exemplary hybridoma technology is described by Niman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, 80:4949-4953. Other methods of producing monoclonal antibodies, a hybridoma cell, or a hybridoma cell culture are also well known. See *e.g.*, *Antibodies: A Laboratory Manual*, Harlow *et al.*, Cold Spring Harbor Laboratory, 1988; or the method of isolating monoclonal antibodies from an immunological repertoire as described by Sasatry, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:5728-5732; and Huse *et al.*, *Science*, 1981, 246:1275-1281. The references cited are hereby incorporated herein by reference.

As used herein, a semi-permeable membrane refers to a bio-compatible material which is impermeable to liquids and capable of allowing the transfer of gases through it. Such gases include, but are not limited to, oxygen, water vapor, and carbon dioxide. Semi-permeable membranes are an example of a material that can be used to form at least a portion of an enclosure defining a flow chamber cavity. The semi-permeable membrane may be capable of excluding microbial contamination (*e.g.*, the pore size is characteristically small enough to exclude the passage of microbes that can contaminate the analyte, such as cells). In a particular aspect, a semi-permeable membrane can have an optical transparency and clarity sufficient for permitting observation of an analyte, such as cells, for color, growth, size, morphology, imaging, and other purposes well known in the art.

As used herein, the term “bind” refers to any physical attachment or close association, which may be permanent or temporary. The binding can result from hydrogen bonding, hydrophobic forces, van der Waals forces, covalent, or ionic bonding, for example.

As used herein, the term “particle” includes insoluble materials of any configuration, including, but not limited to, spherical, thread-like, brush-like, and irregular shapes. Particles can be porous with regular or random channels inside. Particles can be magnetic. Examples of particles include, but are not limited to, silica, cellulose, Sepharose beads, polystyrene (solid, porous, derivatized) beads, controlled-pore glass, gel beads, magnetic beads, sols, biological cells, subcellular particles, microorganisms (protozoans, bacteria, yeast, viruses, and other infectious agents), micelles, liposomes, cyclodextrins, and other insoluble materials.

A “coding sequence” or “coding region” is a polynucleotide sequence that is transcribed into mRNA and/or translated into a polypeptide. For example, a coding sequence may encode a polypeptide of interest. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and recombinant polynucleotide sequences.

As used herein, the term “polypeptide” refers to any polymer comprising any number of two or more amino acids, and is used interchangeably herein with the terms “protein”, “gene product”, and “peptide”.

As used herein, the term “nucleoside” refers to a molecule having a purine or pyrimidine base covalently linked to a ribose or deoxyribose sugar. Exemplary nucleosides include adenosine, guanosine, cytidine, uridine and thymidine.

The term “nucleotide” refers to a nucleoside having one or more phosphate groups joined in ester linkages to the sugar moiety. Exemplary nucleotides include nucleoside monophosphates, diphosphates and triphosphates.

The terms “polynucleotide”, “nucleic acid molecule”, and “nucleotide molecule” are used interchangeably herein and refer to a polymer of nucleotides joined together by a phosphodiester linkage between 5' and 3' carbon atoms. Polynucleotides can encode a polypeptide such as biomarker polypeptide (whether expressed or non-expressed), or may be short interfering RNA (siRNA), antisense nucleic acids (antisense oligonucleotides), aptamers, ribozymes (catalytic RNA), or triplex-forming oligonucleotides (*i.e.*, antigene), for example.

As used herein, the term “RNA” or “RNA molecule” or “ribonucleic acid molecule” refers generally to a polymer of ribonucleotides. The term “DNA” or “DNA molecule” or deoxyribonucleic acid molecule” refers generally to a polymer of deoxyribonucleotides. DNA and RNA molecules can be synthesized naturally (*e.g.*, by DNA replication or transcription of DNA, respectively). RNA molecules can be post-transcriptionally modified. DNA and RNA molecules can also be chemically synthesized. DNA and RNA molecules can be single-stranded (*i.e.*, ssRNA and ssDNA, respectively) or multi-stranded (*e.g.*, double stranded, *i.e.*, dsRNA and dsDNA, respectively). Based on the nature of the invention, however, the term “RNA” or “RNA molecule” or “ribonucleic acid molecule” can also refer to a polymer comprising primarily (*i.e.*, greater than 80% or, preferably greater than 90%) ribonucleotides but optionally including at least one non-ribonucleotide molecule, for example, at least one deoxyribonucleotide and/or at least one nucleotide analog.

As used herein, the term “nucleotide analog” or “nucleic acid analog”, also referred to herein as an altered nucleotide/nucleic acid or modified nucleotide/nucleic acid refers to a non-standard nucleotide, including non-naturally occurring ribonucleotides or deoxyribonucleotides. Preferred nucleotide analogs are modified at any position so as to alter certain chemical properties of the nucleotide yet retain the ability of the nucleotide analog to perform its intended function. For example, locked nucleic acids (LNA) are a class of nucleotide analogs possessing very high affinity and excellent specificity toward complementary DNA and RNA. LNA oligonucleotides have been applied as antisense molecules both *in vitro* and *in vivo* (Jepsen J.S. *et al.*, *Oligonucleotides*, 2004, 14(2):130-146).

As used herein, the term “RNA analog” refers to a polynucleotide (*e.g.*, a chemically synthesized polynucleotide) having at least one altered or modified nucleotide as compared to a corresponding unaltered or unmodified RNA but retaining the same or similar nature or function as the corresponding unaltered or unmodified RNA. As discussed above, the oligonucleotides may be linked with linkages which result in a lower rate of hydrolysis of the RNA analog as compared to an RNA molecule with phosphodiester linkages. Exemplary RNA analogues include sugar- and/or backbone-modified ribonucleotides and/or deoxyribonucleotides. Such alterations or modifications can further include addition of non-nucleotide material, such as to the end(s) of the RNA or internally (at one or more nucleotides of the RNA).

As used herein, the terms “radiation”, “ionizing radiation”, and “IR” refer to electromagnetic radiation (*e.g.*, x-rays and gamma rays) and/or particulate radiation (*e.g.*,

alpha, beta⁺, and beta⁻). Gamma-rays have the highest energy of any of these and are emitted from certain radio-isotopes that may be released from a dirty bomb, nuclear power plant accident, or nuclear bomb. While these radio-isotopes may release other forms of IR, gamma-rays are able to penetrate skin and travel long distances, therefore affecting more people and causing more DNA damage than other types of radiation. It is considered the primary hazard to humans during a radiological emergency.

The terms “comprising”, “consisting of” and “consisting essentially of” are defined according to their standard meaning. The terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

The terms “isolated” or “biologically pure” refer to material that is substantially or essentially free from components which normally accompany the material as it is found in its native state.

As used in this specification, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a biomarker” includes more than one such biomarker. A reference to “an antibody” includes more than one such antibody. A reference to “a molecule” includes more than one such molecule, and so forth.

The practice of the present invention can employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, electrophysiology, and pharmacology that are within the skill of the art. Such techniques are explained fully in the literature (see, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989); DNA Cloning, Vols. I and II (D. N. Glover Ed. 1985); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan Eds., Academic Press, Inc.); *Transcription and Translation* (Hames *et al.* Eds. 1984); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller *et al.* Eds. (1987) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); *Scopes, Protein Purification: Principles and Practice* (2nd ed., Springer-Verlag); and *PCR: A Practical Approach* (McPherson *et al.* Eds. (1991) IRL Press)), each of which are incorporated herein by reference in their entirety.

Following are examples that illustrate materials, methods, and procedures for practicing the invention. The examples are illustrative and should not be construed as limiting.

MATERIALS AND METHODS

Patients and Volunteers. Informed consent for study participation was obtained from four pediatric cancer patients at the Children's Hospital of Birmingham and nine healthy volunteers according to guidelines set forth by the Institutional Review Board (IRB) at the University of Alabama at Birmingham (UAB). All studies were approved by the IRB at UAB. Cancer patients used in this study were diagnosed with Acute Lymphocytic Leukemia (ALL) and were undergoing myeloablative therapy with total body irradiation (TBI). None of these patients were being treated with chemotherapy during the course of TBI. All patients received 2 Gy twice a day for three consecutive days (total dose of 12 Gy). Whole blood was collected prior to irradiation (baseline) and at approximately 5, 23 and 48 hours after the first fraction of TBI. Whole blood collected from nine healthy volunteers served as control samples.

Irradiation of C57BL6 Mouse. C57BL6 mice were obtained from NCI-Frederick (Frederick, MD) and were allowed to acclimate for one to two weeks prior to treatment. 8-week old mice were anesthetized with a ketamine/xylazine mixture (dose of 85 mg/kg ketamine; 10 mg/kg xylazine) followed by sham or total body irradiation using a ⁶⁰Co Teletherapy unit (Picker, Cleveland OH). Mice were euthanized via exsanguination and cervical dislocation at 0, 5, 12, 23, or 48 hours following irradiation. All experiments were approved and conducted under guidelines set forth by the UAB Institutional Animal Care and Use Committee.

A total of 166 mice were included in a training set that was used for development of all statistical models. The training set was comprised of 70 controls (non-irradiated) as well as 8 mice per treatment group (1, 2, or 6 Gy) per time point (5, 12, 23, and 48 hours post-TBI). A total of 45 mice were utilized as an independent validation set for testing statistical models developed in the training set. The validation set contained 9 control (non-irradiated) mice and 3-4 mice per treatment group (1, 2, 4, 6, or 8 Gy) at two time points (12 or 48 hours).

Blood Collection and RNA Isolation. Whole blood samples were collected from four patients and nine healthy volunteers via venipuncture. Mouse blood was collected through intra-cardiac puncture. All blood samples were drawn directly into PAXgene Blood RNA tubes (PreAnalytiX by BD/Qiagen, Franklin Lakes NJ) and total RNA was isolated with the PAXgene Blood RNA kit (Qiagen, Valencia CA). All samples underwent on-column DNA digestion using the Qiagen RNase-free Dnase set (Qiagen, Valencia CA) according to manufacturer's instructions. RNA was reverse transcribed to cDNA with the High Capacity

cDNA Archive kit (Applied Biosystems, Foster City CA) following manufacturer's instructions.

Real-time quantitative Low Density Array (RTQ-LDA) design. To identify potential biodosimetry genes in humans exposed to known amounts of radiation, a rationally-designed RTQ-LDA was utilized to examine differences in expression of 46 independent genes in blood samples collected from healthy volunteers and from ALL patients at baseline (prior to irradiation) and at 5, 23, and 48 hours following the first fraction of TBI. Selection of these genes ensued after an earlier study conducted by the inventors' laboratory which identified seventeen potential biodosimetry genes in blood samples collected from a separate set of six pediatric ALL patients undergoing treatment with TBI (unpublished results). The remaining twenty-nine genes were included because they met one of the following criteria: 1) reported as radiation responsive by at least two independent investigators; 2) demonstrated radiation response in multiple models (*in vivo*, *in vitro* or *ex vivo*); 3) identified in array studies of TBI cancer patients. The complete RTQ-LDA also included two housekeeping genes, RPLPO and 18S, for a total of 48 independent genes (Table 1B). These 48 genes were formatted onto a RTQ-LDA (Applied Biosystems, Foster City CA) as previously described by the inventors' laboratory (33, 34).

Real-time Quantitative PCR (RTQ). Genes identified as potential biodosimetry genes from RTQ-LDA analysis in patients were analyzed via single gene RTQ in blood samples from mice. Individual RTQ primer probe sets for mouse Acta2, Bbc3, Ccng1, Cdkn1a, Gadd45a, Mdk, Serpine1, and Tnfrsf10b were obtained from Applied Biosystems (Foster City CA). PCR amplification for both RTQ-LDA and single gene RTQ analysis was performed on an ABI 7900HT Sequence Detection System. Cycling conditions for RTQ-LDA analysis were as follows: 50°C for 2 minutes and 94.5°C for 10 minutes followed by 40 cycles at 97°C for 15 seconds and 59.7°C for 1 minute. Cycling conditions for single-gene RTQ were as follows: 50°C for 2 minutes and 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All single-gene RTQ reactions, including no template controls, were performed in triplicate and samples were normalized to the mouse Rps9 housekeeping gene. Expression values for all analyses were calculated as previously described (33-36).

Statistical Analysis. The overall approach in the current study was to identify potential biodosimetry genes in patients exposed to TBI and further evaluate these genes in a murine model in which dose of exposure and time of sample collection could be varied. Due to the limited number of patients included in this study (n=4), p-values resulting from

statistical testing of patient gene expression were not considered definitive and the following filtering criteria were applied. Genes with no detectable expression ($C_t=40$) or with only marginal changes in expression following exposure to radiation (<3 standard deviations from average baseline expression) were not examined in the mouse model. In addition, genes whose expression varied (>3 standard deviations) between healthy volunteers and average expression in baseline patient samples were not further examined due to a potential association with cancer diagnosis and/or treatment. Those genes remaining after application of these filtering criteria were further analyzed in mice.

Prior to single-gene RTQ analysis in irradiated mice, a power analysis was performed to ensure the ability to detect statistically significant changes in gene expression. Using a two-sided two-group t-test of equal mean and significance level of 0.05, it was determined that 8 mice per experimental group was sufficient to detect an effect size of 1.5 to 2 standard deviations. Significant differences in mouse gene expression were determined at each time point with One-way ANOVA analysis followed by Tukey's Multiple Comparison Test. Dose-dependent changes in mouse gene expression were assessed with linear regression analysis. All statistical analyses of mouse gene expression values were performed in GraphPad Prism Software with p-values <0.05 considered significant. Only genes whose expression changed significantly following irradiation in mice were included in class prediction and multiple linear regression analyses.

Using expression data from the mouse training set, a weighted voting algorithm (37, 38) was utilized to develop a class prediction model at each time point capable of distinguishing irradiated from non-irradiated mice. Non-irradiated and irradiated mice were defined as classes 0 and 1, respectively. Genes whose expression best distinguished these two classes were identified based on the signal-to-noise statistic defined as: $S_x = (\mu_{\text{class}0} - \mu_{\text{class}1} / s_{\text{class}0} + s_{\text{class}1})$, where μ =mean expression and s =standard deviation. Thereafter, the weighted voting classification algorithm was applied. Briefly, each gene in the class prediction model casts a vote for either class 0 or 1 depending on whether the expression level in the sample is closer to the mean of class 0 or 1. The weight of the vote (V) reflects the deviation of the sample expression level (X) from the mean of each class: $V = |X - (\mu_{\text{class}0} + \mu_{\text{class}1}) / 2|$. The votes are summed to obtain the total number of votes for class 0 and class 1 with the class with the most votes deemed as the winning class. The confidence of the prediction of the winning class was calculated as $(V_{\text{win}} - V_{\text{lose}}) / (V_{\text{win}} + V_{\text{lose}})$. Using leave-one-out cross-validation, the number of class prediction errors in the training set was determined using different combinations of genes to identify relevant marker genes capable of

distinguishing the two classes with the highest accuracy. These molecular class prediction profiles were then applied to expression data obtained from an independent validation set of 45 mice. Prior to analyses, gene expression data in the validation set was corrected based on the medians of each set. Weighted voting analyses were performed using GenePattern software available at <http://www.broadinstitute.org>.

Development of a continuous model capable of predicting dose of radiation exposure was performed using multiple linear regression analysis (39). Modeling was performed using all possible combinations of independent variables and the r-squared statistic and their associated p-values were used to determine the global fit of all models. Optimal models developed using data obtained from the training set were utilized to determine the predicted dose of exposure based on gene expression data within the validation set of mice.

EXAMPLE 1—PATIENT TREATMENT DEMOGRAPHICS

Initial studies were conducted on blood samples obtained from four pediatric patients diagnosed with Acute Lymphocytic Leukemia (ALL) undergoing fractionated therapy with TBI prior to bone marrow transplantation (treatment schema illustrated in Figure 1). Blood collection times corresponded with previously scheduled time points used to monitor white blood cell counts. Sufficient RNA for expression analysis could not be isolated from blood samples collected after the 48-hour time point. As shown in Table 1A, white blood cell counts varied prior to TBI and decreased dramatically following completion of myeloablative therapy.

EXAMPLE 2—RTQ-LDA ANALYSIS IN PATIENTS UNDERGOING TOTAL BODY IRRADIATION

To identify potential biodosimetry genes, RTQ-LDA analysis was performed in blood samples collected from four ALL patients prior to and following TBI. Nine healthy volunteers were also analyzed to examine variation in gene expression in a disease free population. Of the forty-eight genes represented on the RTQ-LDA (forty-six target and two housekeeping genes), the expression of nineteen target genes fell below the limits of detection (Footnote^a in Table 1B). Seven genes demonstrated variability (greater than 3 standard deviations) between baseline patient samples and healthy volunteers (Footnote^b in Table 1B) suggesting that the expression of these genes may be altered by disease state or prior treatment for cancer. Eight genes (bolded in Table 1B) demonstrated increased expression in irradiated patient samples compared to baseline patient samples (Figure 2).

Linear regression analysis demonstrated a significant correlation ($p < 0.05$) between time (post-TBI) and mRNA levels of CDKN1A, MDK, SERPINE1, and TNFRSF10B (Table 2). Due to the fractionated nature of TBI therapy, it could not be determined if the observed changes in gene expression resulted from the accumulating dose of radiation or from increasing time after exposure. To elucidate the effects of radiation dose and time from exposure, these eight genes were further evaluated in an irradiated murine model.

Table 1C lists the 8 genes of Table 2, in humans and mice, including National Center for Biotechnology Information (NCBI) accession numbers (25 sequences, corresponding to SEQ ID NOs:1-25).

EXAMPLE 3—GENE EXPRESSION ANALYSIS FOLLOWING TOTAL BODY IRRADIATION OF C57BL6 MICE

Expression analysis of the mouse homologs of the eight genes identified in patient samples was performed in C57BL6 mice using single-gene RTQ. One gene (Mdk) demonstrated no detectable expression. Two genes (Acta2 and Gadd45a) showed no significant increase in expression between non-irradiated and irradiated mice at any time point (data not shown). Five genes (Bbc3, Ccng1, Cdkn1a, Serpin1, and Tnfrsf10b) demonstrated increased expression in irradiated mice compared to non-irradiated controls (Figure 3). Stable baseline expression (no significant variation) was observed for all five genes in non-irradiated mice over the 48 hours evaluated.

Four genes (Bbc3, Ccng1, Cdkn1a, and Tnfrsf10b) demonstrated significant differences in expression between non-irradiated mice and at least one irradiated group (1, 2, or 6 Gy) at every time point. Expression of Serpin1 in mice was significantly increased 12 hours after exposure to 6 Gy and at 48 hours in both the 2 Gy and 6 Gy exposure groups. While significant increases in expression were observed for most genes at early time points, dose-dependence was more pronounced with increasing time post-exposure. As shown in Table 3, linear regression analysis demonstrated a significant correlation ($p < 0.05$) between dose and expression levels of Bbc3, Ccng1, Cdkn1a, and Serpin1 at 48 hours post-irradiation. Collectively, these data suggests that these five genes may be useful for determining exposure status (non-irradiated vs. irradiated) at earlier time points and level of exposure (doses) at 48 hours.

EXAMPLE 4—DETERMINING EXPOSURE STATUS (NON-IRRADIATED vs.
IRRADIATED) IN C57BL6 MICE

Using the gene expression data obtained from mice in the training set (Figure 3), we employed a weighted voting algorithm to develop class prediction models at each time point that could distinguish non-irradiated from irradiated mice. Mice were grouped into either class 0 (non-irradiated) or class 1 (irradiated) based on their exposure status. Because there was no significant difference in expression between the non-irradiated groups, all seventy non-irradiated mice (n=14 per time point) were included in class 0 regardless of time of sample collection. Mice exposed to 1, 2, or 6 Gy TBI were grouped into class 1 for a total of twenty-four irradiated mice (n=8 per dose) at each independent time point. All five candidate predictor genes (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) were evaluated independently and in combination to identify the optimal profile that could discriminate non-irradiated from irradiated mice at each time point. Highest accuracy was obtained using unique class prediction models comprised of one or two genes per profile. While all three profiles (Ccng1 at 5 and 12 hours; Bbc3 and Ccng1 at 23 hours; and Ccng1 and Cdkn1a at 48 hours) contained Ccng1, none of the four class prediction signatures included Serpine1. Internal examination of this training set via leave-one-out cross validation demonstrated 94.7, 93.6, 95.7, and 92.6% accuracy in correctly classifying mice as non-irradiated or irradiated at 5, 12, 23, or 48 hours, respectively (Table 5). Specificity for all models was =97.1% with no more than two false positives at any time point. One mouse was misclassified as irradiated using the prediction models developed at all four time points indicating abnormally high expression levels. As expected, the sensitivity of each class prediction model increased with increasing dose. There were no more than five false negatives at any time point. Further, all mice in the 6 Gy treatment group were correctly classified as irradiated at 12, 23, and 48 hours post-irradiation. Overall, these data demonstrate that increased expression of these genes is indicative of radiation exposure and mice exposed to higher doses of irradiation can be correctly classified with higher accuracy. Results are summarized in the upper panel of Table 4.

Class prediction models developed at 12 and 48 hours using gene expression data from the mouse training set were independently tested in a separate validation set of mice. This validation set included mice irradiated with 1, 2, 4, 6, or 8 Gy (n=3 or 4 per time point) and nine non-irradiated mice. These mice were classified into corresponding exposure groups with 92.6 and 96.3% accuracy at 12 and 48 hours post-TBI, respectively (Table 5). There were no false positives using either class prediction model. Importantly, the two mice

misclassified as non-irradiated at 12 hours were in the 1 or 2 Gy exposure groups. At 48 hours, one mouse in the 1 Gy exposure group was incorrectly classified but overall sensitivity remained at 94.4%. This data again demonstrates that the sensitivity to predict exposure status increases with dose. Results are summarized in the lower panel of Table 4.

