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(54) Title: BIOMARKERS FOR THE PREDICTION OF RENAL INJURY

(57) Abstract: The present invention relates to means and methods for predicting the onset of renal injury based on measuring the expression of polynucleotides and proteins, particularly on measuring the expression of sets of novel as well as known polynucleotides and proteins, and to kits utilizing same.



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BIOMARKERS FOR THE PREDICTION OF RENAL INJURY

FIELD OF THE INVENTION

5 The present invention relates to means and methods for predicting the onset of renal injury based on measuring the expression of polynucleotides and proteins, particularly measuring the expression of sets of novel as well as known polynucleotides and proteins.

BACKGROUND OF THE INVENTION

10 Renal injury is a general term used to describe any damage to the kidney caused by various conditions including primary renal dysfunction, response to external substances or secondary renal pathology.

Renal injury commonly occurs after administration of pharmaceutical or toxic agents of various types. The process is typically initiated by a toxic injury to tubular epithelial cells in various nephron segments or by injury to specific cell types in the glomerulus. The initial injury is often followed by cellular proliferation and repair processes that attempts to restore normal renal function.

15 Early recognition of renal injury is hampered by the lack of accurate markers and the shortcoming of and over-reliance on serum markers of impaired glomerular filtration rate (i.e., serum creatinine and blood urea nitrogen, see e.g., Schrier et al, J Clin Invest, 114(1):5-14 (2004)). Drugs associated with the development of tubular nephrosis include aminoglycoside antibiotics, antifungals, antineoplastics, immunosuppressants and radiocontrast dyes, among others.

20 Similarly to the human clinical setting, long-term treatment of rats during preclinical drug development with relatively low doses of, for example, aminoglycoside antibiotics, heavy metal toxicants or antineoplastic drugs, leads to the development of degenerative lesions of the renal tubules. However, histopathological or clinical indications of kidney injury are not readily apparent in the early course of treatment, thus necessitating expensive and lengthy studies.

25 Changes in the expression of mRNA specifically expressed in the injured kidney cells are some of the earliest events that accompany renal injury. This is further accompanied by changes in the expression of other genes that contribute either to cellular repair or recovery of renal function or in those that mediate fibrosis and further pathology of the kidney (Matejka GL. et al., Exp Nephrol, 1998 6:253-264; Norman JT. et al. Proc Natl Acad Sci USA, 1988 85:6768-6772; Safirstein R. et al. Kidney Int, 1990 37:1515-1521). For example, elevation in the expression of heme oxygenase I (*HO-1*), kidney injury molecule-1 (*KIM-1*), clusterin, thymosin beta-4, osteopontin, and several growth factors have been reported in various models of renal injury (Hammerman et al, 1998 Curr Opin Nephrol Hypertens 7:419-424; Yoshida et al, 2002 Kidney International 61: 1646-1654; Amin R.P et al. 2004 Environ Health Perspect. 112(4):465-479; Thomas R.S et al. 2001 Mol Pharmacol. 60(6):1189-1194).

35 International Patent Application Publication No. WO 2006/033701 to ICONIX PHARMACEUTICALS, INC. provides gene signatures as well as methods, apparatuses and reagents useful for predicting future renal tubule injury, based on the expression levels of genes in the signatures.

In one particular embodiment that invention provides a method for predicting whether a compound will induce renal tubule injury using gene expression data from sub-acute treatments. However, the WO 2006/033701 Application discloses that the necessary set useful for generating signatures of varying size and performance includes 186 genes, which is a very large number of genes of which expression should be measured.

International Patent Application Publication No. WO 02/095000 to GENE LOGIC, INC. provides toxicity markers identified in tissues or cells exposed to a known renal toxin, based on the elucidation of the global changes in gene expression. The genes may be used as toxicity markers in drug screening and toxicity assays. That Application includes a database of genes characterized by toxin-induced differential expression designed for use with microarrays and other solid-phase probes. The WO 02/095000 Application does not provide specific combination(s) of markers that can be used for toxicity prediction, and thus the methods disclosed require measuring expression of a vast number of genes.

The development of methods to predict the future onset of renal injury and gain a greater understanding of its underlying mechanism would facilitate the development of more reliable clinical diagnostics and safer therapeutic drugs. Moreover, improved preclinical markers for renal injury, particularly of well-defined gene signatures including small number of genes would dramatically reduce the time, cost, and amount of compound required for prioritizing and selecting lead candidates for progression through drug development.

SUMMARY OF THE INVENTION

The present invention provides novel polynucleotides and proteins and sets including combinations of this novel polynucleotides and proteins and known genes useful in predicting the onset of renal injury, particularly an injury resulting from exposure to a toxin or pharmaceutical agent.

The present invention is based in part on the elucidation of the global changes in gene expression in tissues or cells exposed to known toxins, in particular renal toxins, as compared to unexposed, or exposed to control compounds, tissues or cells, as well as on the identification of individual genes that are differentially expressed upon exposure of the cells to a toxin. The present invention is advantageous over hitherto known methods using gene sets for predicting renal injury, providing sets of only few genes necessary for accurate prediction. Moreover, the present invention provides sets based on the expression of unique polynucleotides and proteins associated with renal injury.

Thus, according to one aspect, the present invention provides an isolated polynucleotide encoding a protein having an amino acid sequence as set forth in any one of SEQ ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236.

According to certain embodiments, the polynucleotide has a nucleic acid sequence as set forth in any one of the SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82,84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207, or a sequence homologous thereto. According to one embodiment, the isolated

polynucleotide is at least 85% homologous to any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82,84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207. According to another embodiment, the isolated polynucleotide is at least 95% homologous to any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82,84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178,180-184,189,192-194, 196, 198, 199, 203, 206, 207.

According to certain embodiments, the polynucleotide has a nucleic acid sequence as set forth in any one of the SEQ. ID NOs: 2, 4, 5, 7, 8, 10, 11, 13, 14, 16-19, 21-23, 25, 26, 28, 29, 31, 33, 35, 37, 39, 41.

According to other embodiments, the present invention provides an isolated protein or polypeptide having an amino acid sequence as set forth in any one of SEQ. ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236, or a sequence homologous thereto. According to one embodiment, the isolated protein or polypeptide is at least 85% homologous to any one of SEQ. ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236. According to another embodiment, the isolated polypeptide is at least 95% homologous to any one of SEQ. ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236.

According to certain embodiments, the isolated protein or polypeptide is encoded by an isolated polynucleotide comprising a nucleic acid sequence as set forth in any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82, 84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207, or a sequence homologous thereto.

According to certain embodiments of the present invention, the novel polynucleotides and proteins described herein are non-limiting examples of markers for diagnosing renal injury. The markers of the invention can be employed for various uses, including but not limited to, prognosis, prediction, screening, early diagnosis, determination of progression, staging, therapy selection and treatment monitoring of renal injury.

According to certain embodiments, the presence of at least one novel nucleic acid sequence in a biological sample predicts the onset of renal injury in the subject. According to certain embodiments, the nucleic acid sequence is as set forth in any one of SEQ ID NO: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82, 84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207.

The present invention further provides sets of the novel polynucleotides and proteins of the present invention, sets of other known genes and sets comprising combinations thereof, wherein expression of these nucleic acid sets is indicative of the onset of renal injury. The novel polynucleotides and proteins and genes of the invention are therefore referred to as markers of renal injury.

According to another aspect, the present invention provides a set of markers of renal injury

comprising at least two markers having a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, listed in Table 19, and the corresponding human and mouse homologues thereto as set forth in any one of SEQ ID NOs: 81-137 and 177-207, respectively, listed in Table 21.

5 According to certain embodiments, the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 9, 12, 20, 24, 27, 30, 32, 34, 36, 38, 46, 50, 54, 56, 60 corresponding to markers listed in any one of Table 14 or 15.

10 According to certain embodiments, the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 12, 48, 52 corresponding to markers listed in any one of Table 26 or 27, and the corresponding human and mouse homologues thereto as set forth in any one of SEQ ID NOs: 81-137 and 177-207, respectively, listed in Table 21.

 According to further embodiments, the at least two markers are selected from a combination of novel polynucleotides and known genes having nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 12 corresponding to markers listed in Table 13.

15 According to particular embodiments, the set comprises all four markers having a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 6, 12 corresponding to markers listed in Table 13, wherein expression of the four markers is indicative of the onset of renal injury.

20 According to additional particular embodiments, the set comprises all four markers having a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 48, 52 corresponding to markers listed in Table 26, wherein expression of the four markers is indicative of the onset of renal injury.

 According to further particular embodiments, the set comprises all six markers having a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 6, 12, 48, 52 corresponding to markers listed in Table 27, wherein expression of the six markers is indicative of the onset of renal injury.

25 According to yet further embodiments, the at least two markers are novel polynucleotides selected from the group consisting of a nucleic acid sequence consisting of SEQ ID NOs: 1, 9, 12, 15, 24, 32, 36, 40, 44, 46, 48, 52, 54, 56, 58, 60 corresponding to markers listed in any one of Table 17 and Table 18.

 According to yet additional embodiments, the at least two markers are selected from a combination of novel polynucleotides and known genes having a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 12, 46, 48, 56, 58, 60 corresponding to markers listed in Table 16.

30 According to particular embodiments, the set comprises all seven markers having a nucleic acid sequence set forth in SEQ ID NOs: 1, 12, 46, 48, 56, 58, 60 corresponding to markers listed in Table 16, wherein expression of the seven markers is indicative of the onset of renal injury.

35 According to another aspect, the present invention provides a set of markers of renal injury comprising at least two markers having an amino acid sequence encoded by the nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, listed in Table 12 or 19, or the corresponding human and mouse homologues, SEQ ID NOs: 81-137 and 177-207, respectively, listed in Table 21. According to one

embodiment, the set of markers of renal injury comprises at least two markers having an amino acid sequence selected from the group consisting of SEQ ID NOs: 62-80, listed in Table 19, and the corresponding human and mouse homologues as set forth in SEQ ID NOs: 138-176 and 208-236, respectively, listed in Table 21.

5 According to certain embodiments, the at least two markers are novel polypeptides having an amino acid sequence selected from the group consisting of SEQ ID NO: 62-66, 68-70 corresponding to markers listed in Table 14.

 According to other embodiments, the at least two markers are known proteins having an amino acid sequence selected from the group consisting of SEQ ID NO: 75, 77, 78, 80 corresponding to markers
10 listed in Table 15.

 According to other embodiments, the at least two markers are known proteins having an amino acid sequence selected from the group consisting of SEQ ID NO: 62, 63, 74, 76 corresponding to markers listed in Table 26.

 According to other embodiments, the at least two markers are known proteins having an amino
15 acid sequence selected from the group consisting of SEQ ID NO: 62-64, 66, 74, 76 corresponding to markers listed in Table 27.

 According to further embodiments, the at least two markers are selected from a combination of novel and known proteins having an amino acid sequence selected from the group consisting of SEQ ID NO: 62-64, 66 corresponding to markers listed in Table 13.

20 According to particular embodiments, the set comprises all four markers having an amino acid sequence set forth in SEQ ID NO: 62-64, 66 corresponding to markers listed in Table 13, wherein expression of the four markers is indicative of the onset of renal injury.

 According to particular embodiments, the set comprises all four markers having an amino acid sequence set forth in SEQ ID NO: 62, 63, 74, 76 corresponding to markers listed in Table 26, wherein
25 expression of the four markers is indicative of the onset of renal injury.

 According to particular embodiments, the set comprises all six markers having an amino acid sequence set forth in SEQ ID NO: 62-64, 66, 74, 76 corresponding to markers listed in Table 27, wherein expression of the four markers is indicative of the onset of renal injury.

 According to yet further embodiments, the at least two markers are novel polypeptides having an
30 amino acid sequence selected from the group consisting of SEQ ID NO: 62, 65-67, 69, 71 corresponding to markers listed in Table 17.

 According to additional embodiments, the at least two markers are known proteins having an amino acid sequence selected from the group consisting of SEQ ID NO: 73, 74, 76-80 corresponding to markers listed in Table 18.

35 According to yet additional embodiments, the at least two markers are selected from a combination of novel and known proteins having an amino acid sequence selected from the group consisting of SEQ

ID NO: 62, 66, 74, 78-80 corresponding to markers listed in Table 16.

According to particular embodiments, the set comprises six markers having an amino acid sequence set forth in SEQ ID NO: 62, 66, 74, 78-80 corresponding to markers listed in Table 16, wherein expression of the six markers is indicative of the onset of renal injury.

5 The markers of the invention described herein are information-rich with respect to classifying biological samples for onset of renal injury, even before histopathological or clinical indications are apparent. Thus, the marker sets of the present invention are highly efficient for the early detection of renal injury that may be caused by various conditions and/or treatments.

10 The present invention further provides a method for testing whether a compound will induce renal injury. According to yet another aspect, the present invention provides a method for predicting renal injury in a subject receiving a treatment with a compound, comprising (a) administering a dose of the compound to at least one subject; (b) after a selected time period, obtaining a biological sample from the at least one subject; (c) measuring in the biological sample the expression level of at least two markers selected from those listed in Table 12; and (d) determining whether the sample is in the positive class for
15 onset of renal injury using a classifier comprising at least the two markers for which the expression level is measured.

20 According to certain embodiments, the subject is a mammal selected from the group consisting of a human, cat, dog, monkey, mouse, pig, rabbit, and rat. According to other embodiments, the subject is a test subject. According to typical embodiments the test subject is a rat. According to other embodiments, the test compound is administered to the subject in a form selected from the group consisting of intravenously (IV), orally (PO, per os), intraperitoneally (IP), intranasal, inhalation, eye drops and ointments. According to yet other embodiments, the biological sample is selected from the group consisting of kidney tissue, body fluid and body secretion.

25 The compound may be administered once or the administration may be repeated at any desired regime. It is to be understood that the selected period of time after which the sample is obtained refers to the time after the last compound administration.

30 According to other embodiments, the test compound is nephrotoxic agent. According to one embodiment, the nephrotoxic agent is selected from the group consisting of aminoglycosides; platinum based chemotherapy agents; heavy metals; DNA interacting drugs; antifungal agents; proximal tubule damaging agents; and vasoconstriction compounds.

35 According to one embodiment, the renal injury is associated with at least one kidney disease or pathology, selected from the group consisting of nephrotoxicity, renal toxicity, nephritis, kidney necrosis, kidney damage, glomerular and tubular injury, focal segmental glomerulosclerosis, kidney dysfunction, nephritic syndrome, acute renal failure, chronic renal failure, proximal tubal dysfunction, acute kidney transplant rejection and chronic kidney transplant rejection.

 According to other embodiments, the method is used for predicting at least one toxic effect of the compound; predicting the progression of a toxic effect of the compound; predicting the renal toxicity of

the compound; or identifying an agent that modulates the onset or progression of a toxic response.

According to certain embodiments, the selected time period after which the sample is obtained from the test subject is prior to the appearance of histopathological or clinical indications of renal injury. According to one embodiment, the selected time period is any one of about 1 day, about 5 days, about 7
5 days, about 14 days, about 21 or about 28 days after administering the compound to the at least one test subject. According to typical embodiments, the selected time period is 1 day or less after administration. According to typical embodiments, the selected time period is 5 days after administration. According to other typical embodiments, the selected time period is 7 days after administration.

The at least two markers can be selected as to produce any one of the marker sets disclosed in the
10 present invention. According to certain typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 1, 3, 6, 12 corresponding to markers listed in Table 13 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 1, 12, 46, 48, 56, 58, 60 corresponding to markers listed in Table 16 or
15 polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 1, 3, 6, 9, 12, 20, 24, 27, 30, 32, 34, 36, 38 corresponding to markers listed in Table 14 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 46, 50, 54, 56, 60 corresponding to markers listed in Table 15 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID
20 NO: 1, 9, 12, 15, 24, 32, 36, 40 corresponding to markers listed in Table 17 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 44, 46, 48, 52, 54, 56, 58, 60 corresponding to markers listed in Table 18 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 1, 3, 48, 52 corresponding to markers listed in
25 Table 26 or polypeptide or proteins encoded therefrom. According to other typical embodiments, the method is performed with a marker set comprising SEQ ID NO: 1, 3, 6, 12, 48, 52 corresponding to markers listed in Table 18 or polypeptide or proteins encoded therefrom.

According to certain typical embodiments, the method is performed with a marker set comprising
30 markers listed in any one of Tables 13, 14, 15, 26 or 27, and the selected time period is 5 days after compound administration. According to other typical embodiments, the method is performed with a marker set comprising markers listed in any one of Tables 16, 17 or 18, and the selected time period is 1 day after compound administration.

According to other embodiments, the classifier is a random forest classifier. In alternative
35 embodiments, the classifier may be another linear or non-linear classifier. According to currently typical embodiments the classifier for renal injury is capable of performing with a training log odds ratio of greater than or equal to 3.75.

Any method for detecting the marker expression as is known to a person skilled in the art may be used according to the teachings of the present invention. According to certain embodiments, the

expression level of the at least two markers is detected at the polynucleotide level by an amplification or hybridization assay. According to typical embodiments, the amplification assay is selected from the group consisting of quantitative or semi-quantitative PCR, Northern blot, dot or slot blot, nuclease protection and microarray assays. According to other embodiments, the expression level of the at least two markers is detected at the polypeptide level by an immunoassay. According to typical embodiments the immunoassay is selected from the group consisting of an ELISA, an RIA, a slot blot, immunohistochemical assay, FACS, a radio-imaging assay or a Western blot.

The present invention also provides means for detecting the expression of the markers disclosed herein in a sample. According to certain embodiments, the present invention provides probes and primers for detecting the polynucleotide expression of the markers disclosed herein. According to one embodiment, the probe or the primer comprises a nucleic acid sequence that specifically hybridizes to a marker selected from Table 12 or Table 21.

According to other embodiments, the present invention provides a set of at least two probes or primers, wherein each of the probes or primers comprises a sequence that specifically hybridizes to a marker selected from Table 19 or Table 21.

According to further embodiments, the present invention provides a set of at least two probes or primers, wherein each of the probes or primers comprises a sequence that specifically hybridizes to an amplicon comprising any one of SEQ ID NOs: 251, 254, 257, 260, 263, 266, 269, 272, 275, 278, 281, 284, 287, 290, 293, 296, 299, 302, 305, 308, 311, 314, 317, 320, 323, 326.

According to further embodiments, the set comprises primers comprising a nucleic acid sequence set forth in any one of SEQ ID NO: 249-250, 252-253, 255-256, 258-259, 261-262, 264-265, 267-268, 270-271, 273-274, 276-277, 279-280, 282-283, 285-286, 288-289, 291-292, 294-295, 297-298, 300-301, 303-304, 306-307, 309-310, 312-313, 315-316, 318-319, 321-322, 324-325.

According to further embodiments, the set comprises probes or primers that hybridize to at least a plurality of markers selected from any one of Tables 13, Table 14, Table 15, Table 26 and Table 27. According to certain currently preferred embodiments, the plurality of markers comprises all the markers of Table 13. According to other certain currently preferred embodiments, the plurality of markers comprises all the markers of Table 26. According to other certain currently preferred embodiments, the plurality of markers comprises all the markers of Table 27.

According to yet additional embodiments, the set comprises probes or primers that hybridize to at least a plurality of markers selected from any one of Tables 16, Table 17 and Table 18. According to certain currently preferred embodiments, the plurality of markers comprises all the markers of Table 16.

The hybridization probes of the present invention can be used as free polynucleotides in a solution or can be attached to a solid support as is known to a person skilled in the art. According to certain embodiments, the solid support is selected from the group consisting of a membrane, a glass support and a silicon support.

According to one embodiment, detecting the presence of the polypeptide or polynucleotide is

indicative of renal injury. According to another embodiment, a change in the expression and/or the level of the polynucleotide or polypeptide compared to its expression and/or level in a sample obtained from a healthy subject is indicative of the renal injury. According to a further embodiment, a change in the expression and/or level of the polynucleotide or polypeptide compared to its level and/or expression in a sample obtained from the said subject at earlier stage is indicative of the renal injury. According to still further embodiment, detecting the presence and/or relative change in the expression and/or level of the polynucleotide or polypeptide is useful for selecting a treatment and/or monitoring a treatment of renal injury.

According to additional aspect, the present invention provides a method for selecting a treatment or monitoring a treatment for renal injury comprising (a) obtaining a first sample from a subject suffering from renal injury; (b) administering the treatment to the subject; (c) obtaining a second sample from said subject; (d) measuring in the first and second samples the expression level of at least two markers selected from those listed in Table 12 or Table 21; and (e) determining a change in the expression and/or level of the polynucleotides or polypeptides in said second sample compared to the level and/or expression in said first sample, wherein relative change in said expression and/or level of the polynucleotide or polypeptide is useful for selecting a treatment and/or monitoring a treatment of the renal injury.

The present invention also provides a kit for predicting whether renal injury will occur in a test subject comprising at least one means for detecting the expression of at least two markers as described above, further comprising reagents for performing the detection. According to certain embodiments, the kit comprises at least two primers or probes and reagents for detecting at least two genes listed in Table 12. According to other embodiments, the kit comprises at least two polypeptides and reagents for detecting at least two polypeptides encoded by the genes listed in Table 12.

In one embodiment, the kit comprises at least a plurality of polynucleotides or polypeptides representing a plurality of genes selected from those listed in Table 12. In one embodiment the kit comprising a plurality of genes includes at least 2 genes selected from those listed in Table 12. In another preferred embodiment the plurality of genes are variables in a classifier capable of classifying renal injury with a training log odds ratio of greater than or equal to 3.75. In one typical embodiment, the kit comprises polynucleotides or polypeptides capable of detecting a subset of genes listed in any one of tables 13, 26, 27 and 16. In one preferred embodiment, the kit comprises polynucleotide probes capable of hybridizing to a plurality of genes selected from those listed in Table 12. In one preferred embodiment, the kit comprises polynucleotide probes capable of hybridizing to a plurality of amplicons selected from those listed in Table 20. In one preferred embodiment, the kit comprises polynucleotide probes selected from those listed in Table 20. According to further embodiments, the kit further comprises at least one solid surface, wherein a plurality of polynucleotide probes are bound to the at least one solid surface. In one embodiment, the plurality of probes is bound to a single solid surface in an array. Alternatively, the plurality of probes is bound to the solid surface on a plurality of beads.

According to one embodiment, the kit comprises reagents for detecting the marker expression by employing a NAT-based technology. In one embodiment, the NAT-based assay is selected from the group consisting of a PCR, Real-Time PCR, LCR, Self-Sustained Synthetic Reaction, Q-Beta Replicase,

Cycling Probe Reaction, Branched DNA, RFLP analysis, DGGE/TGGE, Single-Strand Conformation Polymorphism, Dideoxy Fingerprinting, Microarrays, Fluorescence, *In Situ* Hybridization or Comparative Genomic Hybridization.

According to other preferred embodiments, the kit comprises a plurality of antibodies capable of recognizing or interacting with a plurality of polypeptides encoded by genes selected from those listed in Table 12. In certain embodiments, the polypeptides are secreted proteins encoded by genes listed in Table 12. According to other embodiments, the kit further comprises at least one reagent for performing an ELISA, an RIA, a slot blot, an immunohistochemical assay, FACS, *in vivo* imaging, a radio-imaging assay, or a Western blot.

Other objects, features and advantages of the present invention will become clear from the following description and drawings.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 presents chemical structure of T1 drug

Figure 2 presents chemical structure of T2 drug

Figure 3 presents histological section of the kidney, magnified x200. Figure 3A presents a control animal. The arrows represent normal aspects of cortical tubules (eosinophilic cytoplasm). Figure 3B presents 50mg/kg T2-treated animal. The arrows represent basophilic tubules in the cortex, indicating post-necrotic regeneration.

Figure 4 presents histological section of the kidney, magnified x400. Figure 4A presents a control animal. The arrows represent normal aspects of cortical tubules (eosinophilic cytoplasm). Figure 4B presents 50mg/kg T2-treated animal. The arrows represent basophilic tubules in the cortex, indicating post-necrotic regeneration.

Figure 5 presents histological section of the kidney, magnified x600. Figure 5A presents a control animal. The arrows represent normal aspects of cortical tubules (eosinophilic cytoplasm). Figure 5B presents 50mg/kg T2-treated animal. The arrows represent basophilic tubules in the cortex, indicating post-necrotic regeneration.

Figure 6 presents the ROC curve of the Random-Forest classifier based on the Real Time PCR measurements from the validation stage of the labeled samples. The ROC curve is based on the out-of-the-bag method for estimating the performance of a Random-Forest classifier.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel polynucleotides and polypeptides, as well as sets of these novel compounds and set of known genes which are useful as diagnostic markers, particularly for predicting the onset of renal injury.

The present invention further provides methods for predicting whether a treatment with a

compound would induce renal injury, following sub-chronic or long-term treatment. The present invention is based in part on gene expression data obtained from sub-acute or short-term treatments with a certain compound. The present invention now discloses necessary and sufficient sets of genes and specific signatures comprising these genes that allow gene expression data to be used to identify the ability of a compound treatment to induce late onset renal injury before actual histological or clinical indication are apparent. Further, the invention provides kits comprising means for detecting the disclosed gene sets and signatures. The means and methods provided by the present invention enable the detection of a compound toxicity using short term studies, avoiding lengthy and costly long term studies.

Definitions

10 “Marker”: in some embodiments, the phrase “marker” in the context of the present invention is used interchangeably with the phrase “gene” and refers to a nucleic acid fragment, a peptide, or a polypeptide, which is differentially expressed in a sample taken from subjects having one of the herein-described diseases or conditions, as compared to a comparable sample taken from subjects who do not have one the above-described diseases or conditions.

15 As used herein, the term “expression” refers to the amount of a nucleic acid molecule, peptide or polypeptide present in a sample at a certain time point, which amount may be the result of any process taking place in a cell within the sample, including, but not limited to, gene transcription and translation and degradation or stabilization of the gene product.

20 “Renal injury” refers to any damage to the kidney that can be caused by primary renal dysfunction (i.e. Alport's syndrome, response to external substances (nephrotoxics), infections, altered blood supply, malignancies, etc.) or secondary renal pathology (i.e. complications of diabetes mellitus, multiple myeloma, etc.).

25 Renal injury includes but is not limited to: nephritis, kidney necrosis, kidney damage, nephrotoxicity, renal toxicity, glomerular and tubular injury, focal segmental glomerulosclerosis, kidney dysfunction, nephritic syndrome, acute renal failure, chronic renal failure, proximal tubal dysfunction, acute kidney transplant rejection, chronic kidney transplant rejection.

30 “Nephrotoxicant”: in the context of the present invention is used interchangeably with the phrase “nephrotoxic agent” and/or “renal toxin”, and refers to every substance (chemical compound and/or protein, recombinant or endogenous, including toxins or medications) that accumulates or that its clearance is via the renal system and causes renal injury. Examples of substance families that can cause such renal injury include but are not limited to: Aminoglycosides (i.e. Gentamicin Tobramycin, Amikacin, Kanamycin, Neomycin, Netilmicin, Paromomycin, Streptomycin, Tobramycin and Apramycin); Platinum based chemotherapy (i.e. Cisplatin, carboplatin); Heavy metals (i.e. Cadmium Chloride, Chromium, Arsenic, Lead, Mercury, Manganese); DNA interacting drugs (i.e. Doxorubicin, 35 Daunorubicin, Epirubicin, Idarubicin, Mitoxantrone); Antifungal (i.e. Amphotericin B); Proximal tubule damaging agents (i.e. Acyclovir, Foscarnet, Pentamidine, Ifosfamide); and Vasoconstriction compounds (i.e. Radiocontrast agents, Cyclosporine, Tacrolimus).

As used herein, the term "test subject" refers to a subject receiving a compound or a treatment in order to evaluate the effect of the compound or treatment on the subject, including its efficacy, side effects, adverse effects and the like. According to typical embodiments, the test subject is a mammal.

"Multivariate dataset" as used herein, refers to any dataset comprising a plurality of different variables including but not limited to chemogenomic datasets comprising intensity measurements from gene expression experiments, such as those carried out on polynucleotide microarrays, or multiple protein binding affinities measured using a protein chip. Other examples of multivariate data include assemblies of data from a plurality of standard toxicological or pharmacological assays (e.g., blood analytes measured using enzymatic assays, antibody based ELISA or other detection techniques).

"Variable" as used herein, refers to any value that may vary. For example, variables may include relative or absolute amounts of biological molecules, such as mRNA or proteins, or other biological metabolites. Variables may also include dosing amounts of test compounds.

"Classifier" as used herein, refers to a function of a set of variables that is capable of answering a classification question. A "classification question" may be of any type susceptible to yielding a yes or no answer (e.g., "Is the unknown a member of the class or does it belong with everything else outside the class?"). A valid classifier is defined as a classifier capable of achieving a performance for its classification task at or above a selected threshold value. For example, a log odds ratio > 3.75 represents a preferred threshold of the present invention. Higher or lower threshold values may be selected depending of the specific classification task.

"Random Forest" as used herein, refers to a type of non-linear classifier based on the majority decision of a collection of decision trees, each stating the outcome label (e.g. – the answer to the classification question), according to the values of the input variables. (Breiman, Leo "Random Forests". Machine Learning 45 (1), 5-32, 2001). We use the random forest algorithm supplied by the R programming language.

"Signature" as used herein, refers to a combination of variables. As well as, possibly, a classification algorithm that provides a unique value or function capable of answering a classification question. A signature may include as few as one variable. Signatures include but are not limited to Random Forest classifiers.

"Log odds ratio" or "LOR" is used herein to summarize the performance of classifiers or signatures. LOR is defined generally as the natural log of the ratio of predicting a subject to be positive when it is positive, versus the odds of predicting a subject to be positive when it is negative. LOR is estimated herein using a set of training or test cross-validation partitions according to the following equation,

$$LOR = \ln \frac{\left(\sum_{i=1}^c TP_i + 0.5 \right) * \left(\sum_{i=1}^c TN_i + 0.5 \right)}{\left(\sum_{i=1}^c FP_i + 0.5 \right) * \left(\sum_{i=1}^c FN_i + 0.5 \right)}$$

Where c (typically $c = 100$ as described herein) equals the number of partitions, and TP_i , TN_i , FP_i and FN_i represent the number of true positive, true negative, false positive, and false negative occurrences in the test set of the i^{th} partition, respectively.

“Accuracy” as used herein, refers to an alternative mean of summarizing the performance of classifiers of signatures. Accuracy is the percentage of correctly labeled samples. It is estimated herein using a set of training or test cross-validation partitions according to the following equation,

$$ACC = 100 * \frac{\sum_{i=1}^c (TP_i + TN_i)}{\sum_{i=1}^c N_i}$$

Where c (typically $c = 100$ as described herein) equals the number of partitions, TP_i , TN_i , and N_i represent the number of true positive, true negative, and all samples in the test set of the i^{th} partition, respectively.

“Array” as used herein, refers to a set of different biological molecules (e.g., polynucleotides, peptides, carbohydrates, etc.). An array may be immobilized in or on one or more solid substrates (e.g., glass slides, beads, or gels) or may be a collection of different molecules in solution (e.g., a set of PCR primers). An array may include a plurality of biological polymers of a single class (e.g., polynucleotides) or a mixture of different classes of biopolymers (e.g., an array including both proteins and nucleic acids immobilized on a single substrate).

“Array data” as used herein refers to any set of constants and/or variables that may be observed, measured or otherwise derived from an experiment using an array, including but not limited to: fluorescence (or other signaling moiety) intensity ratios, binding affinities, hybridization stringency, temperature, buffer concentrations.

“Proteomic data” as used herein refers to any set of constants and/or variables that may be observed, measured or otherwise derived from an experiment involving a plurality of mPvNA translation products (e.g., proteins, peptides, etc) and/or small molecular weight metabolites or exhaled gases associated with these translation products.

25 General Methods of the Invention

The present invention provides novel polynucleotide and protein and gene signatures useful for detecting renal injury. The invention discloses lists of genes that may be used to create a signature that performs above a certain minimal threshold level for a specific prediction of renal injury. This set of genes also may be used to derive additional signatures with varying numbers of genes and levels of performance for particular applications (e.g., diagnostic assays and devices).

Using Signatures for Predicting Renal Injury

A diagnostic usually consists in performing one or more assays and in assigning a sample to one or more categories based on the results of the assay(s). Desirable attributes of diagnostic assays include high

sensitivity and specificity measured in terms of low false negative and false positive rates and overall accuracy. Because diagnostic assays are often used to assign large number of samples to given categories, the issues of cost per assay and throughput (number of assays per unit time or per worker hour) are of paramount importance. Typically the development of a diagnostic assay involves the following steps: (1) define the end point to diagnose, e.g., renal injury; (2) identify one or more markers whose alteration correlates with the end point, e.g., elevation of expression of a gene set; and (3) develop a specific, accurate, high - throughput and cost-effective assay for that marker. In order to increase throughput and decrease costs several diagnostics are often combined in a panel of assays, especially when the detection methodologies are compatible. For example several ELISA-based assays, each using different antibodies to ascertain different end points may be combined in a single panel and commercialized as a single kit. Even in this case, however, each of the ELISA- based assays had to be developed individually often requiring the generation of specific reagents.

The present invention provides signatures comprising as few as 2 genes, preferably as few as 4 genes, preferably as few as 5 genes, that are useful for determining a therapeutic or toxicological end-point for renal injury. These signatures (and the genes from which they are composed) may also be used in the design of improved diagnostic kits that answer the same questions as a large microarray but using a much smaller fraction of data. Generally, the reduction of information in a large chemogenomic dataset to a simple signature enables much simpler devices compatible with low cost high throughput multi-analyte measurement.

Consequently, the signatures of the present invention provide the ability to produce cheaper, higher throughput, diagnostic measurement methods or strategies. In particular, the invention provides diagnostic marker sets useful in diagnostic assays and the associated diagnostic kits. As used herein, diagnostic assays include assays that may be used for test subjects, patient prognosis and therapeutic monitoring.

Diagnostic marker sets may include markers representing a subset of genes disclosed in Table 12-18, and tables 26-27, and the genes homologous thereto, disclosed in table 21. In one preferred embodiment, the diagnostic marker set is a plurality of polynucleotides or polypeptides representing specific genes in the list contained in these tables. Such biopolymer marker sets are immediately applicable in any of the diagnostic assay methods (and the associate kits) well known for polynucleotides and polypeptides (e.g., DNA arrays, RT-PCR, immunoassays or other receptor based assays for polypeptides or proteins). Thus, a very simple diagnostic array may be designed that answers 3 or 4 specific classification questions and includes only 10-20 polynucleotides representing the approximately 5-10 genes in each of the signatures. Of course, depending on the level of accuracy required the LOR threshold for selecting a sufficient gene signature may be varied.

The diagnostic marker sets of the invention may be provided in kits, wherein the kits may or may not comprise additional reagents or components necessary for the particular diagnostic application in which the marker set is to be employed. Thus, for a polynucleotide array applications, the diagnostic marker sets may be provided in a kit which further comprises one or more of the additional requisite reagents for amplifying and/or labeling a microarray probe or target (e.g., polymerases, labeled

nucleotides, and the a variety of array formats (for either polynucleotides and/or polypeptides) are well-known in the art and may be used with the methods and subsets produced by the present invention. In one embodiment, photolithographic or micromirror methods may be used to spatially direct light-induced chemical modifications of spacer units or functional groups resulting in attachment at specific localized regions on the surface of the substrate. Light-directed methods of controlling reactivity and immobilizing chemical compounds on solid substrates are well-known in the art and described in U.S. Patent Nos. 4,562,157, 5,143,854, 5,556,961, 5,968,740, and 6,153,744, and PCT publication WO 99/42813, each of which is hereby incorporated by reference herein.

Alternatively, a plurality of molecules may be attached to a single substrate by precise deposition of chemical reagents. For example, methods for achieving high spatial resolution in depositing small volumes of a liquid reagent on a solid substrate are disclosed in U.S Patent Nos. 5,474,796 and 5,807,522, both of which are hereby incorporated by reference herein.

It should also be noted that in many cases a single diagnostic device may not satisfy all needs. However, even for an initial exploratory investigation {e.g., classifying drug- treated rats) DNA arrays with sufficient gene sets of varying size (number of genes), each adapted to a specific follow-up technology, can be created. In addition, in the case of drug- treated rats, different arrays may be defined for each tissue.

Alternatively, a single substrate may be produced with several different small arrays of genes in different areas on the surface of the substrate. Each of these different arrays may represent a sufficient set of genes for the same classification question but with a different optimal gene signature for each different tissue. Thus, a single array could be used for particular diagnostic question regardless of the tissue source of the sample (or even if the sample was from a mixture of tissue sources, e.g., in a forensic sample).

According to the present invention, the genes identified in Table 12 may be used as markers or drug targets to evaluate the effects of a candidate drug, chemical compound or other agent on a cell or tissue sample. The genes may also be used as drug targets to screen for agents that modulate their expression and/or activity. In various formats, a candidate drug or agent can be screened for the ability to stimulate the transcription or expression of a given marker or markers or to down-regulate or counteract the transcription or expression of a marker or markers. According to the present invention, one can also compare the specificity of a drug's effects by looking at the number of markers which the drug induces and comparing them. More specific drugs will have less transcriptional targets. Similar sets of markers identified for two drugs may indicate a similarity of effects.

Assays to monitor the expression of a marker or markers as defined in Table 12 may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention if it is capable of up-or down-regulating expression of the nucleic acid in a cell.

Nucleic Acid Assay Formats

The genes identified as being differentially expressed upon exposure to a known renal toxin (Tables 12-18 and 26-27, and Table 21) may be used in a variety of nucleic acid detection assays to detect or quantify the expression level of a gene or multiple genes in a given sample.

5 Any assay format to detect gene expression may be used. For example, traditional Northern blotting, dot or slot blot, nuclease protection, primer directed amplification, RT-PCR, semi-or quantitative PCR, branched-chain DNA and differential display methods may be used for detecting gene expression levels. Those methods are useful for some embodiments of the invention. In cases where smaller numbers of genes are detected, amplification based assays may be most efficient.

10 Methods and assays of the invention, however, may be most efficiently designed with hybridization-based methods for detecting the expression of a large number of genes.

Any hybridization assay format may be used, including solution-based and solid support-based assay formats. Solid supports containing oligonucleotide probes for differentially expressed genes of the invention can be filters, polyvinyl chloride dishes, particles, beads, microparticles or silicon or glass
15 based chips, etc. Such chips, wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755).

Any solid surface, to which oligonucleotides can be bound, either directly or indirectly, either covalently or non-covalently, can be used. A preferred solid support is a high density array or DNA chip. These contain a particular oligonucleotide probe in a predetermined location on the array. Each
20 predetermined location may contain more than one molecule of the probe, but each molecule within the predetermined location has an identical sequence. Such predetermined locations are termed features. There may be, for example, from 2, 10, 100, 1000 to 10,000, 100,000 or 400,000 or more of such features on a single solid support. The solid support or the area within which the probes are attached may be on the order of about a square centimeter. Probes corresponding to the genes of Tables 12-18 and 26-27, or
25 Table 21 may be attached to single or multiple solid support structures, e. g., the probes may be attached to a single chip or to multiple chips to comprise a chip set.

Oligonucleotide probe arrays for expression monitoring can be made and used according to any techniques known in the art (see for example, Lockhart et al. (1996), NatBiotechnol 14: 1675-1680; McGall et al. (1996), Proc Nat Acad Sci USA 93: 13555-13460). Such probe arrays may contain at least
30 two or more oligonucleotides that are complementary to or hybridize to two or more of the genes described in Tables 12-18 and 26-27 or Table 21. In one embodiment, such arrays contain oligonucleotides that are complementary to or hybridize to any subset of the genes in any one or all of Tables 14, 15, 17, 18, 26 and 27. In a preferred embodiment, such arrays contain oligonucleotides that are complementary to or hybridize to all or nearly all of the genes in any one of Tables 13, 16, 26 and 27.
35 Preferred arrays contain all or nearly all of the genes listed in Tables 12-18 and 26-27 or Table 21. In a preferred embodiment, arrays are constructed that contain oligonucleotides to detect all or nearly all of the genes in any one or all of Tables 12-18, 26-27 and Table 21, in particular Tables 13, 14, 16, 27, 26 and 27 on a single solid support substrate, such as a chip.

NAT Assays

Detection of a nucleic acid of interest in a biological sample may also optionally be effected by NAT-based assays, which involve nucleic acid amplification technology, such as PCR for example (or variations thereof such as real-time PCR for example).

5 As used herein, a "primer" defines an oligonucleotide which is capable of annealing to (hybridizing with) a target sequence, thereby creating a double stranded region which can serve as an initiation point for DNA synthesis under suitable conditions.

Amplification of a selected, or target, nucleic acid sequence may be carried out by a number of suitable methods. See generally Kwoh et al., 1990, Am. Biotechnol. Lab. 8:14 Numerous amplification
10 techniques have been described and can be readily adapted to suit particular needs of a person of ordinary skill. Non-limiting examples of amplification techniques include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), transcription-based amplification, the q3 replicase system and NASBA (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86, 1173-1177; Lizardi et al., 1988, BioTechnology 6:1197-1202; Malek et al., 1994, Methods Mol. Biol., 28:253-260; and
15 Sambrook et al., 1989, supra).

The terminology "amplification pair" (or "primer pair") refers herein to a pair of oligonucleotides (oligos) of the present invention, which are selected to be used together in amplifying a selected nucleic acid sequence by one of a number of types of amplification processes, preferably a polymerase chain reaction. Other types of amplification processes include ligase chain reaction, strand displacement
20 amplification, or nucleic acid sequence-based amplification, as explained in greater detail below. As commonly known in the art, the oligos are designed to bind to a complementary sequence under selected conditions.

In one particular embodiment, amplification of a nucleic acid sample from a patient is amplified under conditions which favor the amplification of the most abundant differentially expressed nucleic acid.
25 In one preferred embodiment, RT-PCR is carried out on an mRNA sample from a patient under conditions which favor the amplification of the most abundant mRNA. In another preferred embodiment, the amplification of the differentially expressed nucleic acids is carried out simultaneously. It will be realized by a person skilled in the art that such methods could be adapted for the detection of differentially expressed proteins instead of differentially expressed nucleic acid sequences.

30 The nucleic acid (i.e. DNA or RNA) for practicing the present invention may be obtained according to well known methods.

Oligonucleotide primers of the present invention may be of any suitable length, depending on the particular assay format and the particular needs and targeted genomes employed. Optionally, the oligonucleotide primers are at least 12 nucleotides in length, preferably between 15 and 24 molecules, and
35 they may be adapted to be especially suited to a chosen nucleic acid amplification system. As commonly known in the art, the oligonucleotide primers can be designed by taking into consideration the melting point of hybridization thereof with its targeted sequence (Sambrook et al., 1989, Molecular Cloning -A Laboratory Manual, 2nd Edition, CSH Laboratories; Ausubel et al., 1989, in Current Protocols in

Molecular Biology, John Wiley & Sons Inc., N.Y.).

It will be appreciated that antisense oligonucleotides may be employed to quantify expression of a splice isoform of interest. Such detection is effected at the pre-mRNA level. Essentially the ability to quantitate transcription from a splice site of interest can be effected based on splice site accessibility.

5 Oligonucleotides may compete with splicing factors for the splice site sequences. Thus, low activity of the antisense oligonucleotide is indicative of splicing activity.

The polymerase chain reaction and other nucleic acid amplification reactions are well known in the art (various non-limiting examples of these reactions are described in greater detail below). The pair of oligonucleotides according to this aspect of the present invention are preferably selected to have
10 compatible melting temperatures (T_m), e.g., melting temperatures which differ by less than that 7°C , preferably less than 5°C , more preferably less than 4°C , most preferably less than 3°C , ideally between 3°C and 0°C .

Polymerase Chain Reaction (PCR): The polymerase chain reaction (PCR), as described in U.S. Pat. Nos. 4,683,195 and 4,683,202 to Mullis and Mullis et al., is a method of increasing the
15 concentration of a segment of target sequence in a mixture of genomic DNA without cloning or purification. This technology provides one approach to the problems of low target sequence concentration. PCR can be used to directly increase the concentration of the target to an easily detectable level. This process for amplifying the target sequence involves the introduction of a molar excess of two oligonucleotide primers which are complementary to their respective strands of the double-stranded target
20 sequence to the DNA mixture containing the desired target sequence. The mixture is denatured and then allowed to hybridize. Following hybridization, the primers are extended with polymerase so as to form complementary strands. The steps of denaturation, hybridization (annealing), and polymerase extension (elongation) can be repeated as often as needed, in order to obtain relatively high concentrations of a segment of the desired target sequence.

25 The length of the segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and, therefore, this length is a controllable parameter. Because the desired segments of the target sequence become the dominant sequences (in terms of concentration) in the mixture, they are said to be "PCR-amplified."

Ligase Chain Reaction (LCR or LAR): The ligase chain reaction [LCR; sometimes referred to
30 as "Ligase Amplification Reaction" (LAR)] has developed into a well-recognized alternative method of amplifying nucleic acids. In LCR, four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, which hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules.
35 Importantly, in LCR, two probes are ligated together only when they base-pair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, and ligation amplify a short segment of DNA. LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes: see for example Segev, PCT Publication No. W09001069 A1 (1990). However,

because the four oligonucleotides used in this assay can pair to form two short ligatable fragments, there is the potential for the generation of target-independent background signal. The use of LCR for mutant screening is limited to the examination of specific nucleic acid positions.

5 Self-Sustained Synthetic Reaction (3SR/NASBA): The self-sustained sequence replication reaction (3SR) is a transcription-based in vitro amplification system that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection. In this method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5' end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target
10 sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest. The use of 3SR to detect mutations is kinetically limited to screening small segments of DNA (e.g., 200-300 base pairs).

Q-Beta (Q β) Replicase: In this method, a probe which recognizes the sequence of interest is attached to the replicatable RNA template for Q β replicase. A previously identified major problem with
15 false positives resulting from the replication of unhybridized probes has been addressed through use of a sequence-specific ligation step. However, available thermostable DNA ligases are not effective on this RNA substrate, so the ligation must be performed by T4 DNA ligase at low temperatures (37 degrees C.). This prevents the use of high temperature as a means of achieving specificity as in the LCR, the ligation event can be used to detect a mutation at the junction site, but not elsewhere.

20 A successful diagnostic method must be very specific. A straight-forward method of controlling the specificity of nucleic acid hybridization is by controlling the temperature of the reaction. While the 3SR/NASBA, and Q β systems are all able to generate a large quantity of signal, one or more of the enzymes involved in each cannot be used at high temperature (i.e., > 55°C). Therefore the reaction temperatures cannot be raised to prevent non-specific hybridization of the probes. If probes are shortened
25 in order to make them melt more easily at low temperatures, the likelihood of having more than one perfect match in a complex genome increases. For these reasons, PCR and LCR currently dominate the research field in detection technologies.

The basis of the amplification procedure in the PCR and LCR is the fact that the products of one cycle become usable templates in all subsequent cycles, consequently doubling the population with each
30 cycle. The final yield of any such doubling system can be expressed as: $(1+X)^n = y$, where "X" is the mean efficiency (percent copied in each cycle), "n" is the number of cycles, and "y" is the overall efficiency, or yield of the reaction. If every copy of a target DNA is utilized as a template in every cycle of a polymerase chain reaction, then the mean efficiency is 100%. If 20 cycles of PCR are performed, then the yield will be 2^{20} , or 1,048,576 copies of the starting material. If the reaction conditions reduce
35 the mean efficiency to 85%, then the yield in those 20 cycles will be only 1.85^{20} , or 220,513 copies of the starting material. In other words, a PCR running at 85 % efficiency will yield only 21 % as much final product, compared to a reaction running at 100 % efficiency. A reaction that is reduced to 50% mean efficiency will yield less than 1 % of the possible product.

In practice, routine polymerase chain reactions rarely achieve the theoretical maximum yield, and PCRs are usually run for more than 20 cycles to compensate for the lower yield. At 50% mean efficiency, it would take 34 cycles to achieve the million-fold amplification theoretically possible in 20, and at lower efficiencies, the number of cycles required becomes prohibitive. In addition, any background products that amplify with a better mean efficiency than the intended target will become the dominant products.

Also, many variables can influence the mean efficiency of PCR, including target DNA length and secondary structure, primer length and design, primer and dNTP concentrations, and buffer composition, to name but a few. Contamination of the reaction with exogenous DNA (e.g., DNA spilled onto lab surfaces) or cross-contamination is also a major consideration. Reaction conditions must be carefully optimized for each different primer pair and target sequence, and the process can take days, even for an experienced investigator. The laboriousness of this process, including numerous technical considerations and other factors, presents a significant drawback to using PCR in the clinical setting. Indeed, PCR has yet to penetrate the clinical market in a significant way. The same concerns arise with LCR, as LCR must also be optimized to use different oligonucleotide sequences for each target sequence. In addition, both methods require expensive equipment, capable of precise temperature cycling.

Many applications of nucleic acid detection technologies, such as in studies of allelic variation, involve not only detection of a specific sequence in a complex background, but also the discrimination between sequences with few, or single, nucleotide differences. One method of the detection of allele-specific variants by PCR is based upon the fact that it is difficult for Taq polymerase to synthesize a DNA strand when there is a mismatch between the template strand and the 3' end of the primer. An allele-specific variant may be detected by the use of a primer that is perfectly matched with only one of the possible alleles; the mismatch to the other allele acts to prevent the extension of the primer, thereby preventing the amplification of that sequence. This method has a substantial limitation in that the base composition of the mismatch influences the ability to prevent extension across the mismatch, and certain mismatches do not prevent extension or have only a minimal effect.

A similar 3'-mismatch strategy is used with greater effect to prevent ligation in the LCR. Any mismatch effectively blocks the action of the thermostable ligase, but LCR still has the drawback of target-independent background ligation products initiating the amplification. Moreover, the combination of PCR with subsequent LCR to identify the nucleotides at individual positions is also a clearly cumbersome proposition for the clinical laboratory.

The direct detection method according to various preferred embodiments of the present invention may be, for example a cycling probe reaction (CPR) or a branched DNA analysis.

When a sufficient amount of a nucleic acid to be detected is available, there are advantages to detecting that sequence directly, instead of making more copies of that target, (e.g., as in PCR and LCR). Most notably, a method that does not amplify the signal exponentially is more amenable to quantitative analysis. Even if the signal is enhanced by attaching multiple dyes to a single oligonucleotide, the correlation between the final signal intensity and amount of target is direct. Such a system has an

additional advantage that the products of the reaction will not themselves promote further reaction, so contamination of lab surfaces by the products is not as much of a concern. Recently devised techniques have sought to eliminate the use of radioactivity and/or improve the sensitivity in automatable formats. Two examples are the "Cycling Probe Reaction" (CPR), and "Branched DNA" (bDNA).

5 Cycling probe reaction (CPR): The cycling probe reaction (CPR), uses a long chimeric oligonucleotide in which a central portion is made of RNA while the two termini are made of DNA. Hybridization of the probe to a target DNA and exposure to a thermostable RNase H causes the RNA portion to be digested. This destabilizes the remaining DNA portions of the duplex, releasing the remainder of the probe from the target DNA and allowing another probe molecule to repeat the process.
10 The signal, in the form of cleaved probe molecules, accumulates at a linear rate. While the repeating process increases the signal, the RNA portion of the oligonucleotide is vulnerable to RNases that may be carried through sample preparation.

 Branched DNA: Branched DNA (bDNA), involves oligonucleotides with branched structures that allow each individual oligonucleotide to carry 35 to 40 labels (e.g., alkaline phosphatase enzymes).
15 While this enhances the signal from a hybridization event, signal from non-specific binding is similarly increased.

 The detection of at least one sequence change according to various preferred embodiments of the present invention may be accomplished by, for example restriction fragment length polymorphism (RFLP analysis), allele specific oligonucleotide (ASO) analysis, Denaturing/Temperature Gradient Gel
20 Electrophoresis (DGGE/TGGE), Single-Strand Conformation Polymorphism (SSCP) analysis or Dideoxy fingerprinting (ddF).

 The demand for tests which allow the detection of specific nucleic acid sequences and sequence changes is growing rapidly in clinical diagnostics. As nucleic acid sequence data for genes from humans and pathogenic organisms accumulates, the demand for fast, cost-effective, and easy-to-use tests for as
25 yet mutations within specific sequences is rapidly increasing.

 A handful of methods have been devised to scan nucleic acid segments for mutations. One option is to determine the entire gene sequence of each test sample (e.g., a bacterial isolate). For sequences under approximately 600 nucleotides, this may be accomplished using amplified material (e.g., PCR reaction products). This avoids the time and expense associated with cloning the segment of
30 interest. However, specialized equipment and highly trained personnel are required, and the method is too labor-intensive and expensive to be practical and effective in the clinical setting.

 In view of the difficulties associated with sequencing, a given segment of nucleic acid may be characterized on several other levels. At the lowest resolution, the size of the molecule can be determined by electrophoresis by comparison to a known standard run on the same gel. A more detailed picture of
35 the molecule may be achieved by cleavage with combinations of restriction enzymes prior to electrophoresis, to allow construction of an ordered map. The presence of specific sequences within the fragment can be detected by hybridization of a labeled probe, or the precise nucleotide sequence can be determined by partial chemical degradation or by primer extension in the presence of chain-terminating

nucleotide analogs.

Restriction fragment length polymorphism (RFLP): For detection of single-base differences between like sequences, the requirements of the analysis are often at the highest level of resolution. For cases in which the position of the nucleotide in question is known in advance, several methods have been developed for examining single base changes without direct sequencing. For example, if a mutation of interest happens to fall within a restriction recognition sequence, a change in the pattern of digestion can be used as a diagnostic tool (e.g., restriction fragment length polymorphism [RFLP] analysis).

Single point mutations have been also detected by the creation or destruction of RFLPs. Mutations are detected and localized by the presence and size of the RNA fragments generated by cleavage at the mismatches. Single nucleotide mismatches in DNA heteroduplexes are also recognized and cleaved by some chemicals, providing an alternative strategy to detect single base substitutions, generically named the "Mismatch Chemical Cleavage" (MCC). However, this method requires the use of osmium tetroxide and piperidine, two highly noxious chemicals which are not suited for use in a clinical laboratory.

RFLP analysis suffers from low sensitivity and requires a large amount of sample. When RFLP analysis is used for the detection of point mutations, it is, by its nature, limited to the detection of only those single base changes which fall within a restriction sequence of a known restriction endonuclease. Moreover, the majority of the available enzymes has 4 to 6 base-pair recognition sequences, and cleave too frequently for many large-scale DNA manipulations. Thus, it is applicable only in a small fraction of cases, as most mutations do not fall within such sites.

A handful of rare-cutting restriction enzymes with 8 base-pair specificities have been isolated and these are widely used in genetic mapping, but these enzymes are few in number, are limited to the recognition of G+C-rich sequences, and cleave at sites that tend to be highly clustered. Recently, endonucleases encoded by group I introns have been discovered that might have greater than 12 base-pair specificity, but again, these are few in number.

Allele specific oligonucleotide (ASO): If the change is not in a recognition sequence, then allele-specific oligonucleotides (ASOs), can be designed to hybridize in proximity to the mutated nucleotide, such that a primer extension or ligation event can be used as the indicator of a match or a mismatch. Hybridization with radioactively labeled allelic specific oligonucleotides (ASO) also has been applied to the detection of specific point mutations. The method is based on the differences in the melting temperature of short DNA fragments differing by a single nucleotide. Stringent hybridization and washing conditions can differentiate between mutant and wild-type alleles. The ASO approach applied to PCR products also has been extensively utilized by various researchers to detect and characterize point mutations in ras genes and gsp/gip oncogenes. Because of the presence of various nucleotide changes in multiple positions, the ASO method requires the use of many oligonucleotides to cover all possible oncogenic mutations.

With either of the techniques described above (i.e., RFLP and ASO), the precise location of the suspected mutation must be known in advance of the test. That is to say, they are inapplicable when one

needs to detect the presence of a mutation within a gene or sequence of interest.

Denaturing/Temperature Gradient Gel Electrophoresis (DGGE/TGGE): Two other methods rely on detecting changes in electrophoretic mobility in response to minor sequence changes. One of these methods, termed "Denaturing Gradient Gel Electrophoresis" (DGGE) is based on the observation that slightly different sequences will display different patterns of local melting when electrophoretically resolved on a gradient gel. In this manner, variants can be distinguished, as differences in melting properties of homoduplexes versus heteroduplexes differing in a single nucleotide can detect the presence of mutations in the target sequences because of the corresponding changes in their electrophoretic mobilities. The fragments to be analyzed, usually PCR products, are "clamped" at one end by a long stretch of G-C base pairs (30-80) to allow complete denaturation of the sequence of interest without complete dissociation of the strands. The attachment of a GC "clamp" to the DNA fragments increases the fraction of mutations that can be recognized by DGGE. Attaching a GC clamp to one primer is critical to ensure that the amplified sequence has a low dissociation temperature. Modifications of the technique have been developed, using temperature gradients, and the method can be also applied to RNA:RNA duplexes.

Limitations on the utility of DGGE include the requirement that the denaturing conditions must be optimized for each type of DNA to be tested. Furthermore, the method requires specialized equipment to prepare the gels and maintain the needed high temperatures during electrophoresis. The expense associated with the synthesis of the clamping tail on one oligonucleotide for each sequence to be tested is also a major consideration. In addition, long running times are required for DGGE. The long running time of DGGE was shortened in a modification of DGGE called constant denaturant gel electrophoresis (CDGE). CDGE requires that gels be performed under different denaturant conditions in order to reach high efficiency for the detection of mutations.

A technique analogous to DGGE, termed temperature gradient gel electrophoresis (TGGE), uses a thermal gradient rather than a chemical denaturant gradient. TGGE requires the use of specialized equipment which can generate a temperature gradient perpendicularly oriented relative to the electrical field. TGGE can detect mutations in relatively small fragments of DNA therefore scanning of large gene segments requires the use of multiple PCR products prior to running the gel.

Single-Strand Conformation Polymorphism (SSCP): Another common method, called "Single-Strand Conformation Polymorphism" (SSCP) was developed by Hayashi, Sekya and colleagues and is based on the observation that single strands of nucleic acid can take on characteristic conformations in non-denaturing conditions, and these conformations influence electrophoretic mobility. The complementary strands assume sufficiently different structures that one strand may be resolved from the other. Changes in sequences within the fragment will also change the conformation, consequently altering the mobility and allowing this to be used as an assay for sequence variations.