EXAMPLE 5—DETERMINING DOSE IN C57BL6 MICE

Expression of all five genes demonstrated a significant linear correlation with dose at 48 hours post-irradiation (Table 3) suggesting the potential to develop a continuous model capable of determining dose of exposure at this time point. Multiple linear regression analysis was performed using expression data obtained from mice in the training set. All possible combinations of genes were used to determine the model that was most capable of estimating dose of exposure. The model with the highest adjusted r-squared value was chosen for validation.

Multiple linear regression analysis of gene expression data from the mouse training set revealed that the model defined by expression of *Ccng1* and *Cdkn1a* had the highest adjusted r-squared value. The regression model for this data is predicted dose = $-0.7674 + 0.6343 * [Ccng1] + 0.0804 * [Cdkn1a]$ (adjusted $r^2=0.8400$, $p<0.0001$). This model was initially validated within the training set to determine predicted dose based on expression of *Ccng1* and *Cdkn1a*. The relationship of actual dose versus predicted dose is visualized in Figure 4A. There was a significant linear correlation between these two parameters ($r^2=.8487$, $p<0.0001$). The average predicted doses for the 0, 1, 2, and 6 Gy treatment groups in the training set were 0.1 ± 0.5 , 1.2 ± 0.3 , 2.3 ± 0.5 , 5.3 ± 1.6 Gy, respectively (upper panel of Table 5).

This regression model was independently tested for its ability to predict dose using the gene expression data obtained from the validation set. Importantly, this set contained two additional treatment groups (4 and 8 Gy) to test the boundaries of this continuous model. As shown in Figure 4B, there was a significant linear correlation between actual dose and predicted dose ($r^2=0.7113$, $p<0.0001$). The average predicted doses for the 0, 1, 2, 4, 6, and 8 Gy treatment groups were 0.0 ± 0.2 , 1.6 ± 1.0 , 2.9 ± 1.4 , 5.1 ± 2.0 , 5.3 ± 0.7 , and 10.5 ± 5.6 Gy, respectively (lower panel of Table 5). Variability in the predicted dose was greatest in the 8 Gy treatment group. Overall, the multiple linear regression model slightly overestimated dose.

Currently, chromosome aberration cytogenetics remains the only validated method capable of retrospective radiation dose assessment determined directly from biological

samples. While advances have been made to automate this method (40), it remains both time- and labor-intensive and would not be amenable to mass casualty situations. Recent microarray studies examining the transcriptional response to ionizing radiation have identified potential molecular markers of radiation exposure. With this in mind, we sought to develop statistical models of gene expression with the ability to not only distinguish non-irradiated from irradiated individuals, but also estimate the actual dose of exposure.

Unique to this study, a “reverse-translational” approach was utilized whereby we initially identified potential biodosimetry genes in human samples prior to assessment of dose-dependent radiation responses in other experimental models. Further, gene expression analysis was performed utilizing a rationally-designed RTQ-LDA comprised of genes previously characterized as responsive to ionizing radiation by other investigators in combination with genes we identified in our preliminary studies (Table 1). In the current study, eight potential biodosimetry genes were identified from the whole blood of four cancer patients undergoing treatment with TBI (Figure 2). Interestingly, this is the first report to our knowledge demonstrating radiation response of four genes (CCNG1, MDK, SERPINE1, and TNFRSF10B) in human blood.

While analysis of transcriptional response to radiation in human subjects is imperative for validation of biodosimetry genes, the population of individuals exposed to known amounts of radiation is very limited. Further, the effects of single acute doses of radiation and dose-dependent responses cannot be assessed in patients undergoing stringent treatment protocols, which include fractionated radiotherapy. Although *in vitro* cell lines and *ex vivo* whole blood models could be used to examine mRNA profiles after irradiation, these models lack the endogenous cellular microenvironment which may influence the overall transcriptional response. Therefore, we elected to assess dose-dependent changes in gene expression in C57BL6 mice, a strain of mice with intermediate radiation sensitivity compared to radiosensitive BALB/cJ and radioresistant 129/J mice (41). Mice were irradiated with doses between 1 Gy and 8 Gy, representing exposures that would initiate varying severities of acute radiation syndrome³. Of the eight radiation responsive genes identified in patients, expression of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b (Figure 3) increased significantly in irradiated mice ($p < 0.05$). Most of these genes are involved in apoptosis (Bbc3 and Tnfrsf10b) or cell cycle regulation (Ccng1 and Cdkn1a) and have very well characterized radiation responses. However, while Serpine1 mRNA and protein levels have been shown to increase in numerous tissue types after ionizing radiation (42-45), its response in whole blood has never been characterized. It has been suggested that increased

SERPINE1 levels may contribute to more efficient thrombus formation following vascular injury (46), which may be important following exposure to ionizing radiation.

To determine if changes in gene expression are capable of qualitative (non-irradiated/irradiated) and/or quantitative (dose) assessment of radiation exposure, class prediction (weighted voting) and multiple linear regression models were developed at each time point. Using data obtained from a training set of mice, we developed class prediction models (using the expression of Ccng1 and Cdkn1a) capable of distinguishing non-irradiated from irradiated mice in a separate validation set with an accuracy, specificity and sensitivity of 96.3, 100.0 and 94.4%, respectively, at 48 hours (Table 5). Applying this model back to the original set of human samples (non-irradiated controls and irradiated cancer patients) demonstrates an impressive accuracy, specificity and sensitivity of 93.7, 92.3 and 100%, respectively at 48 hours. Collectively, these data suggest that gene expression analysis may facilitate rapid identification of exposed individuals in need of further evaluation through other dosimetry methods, such as monitoring of clinical symptoms or cytogenetic analysis.

The significant linear dose-dependent increase in the expression of all five genes (Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b) at 48 hours post-irradiation (Table 3) suggested that a continuous model could be developed to predict radiation dose. In the training set of mice, a multiple linear regression model capable of predicting dose of exposure was developed using the expression of Ccng1 and Cdkn1a (predicted dose = $-0.7674 + 0.6343 * [Ccng1] + 0.0804 * [Cdkn1a]$). Examination of this model in an independent validation set of mice predicted doses for the 0, 1, 2, 4, 6, and 8 Gy treatment groups as 0.0 ± 0.2 , 1.6 ± 1.0 , 2.9 ± 1.4 , 5.1 ± 2.0 , 5.3 ± 0.7 , and 10.5 ± 5.6 Gy, respectively. This model could not be directly applied to predict dose in the original set of human samples (non-irradiated controls and irradiated cancer patients) due to the fractionated nature of the radiation therapy administered. However, these studies provide a "proof of principle" suggesting that gene expression may be used for determining dose of radiation exposure.

In conclusion, the current study: 1.) identified biodosimetry genes in cancer patients whose expression changed with exposure to radiation; 2.) utilized these genes in an *in vivo* mouse model to develop classification models capable of distinguishing non-irradiated from irradiated individuals and 3.) utilized a continuous regression model to demonstrate that estimation of dose of exposure was possible. These studies show that incorporating gene expression analysis into current dosimetry protocols may facilitate qualitative (non-irradiated/irradiated) and quantitative (dose) assessment of radiation exposure. Even more,

incorporating the analysis of protein levels of these genes may be used in protein assays that could potentially be self-administered.

5 All patents, patent applications, provisional applications, and publications referred to or cited herein, *supra* or *infra*, are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

10 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Tables

TABLE 1A
Patient Demographics and White Blood Cell Counts

Patient	Age (yrs)	Sex	Race	Prior to TBI	White Blood Cell Counts (1000/uL)				
					Day 1 ^a	Day 2 ^a	Day 3 ^a	Day 4 ^b	Day 5 ^b
A	14	F	White		2.42	3.07		1.58	
B	14	M	Black	2.23	2	1.69			0.74
C	13	F	White	3.41	6.42		3.05		1.76
D	20	F	White	8.33		3.28	3.1	1.12	0.81
Average=	15.25			4.66 ± 3.24	3.61 ± 2.44	2.68 ± 0.86	3.08 ^c	1.35 ^c	1.10 ± 0.57

^a White Blood Cell Counts taken throughout the course of TBI

^b White Blood Cell Counts taken after their completion of TBI

^c Standard deviation not calculated due to inadequate sample number

Table 1B. Genes Previously Showing Transcriptional Responses to Ionizing Radiation

Gene Name	NCBI RefSeq	References
<u>Genes Identified in Our Preliminary Studies</u>		
Bcl-2 Associated X Protein (BAX)	NM_138765	Amundson 1999, Amundson and Bittner 2003, Jen 2003, Jen 2005, Mori 2005, Gruel 2006, Dressman 2007, Lin 2007, Paul 2008
Cadherin 5, Type 2 (CDH5)	NM_001795	
Chemokine (C-C motif) Ligand 26 (CCL26)	NM_006072	
Chemokine (C-X3-C motif) Ligand 1 (CX3CL1)	NM_002996	
Chemokine (C-X-C motif) Ligand 5 (CXCL5)	NM_002994	Amundson 2004
Chemokine (C-X-C motif) Ligand 9 (CXCL9)	NM_002416	
Chemokine (C-X-C motif) Ligand 10 (CXCL10)	NM_001565	
Chemokine (C-X-C motif) Ligand 11 (CXCL11)	NM_005409	
Chemokine (C-X-C motif) Ligand 12 (CXCL12)	NM_000609	Amundson 1999
Colony Stimulating Factor 1 (CSF1)	NM_172211	
Interferon, Alpha 2 (IFNA2)	NM_000605	Amundson 2000
Interleukin 1, Beta (IL1B)	NM_000576	
Leptin (LEP)	NM_000230	Akerman 2005, Paul 2008
Leukemia Inhibitory Factor (LIF)	NM_002309	
Midkine (MDK)	NM_001012333	
Oncostatin M (OSM)	NM_020530	
Serpin peptidase inhibitor, clade E, member 1 (SERPINE1)	NM_000602	
<u>Genes Previously Identified by Other Investigators</u>		
Actin, Alpha 2, Smooth Muscle, Aorta (ACTA2)	NM_001613	Jen 2003, Dressman 2007, Paul 2008
Activating Transcription Factor 3 (ATF3)	NM_001674	Amundson 1999, Amundson and Bittner 2003, Jen 2003, Akerman 2005, Jen 2005, Mori 2005
Bcl-2 Binding Component 3 (BBC3)	NM_014417	Tusher 2001, Jen 2005, Kis 2006, Dressman 2007, Paul 2008, Turtoi 2009
BTG family, member 2 (BTG2)	NM_006763	Ofir 2000, Amundson and Lee 2003, Jen 2003, Jen 2005, Kis 2006, Lin 2007
Cell Division Cycle 20 Homolog (CDC20)	NM_001255	Tusher 2001, Jen 2003, Lin 2007
Cyclin A2 (CCNA2)	NM_001237	Jen 2003, Lin 2007
Cyclin B1 (CCNB1)	NM_031966	Tusher 2001, Jen 2003, Lin 2007, Amundson 2008
Cyclin G1 (CCNG1)	NM_004060	Amundson 2000, Tusher 2001, Jen 2003, Akerman 2005, Jen 2005, Mori 2005, Paul 2008

Table 1B. Genes Previously Showing Transcriptional Responses to Ionizing Radiation

Gene Name	NCBI RefSeq	References
Cyclin-Dependent Kinase Inhibitor 1A (CDKN1A)	NM_000389	Amundson 1999, Amundson 2000, Tusher 2001, Amundson and Bittner 2003, Amundson and Lee 2003, Jen 2003, Amundson 2004, Akerman 2005, Jen 2005, Mori 2005, Kis 2006, Zschenker 2006, Dressman 2007, Lin 2007, Paul 2008, Turtoi 2008, Turtoi 2009
Damage-Specific DNA-Binding Protein 2 (DDB2)	NM_000107	Amundson 2000, Amundson and Bittner 2003, Jen 2003, Amundson 2004, Grace 2005, Jen 2005, Mori 2005, Dressman 2007, Lin 2007, Paul 2008
Fc Fragment of IgG, high affinity 1A, Receptor (FCGR1A)	NM_000566	Amundson 2004
Ferrodoxin Reductase (FDXR)	NM_004110	Jen 2003, Jen 2005, Mori 2005, Kis 2006, Paul 2008
Growth Arrest and DNA-Damage-Inducible, Alpha (GADD45A)	NM_001924	Papathanasiou 1991, Amundson 1999, Tusher 2001, Grace 2002, Amundson and Lee 2003, Jen 2003, Amundson 2004, Akerman 2005, Grace 2005, Jen 2005, Mori 2005, Kis 2006, Lin 2007, Paul 2008, Turtoi 2009
Heat Shock 10 kD Protein 1 (HSPE1)	NM_002157	Jen 2003, Gruel 2006
Interleukin 8 (IL-8)	NM_000584	Amundson 1999, Amundson and Bittner 2003, Amundson 2004
Mdm2 p53 binding protein homolog (MDM2)	NM_006878	Amundson 1999, Tusher 2001, Amundson and Bittner 2003, Amundson and Lee 2003, Jen 2003, Akerman 2005, Zschenker 2006, Paul 2008, Turtoi 2008, Turtoi 2009
Proliferating Cell Nuclear Antigen (PCNA)	NM_199246	Amundson 2000, Tusher 2001, Jen 2003, Akerman 2005, Mori 2005, Kis 2006, Paul 2008, Turtoi 2008, Turtoi 2009
Proprotein Convertase Subtilisin/Kexin Type 1 (PCSK1)	NM_000439	
Protein Kinase C, Eta (PRKCH)	NM_006255	Amundson 1999
Protein Phosphatase 1D Magnesium-Dependent, Delta Isoform (PPM1D)	NM_003620	Dressman 2007
Tumor Necrosis Factor (TNF)	NM_000594	Amundson and Lee 2003, Jen 2003, Jen 2005, Kis 2006, Paul 2008
Tumor Necrosis Factor Ligand Superfamily, Member 4 (TNFSF4)	NM_003326	Tusher 2001, Amundson 2004
Tumor Necrosis Factor Receptor Superfamily, Member 6 (TNFRSF6/FAS)	NM_152873	Amundson 1999, Tusher 2001, Akerman 2005, Paul 2008
		Tusher 2001, Amundson and Lee 2003, Jen 2003, Sakamoto-Hojo 2003, Jen 2005, Mori 2005, Lin 2007, Paul 2008

Table 1B. Genes Previously Showing Transcriptional Responses to Ionizing Radiation

Gene Name	NCBI RefSeq	References
Tumor Necrosis Factor Receptor Superfamily, Member 10B (TNFRSF10B)	NM_003810	Jen 2003, Akerman 2005, Jen 2005, Mori 2005, Lin 2007, Paul 2008, Amundson 2008
Tumor Protein p53 inducible protein 3 (TP53I3)	NM_147184	Jen 2003, Dressman 2007, Lin 2007, Long 2007
Ubiquitin conjugating enzyme E2S (UBE2S)	NM_014501	Tusher 2001, Jen 2003, Lin 2007
V-myc Myelocytomatosis Viral Oncogene Homolog (MYC)	NM_002467	Amundson 1999, Tusher 2001, Jen 2003, Akerman 2005, Lin 2007, Paul 2008, Turtoi 2008
V-rel Reticuloendotheliosis Viral Oncogene Homolog B (REL-B)	NM_006509	Amundson 1999, Amundson and Bittner 2003, Wang 2006, Paul 2008
Xeroderma Pigmentosum, Complementation Group C (XPC)	NM_004628	Amundson 2000, Tusher 2001, Amundson and Bittner 2003, Jen 2003, Akerman 2005, Jen 2005, Mori 2005, Zschenker 2006, Dressman 2007, Long 2007, Paul 2008, Amundson 2008

Housekeeping Genes

RPLPO

18S

Table 1C. Eight genes demonstrating increased expression in irradiated patient samples compared to baseline patient samples (human and mouse)

ABI Assay ID Human	Human RefSeq	ABI Assay ID Mouse	Mouse RefSeq
Hs00426835_g1	NM_001141945.1 and NM_001613.2	Mm00725412_s1	NM_007392.2
Hs00248075_m1	NM_014417.3, NM_001127240.1, and NM_001127241.1	Mm00519268_m1	NM_133234
Hs00171112_m1	NM_004060.3 and NM_199246.1	Mm00438084_m1	NM_009831.2 NM_001111099.1 and NM_007669.4
Hs00355782_m1	NM_078467.1 and NM_000389.3	Mm00432448_m1	NM_007836.1
Hs00169255_m1	NM_001924.2 NM_001012334.1, NM_001012333.1, and	Mm00432802_m1	
Hs00171064_m1	NM_002391.3	Mm00440279_m1	NM_010784.4
Hs01126606_m1	NM_000602.2	Mm00435860_m1	NM_008871.2
Hs00366272_m1	NM_147187.1 and NM_003842.3	Mm00457866_m1	NM_020275.3

TABLE 2
Regression Analysis of Patient Samples^a

Gene	r² value	p-value
ACTA2	0.8182	0.0955
BBC3	0.8555	0.0751
CCNG1	0.8326	0.0875
CDKN1A	0.9589	0.0208
GADD45A	0.8104	0.0998
MDK	0.9855	0.0073
SERPINE1	0.9394	0.0308
TNFRSF10B	0.9133	0.0444

^aCalculated by plotting time from exposure (x-axis) vs. gene expression (y-axis)

TABLE 3
Linear Regression Analysis of Gene Expression vs. Dose in C57BL/6 Mice

Gene	5 hours ^a		12 hours ^a		23 hours ^a		48 hours ^a	
	r ² value ^b	p-value ^b	r ² value ^b	p-value ^b	r ² value ^b	p-value ^b	r ² value ^b	p-value ^b
Bbc3	0.1019	0.6808	0.7252	0.1484	0.2709	0.4795	0.9844	0.0078
Ceng1	0.4435	0.3341	0.9506	0.025	0.9161	0.0429	0.9868	0.0066
Cdkn1a	0.7606	0.1279	0.9251	0.0382	0.6881	0.1705	0.9869	0.0066
Serpine1	0.3758	0.387	0.6927	0.1677	0.9271	0.0372	0.906	0.0481
Tnfrsf10b	0.5193	0.2794	0.9995	0.0003	0.05013	0.7761	0.9687	0.0158

^a Time Post-TBI

^b Calculated by plotting dose (x-axis) vs. gene expression (y-axis)

TABLE 4

Prediction of Exposure in Mice (Nonirradiated vs. Irradiated)

	5 hours ^d		12 hours ^d		23 hours ^e		48 hours ^f	
	%	Ratio	%	Ratio	%	Ratio	%	Ratio
Training Set								
Accuracy ^a	94.7	89/94	93.6	88/94	95.7	90/94	92.6	87/94
Specificity ^b	97.1	68/70	97.1	68/70	98.6	69/70	97.1	68/70
Sensitivity ^c								
1 Gy	87.5	7/8	75	6/8	62.5	5/8	50	4/8
2 Gy	87.5	7/8	75	6/8	100	8/8	87.5	7/8
6 Gy	87.5	7/8	100	8/8	100	8/8	100	8/8
Overall	87.5	21/24	83.3	20/24	87.5	21/24	79.2	19/24
Validation Set								
Accuracy ^a			92.6	25/27			96.3	26/27
Specificity ^b			100	9/9			100	9/9
Sensitivity ^c								
1 Gy			66.7	2/3			66.7	2/3
2 Gy			66.7	2/3			100	3/3
4 Gy			100	4/4			100	4/4
6 Gy			100	4/4			100	4/4
8 Gy			100	4/4			100	4/4
Overall			88.9	16/18			94.4	17/18

^a number of mice correctly classified/total number of mice^b number of mice correctly classified as nonirradiated/total number of nonirradiated mice^c number of mice correctly classified as irradiated/total number of irradiated mice^d prediction based on expression of Ccng1^e prediction based on expression of Bbc3 and Ccng1^f prediction based on expression of Ccng1 and Cdkn1a

TABLE 5

Predicted Dose based on Multiple Linear Regression Analysis

Actual Dose	Mean	SD	n=	Min	Max	Median
Training Set						
0 Gy	0.1	0.5	14	-0.2	1.8	0.0
1 Gy	1.2	0.3	8	0.8	1.6	1.2
2 Gy	2.3	0.5	8	1.5	2.9	2.4
6 Gy	5.3	1.6	8	2.3	7.3	5.4
Validation Set						
0 Gy	0.0	0.2	9	-0.3	0.3	0.0
1 Gy	1.6	1.0	3	0.8	2.7	1.3
2 Gy	2.9	1.4	3	2.1	4.5	2.2
4 Gy	5.1	2.0	4	3.1	7.3	5.0
6 Gy	5.3	0.7	4	4.7	6.4	5.1
8 Gy	10.5	5.6	4	6.7	18.8	8.2

^a predicted dose = 0.7674 + 0.6343 * [Ccng1] - 0.0804 * [Cdkn1a]

TABLE 6
Class Prediction of Dosing Groups in Mice (<2 Gy vs. ≥2 Gy)