The SSCP process involves denaturing a DNA segment (e.g., a PCR product) that is labeled on both strands, followed by slow electrophoretic separation on a non-denaturing polyacrylamide gel, so that intra-molecular interactions can form and not be disturbed during the run. This technique is extremely

sensitive to variations in gel composition and temperature. A serious limitation of this method is the relative difficulty encountered in comparing data generated in different laboratories, under apparently similar conditions.

Dideoxy fingerprinting (ddF): The dideoxy fingerprinting (ddF) is another technique developed to scan genes for the presence of mutations. The ddF technique combines components of Sanger dideoxy sequencing with SSCP. A dideoxy sequencing reaction is performed using one dideoxy terminator and then the reaction products are electrophoresed on nondenaturing polyacrylamide gels to detect alterations in mobility of the termination segments as in SSCP analysis. While ddF is an improvement over SSCP in terms of increased sensitivity, ddF requires the use of expensive dideoxynucleotides and this technique is still limited to the analysis of fragments of the size suitable for SSCP (i.e., fragments of 200-300 bases for optimal detection of mutations).

In addition to the above limitations, all of these methods are limited as to the size of the nucleic acid fragment that can be analyzed. For the direct sequencing approach, sequences of greater than 600 base pairs require cloning, with the consequent delays and expense of either deletion sub-cloning or primer walking, in order to cover the entire fragment. SSCP and DGGE have even more severe size limitations. Because of reduced sensitivity to sequence changes, these methods are not considered suitable for larger fragments. Although SSCP is reportedly able to detect 90 % of single-base substitutions within a 200 base-pair fragment, the detection drops to less than 50 % for 400 base pair fragments. Similarly, the sensitivity of DGGE decreases as the length of the fragment reaches 500 base-pairs. The ddF technique, as a combination of direct sequencing and SSCP, is also limited by the relatively small size of the DNA that can be screened.

According to a presently preferred embodiment of the present invention the step of searching for any of the nucleic acid sequences described here, in cells derived from a cancer patient is effected by any suitable technique, including, but not limited to, nucleic acid sequencing, polymerase chain reaction, ligase chain reaction, self-sustained synthetic reaction, Q β -Replicase, cycling probe reaction, branched DNA, restriction fragment length polymorphism analysis, mismatch chemical cleavage, heteroduplex analysis, allele-specific oligonucleotides, denaturing gradient gel electrophoresis, constant denaturant gel electrophoresis, temperature gradient gel electrophoresis and dideoxy fingerprinting.

Detection may also optionally be performed with a chip or other such device. The nucleic acid sample which includes the candidate region to be analyzed is preferably isolated, amplified and labeled with a reporter group. This reporter group can be a fluorescent group such as phycoerythrin. The labeled nucleic acid is then incubated with the probes immobilized on the chip using a fluidics station.

Once the reaction is completed, the chip is inserted into a scanner and patterns of hybridization are detected. The hybridization data is collected, as a signal emitted from the reporter groups already incorporated into the nucleic acid, which is now bound to the probes attached to the chip. Since the sequence and position of each probe immobilized on the chip is known, the identity of the nucleic acid hybridized to a given probe can be determined.

It will be appreciated that when utilized along with automated equipment, the above described

detection methods can be used to screen multiple samples for a disease and/or pathological condition both rapidly and easily.

Immunoassays

5 Assays to monitor the expression of a marker or markers as defined in Table 12 may utilize any available means of monitoring for changes in the expression level of the polypeptides and/or proteins of the present invention. As used herein, an agent is said to modulate the expression of an amino acid of the invention if it is capable of up-or down-regulating expression of the amino acid in a cell.

10 The genes identified as being differentially expressed upon exposure to a known renal toxin (Tables 12-18, 26-27 and Table 21) may be used in a variety of amino acid detection assays to detect or quantify the level of a polypeptide and/or protein or multiple polypeptides and/or proteins in a given sample.

Any assay format to detect polypeptide and/or protein levels may be used. For example, immunoassay, such as ELISA, an RIA, a slot blot, immunohistochemical assay, FACS, a radio-imaging assay or a Western blot, or other receptor based assays for polypeptides or proteins.

15 "Immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," or "specifically interacts or binds" when referring to a protein or peptide (or other epitope), refers, in some embodiments, to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times greater than the background (non-specific signal) and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to seminal basic protein from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with seminal basic protein and not with other proteins, except for polymorphic variants and alleles of seminal basic protein. This selection may be achieved by subtracting out antibodies that cross-react with seminal basic protein molecules from other species. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

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Exemplary detectable labels, optionally and preferably for use with immunoassays, include but are not limited to magnetic beads, fluorescent dyes, radiolabels, enzymes (e.g., horse radish peroxidase,

alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic beads. Alternatively, the marker in the sample can be detected using an indirect assay, wherein, for example, a second, labeled antibody is used to detect bound marker-specific antibody, and/or in a competition or inhibition assay wherein, for example, a monoclonal antibody which binds to a distinct epitope of the marker are incubated simultaneously with the mixture.

Kits

The invention further includes kits combining, in different combinations, high density oligonucleotide arrays, reagents for use with the arrays, protein encoded by the genes of Table 12 and homologous gene thereto, signal detection and array-processing instruments, gene expression databases and analysis and database management software described above.

The kits may be used, for example, to predict or model the toxic response of a test compound, to monitor the progression of renal disease states, to identify genes that show promise as new drug targets and to screen known and newly designed drugs as discussed above.

The kits may be used in the pharmaceutical industry, where the need for receiving toxicity and other indications relating to a drug as early as possible is strong due to the high costs associated with drug development, but where bioinformatics, in particular gene expression informatics, is still lacking. These kits will reduce the costs, time and risks associated with traditional new drug screening using cell cultures and laboratory animals. The results of large-scale drug screening of pre-grouped patient populations, pharmacogenomics testing, can also be applied to select drugs with greater efficacy and fewer side-effects. The kits may also be used by smaller biotechnology companies and research institutes who do not have the facilities for performing such large-scale testing themselves.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting.

The general means and methods of the invention as described above are exemplified below. The following examples are offered as illustrations of specific embodiments and are not intended to limit the inventions disclosed throughout the whole of the specification.

EXAMPLES

Example 1: Identification of Toxicity Markers

Experimental Design

The renal toxins Gentamycin (antibiotic), Cisplatin (chemotherapeutic agent), Tobramycin (antibiotic), Cadmium chloride (heavy metal in the following industries: plastic, electroplating, batteries, paints, smelter), Doxorubicin (chemotherapeutic antibiotic) and Valproic acid (antiepileptic agent that

was used as a negative control), were administered to male and female Sprague-Dawley rats at various time points and routes of administration as describes below. The Sprague-Dawley Crl:CD(SD) rats were selected for the current investigation, since this strain is frequently used in preclinical investigations including investigations of microarray expression profiling during preclinical drug development.

- 5 The test items (positive and negative controls) were administered to animals in accordance with the usual route of administration in humans (i.e. IP for gentamycin, cisplatin, tobramycin and cadmium; IV for doxorubicin; and oral for valproic acid). Control animals were treated intraperitoneal with saline.

- 10 Each substance was administered to the animals once or daily for 5 days or 28 days at the appropriate dose level. The test substances and dose levels were selected according to the predicted pathological effects of each compound: the selected dose of each compound was expected to cause no histological changes after single dose administration, but to induce significant kidney toxicity after 28 days of daily treatment.

Dose formulation:

- 15 Gentamycin: injection (80 mg/mL)
Cisplatin: injection (10 mg/20mL)
Tobramycin (Brulamycin): injection (40 mg/1mL and 80 mg/2mL)
Cadmium chloride: a stock solution was prepared according to the valid SOP of IDRI (VIG/010 "Preparation of injection").
Doxorubicin: injection (20 mg/10mL),
20 Valproic acid – negative control. A suspension in concentration of 100 mg/mL for oral use.

Randomization and group allocation: prior to treatment, the animals were assigned to the experimental groups based on their body weight. Mean body weight of each group at randomization was not deviate $\pm 20\%$ of the mean population weight. Males and females were randomized separately. For the randomization, a computer program was used.

- 25 The original study groups and doses are shown in Table 1 below. Allocation to the dose group, 3 animals in each group, is shown in Table 2.

Table 1: Original study groups and doses

Groups	Test items	Dose (mg/kg)	Dose Volume (ml/kg)	Conc. of stock (mg/ml)	Route of administration	No. of animals	
						Males	Females
1MF	Control/Saline	-	5	-	IP	9	9
2MF	Gentamycin	40	2	20	IP	9	9
3MF	Cisplatin	0.5	2	0.25	IP	9	9
4MF	Tobramycin	40	2	20	IP	9	9
5MF	Cadmium chloride	2	2	1	IP	9	9
6MF	Doxorubicin	4	2	2	IV	9	9
7MF	Valproic acid	500	5	100	PO	9	9

IV – intravenous, IP – intraperitoneal, PO – per oral

Table 2: Allocation to the dose group, 3 animals in each group

Groups	Duration of treatment (days)
1MF	1, 5, 28
2MF	1, 5, 28
3MF	1, 5, 28
4MF	1, 5, 28
5MF	1, 5, 28
6MF	1, 5, 28
7MF	1, 5, 28

Results

The animals were treated with 6 different test items each for 3 duration of treatment (1, 5 and 28 days). The actual study design is shown in Table 3.

Table 3: Study design

Groups	Test items	Dose (mg/kg)	Dose Volume (ml/kg)	Conc. of stock (mg/ml)	Route of adminis- tration	No. of animals	
						Males	Females
1MF	Control/Saline	-	2	-	IP	9	9
2MF	Gentamycin	40	2	20	IP	9	9
3MF	Cisplatin	0.5	2	0.25	IP	9	9
4MF	Tobramycin	40	2	20	IP	9	9
5MF	Cadmium cl.*	2	2	1	IP	9	9
		0.75*		0.375			
6MF	Doxorubicin**	4	2	2	IV	9	9
		1**		0.5			
7MF	Valproic acid	500	5	100	PO	9	9
8MF***	-	-	-	-	-	3	3

IV – intravenous, IP – intraperitoneal, PO –oral (per os)

Modifications to the original study design:

10 *Animals treated with Cadmium chloride did not tolerate the treatment well, for that reason the dose was modified from 2 mg/kg to 0.75 mg/kg. Since males were in poor condition and excluded from the study, control (naïve) animals were introduced to the 5M dose group and treated with the lower dose for 24 days.

**From day 7 onwards, a sudden decay was observed in the condition of males and females treated with 4 mg/kg Doxorubicin (6MF group). This decay affected the 28-days study group of 6MF. These animals died or became moribund; therefore, naive animals were introduced into this group. The dose of the Doxorubicin treated group was lowered from 4 mg/kg to 1 mg/kg. However, males from Day 19-20 and females from Day 22 received 0.5 mg/kg dose of Doxorubicin, due to their decrease in body weight.

*** 8MF control groups were introduced in the study, including 3 males and 3 females. Animals from these groups did not receive any treatment; however samples (tissue, blood, urine) were taken from them.

During the treatment period the animals were observed daily for clinical symptoms, their body weight was measured weekly and the water consumption was measured daily.

The day before autopsy, urinalysis was performed and blood samples were taken for hematology and clinical chemistry examinations.

On the day of autopsy the animals were anaesthetized with isoflurane, blood was collected for PBMC isolation then subjected to autopsy. Tissues and organs (adrenals, brain, kidneys, liver, spleen and thymus) were weighed and subjected to gross pathological and histopathological examinations.

Sterile kidney and liver samples were taken from the animals, frozen in liquid nitrogen and stored at -80°C. PBMC (Peripheral Blood Mononuclear Cell) and urine samples were stored at -80°C as well. RNA was extracted from Kidney samples for toxicogenomic analysis for prediction of nephrotoxicity.

Finding summary:

1. Toxicants effects

28-DAY

Gentamycin

Signs of nephrotoxicity occurred in all animals of both sexes. During autopsy enlarged and discolored kidneys were observed in males. Kidney weight increased in both sexes. These changes were accompanied by clinical pathological findings: higher creatinine values in males and granular cylinders in the urine of females. The test item affected body weight gain of animals in both sexes. The nephrotoxic effect of the test item was confirmed by histopathology.

Cisplatin

The most severe nephrotoxic changes were found in Cisplatin treated groups of both sexes. Enlarged and discolored kidneys, increased kidneys and decreased thymus weight were found in both sexes. The nephrotoxic effect was detected during clinical pathology: Higher values of creatinine and urea nitrogen in both sexes & mild changes in the urine sediment. The test item affected body weight gain and water consumption in both sexes.

Tobramycin

Enlarged, discolored kidneys, with increased weights were observed in males. Clinical pathology revealed a nephrotoxic effect and higher values of creatinine and urea nitrogen in the blood as well as granular cylinders in the urine sediment of both sexes. Histopathological examinations confirmed nephrotoxic changes in both sexes.

Cadmium chloride

Mild clinical symptoms (hollow psoas, colic) were recorded in both sexes immediately after the administration. The test item affected body weight gain in both sexes. Slightly lower water consumption was recorded in males. Round bordered spleen and liver and thickening of peritoneum were recorded in both sexes, which correspond with higher liver and spleen weight. In addition increased weight of kidneys and decreased thymus were recorded in females. AST (Aspartate Aminotransferase) values were elevated in both sexes. A few transitional epithelial cells were observed in the urine of females. During histopathological examination significant nephrotoxicity was found only in a single female.

Doxorubicin

The test item caused severe clinical symptoms and lethality when administered in a 4 mg/kg dose. Two out of 3 males and 1 out of 3 females died. The lowered dose of 1 mg/kg of the test item was not lethal, but affected body weight gain in both sexes and water consumption in males. An increased weight of kidneys was found in males. The liver weight increased, while the weight of the thymus and spleen decreased significantly in both sexes. Elevated values of AST, ALT and Gamma GT were recorded in the male and decreased values of total protein, Albumin and Globulin were found in both sexes. Mild to moderate lymphoid depletion of the thymus was observed in both sexes.

Valproic acid

No histopathological signs of nephrotoxicity were found. The only toxic effect was a decreased total protein and Globulin value found in males.

Control/Saline

No histopathological signs of nephrotoxicity were found

5-DAY

No lethality was recorded in any groups of both sexes.

Gentamycin

No nephrotoxicity was observed, except elevated urea nitrogen levels found in females. Body weight gain was decreased in females. No renal damage was found at histopathological examination.

Cisplatin

Mild, focal tubular epithelial cell necrosis in the medulla of kidneys was noted in a single male. No remarkable observations were found at autopsy. Decreased body weight gain and water consumption were recorded in both sexes.

Tobramycin

No renal damage was found during histopathology. Elevated urea nitrogen levels were found in females. Cadmium chloride: No renal damage was found. The test item caused mild clinical symptoms (hollow psoas, colic) immediately after the administration. Body weight gain decreased in both sexes.

5 Slightly increased Gamma GT values were recorded in females.

Doxorubicin

No renal damage was found. Decreased body weight gain and water consumption was recorded in both sexes. Minimal to mild thymus atrophy and minimal lymphoid depletion of the spleen were found in males which was in accordance with gross pathological findings. RBC, HBG, HCT, reticulocyte and

10 WBC counts were lower in both sexes.

Valproic acid

No renal damage was found. Slightly lower total protein and Albumin values were found in males.

Control/Saline

No histopathological signs of nephrotoxicity were found

15 **SINGLE DAY**

No renal damage was found.

Cisplatin

Decreased body weight gain was recorded in both sexes. Slightly lower lymphocytes and increased neutrophils counts were recorded in males.

20 **Cadmium chloride**

Mild clinical symptoms (hollow psoas, colic) were observed in both sexes immediately after the administration. Decreased body weight gain was recorded in both sexes. Lower lymphocytes and increased neutrophils counts were found in males.

Doxorubicin

25 Decreased body weight gain was recorded in both sexes. Lower relative weights of spleen were recorded in females. Higher WBC and neutrophil counts, decreasing tendency of total protein, Albumin and Globulin were found in males.

Control/Saline

No histopathological signs of nephrotoxicity were found

30 **2. Histopathology**

At histopathological examination after **28-day treatment**, nephrotoxicity occurred in the following decreasing order: Cisplatin, Gentamycin, Tobramycin and Doxorubicin in males; Cisplatin, Cadmium chloride, Gentamycin, Doxorubicin and Tobramycin in females. Males were more sensitive to

most of the substances.

Valproic acid proved to be non-nephrotoxic.

No renal damage was found after **5-day treatment** in all dosed groups of both sexes, compared to the controls, except a single male treated with Cisplatin, where mild, focal tubular epithelial cell necrosis in the medulla was found.

No test item related renal damage was found after **single treatment** in all dosed groups of both sexes, compared to the controls.

Table 4 presents the summarized findings recorded after the 28-day treatment period in the different dosed groups of both sexes.

10 Table 4: Findings after the 28-day treatment period

Compound	Dose (mg/kg)	Findings			
		Autopsy	Organ weight	Clinical pathology	Histopathology
Gentamycin	40	♂♀: ↑↑, pale kidneys	♂♀: ↑↑ kidneys	♂: ↑CREA ♀: granular cylinders in urine	<u>Kidneys</u> ♂♀: lymphocytic inflammation, tubular basophilia in the cortex. ♂: tubular dilatation in the medulla
Cisplatin	0.5	♂♀: ↑↑, pale kidneys	♂♀: ↑↑ kidneys	♂♀: ↑↑BUN, CREA ♂: trans. epith. cells in urine ♀ (1/3): oval lipid cell in urine	<u>Kidneys</u> ♂♀: lymphocytic inflammation, tubular basophilia in the cortex. Tubular dilatation, bizarre forms in the medulla.
Tobramycin	40	♂: ↑↑, pale kidneys	♂: ↑↑ kidneys	♂♀: ↑↑BUN, CREA ♂♀: granular cylinders in urine ♂: trans. epith. cells in urine	<u>Kidneys</u> : ♂♀: lymphocytic inflammation, tubular basophilia in the cortex. ♂: tubular dilatation and basophilia in the medulla.
Cadmium chloride	0.75	♂♀: ↑↑ liver, spleen, thickening of peritoneum	♂♀: ↑↑ liver, spleen	♂♀: ↑↑ AST ♀: trans. epith. cells in urine	<u>Spleen</u> : ♂: lymphoid depletion
Doxorubicin	1	♂♀: ↑↑ thymus	♂♀: ↑↑ thymus	♂: ↑↑AST, ALT, γGT ♂♀: ↓↓TP, Alb., Glob.	<u>Kidneys</u> : ♂: tubular dilatation and basophilia in the cortex. Tubular dilatation in the medulla. <u>Thymus</u> : ♂♀: lymphoid depletion

CREA – creatinine, BUN - Blood urea nitrogen, AST - aspartate aminotransferase, ALT - alanine aminotransferase, Alb – Albumin

Table 5 presents the summarized findings recorded after the 5-day treatment period in the different dosed groups.

5 Table 5: Findings after the 5-day treatment period

Compound	Dose (mg/kg)	Findings			
		Autopsy	Organ weight	Clinical pathology	Histopathology
Gentamycin	40	No effect	No effect	♀:↑↑BUN	No renal damage
Cisplatin	0.5	No effect	No effect	♂ (No.24): ↑↑PLT, ↓↓reticulocytes, ↑BUN and CREA	♂ (No.24): tubular necrosis in the medulla
Tobramycin	40	No effect	No effect	♀:↑↑BUN	No renal damage
Cadmium chloride	2.	No effect	♂♀: ↓thymus	♀:↑ γGT	No renal damage
Doxorubicin	4	♂♀: ↓↓thymus and spleen	♂♀: ↓↓thymus and spleen	♂♀: ↓↓RBC, HGB, HCT, reticulocytes, WBC	No renal damage. ♂: <u>Thymus</u> : atrophy <u>Spleen</u> : lymphoid depletion

No test item related renal damage was found after single treatment in all dosed groups of both sexes, compared to the controls.

3. Lethality

10 **28-DAYS TREATMENT**

Death occurred only in Doxorubicin treated groups with the originally planned dose. No lethality was recorded after lowering the dose from 4 mg/kg to 1 mg/kg.

Table 6: Lethality in Doxorubicin treated groups

	Died	Moribund
Males	2/3 (Day 8)	1/3 (Day 8)
Females	1/3 (Day 9)	2/3 (Day 9)

15 **SINGLE AND 5-DAYS TREATMENT**

No lethality was recorded in any groups of both sexes.

4. Clinical Symptoms

28-DAYS TREATMENT

Clinical symptoms were observed only in Cadmium chloride groups and in the 4 mg/kg (originally planned dose) Doxorubicin treated groups of both sexes.

5 **SINGLE AND 5-DAY TREATMENT**

Clinical symptoms were observed only in Cadmium chloride groups of both sexes. No clinical symptoms were observed in any other groups of both sexes.

5. Body weight and body gain

28-DAY TREATMENT

10 Effect on body weight and body weight gain was seen in groups of both sexes treated with Doxorubicin, Cadmium chloride, Cisplatin and Gentamycin.

5-DAYS TREATMENT

Decreased body weight gain was recorded in males treated with Cisplatin, Cadmium chloride, Doxorubicin, Valproic acid and in all dosed groups of females.

15 6. Water Consumption

Compared with controls the following observations were made concerning water consumption:

28-DAY TREATMENT

Males

20 Slight decrease during the last week in groups dosed with Cisplatin, Cadmium chloride and Valproic acid; Significant decrease from Week 2 in the Doxorubicin treated group.

Females

Decreased during Week 4 in Cisplatin treated group.

5-DAY TREATMENT

Decrease in Cisplatin and Doxorubicin treated groups of both sexes.

25 Decrease in Tobramycin and Cadmium chloride treated females

Increase in Valproic acid treated females

6. Autopsy

28-DAY TREATMENT

30 At necropsy of animals treated with 4 mg/kg Doxorubicin who died (male No.: 52, 54 and female No.: 154) dehydration and anaemic organs were observed.

Scheduled autopsy

Alterations were found in the kidneys in both sexes treated with Cisplatin and in males treated with Gentamycin and Tobramycin. Pale and enlarged kidneys were found in a single female (No.145) treated with Cadmium chloride. Smaller thymuses were found in Doxorubicin treated groups of both sexes. Round bordered liver, enlarged spleen and thickening of the peritoneum were found in both sexes treated with Cadmium chloride.

5-DAY TREATMENT

No alterations were found in the kidneys in the dosed groups of both sexes. No remarkable observations were found at autopsy in females. Smaller thymus and spleen were recorded in the Doxorubicin treated group of males.

SINGLE TREATMENT

No alterations were found in the kidneys in the dosed groups of both sexes. No remarkable observations were found in females. Hemorrhagic thymus were found with different incidence in the dosed groups in males, including controls.

15 7. Organ Weights

28-DAY TREATMENT

Compared with controls the following observations were made concerning the relative organ weights, taking into consideration the small number of groups (3/sex/group):

Increased liver weights in groups of both sexes treated with Cadmium chloride and Doxorubicin; Increased weights of kidneys in males treated with Gentamycin, Cisplatin, Tobramycin and Doxorubicin; Higher weights of kidneys in females treated with Gentamycin, Cisplatin and Cadmium chloride; Weights of spleen were higher in Gentamycin treated males and Cadmium chloride treated groups of both sexes. Lower weights were recorded in Doxorubicin treated groups of both sexes; Decreased thymus weights were found in Gentamycin and Tobramycin treated males, in Cisplatin and Doxorubicin treated groups of both sexes and in females treated with Cadmium chloride.

5-DAY TREATMENT

No effect was observed in kidneys of the dosed groups of both sexes.

Compared with controls the following observations were made concerning the relative organ weights, taking into consideration the small number of groups (3/sex/group): Lower weights of spleen in Doxorubicin treated groups of both sexes; Lower thymus weights in Doxorubicin, Cadmium chloride and Valproic acid treated groups of both sexes.

SINGLE TREATMENT

No effect was observed in kidneys of the dosed groups of both sexes. No significant alterations were recorded in males.

Compared with controls the following tendencies were observed in females concerning the relative organ weights, taking into consideration the small number of groups (3/sex/group): Lower weights of liver in Cisplatin treated group; Lower weights of thymus in Gentamycin, Cisplatin and Valproic acid treated groups; Lower weights of spleen in Cisplatin and Doxorubicin treated groups.

5 **Expression analysis**

RNA isolation from kidney tissues

RNA was isolated from the kidney tissues using the following procedure:

Step 1: Homogenization of Tissue: Add 200 mg of tissue sample to 7ml of cold TRI Reagent (MRC) on ice. Homogenize tissue using several short pulses (5 sec.) of the homogenizer.

10 Step 2: Phase separation: Add 0.2ml of BCP (1-BROMO-3-CHLORO-PROPANE, SIGMA, Cat. No. B-9673) per 1 ml TRI Reagent. Cover the sample tightly and mix vigorously for 15 sec. Incubate the sample at RT 2-3min. Spin the sample at 12000g for 20min at 4°C.

15 Step 3: RNA precipitation: Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. Remove the top aqueous layer to a fresh tube. Add 0.5ml Isopropanol per 1ml TRI Reagent used in the original homogenization step. Mix the tube by vortex and incubate the sample at RT for 7-8min. Spin the sample at 12000g for 15min at 4°C.

20 Step 4: RNA wash: Remove the sup and add a volume of 80% ethanol (made with DEPC-treated water, stored at -20°C) equal to the original volume of TRI Reagent to rinse the pellet. Centrifuge the sample at 12000g for 5min at 4°C. Remove the ethanol and resuspend the RNA pellet in 400ul DEPC water.

25 Step 5: RNA purification using phenol-chlorophorm extraction and MaXtract low Density tubes: Equilibrate the phenol:chlorophorm:isoamyl alcohol mix (25:24:1) (Ambion, Cat. No. 9732) to room temperature. Pellet the MaXtract tubes (QIAGEN, Cat. No. 129016) prior to use by centrifugation 1 minute at maximum speed. To the RNA dissolved add an equal volume (400ul) of phenol:chlorophorm:isoamyl (25:24:1) alcohol mix and vortex. Transfer the entire Total RNA-phenol/chlorophorm mix to MaXtract tube. Centrifuge at 13000rpm for 2 min. Transfer the aqueous upper phase to a fresh non-stick 1.5 ml tube (Ambion, Cat. No. AM12450). Pay attention not to touch the gel with the pipet tip as it reduces the grade of RNA purity. Repeat steps 5.3-5.5.

30 Step 6: RNA precipitation II: Precipitate the RNA by adding 0.1 vol 3M sodium acetate (RNAse free, Sigma Cat. No. S-7899) and 0.8 volume isopropanol (RNA vol.+sodium acetate vol.) For 400 µl RNA: 40 µl Sodium acetate and 352 µl isopropanol. Vortex 5 sec. Store at -20°C over night. Fast cool the centrifuge. Spin the samples at full speed (14000 RPM) for 20 min at 4°C pellet would be at the bottom.

35 Step 7: RNA wash II and resuspending: Pipette off the supernatant. Rinse twice the pellet with 0.5 ml 80% ethanol (made with DEPC-treated water , stored at -20 °C), centrifuge for 5 min at 9500 RPM, 4

°C. Pipette out the ethanol, then short spin the samples in Eppendorf centrifuge at full speed for 10 sec., and pipette out the remainder of ethanol. Air dry the pellet. Simultaneously, preheat the DEPC treated water to 55-70°C. Check the RNA for dryness. When dry, it is almost completely transparent. Add appropriate volume of DEPC treated water to the pellet (20-150 µl) depending on the size of the pellet –
5 do not mix! Heat the samples 10 min. at 55-70 °C. Mix well by pipetation.

Final RNA product was quantified by spectrophotometric quantification. The quality of the RNA was evaluated by measuring the 260/280 and 260/230 ratios and confirmed by agarose gel. RNA was deemed of a suitable quality for microarray analysis if the 260/280 ratio was between 1.8 and 2.1, 260/230 ratio was higher than 1.5 and the gel showed no visible degradation products lower than the 18S
10 ribosomal band.

Microarray Hybridization

RNA samples extracted from the 1-Day and 5-Days treated animals and were sent to hybridization on Affymetrix array GeneChip® Rat Genome 230 2.0. The hybridization was performed according to the Affymetrix' following protocol:

15 Step 1: Target Preparation: Using protocols in Affymetrix' manual Section 2, double-stranded cDNA is synthesized from total RNA (or purified poly-A messenger RNA) isolated from tissue or cells. An in vitro transcription (IVT) reaction is then done to produce biotin-labeled cRNA from the cDNA. The cRNA is fragmented before hybridization.

20 Step 2: Target Hybridization: A hybridization cocktail is prepared, including the fragmented target, probe array controls, BSA, and herring sperm DNA. It is then hybridized to the probe array during a 16-hour incubation. The hybridization process is describes in Affymetrix' manuals.

25 Step 3: Fluidics Station Setup: Specific experimental information is defined using Affymetrix® Microarray Suite or GeneChip Operating Software (GCOS) on a PC-compatible workstation. The probe array type, sample description, and comments are entered and saved with a unique experiment name. The fluidics station is then prepared for use by priming with the appropriate buffers.

Step 4: Probe Array Washing and Staining: Immediately following hybridization, the probe array undergoes an automated washing and staining protocol on the fluidics station.

30 Step 5: Probe Array Scan: Once the probe array has been hybridized, washed, and stained, it is scanned. Each workstation running Affymetrix Microarray Suite or GCOS can control one scanner. The software defines the probe cells and computes intensity for each cell. Each complete probe array image is stored in a separate data file identified by the experiment name and is saved with a data image file (.dat) extension.

Step 6: Data Analysis: The .dat image is analyzed for probe intensities; results are reported in tabular and graphical formats.

35 The expression levels were extracted using Affymetrix' MAS5 algorithm. Further multiplicative normalization on the data – setting the 95th percentile of the expression vector of each sample to an

arbitrary constant value (1200) – was then performed.

Classifier on Microarray Data

Finding signatures using machine learning algorithms requires two steps – selecting a limited set of features to be used for the signature, and building a classifier using these features. The feature selection stage is especially significant here for several reasons. Large number of features means a low signal to noise ratio, tampering the classifier performance, large number of features, compared to the relatively small number of samples, means that spurious features might exist. These are features that give good classification on the experiment data *by chance*, causing the classifier to be over-fitted to the learning set and have low prediction ability, and finally – a small number of features is important for practical application of the signature.

The initial features selection process is done by performing Mann-Whitney (Wilcoxon) rank-sum test on the data, considering only probesets whose overall normalized expression mean is over 25, to avoid working at noise-level.

Further features selection is done iteratively by building a Random Forest classifier, using the initial list of features, and estimating the importance of features as given by the algorithm's "Out of the Bag" approach. Less important features (those that have low impact on the performance of the classifier) are removed from the features list and the process is repeated.

The performance of the classifier built as just described, is estimated by calculating the LOR and Accuracy after performing a cross validation process – some of the samples are removed from the data. The whole process of features-selection and classifier building is performed on the data, and the prediction is tested on the left-out samples.

The following example illustrates the results of the process - working on the Day-5 samples of animals treated with toxic compounds, not including animals treated with Cadmium-Chloride (see pathological data, above) ("Toxic"), as compared to all controls samples (Saline and Valproic-acid treated animals at Day-1 and Day-5, as well as naïve rats) ("Control"). A list of 20 features (probesets) was initially selected, and then iteratively reduced to 6 features. The random forest classifier was used with the iterative list of features, as well as the gender of the rat used as an extra two-value feature. The random forest classifier was used with 2500 decision trees in each run. The cross validation was performed by leaving-out randomly selected samples – 3 control samples and 3 toxic samples. This was repeated 350 times. The bound for deciding whether a sample is predicted as toxic or control, was chosen as to maximize the Accuracy. The "Confusion matrix" for this optimal bound is given in Table 7.

Table 7: "Confusion matrix"

	Control	Toxic
Predicted as Control	965	85
Predicted as Toxic	151	899

The Accuracy of the signatures as evaluated by this process is 89%, and the LOR is 4.2

Selecting Genes for Validation

Following the analysis performed on the Day-5 data, 20 top-scoring probesets were selected for further validation using qRT-PCR. The probesets were mapped to the transcriptome produced by the LEADS clustering and assembly system (described in Sorek, R., Ast, G. & Graur, D. Alu-containing exons are alternatively spliced. Genome Res 12, 1060-7 (2002); US patent No: 6,625,545; and U.S. Patent Application No. 10/426,002, published as US20040101876 on May 27 2004; all of which are hereby incorporated by reference as if fully set forth herein. Briefly, the software cleans the expressed sequences from repeats, vectors and immunoglobulins. It then aligns the expressed sequences to the genome taking alternatively splicing into account and clusters overlapping expressed sequences into "clusters" that represent genes or partial genes) and the relevant contig and gene (whenever existing) were identified. As evident in table 8 below, the mapping of the probeset 1375422_at was not unique so we selected all relevant contigs.

Novel variants for the relevant genes were also identified. This was done based on the available rat ESTs and mRNAs and, when a human and mouse homologues was identified – the mouse ESTs and mRNAs and human transcripts were added the relevant rat ESTs and mRNAs to construct a combined informative contigs.

Table 8: Probe sets selected for qRT-PCR validation, with their LEADS gene name and internal candidate name

Probe set	Internal Name	Gene Name
1375422_at	BE097535_DB71_T0	BE097535
1390507_at	AI045075_DB71_T0	ISG20
1367764_at	H31883_DB71_T0	CCNG1
1369268_at	RATLRFI_DB71_T0	ATF3
1380692_at	BE104394_DB71_T0	BE104394
1388674_at	RNU24174_DB71_T0	CDKN1A
1371785_at	AA686189_DB71_T0	TNFRSF12A
1393728_at	AA964541_DB71_T0	AA964541
1369814_at	RNU90447_DB71_T0	CCL20
1397637_at	AI502869_DB71_T0	AI502869
1388560_at	H33998_DB71_T0	WDR77
1392102_at	AI454051_DB71_T0	AI454051
1370153_at	BI293562_DB71_T0	GDF15
1388140_at	RATRAB13X_DB71_T0	RAB13
1390833_at	AW919147_DB71_T0	AW919147

Probe set	Internal Name	Gene Name
1384934_at	BQ200887_DB71_T0	BQ200887
1375895_at	AI549068_DB71_T0	AI549068
1388642_at	H31799_DB71_T0	EI24
1383162_at	AA799594_DB71_T0	AA799594
1384070_at	H31045_DB71_T0	H31045

RT Preparation and Real-Time qRT-PCR Analysis

Before RT preparation the RNA was treated with DNA-free (Ambion, cat. No. AM1906) DNase treatment and removal - according to manufacturer protocol. DNase procedure was repeated until no
5 DNA is detected in a PCR reaction using the RNA samples.

RT preparation - Purified RNA (4 μ g) is mixed with 600ng Random Hexamer primers (Invitrogen, Cat. No. 48190-011) and 500 μ M dNTP (Takara, Cat. No. 4030) in a total volume of 62.4 μ l. The mixture is then incubated for 5 min at 65 °C and then quickly chilled on ice. Thereafter, 20 μ l of 5X SuperscriptII first strand buffer (Invitrogen, Cat. No. Y00146), 9.6 μ l 0.1M DTT (Invitrogen, Cat. No. Y00147) and 160
10 units RNasin (Promega, Cat. No. N251A) are added, and the mixture is incubated for 10 min at 25 °C, followed by further incubation at 42°C for 2 min. Then, 4 μ l (800units) of SuperscriptII (Invitrogen, Cat. No. 18064-022) is added and the reaction (final volume of 100 μ l) is incubated for 50 min at 42°C and then inactivated at 70°C for 15min. The resulting cDNA is diluted 1:20 in TE buffer (10 mM Tris pH=8, 1 mM EDTA pH=8).

cDNA (5 μ l), prepared as described above, is used as a template in Real-Time PCR reactions using the SYBR Green I assay (PE Applied Biosystem) with specific primers in 100nM concentration if not indicated otherwise and UNG Enzyme (Eurogentech or equal) Amplification is effected as follows: 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15sec, followed by 60°C for 1 min (if not indicated otherwise). Amplification step is followed by dissociation step. Detection is performed by using
20 the PE Applied Biosystem SDS 7000. The cycle in which the reactions achieved a threshold level (Ct) of fluorescence is registered and is used to calculate the relative transcript quantity in the RT reactions. Non-detected samples are assigned Ct value of 41 and are calculated accordingly. The relative quantity is calculated using the equation $Q = \text{efficiency}^{-Ct}$. The efficiency of the PCR reaction is calculated from a standard curve, created by using serial dilutions of several reverse transcription (RT) reactions. To
25 minimize inherent differences in the RT reaction, the resulting relative quantities are normalized to normalization factor calculated as follows:

The expression of four housekeeping (HSPK) genes from different pathways, ACTB (Entrez Gene ID: 81822), ACTG2 (Entrez Gene ID: 25365), HPRT (Entrez Gene ID: 24465) and YWHAZ (Entrez Gene ID: 25578), is found by SYBR green detection. The relative quantity (Q) of each housekeeping
30 gene in each sample is calculated as described above. In order to calculate the "relative Q relative to

MED” each Q for each HSKP gene is divided by the median quantity of this gene in all panel samples. To finally achieve the normalization factor of each sample the geometric mean of all four “relative Q relative to MED” was calculated. The normalization factor was then used for further calculations.

The sequences of the amplicons derived from the housekeeping genes measured in all the examples were as follows:

ACTB (Entrez Gene ID: 81822),

Forward Primer (SEQ ID NO: 237): GGGAAATCGTGCGTGACATT

Reverse Primer: (SEQ ID NO: 238): GCGGCAGTGGCCATCTC

Amplicon (SEQ ID NO: 239):

GGGAAATCGTGCGTGACATTAAAGAGAAGCTGTGCTATGTTGCCCTAGACTTCGAGC
AAGAGATGGCCACTGCCGC

ACTG2 (Entrez Gene ID: 25365),

Forward Primer (SEQ ID NO: 240): TACCCTATTGAGCACGGCAT

Reverse Primer (SEQ ID NO: 241): CGCAGCTCGTTGTAGAAGGT

Amplicon (SEQ ID NO: 242):

TACCCCATGAACACGGCATCATCACGAACTGGGATGACATGGAGAAGATCTGGCAC
CACTCCTTCTACAACGAGCTGCG

HPRT (Entrez Gene ID: 24465)

Forward Primer (SEQ ID NO: 243): GCGAAAGTGGAAAAGCCAAGT

Reverse Primer (SEQ ID NO: 244): GCCACATCAACAGGACTCTTGTA

Amplicon (SEQ ID NO: 245):

GCGAAAGTGGAAAAGCCAAGTACAAAGCCTAAAAGACAGCGGCAAGTTGAATCTAC
AAGAGTCCTGTTGATGTGGC

YWHAZ (Entrez Gene ID: 25578)

Forward Primer (SEQ ID NO: 246): CAAGCATACCAAGAAGCATTGTA

Reverse Primer (SEQ ID NO: 247): GGGCCAGACCCAGTCTGA

Amplicon (SEQ ID NO: 248):

CAAGCATACCAAGAAGCATTGAAATCAGCAAAAAGGAGATGCAGCCGACACACCCC
ATCAGACTGGGTCTGGCCC

The markers of the present invention were tested with regard to their expression in a panel of kidney tissues samples from treated and control rats. Unless otherwise noted, all experimental data relates to the novel polynucleotides and proteins of the present invention, named according to the segment being tested (as expression was tested through RT-PCR as described). A description of the tissue samples used

in the kidney testing panel is provided in Table 9 below. Tests were then performed as described in the "Materials and Experimental Procedures" section above.

The name comprises of the rat's group, as was described in table 2, the rats ID number, day of treatment and the nephrotoxicant it was exposed to:

- 5 Gent – Gentamycin, Cis – Cisplatin, Tob – Tobramycin, CadCl – Cadmium chloride, Dox – Doxorubicin, ValpA – Valproic Acid.

Table 9: Definitions of the tissue samples included in the panel based on their origin and treatment

#	Toxin used	Day		Name
1	Gentamycin	1	day 1 toxicants M	1_2M10_day1_Gent
2	Gentamycin	1		2_2M11_day1_Gent
3	Gentamycin	1		3_2M12_day1_Gent
4	Cisplatin	1		4_3M19_day1_Cis
5	Cisplatin	1		5_3M20_day1_Cis
6	Cisplatin	1		6_3M21_day1_Cis
7	Tobramycin	1		7_4M28_day1_Tob
8	Tobramycin	1		8_4M29_day1_Tob
9	Tobramycin	1		9_4M30_day1_Tob
10	Cadmium chloride	1		10_5M37_day1_CadCl
11	Cadmium chloride	1		11_5M38_day1_CadCl
12	Cadmium chloride	1		12_5M39_day1_CadCl
13	Doxorubicin	1		13_6M46_day1_Dox
14	Doxorubicin	1		14_6M47_day1_Dox
15	Doxorubicin	1		15_6M48_day1_Dox
16	Gentamycin	1	day 1 toxicants F	16_2F110_day1_Gent
17	Gentamycin	1		17_2F111_day1_Gent
18	Gentamycin	1		18_2F112_day1_Gent
19	Cisplatin	1		19_3F119_day1_Cis
20	Cisplatin	1		20_3F120_day1_Cis
21	Cisplatin	1		21_3F121_day1_Cis
22	Tobramycin	1		22_4F128_day1_Tob
23	Tobramycin	1		23_4F129_day1_Tob
24	Tobramycin	1		24_4F130_day1_Tob
25	Cadmium chloride	1		25_5F137_day1_CadCl
26	Cadmium chloride	1		26_5F138_day1_CadCl
27	Cadmium chloride	1		27_5F139_day1_CadCl
28	Doxorubicin	1		28_6F146_day1_Dox

#	Toxin used	Day		Name
29	Doxorubicin	1		29_6F147_day1_Dox
30	Doxorubicin	1		30_6F148_day1_Dox
31	Gentamycin	5	day 5 toxicants M	31_2M13_day5_Gent
32	Gentamycin	5		32_2M14_day5_Gent
33	Gentamycin	5		33_2M15_day5_Gent
34	Cisplatin	5		34_3M22_day5_Cis
35	Cisplatin	5		35_3M23_day5_Cis
36	Cisplatin	5		36_3M24_day5_Cis
37	Tobramycin	5		37_4M31_day5_Tob
38	Tobramycin	5		38_4M32_day5_Tob
39	Tobramycin	5		39_4M33_day5_Tob
40	Cadmium chloride	5		40_5M40_day5_CadCl
41	Cadmium chloride	5		41_5M41_day5_CadCl
42	Cadmium chloride	5		42_5M42_day5_CadCl
43	Doxorubicin	5		43_6M49_day5_Dox
44	Doxorubicin	5		44_6M50_day5_Dox
45	Gentamycin	5	day 5 toxicants F	45_2F113_day5_Gent
46	Gentamycin	5		46_2F114_day5_Gent
47	Gentamycin	5		47_2F115_day5_Gent
48	Cisplatin	5		48_3F122_day5_Cis
49	Cisplatin	5		49_3F123_day5_Cis
50	Cisplatin	5		50_3F124_day5_Cis
51	Tobramycin	5		51_4F131_day5_Tob
52	Tobramycin	5		52_4F132_day5_Tob
53	Tobramycin	5		53_4F133_day5_Tob
54	Cadmium chloride	5		54_5F141_day5_CadCl
55	Cadmium chloride	5		55_5F142_day5_CadCl
56	Doxorubicin	5		56_6F149_day5_Dox
57	Doxorubicin	5		57_6F150_day5_Dox
58	Doxorubicin	5		58_6F151_day5_Dox
59	Naïve	28	Controls All days	59_8M81_day28_Naïve
60	Naïve	28		60_8M82_day28_Naïve
61	Naïve	28		61_8M83_day28_Naïve
62	Naïve	28		62_8F181_day28_Naïve
63	Naïve	28		63_8F182_day28_Naïve
64	Naïve	28		64_8F183_day28_Naïve

#	Toxin used	Day		Name
65	Control/Saline	1		65_1M1_day1_Saline
66	Control/Saline	1		66_1M2_day1_Saline
67	Control/Saline	1		67_1M3_day1_Saline
68	Control/Saline	1		68_1F101_day1_Saline
69	Control/Saline	1		69_1F102_day1_Saline
70	Control/Saline	1		70_1F103_day1_Saline
71	Control/Saline	5		71_1M4_day5_Saline
72	Control/Saline	5		72_1M5_day5_Saline
73	Control/Saline	5		73_1M6_day5_Saline
74	Control/Saline	5		74_1F104_day5_Saline
75	Control/Saline	5		75_1F105_day5_Saline
76	Gentamycin	28	day 28 toxicants M	76_2M16_day28_Gent
77	Gentamycin	28		77_2M17_day28_Gent
78	Gentamycin	28		78_2M18_day28_Gent
79	Cisplatin	28		79_3M25_day28_Cis
80	Cisplatin	28		80_3M26_day28_Cis
81	Cisplatin	28		81_3M27_day28_Cis
82	Tobramycin	28		82_4M34_day28_Tob
83	Tobramycin	28		83_4M35_day28_Tob
84	Tobramycin	28		84_4M36_day28_Tob
85	Cadmium chloride	28		85_5M43_day28_CadCl
86	Cadmium chloride	28		86_5M44_day28_CadCl
87	Cadmium chloride	28		87_5M45_day28_CadCl
88	Doxorubicin	28		88_6M71_day28_Dox
89	Doxorubicin	28		89_6M72_day28_Dox
90	Doxorubicin	28		90_6M73_day28_Dox
91	Gentamycin	28	day 28 toxicants F	91_2F116_day28_Gent
92	Gentamycin	28		92_2F117_day28_Gent
93	Gentamycin	28		93_2F118_day28_Gent
94	Cisplatin	28		94_3F125_day28_Cis
95	Cisplatin	28		95_3F126_day28_Cis
96	Cisplatin	28		96_3F127_day28_Cis
97	Tobramycin	28		97_4F134_day28_Tob
98	Tobramycin	28		98_4F135_day28_Tob
99	Tobramycin	28		99_4F136_day28_Tob
100	Cadmium chloride	28		100_5F143_day28_CadCl
101	Cadmium chloride	28		101_5F144_day28_CadCl

#	Toxin used	Day		Name
102	Cadmium chloride	28	day 28 toxicants F	102_5F145_day28_CadCl
103	Doxorubicin	28		103_6F171_day28_Dox
104	Doxorubicin	28		104_6F172_day28_Dox
105	Doxorubicin	28		105_6F173_day28_Dox
106	Control/Saline	28		106_1M7_day28_Saline
107	Control/Saline	28	Day 28 control	107_1M8_day28_Saline
108	Control/Saline	28		108_1M9_day28_Saline
109	Control/Saline	28		109_1F107_day28_Saline
110	Control/Saline	28		110_1F108_day28_Saline
111	Control/Saline	28		111_1F109_day28_Saline
112	Valproic acid	1	Valproic acid - all days	112_7M55_day1_ValpA
113	Valproic acid	1		113_7M56_day1_ValpA
114	Valproic acid	1		114_7M57_day1_ValpA
115	Valproic acid	1		115_7F155_day1_ValpA
116	Valproic acid	1		116_7F156_day1_ValpA
117	Valproic acid	1		117_7F157_day1_ValpA
118	Valproic acid	5		118_7M58_day5_ValpA
119	Valproic acid	5		119_7M59_day5_ValpA
120	Valproic acid	5		120_7M60_day5_ValpA
121	Valproic acid	5		121_7F158_day5_ValpA
122	Valproic acid	5		122_7F159_day5_ValpA
123	Valproic acid	5		123_7F160_day5_ValpA
124	Valproic acid	28		124_7M61_day28_ValpA
125	Valproic acid	28		125_7M62_day28_ValpA
126	Valproic acid	28		126_7M63_day28_ValpA
127	Valproic acid	28		127_7F161_day28_ValpA
128	Valproic acid	28		128_7F162_day28_ValpA
129	Valproic acid	28		129_7F163_day28_ValpA

Classifier on qRT-PCR Data

- 5 The following tables summarize the microarray and qRT-PCR data for the markers of the present invention. The mean normalized expression level as measured by the microarray, as well as the mean normalized qRT-PCR expression levels, and the relevant Mann-Whitney scores p-values are given for each candidate.

Table 10: Mean expression levels and Mann-Whitney rank sum score p-values for microarray data of Day-1, Day-5 and control samples

Candidate		Microarray Data				
Probeset	Gene name	Normal mean	Tox Day-1 mean	Tox Day-5 mean	Day-1 p-value	Day-5 p-value
1371785_at	TNFRSF12A	212	312	392	0.002	3.00E ⁻⁰⁵
1383162_at	AA799594	4055	3521	3385	0.002	6.00E ⁻⁰³
1393728_at	AA964541	35	46	56	0.01	6.00E ⁻⁰⁵
1390507_at	ISG20	32	47	48	0.0006	6.00E ⁻⁰⁶
1392102_at	AI454051	85	87	98	0.95	6.00E ⁻⁰⁴
1397637_at	AI502869	65	59	56	0.03	3.00E ⁻⁰⁴
1390833_at	AW919147	32	28	25	0.04	2.00E ⁻⁰⁴
1370153_at	GDF15	38	48	73	0.01	1.00E ⁻⁰⁴
1384070_at	H31045	88	99	108	0.02	3.50E ⁻⁰⁵
1388642_at	EI24	867	860	917	0.38	8.00E ⁻⁰⁴
1367764_at	CCNG1	757	864	1229	0.03	4.50E ⁻⁰⁵
1388560_at	WDR77	532	529	572	0.82	4.00E ⁻⁰⁴
1369268_at	ATF3	30	69	86	0.007	4.00E ⁻⁰⁶
1388140_at	RAB13	171	180	193	0.004	8.00E ⁻⁰⁴
1388674_at	CDKN1A	97	123	192	0.003	5.00E ⁻⁰⁶

Table 11: Mean expression levels and Mann-Whitney rank sum score p-values for qRT-

5 PCR data of Day-1, Day-5 and control samples

Candidate		qRT-PCR Data				
Internal name	Gene name	Normal mean	Tox Day-1 mean	Tox Day-5 mean	Day-1 p-value	Day5 p-value
W41270_DB81_seg11	TNFRSF12A	1.08	1.42	2.27	4.00E ⁻⁰⁴	7.50E ⁻¹⁰
AA686189_DB71_seg6	TNFRSF12A	0.99	1.27	1.44	8.00E ⁻⁰⁴	7.50E ⁻⁰⁶
AA799594_DB71_seg0	AA799594	1.66	1.45	1.48	8.00E ⁻⁰³	0.01
AA964541_DB71_seg0	AA964541	0.87	1.24	1.43	2.00E ⁻⁰⁶	3.00E ⁻⁰⁸
W64472_DB81_seg2	ISG20	1.06	1.14	2.32	0.35	2.00E ⁻¹⁰
AI045075_DB71_seg6	ISG20	1.14	1.58	1.46	2.50E ⁻⁰⁴	6.50E ⁻⁰⁴
AI454051_DB71_seg0	AI454051	1.53	1.46	1.48	0.4	0.7
AI502869_DB71_seg0	AI502869	1.62	1.34	1.21	7.00E ⁻⁰³	2.50E ⁻⁰⁵
AW919147_DB71_seg0	AW919147	1.60	1.52	1.43	0.4	0.01
BI293562_DB71_seg2	GDF15	0.97	1.11	1.64	0.03	7.50E ⁻⁰⁷
H31045_DB71_seg5	H31045	1.51	0.99	1.51	0.015	0.5

Internal name	Gene name	Normal mean	Tox Day-1 mean	Tox Day-5 mean	Day-1 p-value	Day5 p-value
W83813_DB81_seg27	EI24	1.30	1.42	2.18	0.55	1.50E ⁻⁰⁸
H31799_DB71_seg23	EI24	1.48	1.56	1.43	0.25	0.01
MUSCYCG1R_DB81_seg15-17	CCNG1	1.20	1.17	2.18	0.25	1.00E ⁻⁰⁷
MUSCYCG1R_DB81_seg19-20	CCNG1	1.16	1.35	2.2	6.00E ⁻⁰³	3.00E ⁻⁰⁹
H31883_DB71_seg13	CCNG1	1.25	1.41	1.51	5.00E ⁻⁰³	0.04
W33294_DB81_seg23	WDR77	1.44	1.27	1.9	0.05	7.00E ⁻⁰⁴
W33294_DB81_seg44	WDR77	1.37	1.42	2.02	0.6	8.00E ⁻⁰⁶
H33998_DB71_seg19	WDR77	1.31	1.58	2.13	0.04	2.00E ⁻⁰⁷
RATLRFI_DB71_seg9	ATF3	0.84	1.47	1.39	0.025	5.00E ⁻⁰⁴
RATRAB13X_DB81_seg15-17	RAB13	1.32	1.37	1.89	0.05	3.00E ⁻⁰⁷
RATRAB13X_DB81_seg22	RAB13	1.36	1.37	2.12	0.04	3.00E ⁻⁰⁷
RATRAB13X_s DB71_eg11-13	RAB13	1.40	1.59	1.42	1.00E ⁻⁰³	0.6
MMU09507_DB81_seg15	CDKN1A	0.88	0.94	1.74	0.08	2.00E ⁻⁰⁶
RNU24174_DB71_seg8	CDKN1A	0.76	0.91	1.21	2.00E ⁻⁰⁵	3.00E ⁻⁰⁵

The qRT-PCR data was used to construct the optimal Random-Forest classifier for Day-5 (Table 13) and Day-1 (Table 16). The optimal performance of the Day-5 signatures, as measured by cross validation is – Accuracy of 94% and LOR of 5.6. We further tested the performance of the optimal classifier on the Day-5 animals treated with Cadmium Chloride and found that 5 out of 6 samples were classified correctly as toxic. The optimal performance of the Day-1 signature is – Accuracy of 89% and LOR of 4.2

Table 14 and Table 15 further list genes that contribute to signatures for classification of Day-5 data. Table 17 and Table 18 further list genes that contribute to signatures of classification of Day-1. These genes can be used to replace the genes in Table 13 and Table 16 to yield classifiers with LOR of at least 3.75.

Table 12: All genes

Candidate ID	Contig	Internal name	Gene name	NV* / Genbank ID	Reference
1	AA686189	W41270_DB81_seg11	TNFRSF12A	NV of NM181086	
2	AA686189	AA686189_DB71_seg6	TNFRSF12A	NM181086	Peter et al., Physiol. Genomics 25: 375-386, 2006.
3	AA799594	AA799594_DB71_seg0	AA799594	AI008646; DY471185 (ESTS, no RNA)	
4	AA964541	AA964541_DB71_seg0	AA964541	AA964541 (one sequence – EST)	WO02095000

Candidate ID	Contig	Internal name	Gene name	NV* / Genbank ID	Reference
5	AI045075	W64472_DB81_seg2	ISG20	NV of NM001008510	
6	AI045075	AI045075_DB71_seg6	ISG20	NM001008510	WO02095000
7	AI454051	AI454051_DB71_seg0	AI454051	BE118959 (EST, no RNA)	
8	AI502869	AI502869_DB71_seg0	AI502869	AI502869;DV713733; AI145914;CB796640 (ESTs, no RNA)	
9	AW919147	AW919147_DB71_seg0	AW919147	AW91914; BF392593 (ESTs, no RNA)	
10	BI293562	BI293562_DB71_seg2	GDF15	NM01921;BI293562 (BI293562 is an EST that extends the transcript)	Peter et al., Physiol. Genomics 25: 375-386, 2006.
11	H31045	H31045_DB71_seg5	H31045	CO569017;DY312216; CF115262;BM392249; AI556451 (ESTs, no RNA.)	
12	H31799	W83813_DB81_seg27	EI24	NV of NM001025660	
13	H31799	H31799_DB71_seg23	EI24	NM001025660	
14	H31883	MUSCYCG1R_DB81_seg15-17	CCNG1	NV of NM012923	
15	H31883	MUSCYCG1R_DB81_seg19-20	CCNG1	NV of NM012923	
16	H31883	H31883_DB71_seg13	CCNG1	NM012923	Thompson et al., Environ Health Perspect. 2004 Mar;112(4):488-94; WO02095000
17	H33998	W33294_DB81_seg23	WDR77	NV of NM001008771	
18	H33998	W33294_DB81_seg44	WDR77	NV of NM001008771	
19	H33998	H33998_DB71_seg19	WDR77	NM001008771	
20	RATLRFI	RATLRFI_DB71_seg9	ATF3	NM012912	Peter et al., Physiol. Genomics 25: 375-386, 2006. WO2004048598 ; WO200295000
21	RATRAB13X	RATRAB13X_DB81_seg15-17	RAB13	NV of NM031092	
22	RATRAB13X	RATRAB13X_DB81_seg22	RAB13	NV of NM031092	
23	RATRAB13X	RATRAB13X_DB71_seg11-13	RAB13	NM031092	WO02095000
24	RNU24174	MMU09507_DB81_seg15	CDKN1A	NV of NM080782	
25	RNU24174	RNU24174_DB71_seg8	CDKN1A	NM080782	Peter et al., Physiol. Genomics 25: 375-386, 2006. WO2004048598 ; WO0295000

*NV identifies novel polynucleotides and proteins, which may or may be not variants of known proteins.