	5 hours ^a		12 hours ^b		23 hours ^c		48 hours ^b	
	%	Ratio	%	Ratio	%	Ratio	%	Ratio
Training Set								
Accuracy^c	93.6	88/94	93.6	88/94	95.7	90/94	96.8	91/94
Specificity^d								
0 Gy	100	70/70	98.6	69/70	98.6	69/70	100	70/70
1 Gy	62.5	5/8	75	6/8	75	6/8	100	8/8
Overall	96.2	75/78	96.2	75/78	96.2	75/78	100	78/78
Sensitivity^e								
2 Gy	75.0	6/8	62.5	5/8	87.5	7/8	62.5	5/8
6 Gy	87.5	7/8	100	8/8	100	8/8	100	8/8
Overall	81.3	13/16	81.3	13/16	93.8	15/16	81.3	13/16
Validation Set								
Accuracy^c			88.9	24/27			100	27/27
Specificity^d								
0 Gy			100	9/9			100	9/9
1 Gy			66.7	2/3			100	3/3
Overall			91.7	11/12			100	12/12
Sensitivity^e								
2 Gy			66.6	2/3			100	3/3
4 Gy			100	4/4			100	4/4
6 Gy			100	4/4			100	4/4
8 Gy			75	3/4			100	4/4
Overall			86.7	13/15			100	15/15

^aprediction based on expression of Tnf α sfl0b

^bprediction based on expression of Ccng1

^cprediction based on expression of Ccng1 and Tnf α sfl0b

^dnumber of mice correctly classified/total number of mice

^enumber of mice correctly classified as nonirradiated/total number of nonirradiated mice

^fnumber of mice correctly classified as irradiated/total number of irradiated mice

Sequences1. NCBI Reference Sequence: NM_001141945.1

Name: Homo sapiens actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant 1, mRNA

Version: NM_001141945.1, GI:213688374

Source: human

Sequence:

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1 ctctccccgc ccccgcgggg cggcgcgcac tcaccacccc gcgcgggagc ggacctttgg
10 61 cttggcttgt cagggttgt ccaggagtgc cgctcctctc tccaaccggg
gtccccctcc
121 agcgacccta aagcttccca gacttccgct tcaattcctg tccgcacccc acgcccacct
181 caacgtggag cgcagtgggc tccgaggagc gcgggagctg ccccgccctgc ccagcggggg
241 cagcacttcg catcaaggcc caagaaaagc aagtctctca ggtttctgag caccggggcc
15 301 tgagggaagg tctaacagc cccggggagc cagtctccaa cgcctccgcg agcagcccgc
361 cgctcccagg tgcccgctg cgccgctgcc gccgcaatcc cgcacgcgtc ccgcgcccgc
421 cccactttgc ctatcccgg gactaagacg ggaatcctgt gaagcagctc cagctatgtg
481 tgaagaagag gacagcactg ccttgggtgt tgacaatggc tctgggctct gtaaggccgg
541 ctttgctggg gacgatgctc ccagggtgtg tttcccatcc attgtgggac gtcccagaca
20 601 tcagggggtg atgggtggaa tgggacaaaa agacagctac gtgggtgacg aagcacagag
661 caaaagagga atcctgaccc tgaagtaccc gatagaacat ggcatcatca ccaactggga
721 cgacatggaa aagatctggc accactcttt ctacaatgag ctctgtgttg cccctgaaga
781 gcatcccacc ctgctcacgg aggcaccctt gaaccccagg gccaaaccgg agaaaatgac
841 tcaaattatg tttgagactt tcaatgtccc agccatgtat gtggctatcc aggcgggtgt
25 901 gtctctctat gctctggac gcacaactgg catcgtgctg gactctggag atgggtgtac
961 ccacaatgtc cccatctatg agggctatgc cttgcccctt gccatcatgc gtctggatct
1021 ggctggccga gatctcactg actacctcat gaagatcctg actgagcgtg gctattcctt
1081 cgttactact gctgagcgtg agattgtccg ggacatcaag gagaaactgt gttatgtagc
1141 tctggacttt gaaaatgaga tggccactgc cgcatactca tctcctcttg agaagagtta
30 1201 cgagttgcct gatgggcaag tgatcaccat cggaaatgaa cgtttccgct gccagagac
1261 cctgttccag ccatecttca tggggatgga gtctgctggc atccatgaaa ccacctacaa
1321 cagcatcatg aagtgtgata ttgacatcag gaaggacctc tatgctaaca atgtcctatc
1381 agggggcacc actatgtacc ctggcattgc cgaccgaatg cagaaggaga tcacggccct
1441 agcaccagc accatgaaga tcaagatcat tgcccctccg gagcgcaaat actctgtctg
35 1501 gatcggtggc tccatcctgg cctctctgtc cacttccag cagatgtgga tcagcaaaaca
1561 ggaatacgat gaagccgggc cttccattgt ccacgcgaaa tgcttctaaa acactttcct
1621 gctcctctct gtctctagca cacaactgtg aatgtcctgt ggaattatgc cttcagttct
1681 tttccaaatc attcctagcc aaagctctga ctcgttacct atgtgttttt taataaatct
1741 gaaataggct actggtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa
40 (SEQ ID NO:1)
```

2. NCBI Reference Sequence: NM_001613

Name: Homo sapiens actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant 2, mRNA.

Version: NM_001613.2 GI:213688378

Source: human

Sequence:

```
1 gaggagagca ggccaagggg ctatataaac cttcagcttt cagcttccct gaacaccacc
61 cagtgtggag cagcccagcc aagcactgtc aggaatcctg tgaagcagct ccagctatgt
50 121 gtgaagaaga ggacagcact gccttgggtgt gtgacaatgg ctctgggctc tgtaaggccg
181 gctttgctgg ggacgatgct cccagggctg ttttccatc cattgtggga cgtcccagac
241 atcagggggg gatgggtgga atgggacaaa aagacagcta cgtgggtgac gaagcacaga
301 gcaaaaagag aatcctgacc ctgaagtacc cgatagaaca tggcatcatc accaactggg
361 acgacatgga aaagatctgg caccactctt tctacaatga gcttcgtgtt gccctgaag
55 421 agcatcccac cctgctcacg gaggcacccc tgaaccccaa ggccaaccgg gagaaaatga
481 ctcaaattat gtttgagact ttcaatgtcc cagccatgta tgtggctatc caggcgggtg
541 tgtctctcta tgctctgga cgcacaactg gcacgtgctg ggaactctga gatgggtgca
601 cccacaatgt ccccatctat gagggctatg ccttgcccca tgccatcatg cgtctggatc
```

661 tggctggccg agatctcact gactacctca tgaagatcct gactgagcgt ggctattcct
 721 tcgttactac tgctgagcgt gagattgtcc gggacatcaa ggagaaactg tgttatgtag
 781 ctctggactt tgaaaatgag atggccactg ccgcatacctc atccctccctt gagaagagtt
 841 acgagttgcc tgatgggcaa gtgatcacca tcggaaatga acgtttccgc tgcccagaga
 901 cctgtttcca gccatccctc atcgggatgg agtctgctgg catccatgaa accacctaca
 961 acagcatcat gaagtgtgat attgacatca ggaaggacct ctatgctaac aatgtcctat
 1021 cagggggcac cactatgtac cctggcattg ccgaccgaat gcagaaggag atcacggccc
 1081 tagcaccag caccatgaag atcaagatca ttgcccctcc ggagcgcaaa tactctgtct
 1141 ggatcggtgg ctccatccctg gcctctctgt ccaccttcca gcagatgtgg atcagcaaac
 1201 aggaatacga tgaagccggg ccttccattg tccaccgcaa atgcttctaa aacactttcc
 1261 tgctcctctc tgtctctagc acacaactgt gaatgtcctg tgggaattatg ccttcagttc
 1321 ttttccaaat cattcctagc caaagctctg actcgttacc tatgtgtttt ttaataaato
 1381 tgaaataggc tactggtaaa aaaaaaaaaa aaaaa

(SEQ ID NO:2)

3. NCBI Reference Sequence: NM_007392

Name: Mus musculus actin, alpha 2, smooth muscle, aorta (Acta2), mRNA.

Version: NM_007392.2 GI:31982518

Source: Mus musculus (house mouse)

Sequence:

1 ggacaccacc caccagagt ggagaagccc agccagtcgc tgtcaggaac cctgagacgc
 61 tgctccagct atgtgtgaag aggaagacag cacagccctg gtgtgcgaca atggctctgg
 121 gctctgtaag gccggtctcg ctggtgatga tgctcccagg gctgttttcc catccatcgt
 181 gggacgtccc agacatcagg gagtaatggg tggaaatggc caaaaagaca gctatgtggg
 241 ggatgaagcc cagagcaaga gagggatcct gacgctgaag tatccgatag aacacggcat
 301 catcaccaac tgggacgaca tggaaaagat ctggcaccac tctttctata acgagcttcc
 361 tgtggcccct gaagagcatc cgacactgct gacagaggca ccactgaacc ctaaggccaa
 421 ccgggagaaa atgaccaga ttatgtttga gaccttcaat gtccccgcca tgtatgtggc
 481 tttcagggt gtgctgtccc tctatgcctc tggacgtaca actggtattg tgctggactc
 541 tggagatggg gtgactcaca acgtgcctat ctatgagggc tatgccctgc ctcatgccat
 601 catgctctg gacttggctg gccgagatct caccgactac ctcatgaaga tctgactga
 661 gcgtggctat tcttctgtga ctactgccga gcgtgagatt gtccgtgaca tcaaggagaa
 721 gctgtgctat gtagctctgg actttgaaaa tgagatggcc acggccgcct cctcttctcc
 781 cctggagaag agctacgaac tgccctgacg gcaggtgatc accattggaa acgaacgctt
 841 ccgctgcccc gagactctct tccagccatc tttcattggg atggagtccg cgggcatcca
 901 cgaaccacc tataacagca tcatgaagtg tgatattgac atcaggaagg atctctatgc
 961 taacaacgtc ctgtcagggg gtaccaccat gtaccaggc attgctgaca ggtatgcagaa
 1021 ggagatcaca gccctcgcac ccagcaccat gaagatcaag atcattgccc ctccagaacg
 1081 caagtactct gtctggatcg gtggctccat cctggcttcc ctgtctacct tccagcagat
 1141 gtggatcagc aaacaggaat acgacgaagc tgggcccctc atcgccacc gcaaatgctt
 1201 ctaagtcccc cctgctctgc ctctagcaca caactgtgaa cgttttgtgg atcagcgctt
 1261 ccagttcctt tccaaatcat tctgcccaca agctttgatt tgttactcgt ggttttttta
 1321 aaaataaata agacatgtgc tacccttaac ttgtagccct ggccatgcaa cactgattca
 1381 attgtgtttc tgttaggtga gaatcattta ttatagggtg gccccctcta gtggtcagga
 1441 atagatgtga cacaactaga aaagtatatt ccaggcatg gatggcatca atcacttcaa
 1501 cagaggaagg tccacttaaa tgatgtaaac ggggtatttg agagcgtata ggacttctat
 1561 agattacata taataatata tattagtacc aataggaaaa atcgacactg acttcacagc
 1621 tagagtgact attttacttt atacatacag gtgaccagat aggtaaaaaa caatttttacg
 1681 tgtattttaa atttggcaag taatatggtt ttattaaaaa cacatctttt gtattaaagc
 1741 atacacacgt gcatggatat gcatgctgta cttttaactt

(SEQ ID NO:3)

4. NCBI Reference Sequence: NM_014417

Name: Homo sapiens BCL2 binding component 3 (BBC3), transcript variant 4, mRNA.

Version: NM_014417.3 GI:187829711

Source: human

Sequence:

1 ggggagcgag ccacatgcga ggggggcgct gggggcgggc gggggcgccac cagcgatccc
 61 agcagcgggc acgacgcgga cgcgcctgcg gcccggggag cagcagcagc cacagccaca
 121 gcagccgcca ctgcagttag agcggcagca gcagcgacag ccacagcagc agccgcccgc
 5 181 gagagcgggc ctggcggggc ggcgcctcct gaaggaagcc gcccgcctcc caccgcgcgc
 241 ccctccggcg tgttcattgc cccggggccc caggagagcg catggcccgc gcacgccagg
 301 agggcagctc cccggagccc gtagaggggc tggcccgcca cggcccgccc ccttcccgcc
 361 tcggccgcct ggtgcctcgc gcagtgtcct ggcgcctctg cgagcccgcc ctggctgcgc
 421 ccccgccgccc cccaccctgc ctgcccgcgc cctacctctg cggcccccac gcccaccgag
 10 481 ccgtcaccgc cgccttgggg ggttcccgct ggcctggggg tccccgcagc cggcccccag
 541 gcccgcgccc ggaacggtcc cagccctcgc tctcgctggc ggagcagcac ctggagtgc
 601 ccgtgcccag cggcccgggg gctctggcgg gcggtccac ccaggcgccc ccgggagtec
 661 gcggggagga ggaacagtgg gccggggaga tcggggccca gctgcggcgg atggcggaag
 721 acctcaacgc acagtacgag cggcgagagc aagaggagca gcagcgccac cggccctcac
 15 781 cctggagggt cctgtacaat ctcatcatgg gactcctgcc cttaccagag ggccacagag
 841 ccccgagat ggagcccaat taggtgcctg caccgcccgc gtggagctca gggactcggg
 901 gggcagcccc ctcccacctc ctgacacctt ggcagcgcg ggggactttc tctgcacct
 961 gtgcatact ggactcccag cctgcctgtt cccggggggc ggcgggggca gccactccag
 1021 cccagcccca gcttgggtg cactgacgga gatgcggact cctgggtccc tggccaagaa
 20 1081 gccagagag ggacggctga tggactcagc atcggaaggt ggcgggtgac gagggggtgg
 1141 ggactgagcc gccgcctctt gccgcccacc accatctcag gaaaggctgt tgtgctggtg
 1201 ccggttccag ctgcaggggt gacactggga ggggggggct ctcctctcgg tgcctctca
 1261 ctctgggctt ggctcaggcc cctgggtgct tccccccctc ctcctgggag ggggcccgtg
 1321 aagagcaaat gagccaaacg tgaccactag ctcctgggag ccagagagtg gggctcgttt
 25 1381 gccggttgc ccagcccggc gccagccat ctccttgag ccagccggcg ggtggtgggc
 1441 atgctgcct cacttctatc aggggttggc caggaggggc ccagactgtg aatcctgtgc
 1501 tctgcccgtg accgcccccc gcccatcaa tccattgca taggtttaga gagagcacgt
 1561 gtgaccactg gcattcattt ggggggtggg agattttggc tgaagccgcc ccagccttag
 1621 tccccagggc caagcgctgg ggggaagacg gggagtccag gaggggggga aatctcggaa
 30 1681 gagggaggag tctgggagt gggagggatg gccagccctg taagatactg tatatgcgt
 1741 gctgtagata ccggaatgaa tttctgttac atgtttggtt aatttttttt gtacatgatt
 1801 tttgtatgtt tctttttcaa taaatcaga ttggaacagt gga
 (SEQ ID NO:4)

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5. NCBI Reference Sequence: NM_001127240Name: Homo sapiens BCL2 binding component 3 (BBC3), transcript variant 1, mRNA.Version: NM_001127240.1 GI:187829729Source: humanSequence:

40 1 gaggcgattg cgattgggtg agaccagta aggatggaaa gtgtagagga gacaggaatc
 61 caggcctttg gaaaaaggaa ggacaaaact caccaaacca gagcagggca ggaagtaaca
 121 atgagaaact gaaaaagaaa cggaatggaa agctatgaga caggatgaaa tttggcatgg
 181 ggtctgccc ggcattgcca tgccaggtgc ccagggtgc ttccacgacg tgggtccctt
 45 241 gccagatttg tggcccccag gagcgccatg gccgcgcac gccagggagg cagctccccc
 301 gagcccgtag agggcctggc ccgcgacggc ccgcgcctct tcccgtcggc ccgctcgtg
 361 cctcggcag tgtcctgcgg cctctgcgag cccggcctgg ctgcgcctcc cgcgcctccc
 421 accctgctgc ccgctgccta cctctgcgcc cccaccgccc caccgcctgt caccgcgcgc
 481 ctgggggggt cccgctggcc tgggggtccc cgcagccggc cccagggccc gcgcccggac
 541 ggtcctcagc cctcgtcttc gctggcggag cagcacctgg agtcgcccgt gccagcgccc
 50 601 ccgggggctc tggcgggcgg tcccaccag gcggccccgg gagtccggcg ggaggaggaa
 661 cagtgggccc gggagatcgg ggcccagctg cggcggtatg cggacgacct caacgcacag
 721 tacgagcggc ggagacaaga ggagcagcag cggcacccgc cctcaccctg gagggtcctg
 781 tacaatctca tcatgggact cctgccttta cccaggggcc acagagcccc cgagatggag
 841 cccaattagg tgctgcacc cgcgcgggtg acgtcaggga ctcggggggc agggccctcc
 55 901 cactcctga caccctggcc agcgcggggg actttctctg caccatgtag catactggac
 961 tcccagccct gctgtcccg gggcgggggc ggggcagcca ctccagcccc agcccagcct
 1021 ggggtgcaact gacggagatg cggactcctg ggtccctggc caagaagcca ggagagggac
 1081 ggctgatgga ctcagcatcg gaagtgggc gtgaccgagg ggggtggggc tgagccgccc
 1141 gcctctgcgc cccaccacca tctcaggaaa ggctgttgtg ctggtgcccg ttccagctgc

1201 aggggtgaca ctgggagggg ggggctctcc tctcgggtget ccttcactct gggcctggcc
 1261 tcaggccctt ggtgcttccc cccctcctcc tgggaggggg cccgtgaaga gcaaatgagc
 1321 caaacgtgac cactagcctc ctggagccag agagtggggc tcgtttgccc gttgctccag
 1381 cccggcgccc agccatcttc cctgagccag ccggcgggtg gtgggcatgc ctgcctcacc
 5 1441 ttcatacagg ggtggccagg aggggcccag actgtgaatc ctgtgctctg cccgtgaccg
 1501 ccccccggcc catcaatccc attgcatagg tttagagaga gcacgtgtga ccactggcat
 1561 tcattttggg ggtgggagat tttggctgaa gccgccccag ccttagtccc cagggccaaag
 1621 cgctgggggg aagacgggga gtcagggagg gggggaaatc tcggaagagg gaggagtctg
 10 1681 ggagtgggga gggatggccc agcctgtaag atactgtata tgcgctgctg tagataccgg
 1741 aatgaatttt ctgtacatgt ttggttaatt tttttgtac atgatttttg tatgtttcct
 1801 tttcaataaa atcagattgg aacagtg

(SEQ ID NO:5)

6. NCBI Reference Sequence: NM_001127241

Name: Homo sapiens BCL2 binding component 3 (BBC3), transcript variant 2, mRNA.

Version: NM_001127241.1 GI:187829741

Source: human

Sequence:

1 gaggcgattg cgattgggtg agaccagta aggatggaaa gtgtagagga gacaggaatc
 20 61 cacggctttg gaaaaaggaa ggacaaaact caccaaacca gagcaggga ggaagtaaca
 121 atgagaaact gaaaaagaaa cggaatggaa agctatgaga caggatgaaa tttggcatgg
 181 ggtctgccc aagcatgtcca tgccagggtc ccagggtgctc ttccacgacg tgggtccctc
 241 gccagatttg tggctctcag cctcgcctct cgctggcgga gcagcacctg gactgcccg
 301 tgcccagcgc cccgggggct ctggcgggcg gtcccaccca ggccgccccg ggagtcgccc
 25 361 gggaggagga acagtggggc cggagatcgc gggcccagct gcgcgggatg gcggacgacc
 421 tcaacgcaca gtacgagcgg cggagacaag aggagcagca gcggcacgcg cctcacctc
 481 ggagggtcct gtacaatctc atcatgggac tctgcccctt acccaggggc cacagagccc
 541 ccgagatgga gcccatttag gtgctgcac ccgcccgggt gagctcaggg actcgggggg
 601 caggcccttc ccacctctg acacctggc cagcgcgggg gactttctct gcaccatgta
 30 661 gcatactgga ctcccagccc tgctgtccc gggggcgggc cggggcagcc actccagccc
 721 cagcccagcc tgggtgacac tgacggagat gcggactcct ggggtccctg ccaagaagcc
 781 aggagaggga cggctgatgg actcagcatc ggaagggtgc ggtgaccgag ggggtgggga
 841 ctgagccgcc cgcctctgcc gccaccacc atctcaggaa aggctgttgt gctggtgcc
 901 gttccagctg caggggtgac actgggaggg gggggctctc ctctcgggtg tcttcactc
 35 961 tgggcctggc ctacggcccc tgggtgcttc cccctcctc ctgggagggg gcccgtaag
 1021 agcaaatgag ccaaacgtga ccactagcct cctggagcca gagagtggg ctggtttgcc
 1081 ggttgctcca gcccgcgccc cagccatctt cctgagcca gccggcgggg ggtgggcatg
 1141 cctgcctcac cttcatcagg ggtggccag gaggggcca gactgtgaat cctgtgctct
 1201 gcccgtagcc gcccccgccc ccatcaatcc cattgcatag gtttagagag agcacgtgtg
 40 1261 accactggca ttcattttgg ggtgggaga ttttggtga agccgcccc gccttagtcc
 1321 ccaggggcaa gcgctggggg gaagacggg agtcaggag ggggggaaat ctcggaagag
 1381 ggaggagctc gggagtggg aggcctgtaa cagcctgtaa gatactgtat atgcgctgct
 1441 gtagataccg gaatgaattt tctgtacatg tttggttaat tttttttgta catgattttt
 1501 gtatgtttcc ttttcaataa aatcagattg gaacagtg

(SEQ ID NO:6)

7. NCBI Reference Sequence: NM_133234

Name: Mus musculus BCL2 binding component 3 (Bbc3), mRNA.

Version: NM_133234.2 GI:254911068

Source: Mus musculus (house mouse)

Sequence:

1 gagcgagcgg ggcccgggga tctgccggga ggcctgagac gcggcataga gccacatgcg
 61 agcggggagc ccaggaggcg gcggcgacac cagcaagcaa gcagcagcag cggatgatccg
 121 gacacgaaga ctccagaagc agcagcagtc actgcagtta gagcagcagg agcagcagca
 181 aggtgcctca atagcaaccc actcggcggg cgagccctcc agaaggcaac cggccgccac
 241 cccatcgcc cctttctccg gagtgttcat gccccgggg ctccaggagag cggcatggcc
 301 cgcgcacgcc aggaggcag ctctccggag ccgtagagg gtctagccc cgcagatccg
 361 cgccccttcc cgtcggcgg cctgatgcc tccgtgtat cctgcagcct ttgcgagccc
 421 ggcctgccc cgcgccctgc tgcccctgcc ttgctgccc cgcctacct ctgcgcccc

5 481 accgetccac ctgcegtcac cgcgcgcctg gggggccccc gctggcctgg gggtcaccgc
 541 agccggccca gagggccgcg cccggacggg cctcagccct cctgtcacc agcccagcag
 601 cacttagagt cgcctgtgcc cagcgcctcg gaggccttgg caggaggccc caccacagct
 661 gccccgggag tgcgtgtgga ggaggaggag tgggcccggg agatcggggc ccagctgcgg
 721 cggatggcgg acgacctcaa cgcgcagtac gagcggcgga gacaagaaga gcagcatcga
 781 caccgacctt caccctggag ggtcatgtac aatctcttca tgggactcct ccccttacct
 841 agggatcctg gagccccaga aatggagccc aactaggtgc ctacaccgcg ccgggggacg
 901 tcggagactt ggggggcagg accccctccg ccttctgaca ccttggccag cgcgggggac
 961 tttttctgca ccatgtagca tactggactg ccagccttgc cgtcccagg ggcaggcaag
 10 1021 ggatgccact cgagcccggg cagcctgggt gcactgatgg agatacggac ttgggggggac
 1081 cctggcctcc cgaaagccag ggaaggagg gctgaaggac tcatggtgac tgagggggtg
 1141 gggaccgagc cgcctgcctc tgcgcgccac caccatctca ggaaaggctg ctggtgctgg
 1201 ctgcccgttc cagctgcagg ggggacgctg ggggtgtccc cagtgcgcct tcacttttgg
 1261 cctggcctca ggcccctggt gcttcccccc ctccctctga ggaggggggc tgtgaagagc
 15 1321 atatgagcca aactgacca ctagcctcct ggagccagag aatggggggc gtgtgaaggc
 1381 cttcttaacc cagtgccag ccactctccc tgagccgcg gcgggcggtg aacgatgcct
 1441 gcctcacctt catctggggg tgtccaggag ggtccagac tgtgaatcct gtgctctgcc
 1501 cgggaccacc cccccccccc caatcccat ccatctcatt gcataagttt agagagagca
 1561 cgtgtgacca ctggcattca tttggggggg gggagatatt ggcggaagcc accccagcct
 20 1621 tagtccccag ggcaaagcgc tggggaggaa gatggggagt cagggagggg ggaagtctca
 1681 gaagagggag gagtctggga gcggggaggg acggcccagc ctgtaagata ctgtacatgc
 1741 actgctgtag atatactgga atgaattttc tgtacatgtt tggttaattt tttttgtaca
 1801 tgatttttgt atgtttcctt ttcaataaaa tcagattgaa cagtgaacac tctttttgtt
 1861 agctttacca gtgacagagc atctggcact acctgtaagg acatgaaaga aacggtgtgt
 25 1921 gtgtgtatgt gtgtgtgtgt gtgtgtgtgt gtgtgtgaga aatggctcag tggtta
 (SEQ ID NO:7)

8. NCBI Reference Sequence: NM_004060.3

Name: Homo sapiens cyclin G1 (CCNG1), transcript variant 1, mRNA.

Version: NM_004060.3 GI:40805830

Source: human

Sequence:

35 1 attcctcggt agggcaggcg cggccccttc ggctccgagc tgaccctgat cagggccgag
 61 ttgtctcggc ggcgctgccg aggcctccac ccaggacagt cccctccccc gggectctct
 121 cctcttgccct acgagtcctc tctcctcgta ggctctcggg atctgatata gtggggtgag
 181 gtgagcaggc ccggggaggg tggttaccgc tgaggagctg cagtctctgt caagatgata
 241 gaggtactga caacaactga ctctcagaaa ctgctacacc agctgaatgc cctggttgaa
 301 caggagtcta gatgtcagcc aaaggctctgt ggtttgagac taattgagtc tgcacacgat
 361 aatggcctca gaatgactgc aagactaagg gactttgaag taaaagatct tcttagtcta
 40 421 attcagttct ttggctttga cacagagaca tttctctag ctgtgaattt actggacaga
 481 ttcctgtcta aaatgaagg acagcccaag caccttgggt gtgttggaact gagctgcttt
 541 tatttggtcg taaaatcaat agaagaggaa aggaatgtcc cattggcaac tgacttgatc
 601 cgaataagtc aatataggtt tacggtttca gacttgatga gaatggaaaa gattgtattg
 661 gagaagggtg gttggaaagt caaagctact actgccttcc aatttctgca actgtattat
 45 721 tcaactcttc aagagaactt gccacttgaa aggagaaata gcattaattt tgaaagacta
 781 gaagctcaac tgaaggcatg tcattgcagg atcatatttt cttaaagcaaa gccttctgtg
 841 ttggcattgt ctatcattgc attagagatc caagcacaga agtgtgtaga gttaacagaa
 901 ggaatagaat gtcttcagaa acattccaag ataaatggca gagatctgac cttctggcaa
 961 gagcttgtat ccaaatgttt aactgaatat tcatcaaata agtgttccaa accaaatgtt
 50 1021 cagaagttga aatggattgt ttctgggctg actgcacggc aattgaagca tagctactac
 1081 agaataactc accttccaac aattcctgaa atggctccct aactggatta ttacagcacc
 1141 aaaaaacttc tctgaagcct ttctccacaa ccttggttcta tggattccat aatggttaca
 1201 tggattttaag ctatgaagcc tcaaaacatc acgagataag catgatggtc tcagacttgg
 1261 gaaaactgcc taatattatg ctgtagtggg attatgttta gatttgaatt catctgtgaa
 55 1321 gcattcaaat caaagctaaa agcctaaatg tgaaatgcta atgacaagcc tgagaaggta
 1381 aactgtgaat cttcatttct atcattgata taactttaga tattggatca atatatattg
 1441 gtggatattg aaatgctatt ggaggagtca cactaatact atcaataact agtcttccca
 1501 cagcttcaat cactgtcatt attctaact tactcctact taaattttaa gttatgaggt
 1561 ttatgtcaaa agcaacattt cacaaatgta cttttaaggc ataataaggg ttaacattct
 60 1621 aggcagtata aacacacccc ataatgcaag taataggtaa tctagagatg tggactttat

5

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1681 tgctatatgg gaattacatt taaatttgag ggcattttat ataaagaaaa atacagacct
1741 ataaagtttg gcatattcat taagttatct ttttaatttt ttttctagaa aacaggtgac
1801 atttgtatct acgataaaaa tttttatata gaacctactg cctcaaaactg aatcccatca
1861 agaaaactag tttctattgt attagtaact caaaataaat tatcacttcg aaaacttgct
1921 ttcccacact aaggtaagtt cagactagat tgaacactcc agaatttttt actacagact
1981 gtttttaagt tagaagtgat ggcaatttta taaatagaga atatacttcc actgatgccc
2041 ttactgtgcc aaaacaaaaa tcttaagaaa agcaagtaga caccttcata actatgaatg
2101 aagctgctga agtagtggtt aggatcctcc atggcagtta gtgaatgtaa gaagtacagt
2161 gttaaagtgt tgtaaacagt tactcagtgc aatgtatagc ctgagtctat ccattgatggc
2221 tatatccaat ttgacatcac gttatggatc agtacacaat gaaaaaccaa agaaccacag
2281 tatatcttat tcttaacttt tgtaaaccat gttttatggg taacttttta gttttcccaa
2341 aaggctgata aatttcaata ttttgaatac atcattgtta attttgagtt ggcagaggta
2401 aactaaccaa ctaccattat gtttttagtac taagggatat acctttcaat aaagttaatg
2461 aaattcaaaa aaaaaaaaaa aaaa

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(SEQ ID NO:8)

9. NCBI Reference Sequence: NM_199246**Name:** Homo sapiens cyclin G1 (CCNG1), transcript variant 2, mRNA.**Version:** NM_199246.1 GI:40805831

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Source: human**Sequence:**

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```

1 attcctcggt agggcaggcg cggccccttc ggctccgagc tgacctgat cagggccgag
61 ttgtctcggc ggcgtgccc aggcctccac ccggggaggg tggttaccgc tgaggagctg
121 cagtctctgt caagatgata gaggactga caacaactga ctctcagaaa ctgtacacc
181 agctgaatgc cctgttgaa caggagtcta gatgtcagcc aaaggctctg ggtttgagac
241 taattgagtc tgcacacgat aatggcctca gaatgactgc aagactaagg gactttgaag
301 taaaagatct tcttagtcta actcagttct ttggctttga cacagagaca ttttctctag
361 ctgtgaattt actggacaga ttctgttcta aaatgaaggc acagcccaag cacottgggt
421 gtgttggaact gagctgcttt tatttggtcg taaaatcaat agaagaggaa aggaatgtcc
481 cattggcaac tgacttgatc cgaataagtc aatatagggt tacggtttca gacttgatga
541 gaatggaaaa gattgtattg gagaagggtg gttggaaagt caaagctact actgccttcc
601 aatttctgca actgtattat tcaactcctc aagagaactt gccacttgaa aggagaaata
661 gcattaattt tgaaagacta gaagctcaac tgaaggcatg tcattgcagg atcatatttt
721 ctaaagcaaa gccttctgtg ttggcattgt ctatcattgc attagagatc caagcacaga
781 agtgtgtaga gttaacagaa ggaatagaat gtcttcagaa acattccaag ataaatggca
841 gagatctgac cttctggcaa gagcttgtat ccaaattgtt aactgaatat tcatcaataa
901 agtgttccaa accaaatggt cagaagttga aatggattgt ttctgggogt actgcaaggc
961 aattgaagca tagctactac agaataactc accttccaac aattcctgaa atggctccct
1021 aactggatta ttacagcacc aaaaaacttc tctgaagcct ttctccacaa ccttgttcta
1081 tggattccat aatgttacaa tggatttaag ctatgaagcc tcaaaacatc acgagataag
1141 catgatggtc tcagacttgg gaaaactgcc taatattatg ctgagtggga attatgttta
1201 gatttgaatt catctgtgaa gcattcaaat caaagctaaa agcctaaatg tgaatgtcta
1261 atgacaagcc tgagaaggta aactgtgaat cttcatttct atcattgata taactttaga
1321 tattggatca atatatattg gtggtattga aaatgctatt ggaggagtca cactaatact
1381 atcaactatc agtcttccca cagcttcaat cactgtcatt atttataatc tactcctact
1441 taaattttta gttatgaggt ttatgtcaaa agcaacattt cacaatgta cttttaaggc
1501 ataataaggg ttaacattct aggcagtata aacacacccc ataatgcaag taataggtaa
1561 tctagagatg tggactttat tgctatatgg gaattacatt taaatttgag ggcattttat
1621 ataaagaaaa atacagacct ataaagtttg gcatattcat taagttatct ttttaatttt
1681 ttttctagaa aacaggtgac atttgtatct acgataaaaa tttttatata gaacctactg
1741 cctcaaaactg aatcccatca agaaaactag tttctattgt attagtaact caaaataaat
1801 tatcacttcg aaaacttgct ttcccacact aaggtaagtt cagactagat tgaacactcc
1861 agaatttttt actacagact gtttttaagt tagaagtgat ggcaatttta taaatagaga
1921 atatacttcc actgatgccc ttactgtgcc aaaacaaaaa tcttaagaaa agcaagtaga
1981 caccttcata actatgaatg aagctgctga agtagtggtt aggatcctcc atggcagtta
2041 gtgaatgtaa gaagtacagt gttaaagtgt tgtaaacagt tactcagtgc aatgtatagc
2101 ctgagtctat ccattgatggc tatatccaat ttgacatcac gttatggatc agtacacaat
2161 gaaaaaccaa agaaccacag tatatcttat tcttaacttt tgtaaaccat gttttatggg
2221 taacttttta gttttcccaa aaggctgata aatttcaata ttttgaatac atcattgtta
2281 attttgagtt ggcagaggta aactaaccaa ctaccattat gtttttagtac taagggatat

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2341 accttttcaat aaagttaatg aaattcaaaa aaaaaaaaaa aaaa
(SEQ ID NO:9)

10. NCBI Reference Sequence: NM_009831

Name: Mus musculus cyclin G1 (Ccn1), mRNA.

Version: NM_009831.2 GI:70906458

Source: Mus musculus (house mouse)

Sequence:

10	1	accaatcagc	gacgtagatg	cgtcccagct	agagagccag	gtctccagct	tgttccccca
	61	ctgccccggc	cagtccttcg	ctgcgcgctg	accccagga	aggcagtact	tggtcagggc
	121	gctgagggac	cctccaccgg	gacgccggcc	cctccccggg	cctctgctca	cttgaccccc
	181	actccctagt	ccgtccccctt	agtaggcctg	tcggatcggg	gacgtggggc	gagctgagag
	241	caggccccgg	gtgggtggtc	accgtgggtg	agacgtggct	gtcaagatga	tagaagtact
15	301	gacaactgac	tctcagaaac	tgctacacca	gctgaacacc	ctggttgaac	aggagtctag
	361	atgtcagcca	aaggtctgcg	gottgaaact	aattgagtcg	gcccattgata	atggcctcag
	421	aatgactgca	agattacggg	actttgaagt	aaaagatcta	cttagtctaa	ctcagttctt
	481	tggctttgac	acggagacat	tttccctagc	tgtgaattta	ctggacagat	tcttgtctaa
	541	aatgaaggta	caggcgaagc	atcttgggtg	tgttggactg	agctgctttt	atttggctgt
20	601	gaaagcgact	gaagaggaaa	ggaatgtccc	actggcgact	gatttgcattc	gaataagtca
	661	gtataggttc	acggtttcag	acctgatgag	aatggagaag	attgtgttgg	agaaagtgtg
	721	ttggaaagtc	aaagctacta	ctgcctttca	atttctgcag	ctctattatt	cactcgttca
	781	cgacaccttg	ccatttgaga	ggagaaacga	tctgaatttt	gaaagactag	aagcccaact
	841	taaggcctgc	cactgcagga	tcataatttt	taaggcaaag	ccttctgtgc	tggcgtatc
25	901	tatccttgcg	ttggagatcc	aagcactgaa	atacgtagag	ttaacagaag	gagtagaatg
	961	tattcagaaa	cattccaaga	taagtggccg	agatttgacc	ttctggcaag	agcttgtttc
	1021	caagtgttta	actgaatatt	catcaaacaa	gtgctccaaa	cctaacggtc	agaagttgaa
	1081	atggattgtg	tctggacgca	ctgcacggca	actgaagcac	agttattata	gaataactca
	1141	cctcccaacg	attcctgaga	ccatttggtt	gttgataaat	ctggttgtta	ttctctgtat
30	1201	acagaaaatt	ttccagtatg	atcattttct	gctacaactg	aagaattgaa	atactatctt
	1261	caatataaag	aatatgggat	gaaaacataa	aggaaaagtg	aattgttgac	tgggtctagat
	1321	agagaatact	ggaaggcatt	cactgtgtac	agtgcgtagc	agttttaaga	gaaaagacat
	1381	atcaaaccct	tagatatacg	ctaatacttt	tcatacaaa	attagcgtag	tagcaaagag
	1441	aatactttta	actcgaattt	taaaagtagt	tactgaaata	gcacttcttt	aaattacgta
35	1501	ccaccccact	gtagcttatt	taaagttgca	taagcccatg	cagaacaaca	agcaatgtga
	1561	cccataatg	aacaaatttt	aatctgccc	tcgactatga	aaatgaagta	caaacctggg
	1621	tgatggactt	acaaagtaat	atagggcatg	cccatgttag	gtttctggaa	actgccagag
	1681	tgtcttaatt	ctatagctag	tattttacct	ctatagcatt	tggactaata	caaagtaatt
	1741	atatgcatga	aaatataaaa	ttggtctctg	atacatcac	atttttgaca	tctaaatttc
40	1801	ttaatcatag	caaagattta	tctttttatg	attaaaaaca	attttttcct	taatgcatgg
	1861	cagcacatgc	ctttaatccc	agcactagag	aggcagagac	aggtagatct	ctgagttcaa
	1921	agccaggctg	gtctacacag	tgagttccaa	gacagctaga	gacctgtgtc	caaaaacact
	1981	caaaacaacc	aaacaccagg	ggtaaatgtt	gctgggaagt	caggggaagt	ggttttagaag
	2041	ggaagcctga	caacctgact	tcattcttca	ggacccacat	gatggaagga	gagaacgaag
45	2101	aactcccaag	ttctctcaca	tatgcacata	cctcaccacc	ccccgcagga	aatacatgat
	2161	catgcgtctg	agatatcacc	agttcacctt	tagcagctcg	cagttttagt	gcagatttct
	2221	gttaagttgg	gtctgtgttg	tttgccatg	tagcaggatt	acagcagcag	caaaaacggg
	2281	ccctcaagtc	tttctgccac	tctgacctga	gtttcctacg	gtacaggatt	tactctgaga
	2341	aacctcagca	ccttgccaca	gtagccttgg	cagaatggcc	tcacgggttag	ggaaactcct
50	2401	gattctaagc	ttgggagagc	tacgcttaga	tttgaattca	cccaggaagc	attcaaatca
	2461	aggctaaaga	cataaatgtg	aaataaaact	gtgaaccttc	attctaaagt	tcactctgact
	2521	tcccagattt	gatcaatata	ttcttaggtg	gtattaaaaa	tggtaaactg	cttaatttta
	2581	atctcaaaa	ttaaattatg	aggtttacat	aaaaaccaac	atttcatgaa	tgacttttta
	2641	aggtattaaa	aggggtactt	aagcggtaaa	tggtttcttg	gcaccataaa	ccaagtaata
55	2701	gttaatttac	aggtgggatt	tttttttatt	gctatgagaa	ttacttttaa	aattgtgggt
	2761	gttttatata	aagcagatat	cacaagtttt	gaaaatttgt	tacctttaat	atttcttcta
	2821	gagaataggt	gtttgtatcc	ataataaaa	aaaaatttgt	cagaactgct	gcttcaatct
	2881	aatcccattt	gagagaattg	tctttactgt	cttaataact	ggatgaatta	tcactctgaa
	2941	aatgtattta	ttgcactaaa	gttagtttag	gcttgataaa	acactccaga	catttttact
	3001	acagactgtt	tctataaaac	tgccattgct	tctaattggag	aatttttatt	taaaagaaat
60	3061	aaaattgctg	agtttatctg	caaaaccttt	ctctaagtct	tatgggacgt	aaggagacac

3121 ctccataatt ataagagccg ttgtgtcag agtctccatg ctggcttgaa tgtatgatcc
 3181 acactgttaa aacataggca gcagctcagg gccctggaca gcctgagtca acctagagta
 3241 gctggaacca ttttgacatg taatggataa gaaaattatc cattgagaag ctgaacaata
 3301 aaccaaagaa cgggtgtatt ttatccttaa cctctgtaaa ccacgttaca ctgagaacac
 3361 ttcagttctt cctaaagggt gataggcttc agtctgaaaa caatattgat ttggagtggg
 3421 cagaagttaa ctaaccaact accattatgt tttgaatata cctttcaata aaatgattga
 3481 aatgcaaaaa aaaaaaaaaa a

(SEQ ID NO:10)

10 11. NCBI Reference Sequence: NM_078467

Name: Homo sapiens cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), transcript variant 2, mRNA.

Version: NM_078467.1 GI:17978494

Source: human

15 Sequence:

1 agctgaggtg tgagcagctg ccgaagtcag ttccttgtgg agccggagct gggcgcggtat
 61 tcgccgaggg accgaggcac tcagaggagg tgagagagcg gcggcagaca acagggggacc
 121 ccggggccggc ggcccagagc cgagccaagc gtgcccgct gtgtccctgc gtgtccgga
 181 ggatgcgtgt tcgcgggtgt gtgctgcgtt cacagggtgt tctgcggcag gcgccatgtc
 241 agaaccggct ggggatgtcc gtcagaacct atgcggcagc aaggcctgcc gccgcctctt
 301 cggcccagtg gacagcgagc agctgagccg cgaactgtgat gcgctaattg cgggctgcac
 361 ccaggaggcc cgtgagcgat ggaacttcga ctttgtcacc gagacaccac tggagggtga
 421 cttcgccttg gacgctgtgc ggggccttgg cctgccccag ctctaccttc ccacggggcc
 481 ccggcgaggg cgggatgagt tgggaggagg caggcggcct ggcacctcac ctgctctgct
 541 gcaggggaca gcagaggaag accatgtgga cctgtcactg tcttgtacct ttgtgcctcg
 601 ctccagggag caggctgaag ggtccccagg tggacctgga gactctcagg gtgaaaaacg
 661 gcggcagacc agcatgacag atttctacca ctccaaacgc cggctgatct tctccaagag
 721 gaagccctaa tccgccaca ggaagcctgc agtccctgga gcgcgagggc ctcaagggcc
 781 cgtctacat cttctgcctt agtctcagtt tgtgtgtctt aattattatt tgtgttttaa
 841 tttaaacacc tctcatgta cataccttg ccgccccctg cccccagcc tctggcatta
 901 gaattattta acaaaaaact aggcgggtga atgagagggt cctaagagtg ctgggcattt
 961 ttattttatg aaatactatt taaagcctcc tcatcccggt ttctctcttt cctctctccc
 1021 ggaggttggg tgggccggct tcatgccagc taacttctcc tccccacttg tccgctgggt
 1081 ggtacctctt ggaggggtgt ggctccttcc catcgctgtc acaggcgggt atgaaattca
 1141 ccccttttcc tggacactca gacctgaatt ctttttcatt tgagaagtaa acagatggca
 1201 ctttgaaggg gcctcaccga gtgggggcat catcaaaaac tttggagtcc cctcacctcc
 1261 tctaagggtt ggaggggtga ccctgaagtg agcacagcct agggctgagc tggggacctg
 1321 gtacctctct ggctcttgat accccctctt gtcttgtgaa ggcaggggga aggtgggggc
 1381 ctggagcaga ccaccccgcc tgcctctatg gccctctga cctgcactgg ggagcccgtc
 1441 tcagtgttga gccttttccc tctttggctc cctgtacct tttgaggagc ccagctacc
 1501 cttcttctcc agctgggctc tgcaattccc ctctgctgct gtccctcccc cttgtccttt
 1561 cccttcagta ccctctcagc tccaggtggc tctgaggtgc ctgtcccacc cccaccccca
 1621 gctcaatgga ctggaagggg aagggaacac caagaagaag ggcacctag ttctacctca
 1681 ggcagctcaa gcagcgaccg cccctctctc tagctgtggg ggtgaggggc ccagtgtgtg
 1741 gcacaggccc ccttgagtgg ggttatctct gtgttagggg tatatgatgg gggagttagt
 1801 ctttctagga gggagacact ggccctcaa atcgccagc gacctctc atccacccca
 1861 tccctcccca gttcattgca ctttgattag cagcggaaca aggagtcaga cattttaaga
 1921 tgggtggcagt agaggctatg gacagggcat gccacgtggg ctcatatggg gctgggagta
 1981 gttgtctttc ctggcactaa cgttgagccc ctggaggcac tgaagtgtt agtgtacttg
 2041 gagtattggg gtctgacccc aaacaccttc cagctcctgt aacatactgg cctggactgt
 2101 tttctctcgg ctcccatgt gtcttggttc ccgtttctcc acctagactg taaacctctc
 2161 gagggcaggg accacacct gtactgttct gtgtctttca cagctcctcc cacaatgctg
 2221 aatatacagc aggtgctcaa taaatgatcc ttagtgactt taataaaaaa aaaaaaaaaa
 2281 a

(SEQ ID NO:11)

12. NCBI Reference Sequence: NM_000389

Name: Homo sapiens cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A), transcript variant 1, mRNA.

Version: NM_000389.3 GI:169790847

Source: human

Sequence:

5
10
15
20
25
30
35
40
45

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1  gtatatcagg gccgcgctga gctgcgccag ctgaggtgtg agcagctgcc gaagtcagtt
61  ccttgtggag ccggagctgg gcgcggatgc gccgaggcac cgaggcactc agaggaggcg
121 ccattgtcaga accggctggg gatgtccgtc agaaccocat cggcagcaag gcctgccgcc
181 gcctcttcgg ccagtggaac agcagcagc tgagccgcga ctgtgatgcg ctaatggcgg
241 gctgcatcca ggaggcccgat gagcgatgga acttcgactt tgtcaccgag acaccactgg
301 agggtgactt cgctggggag cgtgtgcggg gccttggcct gcccaagctc taccttccca
361 cggggccccc gcgaggccgg gatgagttgg gaggaggcag gcggcctggc acctcacctg
421 ctctgctgca ggggacagca gaggaagacc atgtggacct gtcactgtct tgtacccttg
481 tgcctcgctc aggggagcag gctgaagggt cccaggtgg acctggagac tctcagggtc
541 gaaaacggcg gcagaccagc atgacagatt tctaccactc caaacgcggg ctgatcttct
601 ccaagaggaa gccctaatac gccacagga agcctgcagt cctggaagcg cgagggcctc
661 aaaggcccg cctacatctt ctgccttagt ctcagtttgt gtgtcttaat tattatttgt
721 gttttaattt aaacacctcc tcatgtacat accctggcgg cccctgccc cccagcctct
781 ggcattagaa ttattttaa aaaaactagg cggttgaatg agaggttcct aagagtgtcg
841 ggcattttta ttttatgaaa tactatttaa agcctcctca tcccggtgtc tcttttctct
901 ctctcccgga ggttgggtgg gccggcttca tgccagctac ttcctcctcc ccacttgtcc
961 gctgggtggg accctctgga ggggtgtggc tcttcccat cgtgtgcaca ggcggttatg
1021 aaattcaccc ctttctctgg acactcagac ctgaattctt tttcatttga gaagtaaaca
1081 gatggcactt tgaaggggac tcaccgagtg ggggcatcat caaaaacttt ggagtcacct
1141 cacctcctct aaggttgggc aggttgaccc tgaagtgage acagcctagg gctgagctgg
1201 ggacctggtg cctcctggc tcttgatacc cccctctgtc ttgtgaaggc agggggaagg
1261 tggggtcctg gacgagacca cccgcctgc cctcatggcc cctctgacct gcactgggga
1321 gccgcttca gtgttgagcc ttttccctct ttggctcccc tgtacctttt gaggagcccc
1381 agctacctt cttctccagc tgggtctctg aattccctc tgtgtgtgtc cctccccctt
1441 gtcctttccc ttcagtaccc tctcagctcc aggtggctct gaggtgcctg tcccaccccc
1501 cccccagct caatggactg gaaggggaag ggacacacaa gaagaagggc acctagttc
1561 tacctcaggc agctcaagca gcgaccgcc cctcctctag ctgtgggggt gaggtccca
1621 tgtggtggca caggccccct tgagtgggtt tatctctgtg ttagggttat atgatggggg
1681 agtagatctt tctaggaggg agacactggc cctcaaaatc gtccagcgac ctctctcact
1741 caccocatcc ctccccagtt cattgcactt tgattagcag cggaaacaagg agtcagacat
1801 ttttaagatg tggcagtaga ggctatggac agggcatgcc acgtgggctc atatggggct
1861 gggagtagtt gtctttctg gactaacgt tgagccctg gaggcactga agtgcttagt
1921 gtacttgagg tattggggtc tgacccaaa cacttccag ctctgtaac atactggcct
1981 ggactgtttt ctctcggctc cccatgtgtc ctgggtcccg tttctccacc tagactgtaa
2041 acctctcgag ggcagggacc acaccctgta ctgttctgtg tcttccacag ctctccccc
2101 aatgctgaat atacagcagg tgctcaataa atgattctta gtgactttta aaaaaaaaaa
2161 aaaaaaaaaa

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(SEQ ID NO:12)

13. NCBI Reference Sequence: NM_001111099

Name: Mus musculus cyclin-dependent kinase inhibitor 1A (P21) (Cdkn1a), transcript variant 2, mRNA.

Version: NM_001111099.1 GI:162287332

Source: Mus musculus (house mouse)

Sequence:

50
55

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1  ggtgggtggg acctgatgat acccaactac cagctgtggg gtgaggagga gcatgaatgg
61  agacagagac ccagataat taaggacgtc ccactttgcc agcagaataa aaggtgccac
121 aggcaccatg tccaatcctg gtgatgtccg acctgttccg cacaggagca aagtgtgccg
181 ttgtctcttc ggtcccgtag acagttagca gttgcgcgtg gattgcgatg cgtcatggc
241 gggctgtctc caggaggccc gagaacggtg gaactttgac ttctgcacgg agacgcgcgt
301 ggagggaac ttcgtctggg agcgcgttcg gagcctaggg ctgcccagg tctacctgag
361 cctgggtcc cgcagccgtg acgacctggg aggggacaag agggccagta ctctctctgc