Table 13: Optimal signature for Day-5 data

Candidate ID	Contig	Internal name	Gene name
1	AA686189	W41270_DB81_seg11	TNFRSF12A
5	AI045075	W64472_DB81_seg2	ISG20
12	H31799	W83813_DB81_seg27	EI24
15	H31883	MUSCYCG1R_DB81_seg19-20	CCNG1

Table 14: Novel-Variants for Day-5 signatures

Candidate ID	Contig	Internal name	Gene name
1	AA686189	W41270_DB81_seg11	TNFRSF12A
3	AA799594	AA799594_DB71_seg0	AA799594
5	AI045075	W64472_DB81_seg2	ISG20
7	AI454051	AI454051_DB71_seg0	AI454051
8	AI502869	AI502869_DB71_seg0	AI502869
9	AW919147	AW919147_DB71_seg0	AW919147
12	H31799	W83813_DB81_seg27	EI24
14	H31883	MUSCYCG1R_DB81_seg15-17	CCNG1
15	H31883	MUSCYCG1R_DB81_seg19-20	CCNG1
18	H33998	W33294_DB81_seg44	WDR77
21	RATRAB13X	RATRAB13X_DB81_seg15-17	RAB13
22	RATRAB13X	RATRAB13X_DB81_seg22	RAB13
24	RNU24174	MMU09507_DB81_seg15	CDKN1A

5 Table 15: Wild-Types for Day-5 signatures

Candidate ID	Contig	Internal name	Gene name
4	AA964541	AA964541_DB71_seg0	AA964541
10	BI293562	BI293562_DB71_seg2	GDF15
19	H33998	H33998_DB71_seg19	WDR77
20	RATLRFI	RATLRFI_DB71_seg9	ATF3
25	RNU24174	RNU24174_DB71_seg8	CDKN1A

Table 16: Optimal signature for Day-1 data

Candidate ID	Contig	Internal name	Gene name
1	AA686189	W41270_DB81_seg11	TNFRSF12A
4	AA964541	AA964541_DB71_seg0	AA964541
6	AI045075	AI045075_DB71_seg6	ISG20
15	H31883	MUSCYCG1R_DB81_seg19-20	CCNG1
20	RATLRFI	RATLRFI_DB71_seg9	ATF3
23	RATRAB13X	RATRAB13X_DB71_seg11-13	RAB13
25	RNU24174	RNU24174_DB71_seg8	CDKN1A

Table 17: Novel-Variants for Day-1 signatures

Candidate ID	Contig	Internal name	Gene name
1	AA686189	W41270_DB81_seg11	TNFRSF12A
3	AA799594	AA799594_DB71_seg0	AA799594
8	AI502869	AI502869_DB71_seg0	AI502869
11	H31045	H31045_DB71_seg5	H31045
14	H31883	MUSCYCG1R_DB81_seg15-17	CCNG1
15	H31883	MUSCYCG1R_DB81_seg19-20	CCNG1
17	H33998	W33294_DB81_seg23	WDR77
21	RATRAB13X	RATRAB13X_DB81_seg15-17	RAB13

5 Table 18: Wild-Types for Day-1 signatures

Candidate ID	Contig	Internal name	Gene name
2	AA686189	AA686189_DB71_seg6	TNFRSF12A
4	AA964541	AA964541_DB71_seg0	AA964541
6	AI045075	AI045075_DB71_seg6	ISG20
16	H31883	H31883_DB71_seg13	CCNG1
19	H33998	H33998_DB71_seg19	WDR77
20	RATLRFI	RATLRFI_DB71_seg9	ATF3
23	RATRAB13X	RATRAB13X_DB71_seg11-13	RAB13
25	RNU24174	RNU24174_DB71_seg8	CDKN1A

Table 19 below summarizes the SEQ ID NOs for nodes, detector nodes, other unique nodes, transcripts and proteins for each marker of the invention. Nodes are segments within a transcript that might, according to the predictions made by the LEADS platform, represent a single exon or an alternative extension of an exon. Unique nodes are the ones that appear only in a novel variant or polynucleotide and not in the wild-type. The nodes listed for new variants and polynucleotides are unique nodes. When other unique nodes exist for new-variants and polynucleotides of the same genes, they are also given. The detector nodes are the set of nodes within which the primers were designed. When

10

possible it is the same as the unique node (and not given separately), but when the unique node is too short the detector node might include other neighboring nodes as well.

Table 19: Candidate SEQ IDs

Contig	Internal name	Candidate ID	Node SEQ. ID No.	Detector nodes SEQ. ID No.	Other unique nodes SEQ. ID No.	Transcript SEQ. ID No.	Protein SEQ. ID No.
AA686189	W41270_DB81_seg11	1	2			1	62
AA686189	AA686189_DB71_seg6	2	45			44	73
AA799594	AA799594_DB71_seg0	3	33			32	
AA964541	AA964541_DB71_seg0	4	47			46	
AI045075	W64472_DB81_seg2	5	4		5	3	63
AI045075	AI045075_DB71_seg6	6	49			48	74
AI454051	AI454051_DB71_seg0	7	35			34	
AI502869	AI502869_DB71_seg0	8	37			36	
AW919147	AW919147_DB71_seg0	9	39			38	
BI293562	BI293562_DB71_seg2	10	51			50	75
H31045	H31045_DB71_seg5	11	41			40	71
H31799	W83813_DB81_seg27	12	7		8	6	64
H31799	H31799_DB71_seg23	13	43			42	72
H31883	MUSCYCG1R_DB81_seg15-17	14	10	11		9	65
H31883	MUSCYCG1R_DB81_seg19-20	15	13	14		12	66
H31883	H31883_DB71_seg13	16	53			52	76
H33998	W33294_DB81_seg23	17	16		17 18 19	15	67
H33998	W33294_DB81_seg44	18	21		22 23	20	68
H33998	H33998_DB71_seg19	19	55			54	77
RATLRFI	RATLRFI_DB71_seg9	20	57			56	78
RATRAB13X	RATRAB13X_DB81_seg15-17	21	25	26		24	69
RATRAB13X	RATRAB13X_DB81_seg22	22	28		29	27	70
RATRAB13X	RATRAB13X_DB71_seg11-13	23	59			58	79
RNU24174	MMU09507_DB81_seg15	24	31			30	
RNU24174	RNU24174_DB71_seg8	25	61			60	80

5 Table 20 below summarizes the SEQ ID NOs for primers used to amplify specific amplicons for each marker of the invention.

Table 20: Candidate Primers and Amplicons

Contig	Internal name	Candidate ID	Forward primer SEQ. ID No.	Reverse primer SEQ. ID No.	Amplicon SEQ. ID No.
AA686189	W41270_DB81_seg11	1	252	253	254
AA686189	AA686189_DB71_seg6	2	249	250	251
AA799594	AA799594_DB71_seg0	3	255	256	257
AA964541	AA964541_DB71_seg0	4	258	259	260
AI045075	W64472_DB81_seg2	5	264	265	266
AI045075	W64472_DB81_seg2_F6R1	5	327	328	329
AI045075	AI045075_DB71_seg6	6	261	262	263
AI454051	AI454051_DB71_seg0	7	267	268	269
AI502869	AI502869_DB71_seg0	8	270	271	272
AW919147	AW919147_DB71_seg0	9	273	274	275
BI293562	BI293562_DB71_seg2	10	276	277	278
H31045	H31045_DB71_seg5	11	279	280	281
H31799	W83813_DB81_seg27	12	285	286	287
H31799	H31799_DB71_seg23	13	282	283	284
H31883	MUSCYCG1R_DB81_seg15-17	14	291	292	293
H31883	MUSCYCG1R_DB81_seg19-20	15	294	295	296
H31883	H31883_DB71_seg13	16	288	289	290
H31883	H31883_DB71_seg13_F5R5	16	330	331	332
H33998	W33294_DB81_seg23	17	297	298	299
H33998	W33294_DB81_seg44	18	300	301	302
H33998	H33998_DB71_seg19	19	303	304	305
RATLRFI	RATLRFI_DB71_seg9	20	306	307	308
RATRAB13X	RATRAB13X_DB81_seg15-17	21	309	310	311
RATRAB13X	RATRAB13X_DB81_seg22	22	312	313	314
RATRAB13X	RATRAB13X_DB71_seg11-13	23	315	316	317
RNU24174	MMU09507_DB81_seg15	24	321	322	323
RNU24174	RNU24174_DB71_seg8	25	324	325	326

Table 21 below provides human and mouse orthologous sequences for each rat marker of the invention.

Table 21: Candidates Homologues

Contig	Internal name	Candidate ID	Human homologue transcripts SEQ. ID No.	Human homologue protein SEQ. ID No.	Mouse homologue transcripts SEQ. ID No.	Mouse homologue proteins SEQ. ID No.
AA686189	W41270_DB81_seg11	1	105 106 107 108	157 158 159 160	197 198 199 200	226 227 228 229
AA686189	AA686189_DB71_seg6	2	105 106 107 108	157 158 159 160	197 198 199 200	226 227 228 229
AA799594	AA799594_DB71_seg0	3				
AA964541	AA964541_DB71_seg0	4				190
AI045075	W64472_DB81_seg2	5	112 113 114	163 164	202 203 204	231 232 233
AI045075	AI045075_DB71_seg6	6	112 113 114	163 164	202 203 204	231 232 233
AI454051	AI454051_DB71_seg0	7				185
AI502869	AI502869_DB71_seg0	8				
AW919147	AW919147_D B71_seg0	9				195 196
BI293562	BI293562_DB71_seg2	10	100 101 102 103 104	154 155 156	186	215
H31045	H31045_DB71_seg5	11	134 135 136 137	173 174 175 176	179 180 181 182 183 184	209 210 211 212 213 214
H31799	W83813_DB81_seg27	12	83 84 85 86 87 88 89	140 141 142 143 144	205 206 207	234 235 236
H31799	H31799_DB71_seg23	13	83 84 85 86 87 88 89	140 141 142 143 144	205 206 207	234 235 236
H31883	MUSCYCG1R_DB81_seg15-17	14	109 110 111	161 162	188 189	217 218
H31883	MUSCYCG1R_DB81_seg19-20	15	109 110 111	161 162	188 189	217 218
H31883	H31883_DB71_seg13	16	109 110 111	161 162	188 189	217 218
H33998	W33294_DB81_seg23	17	90 91 92 93 94 95 96 97 98 99	145 146 147 148 149 150 151 152 153	191 192 193 194	220 221 222 223
H33998	W33294_DB81_seg44	18	90 91 92 93 94 95 96 97 98 99	145 146 147 148 149 150 151 152 153	191 192 193 194	220 221 222 223

Contig	Internal name	Candidate ID	Human homologue transcripts SEQ. ID No.	Human homologue protein SEQ. ID No.	Mouse homologue transcripts SEQ. ID No.	Mouse homologue proteins SEQ. ID No.
H33998	H33998_DB71_seg19	19	90 91 92 93 94 95 96 97 98 99	145 146 147 148 149 150 151 152 153	191 192 193 194	220 221 222 223
RATLRFI	RATLRFI_DB71_seg9	20	81 82	138 139	177 178	208
RATRAB13X	RATRAB13X_DB81_seg15-17	21	115 116 117 118 119	165 166 167 168 169	201	230
RATRAB13X	RATRAB13X_DB81_seg22	22	115 116 117 118 119	165 166 167 168 169	201	230
RATRAB13X	RATRAB13X_DB71_seg11-13	23	115 116 117 118 119	165 166 167 168 169	201	230
RNU24174	MMU09507_DB81_seg15	24	120 121 122 123 124 125 126 127 128 129 130 131 132 133	170 171 172	187	216
RNU24174	RNU24174_D B71_seg8	25	120 121 122 123 124 125 126 127 128 129 130 131 132 133	170 171 172	187	216

EXAMPLE 2: Real Time qPCR analysis of the selected markers

Example 2.1: Expression of tumor necrosis factor receptor superfamily, member 12a, AA686189, transcripts which are detectable by amplicon as depicted in sequence name AA686189_DB71_seg6 in kidney tissues of treated or untreated rats

Expression of tumor necrosis factor receptor superfamily, member 12a transcripts detectable by or according to seg6 - AA686189_DB71_seg6_F1R1 (SEQ ID NO: 251) amplicon and primers AA686189_DB71_seg6_F1 (SEQ ID NO: 249) and AA686189_DB71_seg6_R1 (SEQ ID NO: 250) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled AA686189_DB71_seg6 in Table 22 contains the normalized expression values of the above-indicated tumor necrosis factor receptor superfamily, member 12a transcript in treated or untreated kidney samples.

As is evident from the column entitled AA686189_DB71_seg6 in Table 22, the level of expression of the tumor necrosis factor receptor superfamily, member 12a transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-

105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $8.00E^{-04}$ and P-value for day 5: $7.5E^{-06}$.

5 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair AA686189_DB71_seg6_F1 (SEQ ID NO: 249) forward primer; and AA686189_DB71_seg6_R1 (SEQ ID NO: 250) reverse primer.

10 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: AA686189_DB71_seg6_F1R1 (SEQ ID NO: 251).

Forward primer (AA686189_DB71_seg6_F1 (SEQ ID NO: 249)):

CCAAGGACTGGGCTTAGGGT

15 Reverse primer (AA686189_DB71_seg6_R1 (SEQ ID NO: 250)):

AGAGATTCCTTGTGCAAATGC

Amplicon (AA686189_DB71_seg6_F1R1 (SEQ ID NO: 251))

20 CCAAGGACTGGGCTTAGGGTTCAGGGGAGCCTTCCAGGGTGTCTAATTGCCCTGTCTC
TGGTTCTGGGGCAGACAGAGAGCCTCAAGCTAGGTCACAAAGCGACTCATACTAAGGATCT
GCAGCATTTGCACAAGGGAATCTCT

Example 2.2: Expression of tumor necrosis factor receptor superfamily, member 12a, AA686189, transcripts which are detectable by amplicon as depicted in sequence name W41270_DB81_seg11 in kidney tissues of treated or untreated rats

25 Expression of tumor necrosis factor receptor superfamily, member 12a transcripts detectable by or according to seg11 - W41270_DB81_seg11_F2R2 (SEQ ID NO: 254) amplicon and primers W41270_DB81_seg11_F2 (SEQ ID NO: 252) and W41270_DB81_seg11_R2 (SEQ ID NO: 253) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

30 The column entitled W41270_DB81_seg11 in Table 22 contains the normalized expression values of the above-indicated tumor necrosis factor receptor superfamily, member 12a transcript in treated or untreated kidney samples.

35 As is evident from the column entitled W41270_DB81_seg11 in Table 22, the level of expression of the tumor necrosis factor receptor superfamily, member 12a transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105

, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $4.00E^{-04}$, and P-value for day 5: $7.50E^{-10}$.

5 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W41270_DB81_seg11_F2 (SEQ ID NO: 252) forward primer; and W41270_DB81_seg11_R2 (SEQ ID NO: 253) reverse primer.

10 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: W41270_DB81_seg11_F2R2 (SEQ ID NO: 254).

Forward primer (W41270_DB81_seg11_F2 (SEQ ID NO: 252)):

GATCTGGGTAGGTGGTTGTTGG

15 Reverse primer (W41270_DB81_seg11_R2 (SEQ ID NO: 253)):

CGCACACCCTTATAAAAAGTCCC

Amplicon (W41270_DB81_seg11_F2R2 (SEQ ID NO: 254)):

GATCTGGGTAGGTGGTTGTTGGGGCAGAAAGGAGGTCGTAGACTTAGGATATAGGAA
ACCAGGAAAACTGACTGAGGAAGGGACTTTTATAAGGGTGTGCG

20 Example 2.3: Expression of AA799594, transcripts which are detectable by amplicon as depicted in sequence name AA799594_DB71_seg0 in kidney tissues of treated or untreated rats

Expression of AA799594 detectable by or according to seg0 - AA799594_DB71_seg0_F1R1 (SEQ ID NO: 257) amplicon and primers AA799594_DB71_seg0_F1 (SEQ ID NO: 255) and
25 AA799594_DB71_seg0_R1 (SEQ ID NO: 256) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

30 The column entitled AA799594_DB71_seg0 in Table 22 contains the normalized expression values of the above-indicated AA799594 transcript in treated or untreated kidney samples.

As is evident from the column entitled AA799594_DB71_seg0 in Table 22, the level of expression of AA799594 transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-
35 129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $8.00E^{-03}$ and P-value for day 5: 0.01.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair:

AA799594_DB71_seg0_F1 (SEQ ID NO: 255) forward primer; and AA799594_DB71_seg0_R1
5 (SEQ ID NO: 256) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: AA799594_DB71_seg0_F1R1 (SEQ ID NO: 257).

10 Forward primer (AA799594_DB71_seg0_F1 (SEQ ID NO: 255)):

ATCTCGATGGTAACGGGTCTAATC

Reverse primer (AA799594_DB71_seg0_R1 (SEQ ID NO: 256)):

TTTGTGCTGACCTTCATGCC

Amplicon (AA799594_DB71_seg0_F1R1 (SEQ ID NO: 257)):

15 ATCTCGATGGTAACGGGTCTAATCAGCCCATGATCAACATAACTGTGGTGATATACAT
TTGGTATTTTAAATTTTCGGATGCCTTCCTCAACATAGCCGTCAAGGCATGAAGGTCAGCAC
AAA

Example 2.4: Expression of AA964541, transcripts which are detectable by amplicon as depicted in sequence name AA964541_DB71_seg0 in kidney tissues of treated or
20 untreated rats

Expression of AA964541 transcripts detectable by or according to seg0 -
AA964541_DB71_seg0_F2R2 (SEQ ID NO: 260) amplicon and primers AA964541_DB71_seg0_F2
(SEQ ID NO: 258) and AA964541_DB71_seg0_R2 (SEQ ID NO: 259) was measured by real time PCR.
The value of the expression was measured by Real-Time PCR and normalized relative to the expression
25 of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR
ANALYSIS" hereinabove.

The column entitled AA964541_DB71_seg0 in Table 22 contains the normalized expression values of the above-indicated AA964541 transcript in treated or untreated kidney samples.

30 As is evident from the column entitled AA964541_DB71_seg0 in Table 22, the level of expression
of AA964541 transcript detectable by the above amplicon was significantly higher in the samples treated
with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9
hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples
numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical
analysis was applied to verify the significance of these results, P-value for day 1: $2.00E^{-06}$, and P-Value
35 for day5: $3.00E^{-08}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: AA964541_DB71_seg0_F2 (SEQ ID NO: 258) forward primer; and AA964541_DB71_seg0_R2 (SEQ ID NO: 259) reverse primer.

5 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

AA964541_DB71_seg0_F2R2 (SEQ ID NO: 260).

Forward primer (AA964541_DB71_seg0_F2 (SEQ ID NO: 258)):

10 CCAGCCTAGCCTCTCTTTTGC

Reverse primer (AA964541_DB71_seg0_R2 (SEQ ID NO: 259)):

AACATTCCCACAGGGTACATTCA

Amplicon (AA964541_DB71_seg0_F2R2 (SEQ ID NO: 260)):

15 CCAGCCTAGCCTCTCTTTTGCCTGCTGGTTCAGCCCACTGGGCCTCCGTCCTTTCCTC
TGGAAGGGACTTGGCCTTGGGTGACAAATTCCTCTTTGATGAATGTACCCTGTGGGAATGTT

Example 2.5: Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts which are detectable by amplicon as depicted in sequence name AI045075_DB71_seg6 in kidney tissues of treated or untreated rats

20 Expression of Interferon stimulated exonuclease 20 (ISG20) transcripts detectable by or according to seg6 - AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263) amplicon and primers AI045075_DB71_seg6_F2 (SEQ ID NO: 261) and AI045075_DB71_seg6_R2 (SEQ ID NO: 262) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

25 The column entitled AI045075_DB71_seg6 in Table 22 contains the normalized expression values of the above-indicated Interferon stimulated exonuclease 20 (ISG20) transcript in treated or untreated kidney samples.

30 As is evident from the column entitled AI045075_DB71_seg6 in Table 22, the level of expression of the Interferon stimulated exonuclease 20 (ISG20) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-Value for day 1: $2.50E^{-04}$, and P-Value for day5: $6.50E^{-04}$.

35 Primer pairs are also optionally and preferably encompassed within the present invention; for

example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: AI045075_DB71_seg6_F2 (SEQ ID NO: 261) forward primer; and AI045075_DB71_seg6_R2 (SEQ ID NO: 262) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263).

Forward primer (AI045075_DB71_seg6_F2 (SEQ ID NO: 261)):

GGGCCACAATGGAGCTCTAC

Reverse primer (AI045075_DB71_seg6_R2 (SEQ ID NO: 262)):

ACAGGTCTCATTCATGGAAACTATG

Amplicon (AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263)):

GGGCCACAATGGAGCTCTACAAAATCTCTCAGCGACTCAGAGCCCAGCGAGGGCTGC
CCTGCCTGGGAACATCAGCCTGAACTTCATCCTCATCCAGGATCAGAAGCAGCTACTCCTTG
AAGGACCATAGTTTTCCATGAATGAGACCTGT

Example 2.6: Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts which are detectable by amplicon as depicted in sequence name W64472_DB81_seg2_ in kidney tissues of treated or untreated rats

Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts detectable by or according to seg2 - W64472_DB81_seg2_F1R1 (SEQ ID NO: 266) amplicon and primers W64472_DB81_seg2_F1 (SEQ ID NO: 264) and W64472_DB81_seg2_R1 (SEQ ID NO: 265) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled W64472_DB81_seg2 in Table 22 contains the normalized expression values of the above-indicated Interferon stimulated exonuclease 20 (ISG20), AI045075, transcript in treated or untreated kidney samples.

As is evident from the column entitled W64472_DB81_seg2 in Table 22, the level of expression of the Interferon stimulated exonuclease 20 (ISG20), AI045075, transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $2.00E^{-10}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for

example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W64472_DB81_seg2_F1 (SEQ ID NO: 264) forward primer; and W64472_DB81_seg2_R1 (SEQ ID NO: 265) reverse primer.

5 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

W64472_DB81_seg2_F1R1 (SEQ ID NO: 266).

Forward primer (W64472_DB81_seg2_F1 (SEQ ID NO: 264)):

GGAGGAGGGCAGAGCCA

10 Reverse primer (W64472_DB81_seg2_R1 (SEQ ID NO: 265)):

TCTGGTTCATTATCAAGGGAAGTTG

Amplicon (W64472_DB81_seg2_F1R1 (SEQ ID NO: 266)):

GGAGGAGGGCAGAGCCAGAGGAGGGACAGCCTGATGCAGACAGCCCTGACTCAACC
TGCCAGCCCCCTTACCTGTCAGCCTTGAGGAGATGGAACAGCCCAGCCTACTAGGCCTGCCC
15 CCACTCCCCAACTTCCCTTGATAATGAACCAGA

Example 2.7: Expression of AI454051 transcripts which are detectable by amplicon as depicted in sequence name AI454051_DB71_seg0 in kidney tissues of treated or untreated rats

20 Expression of AI454051 transcripts detectable by or according to seg0 - AI454051_DB71_seg0_F1R1 (SEQ ID NO: 269) amplicon and primers AI454051_DB71_seg0_F1 (SEQ ID NO: 267) and AI454051_DB71_seg0_R1 (SEQ ID NO: 268) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

25 The column entitled AI454051_DB71_seg0 in Table 22 contains the normalized expression values of the above-indicated AI454051 transcript in treated or untreated kidney samples.

30 As is evident from the column entitled AI454051_DB71_seg0 in Table 22, the level of expression of the AI454051 transcript detectable by the above amplicon was lower in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove).

35 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: AI454051_DB71_seg0_F1 (SEQ ID NO: 267) forward primer; and AI454051_DB71_seg0_R1 (SEQ ID NO: 268) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: AI454051_DB71_seg0_F1R1 (SEQ ID NO: 269).

5 Forward primer (AI454051_DB71_seg0_F1 (SEQ ID NO: 267)):

ATCCTGTTGGGTCTGCTTCTCA

Reverse primer (AI454051_DB71_seg0_R1 (SEQ ID NO: 268)):

CACAAGTAAATACATAGACTGGAATCAATG

Amplicon (AI454051_DB71_seg0_F1R1 (SEQ ID NO: 269)):

10 ATCCTGTTGGGTCTGCTTCTCACAGCGTATGGGGCTGTGTGCTTTTACTCATGGTGGCA
GCAGCAGTTGTTGTCACTTCTTTGGCCAGGCACAGTGGGTGATCCTTACAGAGCAGCACAGA
TTCCCATTGATTCCAGTCTATGTATTTACTTGTG

Example 2.8: Expression of AI502869 transcripts which are detectable by amplicon as depicted in sequence name AI502869_DB71_seg0 in kidney tissues of treated or

15 untreated rats

Expression of AI502869 transcripts detectable by or according to seg0 - AI502869_DB71_seg0_F1R1 (SEQ ID NO: 272) amplicon and primers AI502869_DB71_seg0_F1 (SEQ ID NO: 270) and AI502869_DB71_seg0_R1 (SEQ ID NO: 271) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled AI502869_DB71_seg0 in Table 22 contains the normalized expression values of the above-indicated AI502869 transcript in treated or untreated kidney samples.

As is evident from the column entitled AI502869_DB71_seg0_F1R1 (SEQ ID NO: 272) in Table 22, the level of expression of the AI502869 transcript detectable by the above amplicon was significantly lower in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $7.00E^{-03}$ and P-value for day 5: $2.50E^{-05}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: AI502869_DB71_seg0_F1 (SEQ ID NO: 270) forward primer; and AI502869_DB71_seg0_R1 (SEQ ID NO: 271) reverse primer.

35 The present invention also preferably encompasses any amplicon obtained through the use of any

Forward primer (AI502869 DB71_seg0_F1 (SEQ ID NO: 270)):

Reverse primer (AI502869 DB71 seg0 R1 (SEQ ID NO: 271)):

Amplicon (AI502869 DB71 seg0 F1R1 (SEQ ID NO: 272)):

10

Expression of AW919147 transcripts detectable by or according to seg0 - AW919147_DB71_seg0_F2R2 (SEQ ID NO: 275) amplicon and primers AW919147_DB71_seg0_F2 (SEQ ID NO: 273) and AW919147_DB71_seg0_R2 (SEQ ID NO: 274) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

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Forward primer (AW919147_DB71_seg0_F2 (SEQ ID NO: 273)):

GCCACAGTAGAGGCTGCACTT

Reverse primer (AW919147_DB71_seg0_R2 (SEQ ID NO: 274)):

GAATCAACTGGAAATGTTCTAGGG

5 Amplicon (AW919147_DB71_seg0_F2R2 (SEQ ID NO: 275)):

GCCACAGTAGAGGCTGCACTTGCCACTGCTGTGTAGCGGCACTCTCCTGACTCACTTA
AAAATTCTCTGGAGGTTTAAGTGAGGACCCAGTTCCATCTCTAGATATCCAGGCTCCCTAGA
ACATTTCCAGTTGATTC

10 Example 2.10: Expression of growth differentiation factor 15 (GDF15), BI293562, transcripts which are detectable by amplicon as depicted in sequence name BI293562_DB71_seg2 in kidney tissues of treated or untreated rats

Expression of growth differentiation factor 15 (GDF15) transcripts detectable by or according to seg2 - BI293562_DB71_seg2_F2R2 (SEQ ID NO: 278) amplicon and primers BI293562_DB71_seg2_F2 (SEQ ID NO: 276) and BI293562_DB71_seg2_R2 (SEQ ID NO: 277) was measured by real time PCR.
15 The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled BI293562_DB71_seg2 in Table 22 contains the normalized expression values of the above-indicated growth differentiation factor 15 (GDF15) transcript in treated or untreated kidney
20 samples.

As is evident from the column entitled BI293562_DB71_seg2 in Table 22, the level of expression of the growth differentiation factor 15 (GDF15) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid
25 treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.03 and P-value for day 5: $7.50E^{-07}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative
30 example only of a suitable primer pair: BI293562_DB71_seg2_F2 (SEQ ID NO: 276) forward primer; and BI293562_DB71_seg2_R2 (SEQ ID NO: 277) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon BI293562_DB71_seg2_F2R2 (SEQ ID NO:
35 278):

Forward primer (BI293562_DB71_seg2_F2 (SEQ ID NO: 276)):

CTGCAACTGAGCATGTGCGT

Reverse primer (BI293562_DB71_seg2_R2 (SEQ ID NO: 277)): TGCATAAGAACCACCGGGG

Amplicon (BI293562_DB71_seg2_F2R2 (SEQ ID NO: 278)):

5 CTGCAACTGAGCATGTGCGTGGGCGAGTGCCCCACCTCTACCGGTCGGCCAACACGC
ATGCGCAGATCAAAGCACGCCTGCATGGCCTGCAGCCCGACAGAGTGCCGGCCCCGTGCTG
TGTCCTCCAGCTACACCCCGGTGGTTCTTATGCA

Example 2.11: Expression H31045 transcripts which are detectable by amplicon as depicted in sequence name H31045_DB71_seg5 in kidney tissues of treated or
10 untreated rats

Expression of H31045 transcripts detectable by or according to seg5 - H31045_DB71_seg5_F3R3 (SEQ ID NO: 281) amplicon and primers H31045_DB71_seg5_F3 (SEQ ID NO: 279) and H31045_DB71_seg5_R3 (SEQ ID NO: 280) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house
15 keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled H31045_DB71_seg5 in Table 22 contains the normalized expression values of the above-indicated H31045 transcript in treated or untreated kidney samples.

As is evident from the column entitled H31045_DB71_seg5 in Table 22, the level of expression of
20 the H31045 transcript detectable by the above amplicon was significantly lower in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.015.

25 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: H31045_DB71_seg5_F3 (SEQ ID NO: 279) forward primer; and H31045_DB71_seg5_R3 (SEQ ID NO: 280) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any
30 suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon H31045_DB71_seg5_F3R3 (SEQ ID NO: 281):

Forward primer (H31045_DB71_seg5_F3 (SEQ ID NO: 279)):

GGACGGCCGAGCACAA

35 Reverse primer (H31045_DB71_seg5_R3 (SEQ ID NO: 280)):

GTGTGCATGTGCATAGGTCAGA

Amplicon (H31045_DB71_seg5_F3R3 (SEQ ID NO: 281)):

GGACGGCCGAGCACAATGGTGCTTGCTGTACAGCCTCATAGACCTTCCTTGGATCTCC
CAGACACACTTAGTGGAAGGAGAGAATCCATTCTTGCAAATTGTCTGACCTATGCACATGCA
5 CAC

Example 2.12: Expression Etoposide induced 2.4 mRNA (EI24) H31799 transcripts which are detectable by amplicon as depicted in sequence name H31799_DB71_seg23 in kidney tissues of treated or untreated rats

Expression of Etoposide induced 2.4 mRNA (EI24) transcripts detectable by or according to seg23
10 - H31799_DB71_seg23_F1R1 (SEQ ID NO: 284) amplicon and primers H31799_DB71_seg23_F1 (SEQ ID NO: 282) and H31799_DB71_seg23_R1 (SEQ ID NO: 283) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

15 The column entitled H31799_DB71_seg23 in Table 22 contains the normalized expression values of the above-indicated Etoposide induced 2.4 mRNA (EI24) transcript in treated or untreated kidney samples.

As is evident from the column entitled H31799_DB71_seg23 in Table 22, the level of expression of the Etoposide induced 2.4 mRNA (EI24) transcript detectable by the above amplicon was
20 higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: 0.01.

Primer pairs are also optionally and preferably encompassed within the present invention; for
25 example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: H31799_DB71_seg23_F1 (SEQ ID NO: 282) forward primer; and H31799_DB71_seg23_R1 (SEQ ID NO: 283) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a
30 non-limiting illustrative example only of a suitable amplicon H31799_DB71_seg23_F1R1 (SEQ ID NO: 284):

Forward primer (H31799_DB71_seg23_F1 (SEQ ID NO: 282)):

TACTGTTTCCTGTGAAGCACATACCT

Reverse primer (H31799_DB71_seg23_R1 (SEQ ID NO: 283)):

35 GCAGAGCAGTAAAGACCAAAACC

Amplicon (H31799_DB71_seg23_F1R1 (SEQ ID NO: 284)):

TACTGTTTCCTGTGAAGCACATACCTTGTATGTGGGAGGTAAAGGAGCACGCCAGCTG
CTCCATGTCACTCCCTCTATAGCCATCACTGTCTTGTGTTTTTTGTAACTCAGGTTAGGTTTTGG
TCTTTACTGCTCTGC

- 5 Example 2.13: Expression Etoposide induced 2.4 (EI24) mRNA, H31799, transcripts which are detectable by amplicon as depicted in sequence name W83813_DB81_seg27 in kidney tissues of treated or untreated rats

Expression of Etoposide induced 2.4 mRNA (EI24) transcripts detectable by or according to seg27 - W83813_DB81_seg27_F1R1 (SEQ ID NO: 287) amplicon and primers W83813_DB81_seg27_F1 (SEQ ID NO: 285) and W83813_DB81_seg27_R1 (SEQ ID NO: 286) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

15 The column entitled W83813_DB81_seg27 in Table 22 contains the normalized expression values of the above-indicated Etoposide induced 2.4 mRNA (EI24) transcript in treated or untreated kidney samples.

As is evident from the column entitled W83813_DB81_seg27 in Table 22, the level of expression of the Etoposide induced 2.4 mRNA (EI24) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $1.50E^{-08}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W83813_DB81_seg27_F1 (SEQ ID NO: 285) forward primer; and W83813_DB81_seg27_R1 (SEQ ID NO: 286) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon W83813_DB81_seg27_F1R1 (SEQ ID NO: 287):

Forward primer (W83813_DB81_seg27_F1 (SEQ ID NO: 285)):

AATCTAGGCTGCCTCCTGGAG

Reverse primer (W83813_DB81_seg27_R1 (SEQ ID NO: 286)):

AGGTCAATTATACAAGGCATGACTTTAG

35 Amplicon (W83813_DB81_seg27_F1R1 (SEQ ID NO: 287)):

AATCTAGGCTGCCTCCTGGAGGAAGATACTTAGGAGTTCAGAAGTGAAGAGATGAGG
CTTATAATACTTTTTCCTAAAGTCATGCCTTGTATAATTGACCT

Example 2.14: Expression of Cyclin-G1 (CCNG1), H31883, transcripts which are detectable by amplicon as depicted in sequence name H31883_DB71_seg13 in kidney tissues of treated or untreated rats

Expression of Cyclin-G1 (CCNG1) transcripts detectable by or according to seg13 - H31883_DB71_seg13_F1R1 (SEQ ID NO: 290) amplicon and primers H31883_DB71_seg13_F1 (SEQ ID NO: 288) and H31883_DB71_seg13_R1 (SEQ ID NO: 289) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled H31883_DB71_seg13 in Table 22 contains the normalized expression values of the above-indicated Cyclin-G1 (CCNG1) transcript in treated or untreated kidney samples.

As is evident from the column entitled H31883_DB71_seg13 in Table 22, the level of expression of the Cyclin-G1 (CCNG1) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $5.00E^{-03}$ and P-value for day 5: 0.04.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: H31883_DB71_seg13_F1 (SEQ ID NO: 288) forward primer; and H31883_DB71_seg13_R1 (SEQ ID NO: 289) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: H31883_DB71_seg13_F1R1 (SEQ ID NO: 290).

Forward primer (H31883_DB71_seg13_F1 (SEQ ID NO: 288)):

TCGTCAGAATTGCTGCCTCA

Reverse primer (H31883_DB71_seg13_R1 (SEQ ID NO: 289)):

TCTGTGGTAAAAACCTCCTGGAGT

Amplicon (H31883_DB71_seg13_F1R1 (SEQ ID NO: 290)):

TCGTCAGAATTGCTGCCTCAATCTAGTCCCATTTGAGAAAATTTGTTTCTACTGTCTCA
ATAACTGGATGAAATATCACTCTGAAAACCTGCCTATTGCACTAAAGCTAGTTTAGGCTTGA

TAAAACTCCAGGAGGTTTTTACCACAGA

Example 2.15: Expression of Cyclin-G1 (CCNG1), H31883, transcripts which are detectable by amplicon as depicted in sequence name MUSCYCG1R_DB81_seg15-17 in kidney tissues of treated or untreated rats

5 Expression of Cyclin-G1 (CCNG1) detectable by or according to seg15-17 - MUSCYCG1R_DB81_seg15-17_F1R1 (SEQ ID NO: 293) amplicon and primers MUSCYCG1R_DB81_seg15-17_F1 (SEQ ID NO: 291) and MUSCYCG1R_DB81_seg15-17_R1 (SEQ ID NO: 292) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT
10 PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled MUSCYCG1R_DB81_seg15-17 in Table 22 contains the normalized expression values of the above-indicated Cyclin-G1 (CCNG1) transcript in treated or untreated kidney samples.

15 As is evident from the column entitled MUSCYCG1R_DB81_seg15-17 in Table 22, the level of expression of the Cyclin-G1 (CCNG1) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $1.00E^{-07}$.

20 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: MUSCYCG1R_DB81_seg15-17_F1 (SEQ ID NO: 291) forward primer; and MUSCYCG1R_DB81_seg15-17_R1 (SEQ ID NO: 292) reverse primer.

25 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: MUSCYCG1R_DB81_seg15-17_F1R1 (SEQ ID NO: 293).

Forward primer (MUSCYCG1R_DB81_seg15-17_F1 (SEQ ID NO: 291)):

ATAAAATCAATCCCACTTTCTTGTTAAAAG

30 Reverse primer (MUSCYCG1R_DB81_seg15-17_R1 (SEQ ID NO: 292)):

CTTTCCTTAGAAAAATATGATCCTGC

Amplicon (MUSCYCG1R_DB81_seg15-17_F1R1 (SEQ ID NO: 293)):

ATAAAATCAATCCCACTTTCTTGTTAAAAGGAGAAACGATCTGAATTTTGAAAGACTA
GAAGCCCACTTAAGGCCTGCCACTGCAGGATCATATTTTCTAAGGCAAAG

Example 2.16: Expression of Cyclin-G1 (CCNG1), H31883, transcripts which are detectable by amplicon as depicted in sequence name MUSCYCG1R_DB81_seg19-20 in kidney tissues of treated or untreated rats

Expression of Cyclin-G1 (CCNG1) transcripts detectable by or according to seg19-20 -
 5 MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296) amplicon and primers MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294) and MUSCYCG1R_DB81_seg19-20_R1 (SEQ ID NO: 295) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

10 The column entitled MUSCYCG1R_DB81_seg19-20 in Table 22 contains the normalized expression values of the above-indicated Cyclin-G1 (CCNG1) transcript in treated or untreated kidney samples.

As is evident from the column entitled MUSCYCG1R_DB81_seg19-20 in Table 22, the level of expression of the Cyclin-G1 (CCNG1) transcript detectable by the above amplicon was significantly
 15 higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $6.00E^{-03}$ and P-value for day 5: $3.00E^{-09}$.

20 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294) forward primer; and MUSCYCG1R_DB81_seg19-20_R1 (SEQ ID NO: 295) reverse primer.

25 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296).

Forward primer (MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294)):

GAGATCCAAGCACTGAAGTATGTAGAGT

30 Reverse primer (MUSCYCG1R_DB81_seg19-20_R1 (SEQ ID NO: 295)):

TCAGGAGTACAGTGGATACATTTCTCTT

Amplicon (MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296)):

GAGATCCAAGCACTGAAGTATGTAGAGTTAACAGAAGGAGTAGAATGTATTCAGAAA
 CATTCCAAGGTATGCCAAGGTGATAGCATTGATCCTATTAGCAAGCTACAAGAGAAATGTAT
 35 CCACTGTACTCCTGA

Example 2.17: Expression of repeat domain 77 (WDR77), H33998, transcripts which are detectable by amplicon as depicted in sequence name W33294_DB81_seg23 in kidney tissues of treated or untreated rats

Expression of repeat domain 77 (WDR77) transcripts detectable by or according to seg23 - W33294_DB81_seg23_F1R1 (SEQ ID NO: 299) amplicon and primers W33294_DB81_seg23_F1 (SEQ ID NO: 297) and W33294_DB81_seg23_R1 (SEQ ID NO: 298) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled W33294_DB81_seg23 in Table 22 contains the normalized expression values of the above-indicated repeat domain 77 (WDR77) transcript in treated or untreated kidney samples.

As is evident from the column entitled W33294_DB81_seg23 in Table 22, the level of expression of the repeat domain 77 (WDR77) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $7.00E^{-04}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W33294_DB81_seg23_F1 (SEQ ID NO: 297) forward primer; and W33294_DB81_seg23_R1 (SEQ ID NO: 298) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: W33294_DB81_seg23_F1R1 (SEQ ID NO: 299).

Forward primer (W33294_DB81_seg23_F1 (SEQ ID NO: 297)):

GGGCACTGCATGGATTATGTC

Reverse primer (W33294_DB81_seg23_R1 (SEQ ID NO: 298)):

CCATGCCCAGAATAAAGGAGC

Amplicon (W33294_DB81_seg23_F1R1 (SEQ ID NO: 299)):

GGGCACTGCATGGATTATGTCGGTTTTACCTAGCTTTGGAGACCATCATTTTTTCCTAA
TAGGTTTGCTTCATTGTTTCAGCTCCTTTATTCTGGGCATGG

Example 2.18: Expression of repeat domain 77 (WDR77), H33998, transcripts which are detectable by amplicon as depicted in sequence name W33294_DB81_seg44 in kidney tissues of treated or untreated rats

Expression of repeat domain 77 (WDR77) transcripts detectable by or according to seg44 - W33294_DB81_seg44_F1R1 (SEQ ID NO: 302) amplicon and primers W33294_DB81_seg44_F1 (SEQ ID NO: 300) and W33294_DB81_seg44_R1 (SEQ ID NO: 301) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled W33294_DB81_seg44 in Table 22 contains the normalized expression values of the above-indicated repeat domain 77 (WDR77) transcript in treated or untreated kidney samples.

As is evident from the column entitled W33294_DB81_seg44 in Table 22, the level of expression of the repeat domain 77 (WDR77) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $8.00E^{-06}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W33294_DB81_seg44_F1 (SEQ ID NO: 300) forward primer; and W33294_DB81_seg44_R1 (SEQ ID NO: 301) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: W33294_DB81_seg44_F1R1 (SEQ ID NO: 302).

Forward primer (W33294_DB81_seg44_F1 (SEQ ID NO: 300)):

CTCCGTTAGCAATTATGGGTCAGT

Reverse primer (W33294_DB81_seg44_R1 (SEQ ID NO: 301)):

GAAGCTCATGGTCCTTCAGGG

Amplicon (W33294_DB81_seg44_F1R1 (SEQ ID NO: 302)):

CTCCGTTAGCAATTATGGGTCAGTAACTGATCTTATTAGAGCTTTACAACTTTGGTTT
GGAAAAGCACATAAAATGGGCGCCCTGAAGGACCATGAGCTTC

Example 2.19: Expression of repeat domain 77 (WDR77), H33998, transcripts which are detectable by amplicon as depicted in sequence name H33998_DB71_seg19 in kidney tissues of treated or untreated rats

Expression of repeat domain 77 (WDR77) transcripts detectable by or according to seg19 - H33998_DB71_seg19_F3R3 (SEQ ID NO: 305) amplicon and primers H33998_DB71_seg19_F3 (SEQ ID NO: 303) and H33998_DB71_seg19_R3 (SEQ ID NO: 304) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled H33998_DB71_seg19 in Table 22 contains the normalized expression values of the above-indicated repeat domain 77 (WDR77) transcript in treated or untreated kidney samples.

As is evident from the column entitled H33998_DB71_seg19 in Table 22, the level of expression of the repeat domain 77 (WDR77) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.04 and P-value for day 5: $2.00E^{-07}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: H33998_DB71_seg19_F3 (SEQ ID NO: 303) forward primer; and H33998_DB71_seg19_R3 (SEQ ID NO: 304) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: H33998_DB71_seg19_F3R3 (SEQ ID NO: 305).

Forward primer (H33998_DB71_seg19_F3R3 (SEQ ID NO: 303)):

CTGTCATTGTCATTTTCCCACC

Reverse primer (H33998_DB71_seg19_F3R3 (SEQ ID NO: 304)):

ATGCCGGATCTTCAATCTTAGG

Amplicon (H33998_DB71_seg19_F3R3 (SEQ ID NO: 305)):

CTGTCATTGTCATTTTCCCACCTAAAAATTCCTGAGGACTGATCTGGGTACTTTGCT
CTGGAGAGCTGAAGTCTGAGCGCTGTATATTTGGACTCCTAAGATTGAAGATCCGGCAT

Example 2.20: Expression of Activating transcription factor 3 (ATF3), RATLRFI, transcripts which are detectable by amplicon as depicted in sequence name RATLRFI_DB71_seg9 in kidney tissues of treated or untreated rats

Expression of Activating transcription factor 3 (ATF3) transcripts detectable by or according to
 5 seg9 - RATLRFI_DB71_seg9_F1R1 (SEQ ID NO: 308) amplicon and primers RATLRFI_DB71_seg9_F1 (SEQ ID NO: 306) and RATLRFI_DB71_seg9_R1 (SEQ ID NO: 307) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

10 The column entitled RATLRFI_DB71_seg9 in Table 22 contains the normalized expression values of the above-indicated Activating transcription factor 3 (ATF3) transcript in treated or untreated kidney samples.

As is evident from the column entitled RATLRFI_DB71_seg9 in Table 22, the level of expression of the Activating transcription factor 3 (ATF3) transcript detectable by the above amplicon was
 15 significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.025 and P-value for day 5: $5.00E^{-04}$.

20 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: RATLRFI_DB71_seg9_F1 (SEQ ID NO: 306) forward primer; and RATLRFI_DB71_seg9_R1 (SEQ ID NO: 307) reverse primer.

25 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: RATLRFI_DB71_seg9_F1R1 (SEQ ID NO: 308).

Forward primer (RATLRFI_DB71_seg9_F1 (SEQ ID NO: 306)):

AACTGGCTGATGACCAGCTGT

30 Reverse primer (RATLRFI_DB71_seg9_R1 (SEQ ID NO: 307)):

TGCTGTGCCCCGGGTTCT

Amplicon (RATLRFI_DB71_seg9_F1R1 (SEQ ID NO: 308)):

AACTGGCTGATGACCAGCTGTGCTACTCTGTGCTGACCGAGGACTGATGCCTCCTTCC
 CCTGTACCCACTGCTGAGGAAGAACCCGGGCACAGCA

Example 2.21: Expression of Ras-related protein Rab-13 (RAB13), RATRAB13X, transcripts which are detectable by amplicon as depicted in sequence name RATRAB13X_DB81_seg15-17 in kidney tissues of treated or untreated rats

5 Expression of Ras-related protein Rab-13 (RAB13) transcripts detectable by or according to seg15-17 - RATRAB13X_DB81_seg15-17_F1R1 (SEQ ID NO: 311) amplicon and primers RATRAB13X_DB81_seg15-17_F1 (SEQ ID NO: 309) and RATRAB13X_DB81_seg15-17_R1 (SEQ ID NO: 310) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

10 The column entitled RATRAB13X_DB81_seg15-17 in Table 22 contains the normalized expression values of the above-indicated Ras-related protein Rab-13 (RAB13) transcript in treated or untreated kidney samples.

15 As is evident from the column entitled RATRAB13X_DB81_seg15-17 in Table 22, the level of expression of the Ras-related protein Rab-13 (RAB13) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.05 and P-value for day 5: $3.00E^{-07}$.

20 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: RATRAB13X_DB81_seg15-17_F1 (SEQ ID NO: 309) forward primer; and RATRAB13X_DB81_seg15-17_R1 (SEQ ID NO: 310) reverse primer.

25 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: RATRAB13X_DB81_seg15-17_F1R1 (SEQ ID NO: 311).

Forward primer (RATRAB13X_DB81_seg15-17_F1 (SEQ ID NO: 309)):

GTGAGTCAGGCCGGGCT

30 Reverse primer (RATRAB13X_DB81_seg15-17_R1 (SEQ ID NO: 310)):

TCAAAAAATCGGATTCTGTGCTC

Amplicon (RATRAB13X_DB81_seg15-17_F1R1 (SEQ ID NO: 311)):

GTGAGTCAGGCCGGGCTGCTGTGGCCGAATGCCTTGCTTCTGCCCTTTATCCAGCTCTC
TCCTTCCTACCTCATCCCCTACAGTTGGCTCGAGAGCACAGAATCCGATTTTTTGA

Example 2.22: Expression of Ras-related protein Rab-13 (RAB13), RATRAB13X, transcripts which are detectable by amplicon as depicted in sequence name RATRAB13X_DB81_seg22 in kidney tissues of treated or untreated rats

Expression of Ras-related protein Rab-13 (RAB13) transcripts detectable by or according to seg22
 5 - RATRAB13X_DB81_seg22_F2R2 (SEQ ID NO: 314) amplicon and primers RATRAB13X_DB81_seg22_F2 (SEQ ID NO: 312) and RATRAB13X_DB81_seg22_R2 (SEQ ID NO: 313) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

10 The column entitled RATRAB13X_DB81_seg22 in Table 22 contains the normalized expression values of the above-indicated Ras-related protein Rab-13 (RAB13) transcript in treated or untreated kidney samples.

As is evident from the column entitled RATRAB13X_DB81_seg22 in Table 22, the level of expression of the Ras-related protein Rab-13 (RAB13) transcript detectable by the above amplicon was
 15 significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: 0.04 and P-value for day 5: $3.00E^{-07}$.

20 Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: RATRAB13X_DB81_seg22_F2 (SEQ ID NO: 312) forward primer; and RATRAB13X_DB81_seg22_R2 (SEQ ID NO: 313) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any
 25 suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: RATRAB13X_DB81_seg22_F2R2 (SEQ ID NO: 314).

Forward primer (RATRAB13X_DB81_seg22_F2 (SEQ ID NO: 312)):

TTCTCCTGAATCTGGCTGGGT

30 Reverse primer (RATRAB13X_DB81_seg22_R2 (SEQ ID NO: 313)):

TGGAAAAGGATGTCCATTCTTC

Amplicon (RATRAB13X_DB81_seg22_F2R2 (SEQ ID NO: 314)):

TTCTCCTGAATCTGGCTGGGTCCCCCTTCCTTACCCCAACTCTTTAACTGGTGATGAAA
 ACAGCAAGGAGAAAGGGCAGCCTGAAGAATGGACATCCTTTTTC

Example 2.23: Expression of Ras-related protein Rab-13 (RAB13), RATRAB13X, transcripts which are detectable by amplicon as depicted in sequence name RATRAB13X_DB71_seg11-13 in kidney tissues of treated or untreated rats

Expression of Ras-related protein Rab-13 (RAB13) transcripts detectable by or according to seg11-13 - RATRAB13X_DB71_seg11-13_F1R1 (SEQ ID NO: 317) amplicon and primers RATRAB13X_DB71_seg11-13_F1 (SEQ ID NO: 315) and RATRAB13X_DB71_seg11-13_R1 (SEQ ID NO: 316) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled RATRAB13X_DB71_seg11-13 in Table 22 contains the normalized expression values of the above-indicated Ras-related protein Rab-13 (RAB13) transcript in treated or untreated kidney samples.

As is evident from the column entitled RATRAB13X_DB71_seg11-13 in Table 22, the level of expression of the Ras-related protein Rab-13 (RAB13) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $1.00E^{-03}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: RATRAB13X_DB71_seg11-13_F1 (SEQ ID NO: 315) forward primer; and RATRAB13X_DB71_seg11-13_R1 (SEQ ID NO: 316) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: RATRAB13X_DB71_seg11-13_F1R1 (SEQ ID NO: 317).

Forward primer (RATRAB13X_DB71_seg11-13_F1 (SEQ ID NO: 315)):

AGAATCCGATTTTTTGAGACAAGTG

Reverse primer (RATRAB13X_DB71_seg11-13_R1 (SEQ ID NO: 316)):

GATCTCCGGCCTCCTGTCTT

Amplicon (RATRAB13X_DB71_seg11-13_F1R1 (SEQ ID NO: 317)):

AGAATCCGATTTTTTGAGACAAGTGCCAAATCCAGTGTGAATGTGGATGAGGCTTTCA
GTTCCCTGGCCCGTGACATCTTGCTCAAGACAGGAGGCCGGAGATC

Example 2.24: Expression of Cyclin-dependent kinase inhibitor 1A (CDKN1A), RNU24174, transcripts which are detectable by amplicon as depicted in sequence name MMU09507_DB81_seg15 in kidney tissues of treated or untreated rats

Expression of Cyclin-dependent kinase inhibitor 1A (CDKN1A) transcripts detectable by or according to seg15 - MMU09507_DB81_seg15_F1R1 (SEQ ID NO: 323) amplicon and primers MMU09507_DB81_seg15_F1 (SEQ ID NO: 321) and MMU09507_DB81_seg15_R1 (SEQ ID NO: 322) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

The column entitled MMU09507_DB81_seg15 in Table 22 contains the normalized expression values of the above-indicated Cyclin-dependent kinase inhibitor 1A (CDKN1A) transcript in treated or untreated kidney samples.

As is evident from the column entitled MMU09507_DB81_seg15 in Table 22, the level of expression of the Cyclin-dependent kinase inhibitor 1A (CDKN1A) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $3.00E^{-03}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: MMU09507_DB81_seg15_F1 (SEQ ID NO: 321) forward primer; and MMU09507_DB81_seg15_R1 (SEQ ID NO: 322) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: MMU09507_DB81_seg15_F1R1 (SEQ ID NO: 323).

Forward primer (MMU09507_DB81_seg15_F1 (SEQ ID NO: 321)):

GGGTGGGGGAATCTAGCCT

Reverse primer (MMU09507_DB81_seg15_R1 (SEQ ID NO: 322)):

CCCCAGACAAAGGGACATGT

Amplicon (MMU09507_DB81_seg15_F1R1 (SEQ ID NO: 323)):

GGGTGGGGGAATCTAGCCTCTCTAGAGCCCTAGCCCTCTGACGAGGAGGAGGTGTAG
TGCCCTGTAGCTTTTCCCCAGGACTCGCCACATGTCCCTTTGTCTGGGG

Expression of Cyclin-dependent kinase inhibitor 1A (CDKN1A) transcripts detectable by or according to seg8 - RNU24174_DB71_seg8_F1R1 (SEQ ID NO: 326) amplicon and primers RNU24174_DB71_seg8_F1 (SEQ ID NO: 324) and RNU24174_DB71_seg8_R1 (SEQ ID NO: 325) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT PREPARATION and Real-TIME RT-PCR ANALYSIS" hereinabove.

As is evident from the column entitled RNU24174_DB71_seg8 in Table 22, the level of expression of the Cyclin-dependent kinase inhibitor 1A (CDKN1A) transcript detectable by the above amplicon was significantly higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 1: $2.00E^{-05}$ and P-value for day 5: $3.00E^{-05}$.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: RNU24174_DB71_seg8_F1R1 (SEQ ID NO: 326).

CGCTGAAGTCCTCAGTGACTTGTCCCATTTCTTAGTAGTTGTACAAGGAGTCAGGCCA
AGATGGTGCCTCGGGGGCTGAGGGAGCTCACAGGAAGTGAAGCAGTGACTGGTCCTTTCCCA
GTATTGAATACTGAGCCCCTGTGGGTGTCGAAGCACTTAGTG

Table 22 presents the normalized Real Time PCR results for all 25 amplicons detailed in examples 2.1-2.25 for all samples checked. The qRT-PCR measurements were normalized according to each samples normalization factor as described in "RT Preparation and Real-Time qRT-PCR Analysis" and further multiplied by a constant factor for ease of viewing.