```

421 cctgctgcag gggccagctc cggaggacca cgtggccttg tcgctgtctt gcactctggt
 481 gtctgagcgg cctgaagatt ccccggttgg gcccggaaca tctcagggcc gaaaacggag
 541 gcagaccagc ctgacagatt tctatcactc caagcgcaga ttggtcttct gcaagagaaa
 601 accctgaagt gccacggga gcccgcctt cttctgctgt gggtcaggag gcctcttccc
 5 661 catcttcggc cttagccctc actctgtgtg tcttaattat ttttgtgtt ttaatttaaa
 721 cgtctcctgt atatacgtg cctgccctct cccagtctcc aaacttaaaag ttattttaaaa
 781 aaagaacaaa acaaaacaaa aaaaaccaa acaaaacaaa cctaaattag taggacggta
 841 gggcccttag tgtgggggat ttctattatg tagattatta ttatttaagc ccctcccaac
 901 ccaagctctg tgtttcctat accggaggaa cagtcctact gatatacaac catctgcac
 10 961 cgtttcaccc aacccccctc ccccatctc ctgcctggtt ccttgccact tcttaacctg
 1021 gggatgacct cagacctgaa tagcactttg gaaaaatgag taggactttg gggctcctt
 1081 gtcacctcta aggccagcta ggatgacagt gaagcagtca cagcctagaa cagggatggc
 1141 agttaggact caaccgtaat atcccgactc ttgacattgc tcagacctgt gaagacagga
 1201 atgggtccca ctctggatcc cctttgccac tcttggggag cccacctctc ctgtgggtct
 15 1261 ctggcagctg cccctctatt ttggagggtt aatctgggtga tctgtgtctc ttttcccca
 1321 cccatactt ccccttctgc aggtcggcag gaggcatac taggcacttg cccacagct
 1381 cagtggactg gaagggaatg tatatgcagg gtacactaag tgggattccc tggctttacc
 1441 ttaggcagct ccagtggcaa cccctgcat tgtgggtcta ggggtgggtc ttgggtgtga
 1501 gacaggcctc ccagagcatt ctatggtgtg tgggtgggtg ggtgggtcta tctgggatgg
 20 1561 ggacccagct tgggggtctc agtgacttct cccatttctt agtagcagtt gtacaaggag
 1621 ccaggccaag atgggtgtct gggggctaag ggagctcaca ggacactgag caatggctga
 1681 tcttttctca gtgttgaata ccgtgggtgt caaagcactt agtgggtctg actccagccc
 1741 caaacatccc tgtttctgta acatcctggt ctggactgtc tacccttagc ccgcacccca
 1801 agaacatgta ttgtggctcc ctccctgtct ccactcagat tgtaagcgtc tcacgagaag
 25 1861 ggacagcacc ctgcattgtc ccgagtcctc acaccgacc ccaaagctgg tgctcaataa
 1921 atacttctcg atgatt
 (SEQ ID NO:13)

14. NCBI Reference Sequence: NM_007669

30 Name: Mus musculus cyclin-dependent kinase inhibitor 1A (P21) (Cdkn1a), transcript variant 1, mRNA.

Version: NM_007669.4 GI:161760647

Source: Mus musculus (house mouse)

Sequence:

35 1 agcagccgag aggtgtgagc cgcgcgggtg tcagagtcta ggggaattgg agtcaggcgc
 61 agatccacag cgatatccag acattcagag ccacaggcac catgtccaat cctggtgatg
 121 tccgacctgt tccgcacagg agcaaagtgt gccgttgtct cttcggctcc gtggacagtg
 181 agcagttgag ccgtgattgc gatgcgctca tggcgggctg tctccaggag gcccgagaac
 241 ggtggaactt tgacttcgtc acggagacgc cgctggaggg caacttcgtc tgggagcgcg
 40 301 ttcggagcct agggctgccc aaggctctacc tgagccctgg gtcccgcagc cgtgacgacc
 361 tgggagggga caagaggccc agtacttctc ctgccctgct gcaggggcca gctccggagg
 421 accacgtggc cttgtcgtgt tcttgactc tgggtgtctga gccgcctgaa gattccccgg
 481 gtgggcccgg aacatctcag ggccgaaaac ggaggcagac cagcctgaca gattttctatc
 541 actccaagcg cagattggtc ttctgcaaga gaaaacctg aagtgccac gggagccccg
 45 601 cctctctctg ctgtgggtca ggaggcctct tccccatctt cggccttagc cctcactctg
 661 tgtgtcttaa ttattatttg tgttttaatt taaacgtctc ctgtatatac gctgcctgcc
 721 ctctcccagt ctccaaactt aaagttatct aaaaaaagaa caaaacaaaa caaaaaaac
 781 caaaacaaaa caaacctaaa ttagtaggac ggtagggcc ttagtgtggg ggattttctat
 841 tatgtagatt attattattt aagccctctc caaccacaag tctgtgtttc ctataccgga
 50 901 ggaacagtcc tactgatata aacccatctg catccgtttc acccaacccc cctcccccca
 961 ttccctgcct ggttccttgc cacttcttac ctgggggtga tctcagacc tgaatagcac
 1021 tttggaaaaa tgagtaggac tttgggggtc ccttgtcacc tctaaggcca gctaggatga
 1081 cagtgaagca gtcacagcct agaacaggga tggcagttag gactcaaccg taatatcccg
 1141 actcttgaca ttgtcagac ctgtgaagac aggaatggtc cccactctgg atcccccttg
 55 1201 ccaactcctg ggagcccaac tctcctgtgg gtctctgcca gctgcccctc tattttggag
 1261 ggttaaatct gtgatctgct gctcttttcc cccaccccat acttccccct ctgcaggctg
 1321 gcaggaggca tatctaggca cttgcccac agctcagttg actggaaggg aatgtatatg
 1381 cagggtacac taagtgggat tccctggtct taccttaggc agctccagtg gcaacccct
 1441 gcattgtggg tctagggtgg gtccttgggt gtgagacagg cctccagag cattctatgg

1501 tgtgtggtgg tgggggtggg cttatctggg atggggaccc cagttgggggt tctcagtgc
 1561 ttctccatt tcttagtagc agttgtacaa ggagccaggc caagatggtg tcttgggggc
 1621 taaggagct cacaggacac tgagcaatgg ctgacccctt ctcagtgttg aataccgtgg
 1681 gtgtcaaagc acttagtggg tctgactcca gcccacaaaca tccctgtttc tgtaacatcc
 5 1741 tggctctggac tgtctaccct tagcccgac cccaagaaca tgtattgttg ctccctccct
 1801 gtctccactc agattgtaag cgtctcacga gaaggacag caccctgcat tgtcccgagt
 1861 cctcacacc gaccccaaag ctggtgctca ataaatactt ctgatgatt
 (SEQ ID NO:14)

10

15. NCBI Reference Sequence: NM_001924.2Name: Homo sapiens growth arrest and DNA-damage-inducible, alpha (GADD45A), mRNA.Version: NM_001924.2 GI:9790904Source: humanSequence:

15

1 cagtggctgg taggcagtgg ctgggaggca gcgcccaat tagtgctgtg cggcccgctgg
 61 cgaggcgagg tccggggagc gagcgagcaa gcaaggcggg aggggtggcc ggagctgcgg
 121 cggctggcac aggaggagga gcccgggcgg gcgagggcg gccggagagc gccagggcct
 181 gagctgccgg agcggcgccct gtgagtgtg gcagaaagca ggcgcccgcg cgctagccgt
 241 ggcaggagca gcccgcacgc cgcgctctct cctggggcga cctgcagttt gcaatatgac
 20 301 tttggaggaa ttctcggtg gagagcagaa gaccgaaagg atggataagg tgggggatgc
 361 cctggaggaa gtgctcagca aagccctgag tcagcgcacg atcactgtcg ggggtgtacga
 421 agcggccaag ctgctcaacg tcgaccccg taacgtggtg ttgtgcctgc tggcggcgga
 481 cgaggacgac gacagagatg tggctctgca gatccacttc accctgatcc aggcgttttg
 541 ctgcgagaac gacatcaaca tctgtgcgct cagcaaccgg ggcgggctgg cggagctcct
 25 601 gctcttgagg accgacgctg gcccgcgggc gagcgagggc gccgagcagc ccccggaact
 661 gcaactgcgtg ctggtgacga atccacattc atctcaatgg aaggatcctg ccttaagtca
 721 acttatttgt ttttgccggg aaagtgcgta catggatcaa tgggttccag tgattaatct
 781 cctgaacgg tgatggcatc tgaatgaaa taactgaacc aaattgcaact gaagtttttg
 841 aaataccttt gtagttactc aagcagttac tccctacact gatgcaagga ttacagaaac
 30 901 tgatgccaag gggctgagtg agttcaacta catgttcttg gggcccgagg atagatgact
 961 ttgcagatgg aaagaggtga aaatgaagaa ggaagctgtg ttgaaacaga aaaataagtc
 1021 aaaaggaaca aaaattacaa agaaccatgc aggaaggaaa actatgtatt aatttagaat
 1081 ggttgagtta cattaaaata aaccaaatat gttaaagttt aagtgtgcag ccatagtttg
 1141 ggtatttttg gtttatatgc cctcaagtaa aagaaaagcc gaaaggggta atcatatttg
 35 1201 aaaaccatat tttattgtat tttgatgaga tattaaattc tcaaagtttt attataaatt
 1261 ctactaagtt attttatgac atgaaaagtt atttatgcta taaatttttt gaaacacaat
 1321 acctacaata aactggtatg aataattgca tcatt
 (SEQ ID NO:15)

40

16. NCBI Reference Sequence: NM_007836Name: Mus musculus growth arrest and DNA-damage-inducible 45 alpha (Gadd45a), mRNA.Version: NM_007836.1 GI:6681148Source: Mus musculus (house mouse)Sequence:

45

1 cagtggcccc gaggcagcag tgcagagttc cccagcgagg ctaggcgagc agcgggcggg
 61 cgggagcgga gaaggagggt tgggagcgag cgcagagccg gcgcccgcga ctgtgggggc
 121 caggagcagc ccgcgcgcgg agggaggagc tcgcacttgc aatatgactt tggaggaatt
 181 ctcggtcgca gagcagaaga ccgaaaggat ggacacggtg ggcgatgccc tggaggaagt
 50 241 gctcagcaag gctcggagtc agcgcaccat tacggtcggc gtgtacgagg ctgccaaagt
 301 gctcaacgta gaccccgata acgtggtact gtgcctgctg gctgctgacg aagacgacga
 361 cggggtgtg gctctgcaga tccatttcac cctcatccgt gcgttctgct gcgagaacga
 421 catcaacatc ctgcgggtca gcaaccggg tcggctagct gagctgctgc tactggagaa
 481 cgacgcgggc ccggcggaga gcgggggcgc cgcgcagacc ccggacctgc actgtgtgct
 55 541 ggtgacgaac ccacattcat cacaatggaa ggatcctgcc ttaagtcaac ttatttgttt
 601 ttgccgggaa agtcgctaca tggatcagtg ggtgcccggtg attaatctcc cggaaacgggtg
 661 atggcatccg aatggaaata actgaaccaa attgcaactga agttttgaaa tacctttgta
 721 gttactcaag cagtcactcc ccacgctgat gcaaggatta cagaaactga tgtcaagggg

781 ccgagttcaa ctgcacgagg gctcagagat gactttgcag agggagagag aggtgagcct
841 gaagaaggaa gctgcgagaa aagagaaatc caaggcaaaa gggacaaaaa ctacaaagca
901 ctgcaagaaa gaaaactgct aatttaggat ggccagggtta ctttcaaata agccaaatat
961 tgctttgttg aaacttttaa tgtatagcaa tagtttgggt attttttttc tttttttttt
1021 ttggtcttta tgccctcaaa taaaaggaaa gtaaaagagg attaatcata ttttcaagcc
1081 acagttttaa tgtattttga tgagatgtta aattctcaga agttttatta taaatcttac
1141 taagttatatt tatgatgtga aaggttatatt atgataaagt ttttgaagca cattatctaa
1201 aataaactgg tatggaataa ttgt

(SEQ ID NO:16)

17. NCBI Reference Sequence: NM_001012334.1

Name: Homo sapiens midkine (neurite growth-promoting factor 2) (MDK), transcript variant 1, mRNA.

Version: NM_001012334.1 GI:59894791

Source: human

Sequence:

1 gtgctccccg aaaggcactg ggcgaccgca cccgtggcct tctctgggag accgggtccc
61 agactcccc cagcacagca gagcgcttcc ctgcccaccc ggggaaaccg cccaggtgg
121 gccgcgcccc ctccccagca gccagcaggg cgccagggtt gagccggccg tggaggggag
181 cgggtccccg gggttataca ggcgccgggg ctccgcggca ggatgcagca ccgaggcttc
241 ctctctctca cctctctcgc cctgctggcg ctacctcgc cggtcgccaa aaagaaagat
301 aaggtgaaga agggcgcccc ggggagcgag tgcgctgagt gggcctgggg gccctgcacc
361 ccagcagca aggattgcgg cgtgggtttc cgcgaggcca cctgcggggc ccagaccag
421 cgcatccggt gcagggtgcc ctgcaactgg aagaaggagt ttggagccga ctgcaagtac
481 aagtttgaga actggggtgc gtgtgatggg ggcacaggca ccaaagtccg ccaaggcacc
541 ctgaagaagg cgcgctacaa tgctcagtgc caggagacca tccgcgtcac caagccctgc
601 accccaaga ccaaagcaaa ggccaaagcc aagaaaggga agggaaagga ctagacgcca
661 agcctggatg ccaaggagcc cctggtgtca catggggcct ggccacgccc ctccctctcc
721 caggcccgag atgtgaccca ccagtgcctt ctgtctgtct gttagcttta atcaatcatg
781 cctgcccctt tccctctcac tcccagccc caccctaag tgcccaaagt ggggagggac
841 aagggattct gggaagcttg agcctcccc aaagcaatgt gagtccaga gcccgctttt
901 gttcttcccc acaattccat tactaagaaa cacatcaaat aaactgactt tttcccccca
961 ataaaagctc ttctttttta atataaaaa aaaaaaa

(SEQ ID NO:17)

18. NCBI Reference Sequence: NM_001012333

Name: Homo sapiens midkine (neurite growth-promoting factor 2) (MDK), transcript variant 2, mRNA.

Version: NM_001012333.1 GI:59894795

Source: human

Sequence:

1 gagcgagtga gcgcgcggcg gcccctggtc cgcccgcccg cggccgatct aggggctggg
61 ggctggaggc gggggtgggg gtctgagctg cgtcctgggc tcgaggcgtc ccccggggag
121 tcgctcttta gcgatgcag caccgagggt tctctctcct caccctctct gccctgctgg
181 cgctcacctc cgcggtcgcc aaaaagaaag ataagggtga gaaggcgccg ccggggagcg
241 agtgcgctga gtgggcttgg gggccctgca ccccagcag caaggattgc ggctgtgggt
301 tccgcgaggg cactgcggg gccagaccc agcgcctcgc gtgcagggtg cctgcaact
361 ggaagaagga gtttgagacc gactgcaagt acaagtttga gaactggggg gcgtgtgatg
421 ggggcacagg caccaaagtc cgccaaggca cctgaagaa ggcgcgctac aatgctcagt
481 gccaggagac catccgcgtc accaagccct gcacccccaa gaccaaagca aaggccaag
541 ccaagaaaag gaagggaag gactagacgc caagcctgga tgccaaggag cccctggtgt
601 cacatggggc ctggcccacg cctcctctct ccaggcccg agatgtgacc cccaggtgcc
661 ttctgtctgc tcgttagctt taatcaatca tgccctgctt tgtccctctc actcccagc
721 cccaccccta agtgcccaaa gtggggaggg acaagggtt ctgggaagct tgagcctccc
781 ccaaagcaat gtgagtccca gagccgctt ttgttcttcc ccacaattcc attactaaga
841 aacacatcaa ataaactgac tttttcccc caataaaagc tcttcttttt taatataaaa
901 aaaaaaaaa

(SEQ ID NO:18)

19. NCBI Reference Sequence: NM_002391

Name: Homo sapiens midkine (neurite growth-promoting factor 2) (MDK), transcript variant 3, mRNA

Version: NM_002391.3 GI:59894794

Source: human

Sequence:

```
1 agaccgggttc tggagacaaa aggggcccggc gcggccgggag cgggacgggc cgggcgcggg
61 agggagcgaa gcagcgcggg cagcgagcga gatgcagcac cgaggcttcc tctcctcac
121 cctcctcgcc ctgctggcgc tcacctccgc ggtcgccaaa aagaaagata aggtgaagaa
181 gggcggcccg gggagcgagt gcgctgagtg ggctggggg ccctgcaccc ccagcagcaa
241 ggattgcggc gtgggtttcc gcgagggcac ctgcggggcc cagacccagc gcctccgggtg
301 cagggtgccc tgcaactgga agaaggagtt tggagccgac tgcaagtaca agtttgagaa
361 ctggggtgcg tgtgatgggg gcacaggcac caaagtccgc caaggcacc cgaagaaggc
421 gcgctacaat gctcagtgcc aggagaccat ccgcgtcacc aagccctgca cccccaagac
481 caaagcaaag gccaaagcca agaaaggga gggaaaggac tagacgccaa gcctggatgc
541 caaggagccc ctggtgtcac atggggcctg gccacgccc tccctctccc agggccgaga
601 tgtgaccac cagtgccttc tgtctgctcg ttagctttaa tcaatcatgc cctgccttgt
661 cctctctact cccagcccc acccctaagt gcccaaagt gggagggaca agggattctg
721 ggaagcttga gcctccccc aagcaatgtg agtcccagag ccgccttttg ttcttcccca
781 caattccatt actaagaaac acatcaaata aactgacttt ttcccccaa taaaagctct
841 tcttttttaa tataaaaaaa aaaaaa
```

(SEQ ID NO:19)

20. NCBI Reference Sequence: NM_010784

Name: Mus musculus midkine (Mdk), transcript variant 1, mRNA.

Version: NM_010784.4 GI:59894793

Source: Mus musculus (house mouse)

Sequence:

```
1 ccaggtacc cagctccctg ccacatcaga gacctttctt gcactctgag tgaactgatt
61 aaaaaaaaaa aaaaaaaaaa aaaaccaagc cggaggtgag ccgggcctcg aagggaaggt
121 tcgcggtgac ggtggccgcc ccgagcctgt gacaccagga cactactccc gggcccgcg
181 tgggcaagcg aagtgggtgac ctgagagctg acaggctgag agagggaaaa gtatagacag
241 gcctagacca ggggaagggg aggggataga gagctgggac tgctacgagg ggacctgagc
301 cagaagcgca ctggtaaaac cgaactccag gaccagagac ccagagatca gaggatgcag
361 caccgaggct tcttcttctt cgcctttctt gccctcttgg ttgctacgct cgcggtggcc
421 aaaaaaaaaa agaaggtgaa gaaggcgagc gagtgttcgg agtggacctg ggggccttgc
481 accccagca gcaaggactg cggcatgggc ttccgcgagg gtacctgtgg ggcccagacc
541 cagcgcgtcc attgcaaggt gccctgcaac tggagaagg aatttggagc cgactgcaaa
601 tacaagtttg agagctgggg ggcgtgtgat gggagcactg gcaccaaagc ccgccaaggg
661 accctgaaga aggcgcggta caatgccag tgccaggaga ccacccgctg gactaagccc
721 tgcacctcca agaccaagtc aaagaccaa gccaaagaa gaaaaggaaa ggactaagtc
781 aggaggccag agagcctccg gcctgcgctg gagcctgaac ggagccctcc tctcccacag
841 gcccaagata taaccacca gtgccttttg tcttctgtgc agctctgtca atcacgctg
901 tctctcacg cccacaccaa gtgcccagg tggggaggga caagagattc tggaaagtga
961 gcctcccat accctctttt gttctccca cctgatact tgttattaag aaatgaataa
1021 aataaactca cttttttcca ataaaaaaaa aaaaaaaaaa aa
```

(SEQ ID NO:20)

21. NCBI Reference Sequence: NM_000602.2

Name: Homo sapiens serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (SERPINE1), mRNA.

Version: NM_000602.2 GI:169790801

Source: human

Sequence:

```
1 aggagcacag ctgtgttttg ctgcagggcc aagagcgctg tcaagaagac ccacacgccc
```

61 cccctccagca gctgaattcc tgcagctcag cagccgcgcg cagagcagga cgaaccgcca
 121 atcgcaaggc acctctgaga acttcaggat gcagatgtct ccagccctca cctgcctagt
 181 cctgggcctg gcccttgtct ttggtgaagg gtctgctgtg caccatcccc catcctacgt
 241 ggccacactg gcctcagact tgggggtgag ggtgtttcag caggtggcgc aggcctccaa
 5 301 ggaccgcaac gtggttttct caccctatgg ggtggcctcg gtgttggcca tgcctcagct
 361 gacaacagga ggagaaaccc agcagcagat tcaagcagct atgggattca agattgatga
 421 caaggcatg gccccgcgcc tccggcatct gtacaaggag ctcatggggc catggaacaa
 481 ggatgagatc agcaccacag acgcgatctt cgtccagcgg gatctgaagc tgggtccaggg
 10 541 cttcatgccc cacttcttca ggctgttcg gagcacggtc aagcaagtgg acttttcaga
 601 ggtggagaga gccagattca tcatcaatga ctgggtgaag acacacacaa aaggatgat
 661 cagcaacttg cttgggaaag gagccgtgga ccagctgaca cggctgggtg tgggtgaatgc
 721 cctctacttc aacggccagt ggaagactcc ctccccgac tccagcacc accgcgcct
 781 cttccacaaa tcagacggca gcaactgtct tgtgcccatt atggctcaga ccaacaagtt
 841 caactatact gatttcacca cggccgatgg ccattactac gacatcctgg aactgcctta
 15 901 ccacggggac accctcagca tgttcattgc tggcccttat gaaaaagagg tgcctctctc
 961 tgcctcacc aacattctga gtgccagct catcagccac tggaaaggca acatgaccag
 1021 gctgccccgc ctctgtgttc tgcctcaagt ctccctggag actgaagtgc acctcaggaa
 1081 gccctagag aacctgggaa tgaccgacat gttcagacag tttcaggctg acttcacgag
 1141 tctttcagac caagagcctc tccacgtcgc gcaggcgtg cagaaagtga agatcgagggt
 20 1201 gaacgagagt ggcacggtgg cctctctatc cacagctgtc atagtctcag cccgcattggc
 1261 ccccgaggag atcatcatgg acagaccctt cctctttgtg gtccggcaca accccacagg
 1321 aacagtcctt ttcattgggc aagtgatgga accctgacct tggggaaaga cgccttcctc
 1381 tgggacaaaa ctggagatgc atcgggaaag aagaaactcc gaagaaaaga attttagtgt
 1441 taatgactct ttctgaagga agagaagaca tttgcctttt gttaaaagat ggtaaacag
 25 1501 atctgtctcc aagaccttgg cctctccttg gaggacctt aggtcaaact ccttagtctc
 1561 cacctgagac cctgggagag aagtttgaag cacaactccc ttaaggtctc caaaccagac
 1621 ggtgacgct gcgggaccat ctggggcacc tgcctccacc cgtctctctg cccactcggg
 1681 tctgcagacc tggttccac tgaggccctt tgcaggatgg aactacgggg cttacaggag
 1741 cttttgtgtg cctggtagaa actattttctg ttccagtcac attgccatca ctcttgtact
 30 1801 gccctgccac gcggaggagg ctggtgacag gccaaaggcc agtggaaaga acacctttc
 1861 atctcagact ccactgtggc actggccacc cctccccagt acaggggtgc tgcagggtgc
 1921 agagtgaatg tcccccatca tgtggcccaa ctctcctggc ctggccatct cctctccag
 1981 aaacagtgtg catgggttat tttggagtgt aggtgacttg tttactcatt gaagcagatt
 2041 tctgcttctt tttattttta taggaataga ggaagaaatg tcagatgcgt gccagctct
 35 2101 tcacccccca atctcttggg ggggaggggt gtacctaaat atttatcata tcttgcctc
 2161 tgagtgtctg ttagagagaa agagaactac taaggaaaat aatattattt aaactcgtc
 2221 ctagtgtttc tttgtgtctt gtgtcaccgt atctcaggaa gtccagccac ttgactggca
 2281 cacacccctc cggacatcca gcgtgacgga gccacactg ccaccttgtg gccgcctgag
 40 2341 accctcgcgc cccccgcgcc cctctttttc cccttgatgg aaattgacca tacaatttca
 2401 tctctcttca ggggatcaaa aggacggagt ggggggacag agactcagat gaggacagag
 2461 tggtttccaa tgtgttcaat agatttagga gcagaaatgc aaggggctgc atgacctacc
 2521 aggacagaac tttccccaat tacagggtga ctacagccg cattgggtgac tcaattcaat
 2581 gtgtcatttc cggctgctgt gtgtgagcag tggacacgtg aggggggggt ggggtgagaga
 45 2641 gacaggcagc tcggattcaa ctaccttaga taatatttct gaaaacctac cagccagagg
 2701 gtagggcaca aagatggatg taatgcactt tgggaggcca aggcgggagg attgcttgag
 2761 ccaggtgatt caagaccagc ctgggcaaca tacciaagacc cccgtctctt taaaaatata
 2821 tatattttta atatacttaa atatatattt ctaatatctt taaatatata tatatatatt
 2881 aaagaccaat ttatgggaga attgcacaca gatgtgaaat gaatgtaatc taatagaagc
 2941 ctaatcagcc caccatgttc tccactgaaa aatcctcttt ctttgggggt tttctttctt
 50 3001 tcttttttga ttttgcactg gacggtgacg tcagccatgt acaggatcca caggggtggt
 3061 gtcaaatgct attgaaattg tgttgaattg tatgcttttt cacttttgat aaataaacat
 3121 gtaaaaatgt ttcaaaaaaa taataaaata aataaatagc aagaatatgt caggac

(SEQ ID NO:21)

55 22. NCBI Reference Sequence: NM_008871Name: Mus musculus serine (or cysteine) peptidase inhibitor, clade E, member 1 (Serpine1), mRNA.Version: NM_008871.2 GI:170172561Source: Mus musculus (house mouse)

Sequence:

1 gagcacagct ggatcaggct gcagcagagc cccgagagct ttgtgaagga ggaccgcgcg
 61 acaccgcgct ccggcagcac acagccaacc acagctgagc gacagccaac aagagccaat
 121 cacaaggcac ctttgaatac tcaggatgca gatgtcttca gcccttgctt gcctcatcct
 5 181 gggcctgggt ctggtctctg ggaagggtt cactttaccc ctccgagaat cccacacagc
 241 ccacaggcc accgacttcg gagtaaaagt gtttcagcag gtggtccagg cctccaaaga
 301 ccggaatgtg gtcttctctc cctatggcgt gtccctcggt ctggctatgc tgcagatgac
 361 cacagcgggg aaaaccggc ggcagatcca agatgctatg ggattcaaag tcaatgagaa
 421 gggcacagct catgccctcc gccagctctc caaggagctc atggggcgct ggaacaagaa
 10 481 tgagatcagt actgcggtg ccactcttct ccagcgggac ctgagagctg tccagggtct
 541 catgccccac ttcttcaagc tcttcagac tatggtgaaa cagggtggact tctcagaagt
 601 ggaaagagcc agatttatca tcaatgactg ggtggaaagg cataccaaag gtatgatcag
 661 tgacttactg gccaaagggg ctgtagacga gctgacacgc ctggtgctgg tgaatgccct
 721 ctacttcagt ggccaatgga agactccttt cttagaggcc agcaccacc agcgctctt
 15 781 ccacaagtct gatggcagca ccgtctctgt gccatgatg gctcagagca acaagttcaa
 841 ctacactagc ttcaccacc ccgatgggtc cgagtatgac gttgtggaac tgcctacca
 901 gggcgacacc ctcagcatgt tcactctgct accctttgag aaagatgtgc acctctcgc
 961 cctcaccaac atcttggtat ctgaactcat cagacaatgg aaggccaaca tgaccaggct
 1021 gccccgcctc ctcatcctgc ctaagtctc tctggagact gaagtggacc tcagagggcc
 20 1081 cctggagaag ttgggcatgc ctgacatgtt tagtgcaacc ctggcgact tcacaagtct
 1141 ttccgacca gacagctct ctgtagcaca ggcactgcaa aaggtcagga tcgaggtaaa
 1201 cgagagcggc acagtggcgt ctctctccac agcctttgtc atctcagccc gcatggcccc
 1261 cacggagatg gttatagacc gatcctttct ctttgtggtt cggcacaacc cgacagagac
 1321 aatcctcttc atgggccaag tgatggagcc ttgacagtgg gaagagacgc cttcatttgg
 25 1381 gacgaaactg gagatgttat aagcagaaac tctgaagaaa aggattatct aaaggactct
 1441 atggggagaa agagaaggca actcctcctt acccccaca ctggtaatct ttccaaccag
 1501 catcccagac ctcgactct tgaagggaag agagtctaac tccctcctcc ctagggatcc
 1561 ctaccccaca aaggtctcat ggaccataga actcacagta cctggatctg cccagcatgc
 1621 cctttggacc cagttccac cgaggcccca gcagagtga gggcacaaca ctttcattca
 30 1681 gcaaaatcgt ttgtgttcca gtcacactgt gggcacctct tgcatcgctt gccattgctg
 1741 tggaggggtg ccatgggcca aaggaaaaag cactgtccta tctcaaggte cactgtggaa
 1801 atgtccacct tgcccacct caaggggcaa cggatagaca gatcaaatgg tggccaata
 1861 gcgagccttc tccctgctcc ctcccttgac acagcttgct tatgttatct cagagtgtag
 1921 gtgacttggt tacacagctt ttttcgacc acaactttt ttcatttggg aagggtgtaa
 35 1981 gaaaagtcgg acgtgtgtgt gctgctctct tcgtcccag tctcccagtg ggggggcct
 2041 ggggagattc caggggtgtg attgaatatt tatctcttgc tcttgatgt ttgttgggga
 2101 gaagaagcac ttttaaggaa aatgcttctt atttaaaccg tggcatacgg catcccattt
 2161 ggggtctgca tccctgtatg tcaggggtgc atcactccac aaacotgccc ctctgggtag
 2221 cctcgtgatg gggctcacac tgccgcctag tggcagcga acacaccctt acccggtccc
 40 2281 tccctcctc ccccccccc ccccccccc ccgtggctct tttccttagg gaccttgcca
 2341 aggtgatgct tggcaaccac cgttaaagga aggggggaaa aaagattaga tggagagag
 2401 agagatttga gagagggcaa agtggtttca aatttttcca aggcattcag aagcagagag
 2461 ggaaaagggg ctgtgtgacc taacaggaca gaactttct caattactgg gtgagtcaga
 2521 gctgcactgg tgactcactt caatgtgtca tttccggctg ctgtatgtga gcagtggaca
 45 2581 cgtggggggg cggggggggg atgaaagaga cagcagctcc tgggtcaacca ccttagttag
 2641 ataactttt ttgaaagctt cctagctgga ggtatgatca gaaaaccaat ttactgaaaa
 2701 actgcacaag aaggtaccgt gaatgtaatt tcttagcagg ccaactctgca tctgttatgt
 2761 ctccaccgga aaaaaataa tcatgttggg gtttttgctt ttctctctct cctctttct
 2821 ctctgatttt ttttctctct cttttcatta tgcactggac agccacacac cgtgtaccca
 50 2881 tagggcccca aatgtgggtt cacatggtct tgaattttgt tggttacata tgcctttttg
 2941 ttgttgtttg tcttcacttt tgatatataa acaggtaaat atgtttttta aaaaatacta
 3001 aatatagaga atatgcaaac aaaa
 (SEQ ID NO:22)

55

23. NCBI Reference Sequence: NM_147187.1

Name: Homo sapiens tumor necrosis factor receptor superfamily, member 10b(TNFRSF10B), transcript variant 2, mRNA.