5 Table 22: normalized Real Time PCR

Sample	AA686189_ DB71_seg6	AA799594_ DB71_seg0	AA964541_ DB71_seg0	AI045075_ DB71_seg6
68_1F101_day1_Saline	0.44	1.17	0.25	0.51
69_1F102_day1_Saline	0.38	1.16	0.28	0.44
70_1F103_day1_Saline	0.64	0.92	0.77	0.54
74_1F104_day5_Saline	0.63	1.69	0.41	0.42
75_1F105_day5_Saline	0.54	1.56	0.42	0.59
109_1F107_day28_Saline	0.78	1.6	0.49	0.79
110_1F108_day28_Saline	0.62	1.08	0.46	0.65
111_1F109_day28_Saline	0.68	0.9	0.45	0.9
65_1M1_day1_Saline	0.72	1.02	0.54	0.79
66_1M2_day1_Saline	0.65	0.87	0.65	0.36
67_1M3_day1_Saline	0.55	1.17	0.4	0.84
71_1M4_day5_Saline	0.45	1.28	0.49	0.6
72_1M5_day5_Saline	0.54	0.67	0.38	0.56
73_1M6_day5_Saline	0.41	0.86	0.41	0.45
106_1M7_day28_Saline	0.81	1.06	0.48	0.64
107_1M8_day28_Saline	0.36	1.24	0.25	0.42
108_1M9_day28_Saline	0.73	1.36	0.3	0.55
16_2F110_day1_Gent	1.19	1.25	1.35	1.5
17_2F111_day1_Gent	0.65	0.72	0.74	0.93
18_2F112_day1_Gent	1.07	0.93	1.29	0
45_2F113_day5_Gent	0.64	0.99	0.86	0.76
46_2F114_day5_Gent	0.72	0.89	0.58	1.04
47_2F115_day5_Gent	1.21	1.52	1.04	0.62
91_2F116_day28_Gent	2.7	0.84	3.19	2.09
92_2F117_day28_Gent	2.09	0.8	1.77	3.14
93_2F118_day28_Gent	2.48	0.89	2.16	1.74
1_2M10_day1_Gent	0.94	0.57	0.9	1.07
2_2M11_day1_Gent	1.04	0.95	1.19	1.03
3_2M12_day1_Gent	0.6	0.92	0.44	0.78
31_2M13_day5_Gent	0.81	0.89	0.91	1.1
32_2M14_day5_Gent	0.83	0.56	0.76	0
33_2M15_day5_Gent	0.75	1.04	0.87	1.04
76_2M16_day28_Gent	1.98	0.54	3.33	1.25
77_2M17_day28_Gent	1.23	0.5	1.45	1.99
78_2M18_day28_Gent	2.51	0.67	3.18	1.29
19_3F119_day1_Cis	0.7	1.17	0.88	1.01

Sample	AA686189_ DB71_seg6	AA799594_ DB71_seg0	AA964541_ DB71_seg0	AI045075_ DB71_seg6
20_3F120_day1_Cis	0.44	0.39	0.58	0.68
21_3F121_day1_Cis	0.66	0.98	0.41	0.79
48_3F122_day5_Cis	1.08	1.16	0.99	0.86
49_3F123_day5_Cis	0.93	1.34	0.74	0.53
50_3F124_day5_Cis	0.76	1.11	0.76	0.76
94_3F125_day28_Cis	0	0.74	2.63	2.17
95_3F126_day28_Cis	3.57	0.64	5.28	3.09
96_3F127_day28_Cis	6.06	0.52	6.85	3.37
4_3M19_day1_Cis	0.48	0.59	0.4	0.64
5_3M20_day1_Cis	0.42	0.99	0.29	0.62
6_3M21_day1_Cis	0.68	0.87	0.56	1.09
34_3M22_day5_Cis	1.5	0.93	1.94	1.93
35_3M23_day5_Cis	0.69	0.71	0.67	0.81
36_3M24_day5_Cis	4.48	0.75	4.8	1.33
79_3M25_day28_Cis	3.15	0.36	3.72	3.18
80_3M26_day28_Cis	4.29	0.97	4.71	2.72
81_3M27_day28_Cis	3.86	0.62	3.97	1.57
22_4F128_day1_Tob	1.19	0.87	0.56	1.66
23_4F129_day1_Tob	0.77	0.83	0.82	0.88
24_4F130_day1_Tob	2.14	1.07	2.02	3.16
51_4F131_day5_Tob	0.85	1.38	0.82	0.92
52_4F132_day5_Tob	1.04	0.88	0.77	1.61
53_4F133_day5_Tob	0.78	0.88	0.65	1.56
97_4F134_day28_Tob	2.1	0.71	1.68	0.94
98_4F135_day28_Tob	1.65	0.75	1.37	0.79
99_4F136_day28_Tob	2.23	0.84	1.59	1.19
7_4M28_day1_Tob	0.65	0.94	0.47	0.56
8_4M29_day1_Tob	0.85	1	0.75	1.03
9_4M30_day1_Tob	1.11	0.61	1.28	1.07
37_4M31_day5_Tob	0.53	0.81	0.53	0.74
38_4M32_day5_Tob	0.6	0.74	0.59	0.63
39_4M33_day5_Tob	0.44	1.03	0.34	0.69
82_4M34_day28_Tob	2.37	0.63	2.42	1.04
83_4M35_day28_Tob	3.35	0.66	3.33	1.76
84_4M36_day28_Tob	2.4	0.62	2.07	0.99
25_5F137_day1_CadCl	0.38	1.25	0.41	0.6
26_5F138_day1_CadCl	0.52	1.43	0.55	0.54
27_5F139_day1_CadCl	0.36	1.27	0.3	0.5
54_5F141_day5_CadCl	0.47	1.12	0.29	0.98
55_5F142_day5_CadCl	0.29	0.9	0.27	0.49
100_5F143_day28_CadCl	0.64	1.14	0.32	1.72
101_5F144_day28_CadCl	0.58	0.88	0.42	1.15
102_5F145_day28_CadCl	1.94	0.37	1.72	0.86

Sample	AA686189_ DB71_seg6	AA799594_ DB71_seg0	AA964541_ DB71_seg0	AI045075_ DB71_seg6
10_5M37_day1_CadCl	0.38	1.09	0.28	0.66
11_5M38_day1_CadCl	0.59	1.15	0.6	0.87
12_5M39_day1_CadCl	0.64	1.05	0.56	0.84
40_5M40_day5_CadCl	0.39	1.23	0.42	0.85
41_5M41_day5_CadCl	0.5	0.98	0.41	0.81
42_5M42_day5_CadCl	0.38	1.01	0.42	0.83
85_5M43_day28_CadCl	0.4	0.66	0.32	0.75
86_5M44_day28_CadCl	0.51	0.77	0.55	0.63
87_5M45_day28_CadCl	0.82	0.83	0.72	0.71
28_6F146_day1_Dox	0.78	1.1	0.62	0.61
29_6F147_day1_Dox	0.4	1.52	0.46	0.64
30_6F148_day1_Dox	0.26	1.52	0.33	3.12
56_6F149_day5_Dox	0.51	0.91	0.5	0.76
57_6F150_day5_Dox	0.48	0.84	0.46	0.93
58_6F151_day5_Dox	0.38	1.24	0.4	0.57
103_6F171_day28_Dox	1.5	1.24	1.04	0.66
104_6F172_day28_Dox	0.62	1.39	0.55	0.82
105_6F173_day28_Dox	0.04	1.29	1.3	1.6
13_6M46_day1_Dox	0.59	1.26	0.54	1.12
14_6M47_day1_Dox	0.49	0.92	0.55	1.21
15_6M48_day1_Dox	0.39	0.99	0.38	0.78
43_6M49_day5_Dox	1.2	0.84	1.14	0.93
44_6M50_day5_Dox	0.6	1.22	0.48	2.05
88_6M71_day28_Dox	6.37	0.45	5.73	1.49
89_6M72_day28_Dox	0.92	1.06	0.96	0.9
90_6M73_day28_Dox	1.28	0.92	1.43	0.87
115_7F155_day1_ValpA	0.45	1.42	0.4	1.22
116_7F156_day1_ValpA	0.42	1.1	0.31	0.01
117_7F157_day1_ValpA	0.37	1.1	0.32	0.57
121_7F158_day5_ValpA	0.36	1.33	0.38	0.79
122_7F159_day5_ValpA	0.02	1.21	0.36	0.66
123_7F160_day5_ValpA	0.53	1.37	0.28	0.66
127_7F161_day28_ValpA	0.45	1.17	0.28	0.68
128_7F162_day28_ValpA	0.58	1.55	0.32	1.12
129_7F163_day28_ValpA	0.42	1.32	0.28	1.23
112_7M55_day1_ValpA	0.4	0.92	0.39	0.88
113_7M56_day1_ValpA	0.5	1.28	0.33	0.83
114_7M57_day1_ValpA	0.48	0.84	0.42	0.72
118_7M58_day5_ValpA	0.37	0.99	0.15	0.92
119_7M59_day5_ValpA	0.36	0.86	0.25	0.47
120_7M60_day5_ValpA	0.29	0.91	0.18	0.42
124_7M61_day28_ValpA	0.21	1.05	0.15	0.62
125_7M62_day28_ValpA	0.45	0.81	0.36	0.86

Sample	AA686189_ DB71_seg6	AA799594_ DB71_seg0	AA964541_ DB71_seg0	AI045075_ DB71_seg6
126_7M63_day28_ValpA	0.4	1.23	0.25	0.59
62_8F181_day28_Naive	0.47	1.59	0.39	0.55
63_8F182_day28_Naive	0.6	1.23	0.37	0.51
64_8F183_day28_Naive	0.42	0.84	0.38	0.52
59_8M81_day28_Naive	0.74	1.46	0.68	0.7
60_8M82_day28_Naive	0.46	1.26	0.3	0.33
61_8M83_day28_Naive	0.45	1.18	0.34	0.53

Sample	AI454051_DB71_seg0	AW919147_DB71_seg0	BI293562_DB71_seg2
68_1F101_day1_Saline	0.96	0.9	0.61
69_1F102_day1_Saline	0.99	0.96	0.67
70_1F103_day1_Saline	0.88	1.03	0.68
74_1F104_day5_Saline	1.08	0.92	0.2
75_1F105_day5_Saline	0.93	0.9	0.41
109_1F107_day28_Saline	1.3	1.09	0.29
110_1F108_day28_Saline	1.33	1.12	0.37
111_1F109_day28_Saline	0.7	0.84	0.16
65_1M1_day1_Saline	0.91	0.9	1.45
66_1M2_day1_Saline	0.11	0.73	0.42
67_1M3_day1_Saline	1.13	1.05	0.7
71_1M4_day5_Saline	1.01	0.86	0.83
72_1M5_day5_Saline	0.73	1.17	0.4
73_1M6_day5_Saline	0.49	0.92	0.33
106_1M7_day28_Saline	0.86	1.3	0.63
107_1M8_day28_Saline	0.88	1.28	0.32
108_1M9_day28_Saline	1.16	1.7	0.87
16_2F110_day1_Gent	0.9	1.72	0.59
17_2F111_day1_Gent	0.75	0.72	0.94
18_2F112_day1_Gent	0.57	0.76	1.04
45_2F113_day5_Gent	0.63	0.86	0.95
46_2F114_day5_Gent	1.04	0.88	0.76
47_2F115_day5_Gent	1.19	0.95	2.8
91_2F116_day28_Gent	0.67	0.73	2.01
92_2F117_day28_Gent	1	1.12	1.19
93_2F118_day28_Gent	0.95	0.89	0.81
1_2M10_day1_Gent	0.4	0.72	0.93
2_2M11_day1_Gent	0.54	1.1	0.55
3_2M12_day1_Gent	0.79	0.99	0.41
31_2M13_day5_Gent	0.8	0.71	2.32
32_2M14_day5_Gent	0.63	0.79	1.61
33_2M15_day5_Gent	0.82	0.78	0.93
76_2M16_day28_Gent	1.14	0.75	0.63

Sample	AI454051_DB71_seg0	AW919147_DB71_seg0	BI293562_DB71_seg2
77_2M17_day28_Gent	0.64	0.57	0.72
78_2M18_day28_Gent	0.71	0.93	1.16
19_3F119_day1_Cis	0.94	0.8	0.74
20_3F120_day1_Cis	1.22	1.09	0.5
21_3F121_day1_Cis	1	0.87	0.38
48_3F122_day5_Cis	1.05	1.39	0.74
49_3F123_day5_Cis	1.43	1.05	0.67
50_3F124_day5_Cis	1.39	0.83	0.7
94_3F125_day28_Cis	0.68	1.08	5.98
95_3F126_day28_Cis	0.8	0.54	12.27
96_3F127_day28_Cis	0.87	0.72	8.01
4_3M19_day1_Cis	0.65	0.87	0.7
5_3M20_day1_Cis	0.96	1.21	0.39
6_3M21_day1_Cis	1.45	0.88	0.71
34_3M22_day5_Cis	0.92	1.08	3.45
35_3M23_day5_Cis	1.16	0.8	0.81
36_3M24_day5_Cis	0.86	0.88	1.76
79_3M25_day28_Cis	0.73	0.66	6.24
80_3M26_day28_Cis	0.82	0.84	2.93
81_3M27_day28_Cis	0.78	0.54	3.33
22_4F128_day1_Tob	1.16	0.8	0.29
23_4F129_day1_Tob	1.36	1.06	0.42
24_4F130_day1_Tob	0.8	0.98	0.73
51_4F131_day5_Tob	1.26	0.88	0.71
52_4F132_day5_Tob	1.23	0.99	1.62
53_4F133_day5_Tob	1.32	1.05	0.84
97_4F134_day28_Tob	0.94	0.8	1.32
98_4F135_day28_Tob	1.29	0.86	1.02
99_4F136_day28_Tob	0.72	0.72	0.35
7_4M28_day1_Tob	0.86	0.82	0.58
8_4M29_day1_Tob	1.29	1.33	1.38
9_4M30_day1_Tob	1.17	1.22	1.04
37_4M31_day5_Tob	0.61	0.58	0.3
38_4M32_day5_Tob	0.57	0.63	0.44
39_4M33_day5_Tob	0.63	1.14	0.59
82_4M34_day28_Tob	1	0.76	0.84
83_4M35_day28_Tob	0.94	1.23	1.78
84_4M36_day28_Tob	1	0.63	1.07
25_5F137_day1_CadCl	1.47	1	0.36
26_5F138_day1_CadCl	1.46	1.21	0.4
27_5F139_day1_CadCl	1.08	0.76	0.61
54_5F141_day5_CadCl	1.54	1.06	0.43
55_5F142_day5_CadCl	1.35	1.86	0.63

Sample	AI454051 DB71_seg0	AW919147 DB71_seg0	BI293562 DB71_seg2
100_5F143_day28_CadCl	0.79	0.77	0.4
101_5F144_day28_CadCl	1.05	0.91	0.81
102_5F145_day28_CadCl	0.54	0.42	2.59
10_5M37_day1_CadCl	1.48	1	0.5
11_5M38_day1_CadCl	2.13	1.1	0.51
12_5M39_day1_CadCl	0.77	1.02	0.39
40_5M40_day5_CadCl	0.84	1.3	0.48
41_5M41_day5_CadCl	0.68	0.94	0.88
42_5M42_day5_CadCl	0.92	1	0.78
85_5M43_day28_CadCl	1.29	1.31	0.72
86_5M44_day28_CadCl	1.31	1.18	1.35
87_5M45_day28_CadCl	0.75	0.68	0.31
28_6F146_day1_Dox	1.2	1.35	0.38
29_6F147_day1_Dox	1.05	1.2	0.39
30_6F148_day1_Dox	1.54	1.09	0.42
56_6F149_day5_Dox	1.17	0.99	0.68
57_6F150_day5_Dox	1.35	0.89	0.51
58_6F151_day5_Dox	1.44	1.1	0.7
103_6F171_day28_Dox	0.85	0.88	0.99
104_6F172_day28_Dox	0.92	1.05	0.58
105_6F173_day28_Dox	0.87	0.82	0
13_6M46_day1_Dox	0.8	1.1	0.53
14_6M47_day1_Dox	0.8	1.11	0.42
15_6M48_day1_Dox	1.03	0.91	0.32
43_6M49_day5_Dox	0.3	1.01	1.39
44_6M50_day5_Dox	0.95	1.16	1.04
88_6M71_day28_Dox	0.96	0.48	1.81
89_6M72_day28_Dox	1.11	1.42	0.55
90_6M73_day28_Dox	0.72	1.2	0.71
115_7F155_day1_ValpA	1.56	1.43	0.45
116_7F156_day1_ValpA	1.08	0.84	0.38
117_7F157_day1_ValpA	1.17	1.84	0.4
121_7F158_day5_ValpA	1.49	1.69	0.36
122_7F159_day5_ValpA	1.52	1.83	0.39
123_7F160_day5_ValpA	1.72	0.94	0.12
127_7F161_day28_ValpA	1.16	0.92	0.18
128_7F162_day28_ValpA	1.31	1.31	0.59
129_7F163_day28_ValpA	1.32	1.06	0.5
112_7M55_day1_ValpA	1.08	0.83	0.46
113_7M56_day1_ValpA	0.97	0.96	0.52
114_7M57_day1_ValpA	0.86	1.02	0.5
118_7M58_day5_ValpA	1	0.97	0.59
119_7M59_day5_ValpA	0.99	1.27	0.45

Sample	AI454051 DB71_seg0	AW919147 DB71_seg0	BI293562 DB71_seg2
120 7M60 day5 ValpA	0.81	0.79	0.62
124 7M61 day28 ValpA	0.91	1.12	0.21
125 7M62 day28 ValpA	1.16	1.16	0.31
126 7M63 day28 ValpA	0.95	1.11	0.35
62 8F181 day28 Naive	0.92	1.02	0.41
63 8F182 day28 Naive	1.07	1.08	0.49
64 8F183 day28 Naive	0.81	1.23	0.38
59 8M81 day28 Naive	1.75	1.27	0.72
60 8M82 day28 Naive	0.75	0.8	0.3
61 8M83 day28 Naive	0.66	0.94	0.39

Sample	H31045_ DB71_seg5	H31799_ DB71_seg23	H31883_ DB71_seg13	AI502869_ DB71_seg0
68 1F101 day1 Saline	0.16	1.05	0.88	0.98
69 1F102 day1 Saline	1.03	0.96	0.36	0.68
70 1F103 day1 Saline	0.24	1.06	0.75	0.73
74 1F104 day5 Saline	0.15	1.16	0.71	0.99
75 1F105 day5 Saline	0.67	0.7	0.59	0.67
109 1F107 day28 Saline	0.81	1.21	0.83	1.25
110 1F108 day28 Saline	1	1.13	0.89	2.1
111 1F109 day28 Saline	2.96	1.06	0.83	1.32
65 1M1 day1 Saline	0.93	1.12	0.68	0.62
66 1M2 day1 Saline	0.69	0.87	0.69	0.6
67 1M3 day1 Saline	0.19	1.06	0.78	0.62
71 1M4 day5 Saline	0.28	0.98	0.63	0.82
72 1M5 day5 Saline	0.18	1.11	0.56	0.86
73 1M6 day5 Saline	0.25	0.88	0.68	0.52
106 1M7 day28 Saline	0.42	0.82	0.88	1.24
107 1M8 day28 Saline	2.19	1.13	0.73	1.14
108 1M9 day28 Saline	0.36	1.06	0.84	1.31
16 2F110 day1 Gent	0.27	1.19	0.99	0.83
17 2F111 day1 Gent	0.22	0.88	0.92	0.61
18 2F112 day1 Gent	0.73	0.93	0.82	0.53
45 2F113 day5 Gent	0.52	0.92	0.73	0.73
46 2F114 day5 Gent	1.01	0.91	0.55	0.62
47 2F115 day5 Gent	0.4	1.36	0.91	0.96
91 2F116 day28 Gent	0.79	0.88	0.88	0.66
92 2F117 day28 Gent	0.84	1.05	0.98	0.74
93 2F118 day28 Gent	0.67	1.17	0.96	0.73
1 2M10 day1 Gent	0.16	1	0.82	1.53
2 2M11 day1 Gent	0.19	1.04	0.61	0.99
3 2M12 day1 Gent	0.15	1.2	0.73	0.76

Sample	H31045_ DB71_seg5	H31799_ DB71_seg23	H31883_ DB71_seg13	A1502869_ DB71_seg0
31 2M13 day5 Gent	1.57	1.08	1.01	0.51
32 2M14 day5 Gent	1.47	0.76	0.7	0.43
33 2M15 day5 Gent	0.33	0.88	0.65	0.53
76 2M16 day28 Gent	1.11	1.17	1.05	1.36
77 2M17 day28 Gent	4.65	0.37	0.64	0.92
78 2M18 day28 Gent	0.45	1.06	0.8	0.77
19 3F119 day1 Cis	0.24	1.03	2.03	0.67
20 3F120 day1 Cis	0.28	1.09	1.06	0.78
21 3F121 day1 Cis	0.22	1.04	1.07	0.74
48 3F122 day5 Cis	1.8	1.1	1.17	0.8
49 3F123 day5 Cis	0.34	0.74	1.44	0.93
50 3F124 day5 Cis	0.51	1.23	1.2	0.74
94 3F125 day28 Cis	0.73	1.05	2.72	1.03
95 3F126 day28 Cis	0.73	1.15	4.07	1.03
96 3F127 day28 Cis	0.81	0.98	3.83	1.25
4 3M19 day1 Cis	0.82	1.22	0.74	0.87
5 3M20 day1 Cis	0.21	1.27	0.71	0.87
6 3M21 day1 Cis	1.22	1.12	1.52	0.84
34 3M22 day5 Cis	3.25	0.92	1.73	0.52
35 3M23 day5 Cis	0.23	1.05	1.3	1.13
36 3M24 day5 Cis	1.66	1.06	2.43	0.61
79 3M25 day28 Cis	0.88	0.88	2.41	0.74
80 3M26 day28 Cis	2.92	1.21	3.37	1.35
81 3M27 day28 Cis	2.96	0.9	2.23	0.95
22 4F128 day1 Tob	0.2	0.91	0.87	0.52
23 4F129 day1 Tob	0.18	0.87	0.68	1.32
24 4F130 day1 Tob	0.74	1.2	0.87	0.79
51 4F131 day5 Tob	1.19	1.1	0.82	0.81
52 4F132 day5 Tob	0.42	0.92	0.69	0.74
53 4F133 day5 Tob	0.38	0.83	0.52	0.74
97 4F134 day28 Tob	0.76	1.03	0.84	1.08
98 4F135 day28 Tob	1	0.71	0.63	2.38
99 4F136 day28 Tob	0.63	1.15	0.84	1.04
7 4M28 day1 Tob	0.2	1.09	0.83	0.87
8 4M29 day1 Tob	1.03	1.14	0.69	1.02
9 4M30 day1 Tob	0.22	1.17	0.61	1.28
37 4M31 day5 Tob	0.9	0.71	0.48	0.42
38 4M32 day5 Tob	0.29	0.72	0.47	0.5
39 4M33 day5 Tob	1.65	0.96	0.82	0.72
82 4M34 day28 Tob	2.08	0.94	0.6	1.22
83 4M35 day28 Tob	2.68	0.88	0.73	0.99
84 4M36 day28 Tob	2.92	1.12	0.73	1.22
25 5F137 day1 CadCl	1.97	1	0.73	0.54

Sample	H31045_ DB71_seg5	H31799_ DB71_seg23	H31883_ DB71_seg13	AI502869_ DB71_seg0
26 5F138 day1 CadCl	0.39	1.24	0.63	1.37
27 5F139 day1 CadCl	0.39	1	0.87	0.56
54 5F141 day5 CadCl	0.56	0.83	0.87	0.94
55 5F142 day5 CadCl	0.32	0.99	0.86	0.73
100 5F143 day28 CadCl	2.88	1.59	0.97	0.73
101 5F144 day28 CadCl	0.83	0.94	1.02	1.22
102 5F145 day28 CadCl	0.57	0.67	0.86	0.44
10 5M37 day1 CadCl	0.19	0.91	0.65	1.14
11 5M38 day1 CadCl	0.19	0.9	0.71	2.59
12 5M39 day1 CadCl	0.18	1.15	0.78	0.86
40 5M40 day5 CadCl	0.42	1.17	0.83	0.69
41 5M41 day5 CadCl	0.4	0.98	0.78	0.68
42 5M42 day5 CadCl	0.46	0.94	0.64	0.85
85 5M43 day28 CadCl	2.23	1.05	0.95	2.47
86 5M44 day28 CadCl	2.41	1.02	0.8	2.93
87 5M45 day28 CadCl	2	0.76	0.86	1.18
28 6F146 day1 Dox	1.15	1.04	0.93	0.71
29 6F147 day1 Dox	0.3	0.99	0.89	0.81
30 6F148 day1 Dox	1.95	0.87	0.67	0.67
56 6F149 day5 Dox	1.86	0.95	1.16	0.51
57 6F150 day5 Dox	1.19	1.08	1.03	1
58 6F151 day5 Dox	0.52	0.66	0.9	1.07
103 6F171 day28 Dox	2.16	1.16	1.48	1.33
104 6F172 day28 Dox	2.58	1.25	1.77	1.48
105 6F173 day28 Dox	0.51	1.57	2.29	0.98
13 6M46 day1 Dox	0.23	1.07	1.02	0.77
14 6M47 day1 Dox	0.32	1.26	1.07	0.68
15 6M48 day1 Dox	0.75	0.92	0.9	0.78
43 6M49 day5 Dox	0.35	0.56	1.21	0.73
44 6M50 day5 Dox	1.41	0.9	1.38	0.71
88 6M71 day28 Dox	1.57	0.64	1.45	0.42
89 6M72 day28 Dox	2.33	1.11	1.59	1.49
90 6M73 day28 Dox	2.12	1.06	1.93	1.28
115 7F155 day1 ValpA	0.68	1.29	1.02	0.86
116 7F156 day1 ValpA	1.71	0.9	0.01	1.09
117 7F157 day1 ValpA	0.53	0	0.66	1.13
121 7F158 day5 ValpA	0.56	0.94	1.54	1.81
122 7F159 day5 ValpA	1	1.19	1.1	2.53
123 7F160 day5 ValpA	0.57	1.15	1.05	1.94
127 7F161 day28 ValpA	4.35	0.98	0.92	1.24
128 7F162 day28 ValpA	0.58	0.67	0.9	1.19
129 7F163 day28 ValpA	1.41	0.99	0.81	1.18
112 7M55 day1 ValpA	3.33	1.11	0.75	1.33

Sample	H31045_ DB71_seg5	H31799_ DB71_seg23	H31883_ DB71_seg13	AI502869_ DB71_seg0
113 7M56 day1 ValpA	2.68	1.23	0.9	1
114 7M57 day1 ValpA	0.73	1.16	0.95	1.43
118 7M58 day5 ValpA	2.42	0.51	1	1.37
119 7M59 day5 ValpA	0.83	1.06	0.48	1.06
120 7M60 day5 ValpA	2.53	1.02	0.63	1.29
124 7M61 day28 ValpA	1.58	0.98	0.71	0.97
125 7M62 day28 ValpA	0.2	1.03	1.11	1.46
126 7M63 day28 ValpA	0.47	0.75	0.72	1.43
62 8F181 day28 Naive	1.21	1.05	0.79	1.24
63 8F182 day28 Naive	0.25	0.94	0.73	0.78
64 8F183 day28 Naive	0.25	1	0.66	0.77
59 8M81 day28 Naive	0.54	1.26	0	1
60 8M82 day28 Naive	0.26	1.05	0.58	0.79
61 8M83 day28 Naive	0.29	0.82	0.68	0.64

Sample	H33998 DB71_seg19	RATLRFI DB71_seg9	RATRAB13X DB71_seg11-13
68 1F101 day1 Saline	0.73	0.37	0.97
69 1F102 day1 Saline	1.01	0.28	0.78
70 1F103 day1 Saline	0.88	0.09	0.98
74 1F104 day5 Saline	0.93	0.36	0.97
75 1F105 day5 Saline	1.1	0.49	0.94
109 1F107 day28 Saline	0.41	0.44	1
110 1F108 day28 Saline	0.59	0.37	1.11
111 1F109 day28 Saline	0.57	0.58	0.92
65 1M1 day1 Saline	0.51	0.01	0.83
66 1M2 day1 Saline	0.98	0.32	1.06
67 1M3 day1 Saline	0.51	0.36	0.87
71 1M4 day5 Saline	1.26	0.21	0.76
72 1M5 day5 Saline	1.13	0.25	0.76
73 1M6 day5 Saline	0.98	0.22	0.64
106 1M7 day28 Saline	0.6	0.32	0.82
107 1M8 day28 Saline	0.36	0.12	0.77
108 1M9 day28 Saline	0.73	0.43	0.91
16 2F110 day1 Gent	1.31	0.98	1.16
17 2F111 day1 Gent	0.83	1.54	0.7
18 2F112 day1 Gent	0.56	1.09	0.77
45 2F113 day5 Gent	1.9	0.48	0.9
46 2F114 day5 Gent	1.5	0.43	0.82
47 2F115 day5 Gent	2.19	4.15	1.12
91 2F116 day28 Gent	0.4	5.63	0.83
92 2F117 day28 Gent	0.7	4.17	1.12
93 2F118 day28 Gent	0.56	2.43	0.88

Sample	H33998 DB71_seg19	RATLRFI DB71_seg9	RATRAB13X DB71_seg11-13
1 2M10 day1 Gent	1.51	2.74	2.79
2 2M11 day1 Gent	1.35	1.72	1.14
3 2M12 day1 Gent	0.99	0.27	1.03
31 2M13 day5 Gent	1.91	1.35	0.9
32 2M14 day5 Gent	0.83	1.13	0.61
33 2M15 day5 Gent	1.16	0.59	0.8
76 2M16 day28 Gent	0.4	2.56	1.17
77 2M17 day28 Gent	0.49	1.59	0.76
78 2M18 day28 Gent	0.42	2.51	1.22
19 3F119 day1 Cis	0.85	1.49	0.91
20 3F120 day1 Cis	1	0.3	1.12
21 3F121 day1 Cis	0.87	0.57	1.09
48 3F122 day5 Cis	1.79	0.79	1.06
49 3F123 day5 Cis	1.84	0.44	1.01
50 3F124 day5 Cis	1.95	0.32	1.02
94 3F125 day28 Cis	0.55	2.33	1.29
95 3F126 day28 Cis	0.69	3.05	1.62
96 3F127 day28 Cis	0.85	3.8	1.78
4 3M19 day1 Cis	0.65	0.24	0.87
5 3M20 day1 Cis	0.75	0.2	1.15
6 3M21 day1 Cis	1.14	0.37	1.16
34 3M22 day5 Cis	1.49	0.65	1.13
35 3M23 day5 Cis	0.46	0.34	0.74
36 3M24 day5 Cis	2.14	1.97	1.27
79 3M25 day28 Cis	0.61	3.03	1.57
80 3M26 day28 Cis	0.62	2.4	1.24
81 3M27 day28 Cis	0.41	2.26	1.31
22 4F128 day1 Tob	0.75	1.03	1.11
23 4F129 day1 Tob	0.76	0.68	1.06
24 4F130 day1 Tob	0.77	3.08	1.22
51 4F131 day5 Tob	1.65	0.75	1.21
52 4F132 day5 Tob	1.88	3.08	0.98
53 4F133 day5 Tob	1.61	0.79	0.87
97 4F134 day28 Tob	0.99	1.94	1.25
98 4F135 day28 Tob	0.44	2.39	0.9
99 4F136 day28 Tob	0.74	1.98	1.29
7 4M28 day1 Tob	0.7	0.22	1.12
8 4M29 day1 Tob	0.98	1.63	1.01
9 4M30 day1 Tob	0.71	3.36	1.08
37 4M31 day5 Tob	1.1	0.88	0.59
38 4M32 day5 Tob	0.88	0.72	0.71
39 4M33 day5 Tob	1.3	0.17	0.81
82 4M34 day28 Tob	0.61	2.01	1.12