Version: NM_147187.1 GI:22547118

Source: human

Sequence:

	1	gcgcttgccg	aggattgcgt	tgacgagact	cttattttatt	gtcaccacac	tgtggtggaa
	61	tttgcagttg	cacattggat	ctgattcgcc	ccgccccgaa	tgacgcctgc	ccggaggcag
5	121	tgaaagtaca	gcccgcgcgc	cccaagtcag	cctggacaca	taaatcagca	cgccggccgga
	181	gaaccccgca	atctctgcgc	ccacaaaata	caccgacgat	gcccgatcta	ctttaagggc
	241	tgaaacccac	gggcctgaga	gactataaga	gcgttcccta	ccgccatgga	acaacggggga
	301	cagaacgccc	cgcccgcttc	gggggccccg	aaaaggcacg	gcccaggacc	caggaggggcg
	361	cggggagcca	ggcctgggct	ccgggtcccc	aagacccttg	tgctcgttgt	cgccgcggtc
10	421	ctgctgttgg	tctcagctga	gtctgtcttg	atcacccaac	aagacctagc	tccccagcag
	481	agagcggccc	cacaacaaaa	gagggtccagc	ccctcagagg	gattgtgtcc	acctggacac
	541	catatctcag	aagacggtag	agattgcata	tccctgcaaat	atggacagga	ctatagcact
	601	cactggaatg	acctcctttt	ctgcttgccg	tgccaccagg	gtgattcagg	tgaagtggag
	661	ctaagtcctt	gcaccacgac	cagaaacaca	gtgtgtcagt	gcgaagaagg	caccttccgg
15	721	gaagaagatt	ctcctgagat	gtgccgggaag	tgccgcacag	ggtgtcccag	agggatggtc
	781	aaggctcgtg	attgtacacc	ctggagtgac	atcgaatgtg	tccacaaaga	atcaggcatc
	841	atcataggag	tcacagttgc	agccgtagtc	ttgattgtgg	ctgtgtttgt	ttgcaagtct
	901	ttactgtgga	agaaagtcct	tccttacctg	aaaggcatct	gtcagggtgg	tgggtggggac
	961	cctgagcgtg	tgacacagaag	ctcacacaga	cctggggctg	aggacaatgt	cctcaatgag
20	1021	atcgtgagta	tcttgacgcc	caccacaggtc	cctgagcagg	aatggaatgt	ccaggagcca
	1081	gcagagccaa	cagggtgtcaa	catgttgtcc	cccggggagt	cagacaatgt	gctggaaccg
	1141	gcagaagctg	aaaggtctca	gaggaggagg	ctgctgggtc	cagcaaatga	agtgatctcc
	1201	actgagactc	tgagacagtg	cttcgatgac	tttgacagact	tggtgccctt	tgactcctgg
	1261	gagccgctca	tgaggaagtt	gggcctcatg	gacaatgaga	taaaggtggc	taaagctgag
25	1321	gcagcggggc	acagggacac	cttgtacacg	atgctgataa	agtgggtcaa	caaaaccggg
	1381	cgagatgcct	ctgtccacac	cctgctggat	gccttgagga	cgctgggaga	gagacttgcc
	1441	aagcagaaga	ttgaggacca	cttgttgagc	tctggaaaagt	tcattgtatct	agaaggtaat
	1501	gcagactctg	ccatgtccta	agtgtgatcc	tcttcaggaa	gtcagacctt	ccttggttta
	1561	ccttttttct	ggaaaaagcc	caactggact	ccagtcagta	ggaaagtgcc	acaattgtca
30	1621	catgaccggt	actggaagaa	actctcccat	ccaacatcac	ccagtggatg	gaacatcctg
	1681	taacttttca	ctgcacttgg	cattattttt	ataagctgaa	tgtgataata	aggacactat
	1741	ggaaatgtct	ggatcattcc	gtttgtgcgt	actttgagat	ttggtttggg	atgtcattgt
	1801	tttcacagca	ctttttttatc	ctaattgtaa	tgctttatct	atttatttgg	gtacatttgt
	1861	aagatccatc	tacacagtgc	ttgtccgact	tcacttgata	ctatatgata	tgaacctttt
35	1921	ttgggtgggg	ggtgcggggc	agttcactct	gtctcccagg	ctggagtgc	atgggtgcaat
	1981	cttggtcac	tatagccttg	acctctcagg	ctcaagcgat	tctcccacct	cagccatcca
	2041	ataagctggg	accacaggtg	tgccaccaca	cgccgggcta	attttttgta	ttttgtctag
	2101	atataggggc	tctctatgtt	gctcagggtg	gtctogaatt	cctggactca	agcagtgctg
	2161	ccacctcaga	ctcccaaagc	ggtggaatta	gaggcgtgag	cccccttacc	tggccttaac
40	2221	tttctacttt	tataattctg	tatgttatta	ttttatgaac	atgaagaaac	tttagtaaat
	2281	gtacttgttt	acatagtatt	gtgaatagat	tagataaaca	taaaaggagg	agacatacaa
	2341	tgggggaaga	agaagaagtc	ccctgtaaga	tgtaactgtc	tggtttccag	ccctccctca
	2401	gatgtacttt	ggcttcaatg	attggcaact	tctacagggg	ccagtctttt	gaactggaca
	2461	accttacaag	tatatgagta	ttattttatag	gtagttgttt	acatatgagt	cgggaccaaa
45	2521	gagaactgga	tcacagtgaa	gtcctgtgtg	tggtctggtc	ctacctgggc	agtctcattt
	2581	gcacccatag	cccccatcta	tgacacaggc	gggacagagg	cagatgggtt	agatcacaca
	2641	taacaatagg	gtctatgtca	tatcccaagt	gaacttgagc	cctgtttggg	ctcaggagat
	2701	agaagacaaa	atctgtctcc	cacgtctgcc	atggcatcaa	gggggaagag	tagatgggtg
	2761	ttgagaatgg	tgtgaaatgg	ttgccatctc	aggagtagat	ggcccggtcc	acttctgggt
50	2821	atctgtcacc	ctgagcccat	gagctgcctt	ttagggtaca	gattgcctac	ttgaggacct
	2881	tgcccgctct	gtaagcatct	gactcatctc	agaaatgtca	attcttaaac	actgtggcaa
	2941	caggacctag	aatggctgac	gcatttaagg	tttcttcttg	tgctctgttc	tattattgtt
	3001	ttaagacctc	agtaaccatt	tcagcctctt	tccagcaaac	ccttctccat	agtatttccg
	3061	tcattggaag	atcatttatg	caggtagtca	ttccaggagt	ttttggtctt	ttctgtctca
55	3121	aggcattgtg	tgttttgttc	cgggactgtt	ttgggtggga	caaagttaga	attgcctgaa
	3181	gatcacacat	tcagactgtt	gtgctgtggg	agtttttagga	gtgggggggtg	acctttctgg
	3241	tctttgcact	tccatcctct	cccacttcca	tctggcatcc	cacgggttgt	ccccgtcact
	3301	tctggaaggc	acaggggtgt	gctgcctcct	ggtctttgcc	tttgctgggc	cttctgtgca
	3361	ggacgctcag	cctcagggct	cagaaggtgc	cagtcgggtc	ccaggtccct	tgctccctcc
60	3421	acagaggcct	tcctagaaga	tgcatctaga	gtgtcagcct	tatcagtgtt	taagatttgt
	3481	cttttatttt	taattttttt	gagacagaat	ctcactctct	cgcccgaggc	ggagtgcac
	3541	ggtacgatct	tggtcagtg	caacctccgc	ctcctgggtt	caagcgattc	tcgtgcctca

3601 gcctccggag tagctgggat tgcaggcacc cgcaccacag cctggotaat ttttgtatatt
 3661 ttagtagaga cgggggtttca ccatgtttggt caggctgggtc tgaactcct gacctcaggt
 3721 gatccacctt ggcctccgaa agtgctggga ttacaggcgt gagccaccag ccaggccaag
 3781 ctattctttt aaagtaagct tcctgacgac atgaaataat tgggggtttt gttgttttagt
 5 3841 tacattaggt tttgctatat ccccaggcca aatagcatgt gacacaggac agccatagta
 3901 tagtgtgtca ctctgtggtg gtgtcctttc atgcttctgc cctgtcaaag gtccctatatt
 3961 gaaatgtgtt ataatacaaa caaggaagca catttgtgtac aaaatactta tgtatttatg
 4021 aatccatgac caaatataat atgaaacctt atataaaaaa aaaaaaaaaa aaa

(SEQ ID NO:23)

24. NCBI Reference Sequence: NM_003842

Name: Homo sapiens tumor necrosis factor receptor superfamily, member 10b
 (TNFRSF10B), transcript variant 1, mRNA.

Version: NM_003842.3 GI:22547115

Source: human

Sequence:

1 gcgcttgccg aggattgcgt tgacgagact cttattttatt gtcaccaacc tgtggtggaa
 61 tttgcagttg cacattggat ctgattcgcc ccgccccgaa tgacgcctgc ccggaggcag
 20 121 tgaaagtaca gccgcgcgc cccaagtcag cctggacaca taaatcagca cgcggccgga
 181 gaaccccgca atctctgcgc ccacaaaata caccagcagat gccgatcta ctttaagggc
 241 tgaaacccac ggcctgaga gactataaga gcgttcccta ccgccatgga acaacgggga
 301 cagaacgcc cggcgccttc gggggcccg aaaggcacc gcccaggacc cagggaggcg
 361 cggggagcca ggctgggct ccgggtcccc aagacccttg tgctcgttgt cgcgcgggtc
 25 421 ctgctgttgg tctcagctga gtctgctctg atcaccacaac aagacctagc tcccagcag
 481 agagcggccc cacaacaaaa gaggtccagc cctcagagg gatttgtgtc acctggacac
 541 catactctcag aagacggtag agattgcata cctgcacaa atggacagga ctatagcact
 601 cactggaatg acctcctttt ctgcttgccg tgaccagggt gtgattcagg tgaagtggag
 661 ctaagtcctt gcaccacgac cagaaacaca gtgtgtcagt gcgaagaagg caccttccgg
 30 721 gaagaagatt ctcttgagat gtgcccgaag tgccgcacag ggtgtcccag agggatggtc
 781 aaggtcgggt attgtacacc ctggagtgc atcgaatgtg tccacaaaga atcaggatca
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 961 atttgtggtg tgtttgtttg caagtcttta ctgtggaaga aagtccttcc ttacctgaaa
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 1201 ggggagtcag agcatctgct ggaaccggca gaagctgaaa ggtctcagag gaggaggctg
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 1321 gcagacttgg tgccctttga ctctgggag ccgctcatga ggaagttggg cctcatggac
 1381 aatgagataa aggtggctaa agctgaggca gcgggccaca gggacacctt gtacacgatg
 1441 ctgataaagt gggtaacaa aaccgggcga gatgcctctg tccacacctt gctggatgcc
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 45 1621 tcaggaagtc agaccttccc tgggtttacct tttttctgga aaaagcccaa ctggactcca
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 1741 acatcaccca gtggatggaa catcctgtaa cttttcactg cacttggcat tatttttata
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 1861 ttgagatttg gtttgggatg tcattgtttt cacagcactt ttttatacta atgtaaatgc
 50 1921 tttattttatt tatttgggct acattgtaag atccatctac acagtctgtt tccgacttca
 1981 cttgatacta tatgatatga accttttttg ggtggggggt gcggggcagt tcaactctgtc
 2041 tcccaggctg gagtgcattg gtgcaatctt ggctcactat agccttgacc tctcaggctc
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 2161 ccggctaatt ttttgtattt tgtctagata taggggctct ctatgtttgt cagggtgggtc
 55 2221 tcgaattcct ggactcaagc agtctgccc cctcagactc ccaaagcggg ggaattagag
 2281 gcgtgagccc ccattgcttg ccttaccttt ctacttttat aattctgtat gttatttttt
 2341 tatgaacatg aagaaacttt agtaaagtga cttgtttaca tagttatgtg aatagattag
 2401 ataaacataa aaggaggaga catacaatgg gggaagaaga agaagtcctc tgtaagatgt
 2461 cactgtctgg gttccagccc tccctcagat gtactttggc ttcaatgatt ggcaacttct

5 2521 acagggggcca gtctttttgaa ctggacaacc ttacaagtat atgagtatta tttataggta
 2581 gttgttttaca tatgagtcgg gaccaaagag aactggatcc acgtgaagtc ctgtgtgtgg
 2641 ctgggtcccta cctgggcagt ctcatattgca cccatagccc ccattctatgg acaggctggg
 2701 acagaggcag atgggttaga tcacacataa caatagggtc tatgtcatat cccaagtga
 2761 cttgagccct gtttgggctc aggagataga agacaaaatc tgtctcccac gtctgccatg
 2821 gcatcaaggg ggaagagtag atggtgcttg agaatggtgt gaaatggttg ccattctcagg
 2881 agtagatggc cgggctcact tctggttatc tgtcaccctg agcccatgag ctgccttttta
 2941 gggtagacagat tgcctacttg aggaccttgg ccgctctgta agcatctgac tcattctcaga
 3001 aatgtcaatt cttaaactct gtggcaacag gacctagaat ggctgacgca ttaagggtttt
 10 3061 cttcttctgtg cctgttctat tattgtttta agacctcagt aacctattca gcctctttcc
 3121 agcaaaccct tctccatagt atttcagtc tgggaaggatc atttatgcag gtagtcattc
 3181 caggagtttt tgggtctttt tgtctcaagg cattgtgtgt tttgttccgg gactggtttg
 3241 ggtgggacaa agttagaatt gcctgaagat cacacattca gactgttctg tctgtggagt
 3301 tttaggagtg gggggtgacc tttctggtct ttgcacttcc atctctctcc acttccatct
 15 3361 ggcattccac gcgttgtccc ctgcacttct ggaaggcaca ggggtctgct gcctcctggt
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 3481 tccgggtccca ggtcccttgt ccttccaca gaggccttcc tagaagatgc atctagagtg
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 3601 actctctctgc ccaggctgga gtgcaacggt acgatcttgg ctcagtgcac cctccgcctc
 20 3661 ctgggttcaa gcgattctcg tgcctcagcc tccggagttag ctgggattgc aggcacccgc
 3721 caccacgctt ggctaatttt tgtattttta gtagagacgg ggtttcacca tgttggctag
 3781 gctggctctg aactcctgac ctccaggtgat ccaccttggc ctccgaaagt gctgggatta
 3841 caggcgtgag ccaccagcca ggccaagcta ttctttttaa gtaagcttcc tgacgacatg
 3901 aaataattgg gggttttgtt gtttagttac attaggcttt gctatatccc caggccaaat
 25 3961 agcatgtgac acaggacagc catagtatag tgtgtcactc gtggttggtg tcttttcatg
 4021 cttctgccct gtcaaaggct cctattttgaa atgtgttata atacaaacaa ggaagcacat
 4081 tgtgtacaaa atacttatgt atttatgaat ccattgacaa attaaatatg aaaccttata
 4141 taaaaaaaaa aaaaaaaaaa
 (SEQ ID NO:24)

25. NCBI Reference Sequence: NM_020275

Name: Mus musculus tumor necrosis factor receptor superfamily, member 10b (Tnfrsf10b), mRNA.

Version: NM_020275.3 GI:40789303

Source: Mus musculus (house mouse)

Sequence:

1 gtcgcccgcga caagaatcca gaactttttct gggagtgagg aaatccagag aacttttttta
 61 ggagtgaggg gacagccatc ctctcgtggc tttgggagct gaagccgcag ggttttcggat
 121 gagctgacac catggagcct ccaggaccca gcacgcccac agcctctgcc gctgcccggg
 40 181 cagatcacta cccccaggc ctccggccac tcccgaaagg cagacttcta tatagctttg
 241 cgttgctgct tgcctgtgta caggctgtct ttgttccagt aacagctaac ccagcccata
 301 atcgtccagc tggcctacag cggccggagg agagcccac aagaggaccc tgtctagcag
 361 gccagtacct gtcagaaggg aactgcaagc ctgtcagaga gggatttgac tacaccagcc
 421 attccaacca ttctctggat tcatgtatct tctgcacagt ctgtaaggaa gataaagtctg
 45 481 tagaaacccg atgcaacata accacaaata cgggtgtgtg atgcaaacca ggcacctttg
 541 aagataaaga ctcccctgag atctgccaat catgctctaa ctgcactgac ggggaagagg
 601 aactgacttc ctgtaccccc agagaaaacc ggaagtgtgt ctccaaaacg gcttgggcat
 661 cttggcataa gctaggcctc tggataggac tcttggttcc agtagtgctg ctgattggag
 721 ctctgcttgt ctggaagact ggagcatgga ggcaatgggt gctctgtata aaaagaggct
 50 781 gtgaacggga tcccgaaagt gcgaactctg tgcattcgtc tctcttggac cgacagacat
 841 ctgacacgac aaatgactct aaccacaaca cggaacctgg caagactcag aaaacaggaa
 901 agaagttgct ggttccggta aacggaaacg actcagctga cgacctgaag tttatcttctg
 961 agtattgttc ggacatagtg ccttttgact cctggaacgg tctcatgagg cagttgggccc
 1021 tcacagacaa tcaaatccaa atggtcaaag ccgaaacact ggtcacacgt gaggcctctg
 55 1081 accaaatgct gctcaagtgg cgccaccaga ctgggcgaag tgctccatc aacctctgct
 1141 tggatgcctt ggaagccgtg gaagagagag atgccatgga gaaaattgaa gactacgcag
 1201 tgaaatccgg gaggtttact tatcagaacg ctgcagccca accagagaca gggccaggag
 1261 gatctcagtg cgtttgaagt cagcctgata tacttagtga actcaggaca gccaaaggcta
 1321 tgtagagagc ccgaagatg caggctcttc agtattatga gaatgtactt aattttttct

5 1381 tgtagtagtt agtgtatcat attattgtat tatttatatt attactgtta agtactatgt
1441 tctcttatta gaagttgaac acagaacctc tgagaacaca tatgctacaa gtgttctaac
1501 acacctccag catcccgat tacctttgtt cctgaacaag gcacaattgg tagggtaga
1561 tagggcctgc ctatcatcct aacactccgg tgatggagcc aggaagatca agagttcgag
1621 gccagctggg tcacataaga tcccatataa tgtgcaggat ggctaaactt gctgagagct
1681 gactctgtgg tctcctgtcc cagattctag cgatatccat tactaagacc cttgtccaga
1741 gacaaaagac cacctctgta acagagggaa gaataaaaca gccctagggg ggaaactcct
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10 1861 gcacctccct gtgccagggt ctagtgggca gccactgag ggtacatcta acttgaatct
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15 2161 atacagattt ttgtcctgtg tgaattccta ttgttttttg tttgttttta attttttatt
2221 atttattttt ttcatcaca ttccaaatgc tatcccaaaa gtcccctatg cctcccccct
2281 cctgcccccc cgccctgctc ccctaccac tcaactcact ccactttttt tttttttttt
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2521 ccaatgctat cccaaaagtc cccacatgc tccccaccc actccccacc tgactttata
2581 tgccctcact ccacttcttg gccctgggtg tcccctgtac tggggcataa aacgtttgca
2641 agaccaaggg gcgtctcttt ccactgatgg ccgactaggc catcttctga tacatatgca
2701 gctagagaca cgagctctgg gggggtagtg gttagtccat attgttgttc cacctatagg
25 2761 gttgccgacc ccttcagctc cttgggtact ttctctagct cctccactag gggccctgtg
2821 ttctatccaa tagatgactg tgagcatcca cttctgtatt tgccaggcac tggcatagcc
2881 tcatacgaga cagctatata aggggccagt tttgttttgt tttgttttaa actagcatgc
2941 tggagaggta gctcagcggg taagagcagt ggctgctctt ccagagggtc tgagttccaa
3001 ttcccagcag ctacatagtg gctcacaacc ttctctaatg ggatctgatg tccctttcta
3061 gtgtgtttga agccagtggc agtgtaatta catacacaaa ataaataaat ctttttaaaa
30 3121 aagaaaaaaa aaaaaaaaaa aa

(SEQ ID NO:25)

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CLAIMS

We claim:

1. A method of identifying a mammalian subject exposed to ionizing radiation, comprising: detecting the presence of, and/or quantifying the level of, one or more biomarker nucleic acids or proteins in a blood sample or blood derived sample from the subject, wherein the presence of the biomarker, or a level or concentration of the biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject, and wherein the one or more biomarkers comprise or consist of one or more among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.
2. The method according to claim 1, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.
3. The method according to claim 1, wherein the one or more biomarkers comprise or consist of Bbc3 and Ccng1.
4. The method according to claim 1, wherein the one or more biomarkers comprise or consist of Ccng1.
5. The method according to claim 1, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.
6. The method according to claim 1, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.
7. The method according to any preceding claim, wherein the presence of the biomarker, or a level or concentration of the biomarker above a pre-determined threshold is further indicative of ionizing radiation exposure in the subject at a point in time.
8. The method according to any preceding claim, wherein the presence of the biomarker, or a level or concentration of the biomarker above a pre-determined threshold is further indicative of a quantitative dose of ionizing radiation exposure in the subject.
9. The method according to claim 8, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1a.

10. The method according to claim 1, wherein a level or concentration of the biomarker(s) above a pre-determined threshold is indicative of ionizing radiation exposure of equal to or greater than 2 grays (Gy) in the subject, and wherein a level or concentration of the biomarker(s) below a pre-determined threshold is indicative of ionizing radiation exposure of less than 2 Gy in the subject.

11. The method according to claim 10, wherein the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

12. The method according to claim 1, wherein a level or concentration of the biomarker above a pre-determined threshold is indicative of ionizing radiation exposure in the subject within 5 to 48 hours.

13. The method according to any preceding claim, wherein the subject is human.

14. The method according to any preceding claim, wherein the sample is a blood sample.

15. The method according to claim 14, wherein the blood sample is a peripheral blood sample.

16. The method according to claim 1, wherein the sample comprises whole blood, serum, or plasma.

17. The method according to claim 1, wherein the sample is obtained within 48 hours of exposure of the individual to radiation.

18. The method according to claim 1, wherein the sample is obtained within 5 hours of exposure of the individual to radiation.

19. The method according to claim 1, wherein the sample is obtained within 5 hours to 48 hours of exposure of the individual to radiation.

20. The method according to any preceding claim, wherein said method comprises detecting the presence of, and/or quantifying the level of, one or more biomarker proteins.

21. The method according to any preceding claim, wherein said method comprises detecting the presence of, and/or quantifying the level of, one or more biomarker nucleic acids.

22. The method according to claim 21, wherein the one or more biomarker nucleic acids is RNA.

23. The method according to claim 22, further comprising extracting the target RNA from blood cells of the subject, and analyzing the RNA for expression of one or more genes comprising or consisting of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

24. The method according to claim 23, wherein the extracted target RNA is amplified.

25. The method according to claim 1, wherein the detecting comprises: (a) contacting the sample with a binding agent that binds a biomarker protein to form a complex; and (b) detecting and/or quantifying the complex; and correlating the detected complex to the amount of biomarker protein in the sample, wherein the presence of one or more biomarkers, or the presence of elevated levels of the biomarker protein, is indicative of ionizing radiation exposure.

26. The method according to claim 25, wherein the detecting of step (b) further comprises linking or incorporating a label onto the agent, or using ELISA-based immunoenzymatic detection.

27. The method according to claim 1, wherein said method further comprises comparing the level of one or more biomarkers in the sample with the level of biomarker(s) present in a normal control sample, wherein a higher level of biomarker in the sample as compared to the level in the normal control sample is indicative of exposure to ionizing radiation.

28. The method according to claim 1, wherein the nucleic acid is detected using an oligonucleotide that is complementary with at least a portion of the nucleic acid.

29. The method according to claim 1, wherein the nucleic acid is detected using a nucleic acid hybridization method.

30. The method according to claim 29, wherein the nucleic acid hybridization method is Northern blot analysis, dot blotting, Southern blot analysis, fluorescence *in situ* hybridization (FISH), or polymerase chain reaction (PCR) analysis.

31. The method according to claim 30, wherein the PCR is reverse transcription PCR (RT-PCR).

32. The method according to claim 1, wherein the protein is detected using an aptamer, peptide, or antibody that binds to the protein.

33. The method according to claim 1, wherein the protein is detected using an electrophoretic, chromatographic, or spectrometric method.

34. The method according to claim 1, wherein the protein is detected using mass spectrometry.

35. The method according to claim 1, wherein the presence of the biomarker, or a level or concentration of the biomarker is indicative of ionizing radiation exposure in the subject, and wherein said method further comprises treating the subject for radiation exposure.

36. The method according to claim 1, wherein the presence of the biomarker, or a level or concentration of the biomarker is indicative of ionizing radiation exposure in the subject, and wherein said method further comprises verifying that the mammalian subject is suffering from radiation exposure.

37. The method according to claim 1, wherein the subject is human, wherein the presence of the biomarker, or a level or concentration of the biomarker is indicative of ionizing radiation exposure in the subject, and wherein said method further comprises informing the subject of the exposure or potential exposure to ionizing radiation.

38. A kit comprising an array of nucleic acids attached to a solid substrate, wherein said nucleic acids comprise or consist of one or more oligonucleotides that will hybridize to

one or more target nucleic acid molecules from among one or more of the biomarkers Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

39. The kit according to claim 38, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.

40. The kit according to claim 38, wherein the one or more biomarkers comprise or consist of Bbc3 and Ccng1.

41. The kit according to claim 38, wherein the one or more biomarkers comprise or consist of Ccng1.

42. The kit according to claim 38, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

43. The kit according to claim 38, wherein the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

44. The kit according to claim 38, further comprising one or more oligonucleotides that hybridize to nucleic acid molecules of other radiation-responsive genes.

45. The kit according to claim 44, wherein said other radiation-responsive genes are dose-dependent radiation responsive genes.

46. The kit according to claim 38, wherein said array further comprises one or more oligonucleotides that hybridize to nucleic acids from among those in Table 1B.

47. The kit according to claim 38, further comprising printed instructions for detecting hybridization of said one or more oligonucleotides to said one or more target nucleic acid molecules.

48. A kit comprising primers for amplifying target nucleic acid sequences within one or more biomarkers comprising or consisting of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

49. The kit according to claim 48, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.

50. The kit according to claim 48, wherein the one or more biomarkers comprise or consist of Bbc3 and Ccng1.

51. The kit according to claim 48, wherein the one or more biomarkers comprise or consist of Ccng1.

52. The kit according to claim 48, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

53. The kit according to claim 48, wherein the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

54. The kit according to claim 48, further comprising primers for one or more other radiation-responsive genes.

55. The kit according to claim 54, wherein said other radiation-responsive genes are dose-dependent radiation responsive genes.

56. The kit according to claim 48, wherein said kit further comprises one or more primers for one or more genes from among those listed in Table 1B.

57. The kit according to claim 48, further comprising printed instructions for detecting amplicons produced from amplification of the target nucleic acids.

58. A device comprising an application zone for receiving a sample of blood; a labeling zone containing a binding agent that binds to one or more biomarkers in the sample; and a detection zone where a biomarker-bound binding agent is retained to give a signal, wherein the signal given for a sample from a subject with a biomarker level lower than a threshold concentration is different from the signal given for a sample from a subject with a biomarker level equal to or greater than a threshold concentration, and wherein the one or more biomarkers comprise or consist of one or more from among Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b.

59. The device according to claim 58, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.

60. The device according to claim 58, wherein the one or more biomarkers comprise or consist of Bbc3 and Ccng1.

61. The device according to claim 58, wherein the one or more biomarkers comprise or consist of Ccng1.

62. The device according to claim 58, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

63. The device according to claim 58, wherein the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

64. A method of identifying an expression profile in a sample from a mammalian subject that is indicative of the subject's exposure or non-exposure to ionizing radiation, comprising:

providing a subject expression profile from the mammalian subject; providing one or more reference profiles, wherein each reference profile is associated with exposure or non-exposure to ionizing radiation, wherein the subject expression profile and each reference profile has a value, wherein each value represents the expression level of one or more biomarkers comprising or consisting of Bbc3, Ccng1, Cdkn1a, Serpine1, and Tnfrsf10b; and

identifying the reference profile most similar to the subject expression profile, to thereby identify an expression profile that is indicative of exposure or non-exposure to ionizing radiation.

65. The method according to claim 64, wherein the most similar reference profile is identified by weighting a comparison value for each value of each reference profile using a weight value associated with the corresponding biomarker.

66. The method according to claim 64, wherein the identified reference profile is associated with exposure to ionizing radiation, and wherein said method further comprises treating the mammalian subject for radiation exposure.

67. The method according to claim 64, wherein the identified reference profile is associated with exposure to ionizing radiation, and wherein said method further comprises verifying that the mammalian subject is suffering from radiation exposure.

68. The method according to claim 64, wherein the mammalian subject is human, wherein the identified reference profile is associated with exposure to ionizing radiation, and wherein said method further comprises informing the subject of the exposure or potential exposure to ionizing radiation.

69. The method according to claim 64, wherein the one or more reference profiles comprises a plurality of reference profiles.

70. The method according to claim 69, wherein each reference profile of the plurality of reference profiles is associated with exposure to a different dose of ionizing radiation.

71. The method according to claim 64, wherein the one or more biomarkers comprise or consist of one or two from among Bbc3, Ccng1, and Cdkn1a.

72. The method according to claim 64, wherein the one or more biomarkers comprise or consist of Bbc3 and Ccng1.

73. The method according to claim 64, wherein the one or more biomarkers comprise or consist of Ccng1.

74. The method according to claim 64, wherein the one or more biomarkers comprise or consist of Ccng1 and Cdkn1.

75. The method according to claim 64, wherein the one or more biomarkers comprise or consist of one or both biomarkers from among Ccng1 and Tnfrsf10b.

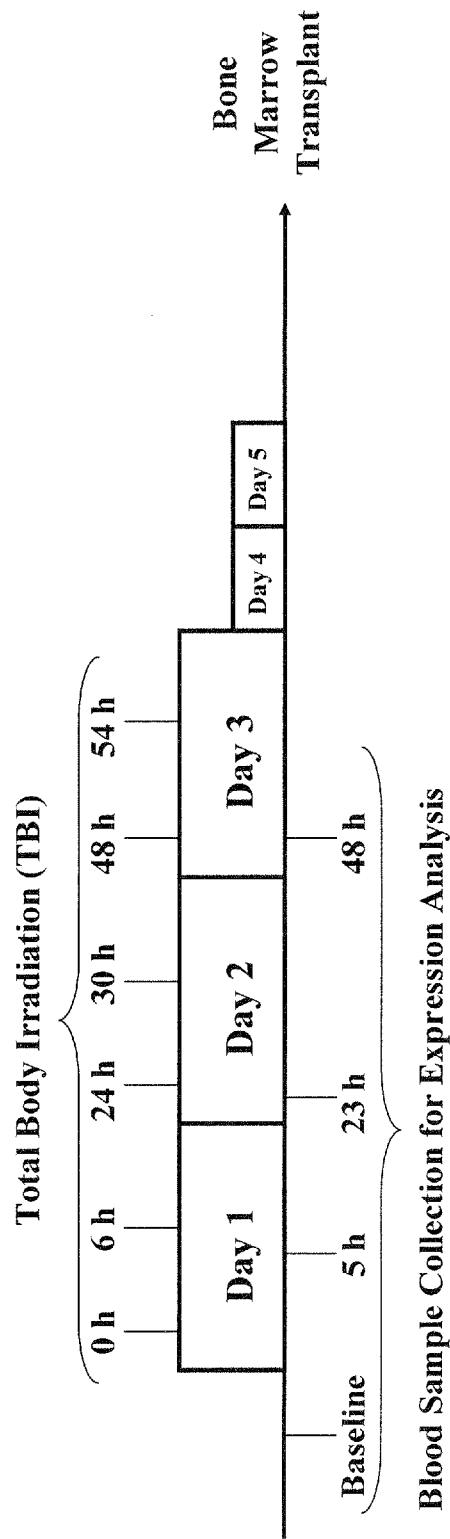


Fig. 1

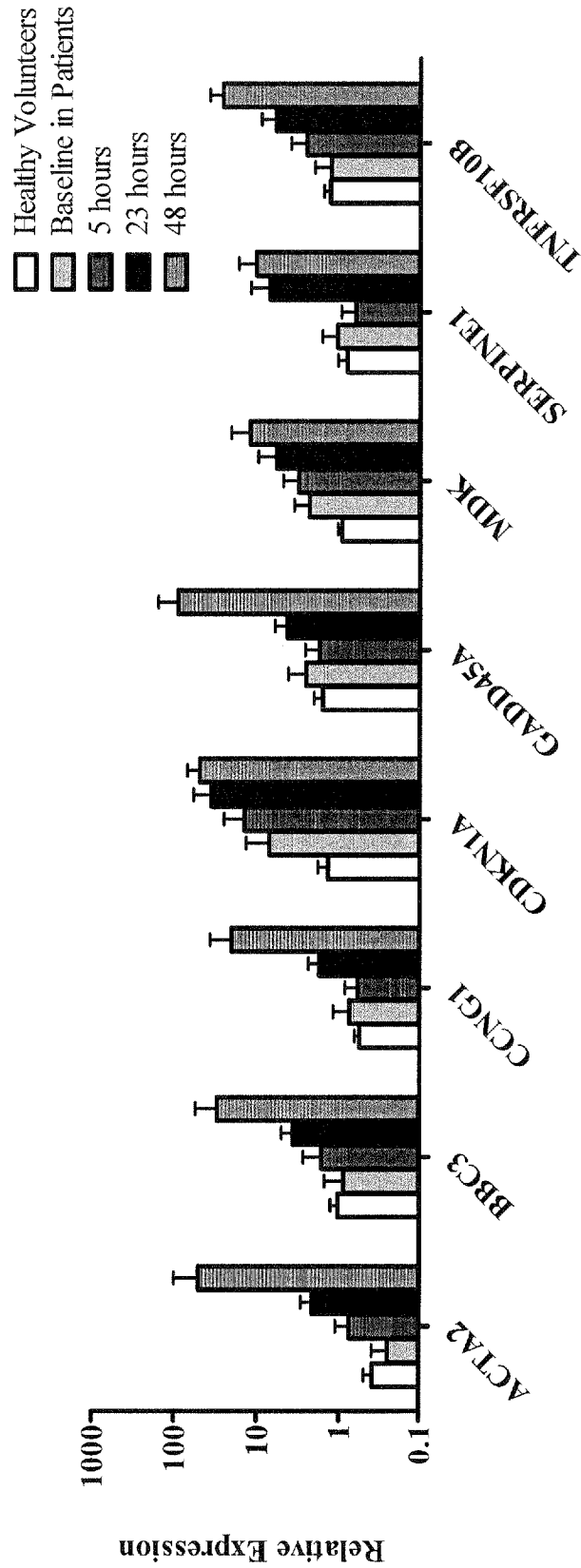


Fig. 2

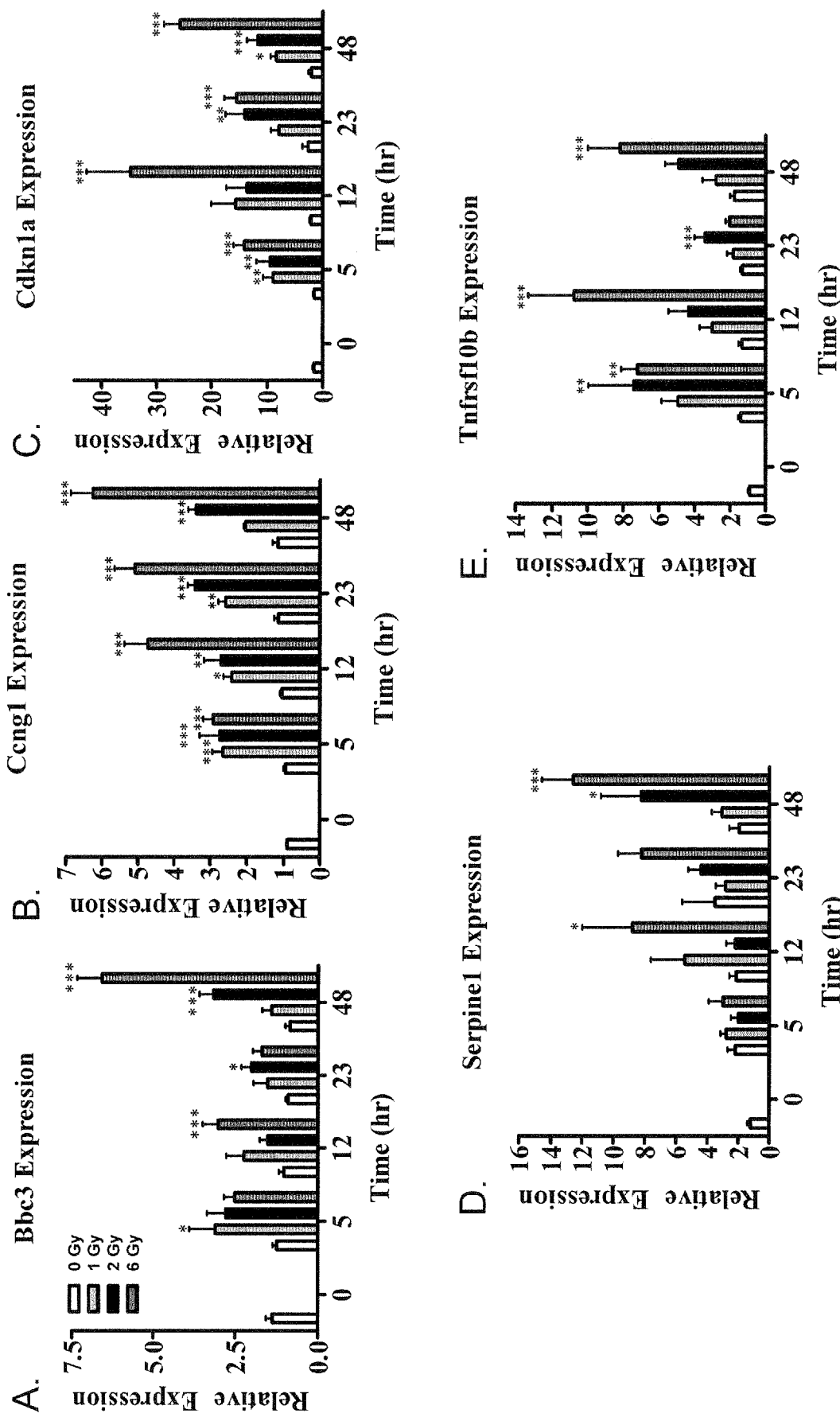
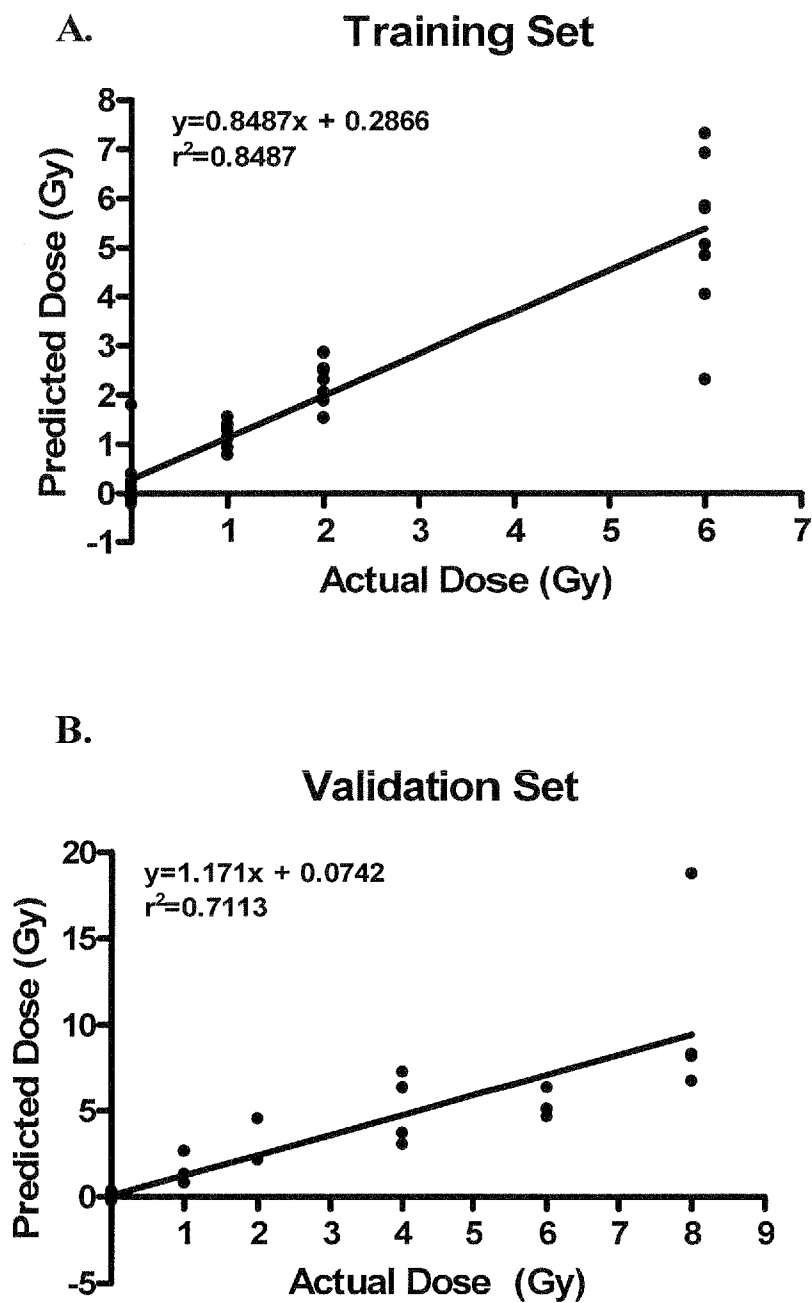


Fig. 3

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**Fig. 4**

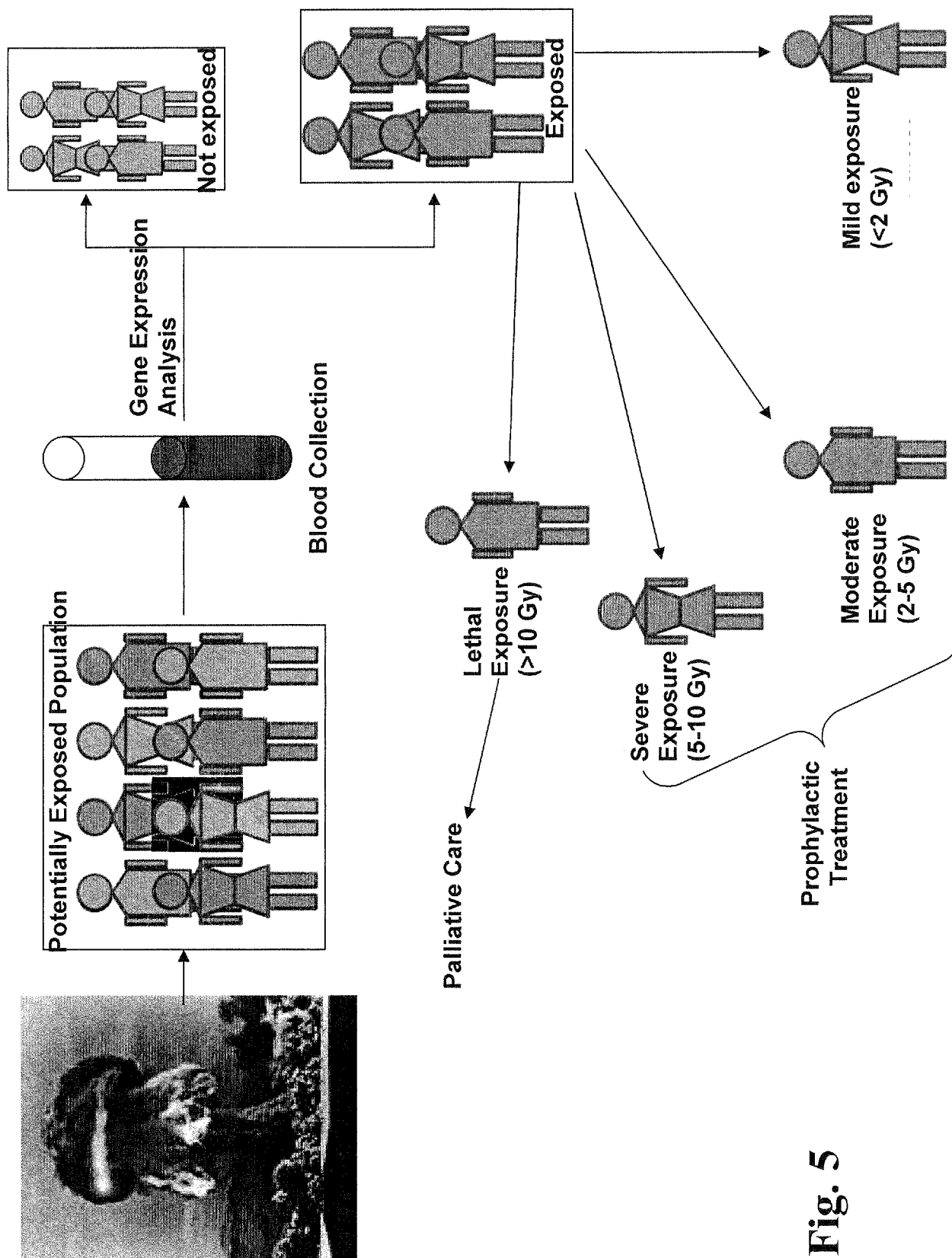


Fig. 5