Sample	H33998 DB71_seg19	RATLRFI DB71_seg9	RATRAB13X DB71_seg11-13
83 4M35 day28 Tob	0.54	3.78	1.34
84 4M36 day28 Tob	0.69	2.37	1.31
25 5F137 day1 CadCl	2.29	0.27	0.95
26 5F138 day1 CadCl	2.51	0.33	1.2
27 5F139 day1 CadCl	0.01	0.32	0.95
54 5F141 day5 CadCl	2	0.35	0.95
55 5F142 day5 CadCl	0.82	0.31	0.9
100 5F143 day28 CadCl	1.26	0.66	1.37
101 5F144 day28 CadCl	0.59	0.85	0.92
102 5F145 day28 CadCl	0.43	1.78	0.96
10 5M37 day1 CadCl	0.76	0.27	1.05
11 5M38 day1 CadCl	0.66	0.36	1.22
12 5M39 day1 CadCl	0.69	0.23	1.14
40 5M40 day5 CadCl	1.39	0.17	0.75
41 5M41 day5 CadCl	1.36	0.18	0.75
42 5M42 day5 CadCl	1.64	0.29	0.62
85 5M43 day28 CadCl	0.45	0.28	1.11
86 5M44 day28 CadCl	0.26	0.67	1.08
87 5M45 day28 CadCl	0.41	0.56	0.88
28 6F146 day1 Dox	1.25	0.25	1.1
29 6F147 day1 Dox	1.34	0.21	0.9
30 6F148 day1 Dox	2.14	0.31	1
56 6F149 day5 Dox	2.39	0.46	1.17
57 6F150 day5 Dox	1.29	0.29	1.16
58 6F151 day5 Dox	3.68	0.18	0.94
103 6F171 day28 Dox	0.48	0.72	0.74
104 6F172 day28 Dox	0.58	0.4	0.95
105 6F173 day28 Dox	0.96	1.47	1.05
13 6M46 day1 Dox	0.97	0.46	0.88
14 6M47 day1 Dox	1.07	0.48	1.09
15 6M48 day1 Dox	2.75	0.24	0.86
43 6M49 day5 Dox	1.19	0.47	0.7
44 6M50 day5 Dox	1.37	0.25	0.83
88 6M71 day28 Dox	0.64	8.34	0.91
89 6M72 day28 Dox	0.44	0.53	0.77
90 6M73 day28 Dox	0.54	1.08	0.84
115 7F155 day1 ValpA	1.48	0.55	1.32
116 7F156 day1 ValpA	0.82	0.32	0.96
117 7F157 day1 ValpA	1.15	0.45	1.01
121 7F158 day5 ValpA	0.76	0.39	0.79
122 7F159 day5 ValpA	0.88	0.41	1.26
123 7F160 day5 ValpA	1.19	0.39	1.41
127 7F161 day28 ValpA	0.71	0.36	0.91

Sample	H33998_DB71_seg19	RATLRFI_DB71_seg9	RATRAB13X_DB71_seg11-13
128_7F162_day28_ValpA	0.56	0.54	0.91
129_7F163_day28_ValpA	0.58	0.53	0.96
112_7M55_day1_ValpA	0.37	0.39	0.79
113_7M56_day1_ValpA	0.77	0.36	0.93
114_7M57_day1_ValpA	0.53	0.44	0.83
118_7M58_day5_ValpA	1.36	0.22	0.67
119_7M59_day5_ValpA	1.11	0.21	0.8
120_7M60_day5_ValpA	1.03	0.3	0.65
124_7M61_day28_ValpA	0.69	0.23	0.88
125_7M62_day28_ValpA	0.78	0.48	0.99
126_7M63_day28_ValpA	0.52	0.33	0.69
62_8F181_day28_Naive	0.69	0.39	1.07
63_8F182_day28_Naive	1.23	0.45	1.07
64_8F183_day28_Naive	0.85	0.34	0.79
59_8M81_day28_Naive	1.25	0.42	1.08
60_8M82_day28_Naive	0.99	0.25	0.66
61_8M83_day28_Naive	0	0.26	0.77

Sample	RNU24174_DB71_seg8	W41270_DB81_seg11
68_1F101_day1_Saline	0.57	0.6
69_1F102_day1_Saline	0.41	0.86
70_1F103_day1_Saline	0.32	1.31
74_1F104_day5_Saline	0.26	0.59
75_1F105_day5_Saline	0.28	0.83
109_1F107_day28_Saline	0.33	0.36
110_1F108_day28_Saline	0.24	0.41
111_1F109_day28_Saline	0.33	0.39
65_1M1_day1_Saline	0.24	0.57
66_1M2_day1_Saline	0.15	0.99
67_1M3_day1_Saline	0.29	0.54
71_1M4_day5_Saline	0.15	0.68
72_1M5_day5_Saline	0.33	1
73_1M6_day5_Saline	0.12	0.46
106_1M7_day28_Saline	0.24	0.33
107_1M8_day28_Saline	0.15	0.26
108_1M9_day28_Saline	0.27	0.33
16_2F110_day1_Gent	0.47	1.24
17_2F111_day1_Gent	0.5	0.67
18_2F112_day1_Gent	0.47	1.19
45_2F113_day5_Gent	0.31	2.04
46_2F114_day5_Gent	0.22	1.34

Sample	RNU24174_DB71_seg8	W41270_DB81_seg11
47_2F115_day5_Gent	0.81	2.42
91_2F116_day28_Gent	1.53	0.94
92_2F117_day28_Gent	1.2	0.81
93_2F118_day28_Gent	0.59	0.81
1_2M10_day1_Gent	0.74	0.46
2_2M11_day1_Gent	0.37	0.68
3_2M12_day1_Gent	0.26	0.57
31_2M13_day5_Gent	0.65	2.03
32_2M14_day5_Gent	0.36	1.08
33_2M15_day5_Gent	0.4	1.2
76_2M16_day28_Gent	1.91	1.42
77_2M17_day28_Gent	0.69	0.75
78_2M18_day28_Gent	0.65	0.84
19_3F119_day1_Cis	0.57	1.11
20_3F120_day1_Cis	0.32	0.86
21_3F121_day1_Cis	0.36	0.76
48_3F122_day5_Cis	0.97	1.97
49_3F123_day5_Cis	0.7	1.9
50_3F124_day5_Cis	0.6	1.79
94_3F125_day28_Cis	8.38	1.45
95_3F126_day28_Cis	12.63	1.8
96_3F127_day28_Cis	12.14	1.48
4_3M19_day1_Cis	0.2	0.33
5_3M20_day1_Cis	0.27	0.63
6_3M21_day1_Cis	0.69	0.71
34_3M22_day5_Cis	1.3	1.51
35_3M23_day5_Cis	0.31	1.07
36_3M24_day5_Cis	3.6	3.12
79_3M25_day28_Cis	10.21	1.94
80_3M26_day28_Cis	7.3	1.11
81_3M27_day28_Cis	8.08	1.45
22_4F128_day1_Tob	0.29	1.01
23_4F129_day1_Tob	0.32	0.44
24_4F130_day1_Tob	0.53	0.83
51_4F131_day5_Tob	0.46	2.6
52_4F132_day5_Tob	0.76	3.25
53_4F133_day5_Tob	0.47	2.67
97_4F134_day28_Tob	0.55	1.01
98_4F135_day28_Tob	0.65	0.73
99_4F136_day28_Tob	0.69	0.9
7_4M28_day1_Tob	0.29	0.57

Sample	RNU24174_DB71_seg8	W41270_DB81_seg11
8_4M29_day1_Tob	0.49	0.79
9_4M30_day1_Tob	0.51	0.76
37_4M31_day5_Tob	0.09	0.62
38_4M32_day5_Tob	0.2	0.81
39_4M33_day5_Tob	0.14	1.21
82_4M34_day28_Tob	0.54	0.92
83_4M35_day28_Tob	0.93	1.03
84_4M36_day28_Tob	0.62	0.79
25_5F137_day1_CadCl	0.3	1.44
26_5F138_day1_CadCl	0.29	1.78
27_5F139_day1_CadCl	0.49	1.48
54_5F141_day5_CadCl	0.33	2.49
55_5F142_day5_CadCl	0.27	1.17
100_5F143_day28_CadCl	0.21	0.86
101_5F144_day28_CadCl	0.45	0.55
102_5F145_day28_CadCl	1.21	0.83
10_5M37_day1_CadCl	0.2	0.5
11_5M38_day1_CadCl	0.4	0.62
12_5M39_day1_CadCl	0.22	0.43
40_5M40_day5_CadCl	0.12	1.02
41_5M41_day5_CadCl	0.13	1.43
42_5M42_day5_CadCl	0.12	1.52
85_5M43_day28_CadCl	0.25	0.33
86_5M44_day28_CadCl	0.39	0.27
87_5M45_day28_CadCl	0.25	0.39
28_6F146_day1_Dox	0.29	2.24
29_6F147_day1_Dox	0.35	1.2
30_6F148_day1_Dox	0.31	1.56
56_6F149_day5_Dox	0.83	1.71
57_6F150_day5_Dox	0.59	1.37
58_6F151_day5_Dox	0.63	2.13
103_6F171_day28_Dox	1.99	0.84
104_6F172_day28_Dox	2.06	0.44
105_6F173_day28_Dox	4.39	0.77
13_6M46_day1_Dox	0.48	1.74
14_6M47_day1_Dox	0.49	0.95
15_6M48_day1_Dox	0.36	0.91
43_6M49_day5_Dox	1.08	1.64
44_6M50_day5_Dox	0.87	1.35
88_6M71_day28_Dox	3.5	1.64
89_6M72_day28_Dox	2.2	0.54

Sample	RNU24174_DB71_seg8	W41270_DB81_seg11
90_6M73_day28_Dox	2.91	0.5
115_7F155_day1_ValpA	0.45	0.87
116_7F156_day1_ValpA	0.28	0.42
117_7F157_day1_ValpA	0.28	0.64
121_7F158_day5_ValpA	0.35	0.54
122_7F159_day5_ValpA	0.51	0.55
123_7F160_day5_ValpA	0.36	0.37
127_7F161_day28_ValpA	0.23	0.65
128_7F162_day28_ValpA	0.28	0.3
129_7F163_day28_ValpA	0.29	0.4
112_7M55_day1_ValpA	0.16	0.57
113_7M56_day1_ValpA	0.19	0.49
114_7M57_day1_ValpA	0.25	0.57
118_7M58_day5_ValpA	0.2	0.7
119_7M59_day5_ValpA	0.14	0.86
120_7M60_day5_ValpA	0.17	0.62
124_7M61_day28_ValpA	0.09	0.39
125_7M62_day28_ValpA	0.19	0.42
126_7M63_day28_ValpA	0.09	0.36
62_8F181_day28_Naive	0.26	0.62
63_8F182_day28_Naive	0.36	0.01
64_8F183_day28_Naive	0.37	0.83
59_8M81_day28_Naive	0.2	1.24
60_8M82_day28_Naive	0.15	0.59
61_8M83_day28_Naive	0.24	0.37

Sample	W64472_DB81_seg2	W83813_DB81_seg27	MUSCYCG1R_DB81_seg15-17
68_1F101_day1_Saline	0.45	0.94	0.67
69_1F102_day1_Saline	0.35	0.77	0.94
70_1F103_day1_Saline	0.43	1.03	0.6
74_1F104_day5_Saline	0.35	1.03	0.76
75_1F105_day5_Saline	0.56	0.99	0.75
109_1F107_day28_Saline	0.24	0.48	0.57
110_1F108_day28_Saline	0.38	0.66	0.76
111_1F109_day28_Saline	0.61	0.67	0.7
65_1M1_day1_Saline	0.65	0.68	0.47
66_1M2_day1_Saline	0.56	0.87	0.41
67_1M3_day1_Saline	1.02	0.94	0.4
71_1M4_day5_Saline	1.02	1.29	0.86
72_1M5_day5_Saline	0.75	1.2	0.92
73_1M6_day5_Saline	0.62	1.07	0.54

Sample	W64472_DB81_seg2	W83813_DB81_seg27	MUSCYCG1R_DB81_seg15-17
106 1M7 day28 Saline	0.4	0.5	0.41
107 1M8 day28 Saline	0.23	0.38	0.45
108 1M9 day28 Saline	0.53	0.43	0.43
16 2F110 day1 Gent	0.63	0.84	0.63
17 2F111 day1 Gent	0.45	0.79	0.53
18 2F112 day1 Gent	0.61	0.78	0.66
45 2F113 day5 Gent	1.5	2.07	1.51
46 2F114 day5 Gent	1.2	1.82	1.61
47 2F115 day5 Gent	0.94	1.7	1.51
91 2F116 day28 Gent	0.92	0.45	0.69
92 2F117 day28 Gent	0.66	0.34	0.62
93 2F118 day28 Gent	0.54	0.62	0.57
1 2M10 day1 Gent	0.4	0.53	0.5
2 2M11 day1 Gent	0.54	0.58	0.49
3 2M12 day1 Gent	0.54	0.71	0.41
31 2M13 day5 Gent	1.95	1.35	0.95
32 2M14 day5 Gent	1.62	0.88	0.79
33 2M15 day5 Gent	1.61	1.4	1.02
76 2M16 day28 Gent	1.49	0.78	0.65
77 2M17 day28 Gent	0.84	0.54	0.56
78 2M18 day28 Gent	0.81	0.38	0.54
19 3F119 day1 Cis	0.8	1.1	0.77
20 3F120 day1 Cis	0.54	0.97	0.85
21 3F121 day1 Cis	0.55	0.99	0.72
48 3F122 day5 Cis	2.23	2.12	3.12
49 3F123 day5 Cis	2.1	2.25	3.59
50 3F124 day5 Cis	1.99	2.12	2.07
94 3F125 day28 Cis	1.81	0.71	1.96
95 3F126 day28 Cis	3.16	0.7	2.57
96 3F127 day28 Cis	2.87	0.78	2.7
4 3M19 day1 Cis	0.3	0.5	0.54
5 3M20 day1 Cis	0.51	0.66	0.35
6 3M21 day1 Cis	0.92	0.94	0.67
34 3M22 day5 Cis	2.64	1.39	2.31
35 3M23 day5 Cis	0.95	0.94	0.92
36 3M24 day5 Cis	4.55	1.41	2.34
79 3M25 day28 Cis	2.8	0.51	2.09
80 3M26 day28 Cis	3.04	0.51	2.31
81 3M27 day28 Cis	1.65	0.57	1.93
22 4F128 day1 Tob	0.35	0.86	0.72
23 4F129 day1 Tob	0.26	0.61	0.46
24 4F130 day1 Tob	0.49	1	0.61
51 4F131 day5 Tob	1.4	2.61	1.95

Sample	W64472_DB81_seg2	W83813_DB81_seg27	MUSCYCG1R_DB81_seg15-17
52 4F132 day5 Tob	1.55	1.93	1.44
53 4F133 day5 Tob	1.27	1.76	1.15
97 4F134 day28 Tob	0.48	0.75	0.77
98 4F135 day28 Tob	0.43	0.49	0.59
99 4F136 day28 Tob	0.62	0.63	0.73
7 4M28 day1 Tob	0.45	0.65	0.43
8 4M29 day1 Tob	0.66	0.73	0.49
9 4M30 day1 Tob	0.44	0.48	0.47
37 4M31 day5 Tob	0.93	0.7	0.4
38 4M32 day5 Tob	0.96	1.13	0.69
39 4M33 day5 Tob	1.3	1.4	1.07
82 4M34 day28 Tob	0.44	0.48	0.72
83 4M35 day28 Tob	0.85	0.56	0.59
84 4M36 day28 Tob	0.79	0.55	0.71
25 5F137 day1 CadCl	1.07	2.07	1.91
26 5F138 day1 CadCl	1.07	2.19	1.36
27 5F139 day1 CadCl	1.1	1.66	1.68
54 5F141 day5 CadCl	1.65	3.79	1.72
55 5F142 day5 CadCl	0.78	1.59	1.13
100 5F143 day28 CadCl	0.89	0.95	0.88
101 5F144 day28 CadCl	0.55	0.64	0.71
102 5F145 day28 CadCl	0.43	0.46	0.65
10 5M37 day1 CadCl	0.55	0.47	0.61
11 5M38 day1 CadCl	1.45	0.51	0.73
12 5M39 day1 CadCl	0.41	0.65	0.36
40 5M40 day5 CadCl	2.23	1.48	1.2
41 5M41 day5 CadCl	1.89	1.67	0.97
42 5M42 day5 CadCl	1.63	1.76	1.15
85 5M43 day28 CadCl	0.67	0.46	0.72
86 5M44 day28 CadCl	0.47	0.34	0.44
87 5M45 day28 CadCl	0.57	0.53	0.58
28 6F146 day1 Dox	1.58	2.9	1.66
29 6F147 day1 Dox	1.09	1.56	1.12
30 6F148 day1 Dox	1.15	2	1.36
56 6F149 day5 Dox	1.97	2.28	1.51
57 6F150 day5 Dox	1.36	1.99	1.97
58 6F151 day5 Dox	2.36	2.9	3.17
103 6F171 day28 Dox	0.8	0.58	1.07
104 6F172 day28 Dox	0.69	0.61	1.52
105 6F173 day28 Dox	1.73	0.63	1.26
13 6M46 day1 Dox	0.86	0.68	0.56
14 6M47 day1 Dox	0.77	0.78	0.64
15 6M48 day1 Dox	0.56	0.64	0.66

Sample	W64472_DB81_seg2	W83813_DB81_seg27	MUSCYCG1R_DB81_seg15-17
43 6M49 day5 Dox	3	1.3	1.65
44 6M50 day5 Dox	2.54	1.41	2
88 6M71 day28 Dox	1.71	0.51	0.99
89 6M72 day28 Dox	0.83	0.55	0.98
90 6M73 day28 Dox	1.15	0.51	1.28
115 7F155 day1 ValpA	0.77	0.98	0.86
116 7F156 day1 ValpA	0.36	0.52	0.61
117 7F157 day1 ValpA	0.47	0.74	0.8
121 7F158 day5 ValpA	0.49	0.59	0.94
122 7F159 day5 ValpA	0.57	0.55	0.77
123 7F160 day5 ValpA	0.33	0.74	1.07
127 7F161 day28 ValpA	0.47	0.69	0.89
128 7F162 day28 ValpA	0.2	0.41	0.61
129 7F163 day28 ValpA	0.24	0.58	0.68
112 7M55 day1 ValpA	0.85	0.82	0.67
113 7M56 day1 ValpA	0.73	0.64	0.42
114 7M57 day1 ValpA	0.44	0.53	0.51
118 7M58 day5 ValpA	0.99	0.68	0.8
119 7M59 day5 ValpA	0.83	1.11	1.1
120 7M60 day5 ValpA	0.93	0.93	1.03
124 7M61 day28 ValpA	0.66	0.52	0.77
125 7M62 day28 ValpA	0.55	0.65	0.71
126 7M63 day28 ValpA	0.24	0.39	0.39
62 8F181 day28 Naive	0.47	1.03	0.68
63 8F182 day28 Naive	0.57	1.24	0.65
64 8F183 day28 Naive	0.46	1.03	0.58
59 8M81 day28 Naive	1.26	2	1.31
60 8M82 day28 Naive	0.69	0.94	0.83
61 8M83 day28 Naive	0.39	0.58	0.45

Sample	MUSCYCG1R_DB81_seg19-20	W33294_DB81_seg23	W33294_DB81_seg44
68 1F101 day1 Saline	0.74	0.78	0.8
69 1F102 day1 Saline	0.84	0.65	0.96
70 1F103 day1 Saline	0.64	0.8	0.72
74 1F104 day5 Saline	1.28	0.86	0.65
75 1F105 day5 Saline	0.75	0.97	0.88
109 1F107 day28 Saline	0.57	1	0.88
110 1F108 day28 Saline	0.5	0.79	0.59
111 1F109 day28 Saline	0.79	1.24	1
65 1M1 day1 Saline	0.44	0.69	0.64
66 1M2 day1 Saline	0.43	0.3	0.69
67 1M3 day1 Saline	0.36	0.46	0.53
71 1M4 day5 Saline	0.87	0.78	1.69

Sample	MUSCYCG1R_DB81_seg19-20	W33294_DB81_seg23	W33294_DB81_seg44
72 1M5 day5 Saline	0.85	1.18	1
73 1M6 day5 Saline	0.78	0.9	0.86
106 1M7 day28 Saline	0.44	0.61	0.63
107 1M8 day28 Saline	0.34	0.62	0.48
108 1M9 day28 Saline	0.51	0.84	0.55
16 2F110 day1 Gent	0.67	1	1.02
17 2F111 day1 Gent	0.55	0.51	0.8
18 2F112 day1 Gent	0.44	0.94	0.77
45 2F113 day5 Gent	1.23	1.64	1.64
46 2F114 day5 Gent	1.41	1.96	1.72
47 2F115 day5 Gent	1.42	1.51	2.13
91 2F116 day28 Gent	0.56	0.64	0.66
92 2F117 day28 Gent	0.74	1.3	0.71
93 2F118 day28 Gent	0.42	0.85	1.04
1 2M10 day1 Gent	1.7	0.57	0.77
2 2M11 day1 Gent	0.48	0.74	0.5
3 2M12 day1 Gent	0.92	0.61	0.64
31 2M13 day5 Gent	1.43	1.25	1.12
32 2M14 day5 Gent	1.04	0.82	0.93
33 2M15 day5 Gent	1	0.95	0.94
76 2M16 day28 Gent	0.79	0.57	0.42
77 2M17 day28 Gent	0.4	0.8	0.85
78 2M18 day28 Gent	0.55	0.67	0.69
19 3F119 day1 Cis	0.86	0.36	1
20 3F120 day1 Cis	0.81	0.28	0.95
21 3F121 day1 Cis	0.81	1.11	1.04
48 3F122 day5 Cis	2.77	2.03	2.31
49 3F123 day5 Cis	3.44	1.99	2.19
50 3F124 day5 Cis	2.45	1.58	2.15
94 3F125 day28 Cis	1.64	0.95	0.35
95 3F126 day28 Cis	2.47	0.97	0.67
96 3F127 day28 Cis	1.67	0.82	0.73
4 3M19 day1 Cis	0.85	0.59	0.6
5 3M20 day1 Cis	0.71	0.49	0.84
6 3M21 day1 Cis	1.18	0.64	0.96
34 3M22 day5 Cis	1.83	0.84	1.47
35 3M23 day5 Cis	1.47	0.59	1.16
36 3M24 day5 Cis	2.33	1.34	1.51
79 3M25 day28 Cis	2.13	0.95	0.83
80 3M26 day28 Cis	1.97	0.82	0.89
81 3M27 day28 Cis	2.21	0.88	0.83
22 4F128 day1 Tob	0.73	0.85	0.98
23 4F129 day1 Tob	0.63	0.56	0.57

Sample	MUSCYCG1R_DB81_seg19-20	W33294_DB81_seg23	W33294_DB81_seg44
24 4F130 day1 Tob	0.58	0.59	0.62
51 4F131 day5 Tob	1.42	2.16	2.01
52 4F132 day5 Tob	1.31	2.37	2.06
53 4F133 day5 Tob	1.2	1.2	1.72
97 4F134 day28 Tob	0.45	0.91	0.95
98 4F135 day28 Tob	0.62	1.16	0.75
99 4F136 day28 Tob	0.58	0.91	0.7
7 4M28 day1 Tob	0.72	0.52	0.78
8 4M29 day1 Tob	0.84	0.91	0.88
9 4M30 day1 Tob	0.63	0.35	0.46
37 4M31 day5 Tob	0.72	0.61	0.48
38 4M32 day5 Tob	0.72	0.81	0.69
39 4M33 day5 Tob	1.53	1.25	1.27
82 4M34 day28 Tob	0.49	1.02	0.75
83 4M35 day28 Tob	0.51	0.78	0.86
84 4M36 day28 Tob	0.6	0.73	0.86
25 5F137 day1 CadCl	2.22	1.9	1.79
26 5F138 day1 CadCl	1	1.74	1.39
27 5F139 day1 CadCl	1.68	1.36	1.57
54 5F141 day5 CadCl	1.21	1.76	2.05
55 5F142 day5 CadCl	1.01	1.7	1.37
100 5F143 day28 CadCl	0.54	0.94	0.88
101 5F144 day28 CadCl	0.62	0.97	0.69
102 5F145 day28 CadCl	0.56	0.85	0.7
10 5M37 day1 CadCl	0.58	0.62	0.63
11 5M38 day1 CadCl	1.03	0.31	0.26
12 5M39 day1 CadCl	0.78	0.37	0.63
40 5M40 day5 CadCl	1.22	1.41	1.32
41 5M41 day5 CadCl	1.12	1.45	1.43
42 5M42 day5 CadCl	1.04	1.03	1.42
85 5M43 day28 CadCl	0.31	0.7	0.73
86 5M44 day28 CadCl	0.75	0.65	0.53
87 5M45 day28 CadCl	0.44	0.65	0.53
28 6F146 day1 Dox	1.17	2.47	2.1
29 6F147 day1 Dox	1.12	1.04	1.3
30 6F148 day1 Dox	1.16	1.36	2.02
56 6F149 day5 Dox	1.94	1.06	1.19
57 6F150 day5 Dox	1.79	2.06	1.76
58 6F151 day5 Dox	3.43	2.03	2.76
103 6F171 day28 Dox	1.24	0.82	0.87
104 6F172 day28 Dox	1.29	0.97	1.09
105 6F173 day28 Dox	0.93	0.62	0.65
13 6M46 day1 Dox	0.98	0.74	0.74

Sample	MUSCYCG1R_DB81_seg19-20	W33294_DB81_seg23	W33294_DB81_seg44
14_6M47_day1_Dox	1.04	0.61	1.01
15_6M48_day1_Dox	0.95	0.82	0.83
43_6M49_day5_Dox	1.7	1.25	1
44_6M50_day5_Dox	1.66	1.06	0.95
88_6M71_day28_Dox	0.88	0.96	0.45
89_6M72_day28_Dox	1.33	1	0.77
90_6M73_day28_Dox	1.26	0.82	0.66
115_7F155_day1_ValpA	0.68	1.67	0.86
116_7F156_day1_ValpA	0.43	0.84	0.72
117_7F157_day1_ValpA	0.77	1.21	1.19
121_7F158_day5_ValpA	0.6	1.14	1.06
122_7F159_day5_ValpA	0.45	1.13	0.85
123_7F160_day5_ValpA	1.11	1.24	0.79
127_7F161_day28_ValpA	0.55	0.98	0.61
128_7F162_day28_ValpA	0.62	0.89	0.74
129_7F163_day28_ValpA	0.5	1.16	0.95
112_7M55_day1_ValpA	0.63	1.32	1.01
113_7M56_day1_ValpA	0.53	0.94	0.7
114_7M57_day1_ValpA	0.44	0.92	0.69
118_7M58_day5_ValpA	0.88	1.09	1.66
119_7M59_day5_ValpA	1.1	1.32	1.33
120_7M60_day5_ValpA	1.08	1.01	1.31
124_7M61_day28_ValpA	0.56	0.52	0.75
125_7M62_day28_ValpA	0.44	0.85	0.85
126_7M63_day28_ValpA	0.33	0.88	0.34
62_8F181_day28_Naive	0.55	0.94	0.73
63_8F182_day28_Naive	0.7	1.02	0.76
64_8F183_day28_Naive	0.79	0.82	0.77
59_8M81_day28_Naive	1.24	1.65	2.01
60_8M82_day28_Naive	0.86	0.85	1.12
61_8M83_day28_Naive	0.44	0.79	0.39

Sample	RATRAB13X_DB81_seg15-17	RATRAB13X_DB81_seg22	MMU09507_DB81_seg15
68_1F101_day1_Saline	0.7	1.29	1.03
69_1F102_day1_Saline	0.59	0.81	1.13
70_1F103_day1_Saline	0.68	0.66	0.51
74_1F104_day5_Saline	0.64	0.82	0.42
75_1F105_day5_Saline	0.77	1.04	0.01
109_1F107_day28_Saline	1.08	0.69	0.67
110_1F108_day28_Saline	0.95	0.61	0.2
111_1F109_day28_Saline	1.03	0.97	0.33
65_1M1_day1_Saline	0.48	1	0.14
66_1M2_day1_Saline	0.55	0.65	0.24

Sample	RATRAB13X_DB81_seg 15-17	RATRAB13X_DB81 seg22	MMU09507_DB81 seg15
67_1M3_day1_Saline	0.48	0.44	0.24
71_1M4_day5_Saline	1.11	0.86	1
72_1M5_day5_Saline	1.02	1.06	0.37
73_1M6_day5_Saline	0.78	0.75	0.25
106_1M7_day28_Saline	0.58	0.68	0.23
107_1M8_day28_Saline	0.5	0.51	0.17
108_1M9_day28_Saline	0.7	0.8	0.19
16_2F110_day1_Gent	0.9	0.98	0.41
17_2F111_day1_Gent	0.63	0.71	0.43
18_2F112_day1_Gent	0.55	0.51	0.23
45_2F113_day5_Gent	1.68	2.16	0.78
46_2F114_day5_Gent	1.5	2.11	0.62
47_2F115_day5_Gent	1.59	1.91	1.13
91_2F116_day28_Gent	0.84	0.68	1.38
92_2F117_day28_Gent	0.9	0.56	0.96
93_2F118_day28_Gent	0.88	0.57	0.7
1_2M10_day1_Gent	0	0.8	0.56
2_2M11_day1_Gent	1.16	0.99	0.32
3_2M12_day1_Gent	0.82	0.6	0.37
31_2M13_day5_Gent	1.25	1.25	0.71
32_2M14_day5_Gent	0.76	0.77	0.41
33_2M15_day5_Gent	1.35	1.16	0.68
76_2M16_day28_Gent	0.77	0.91	0.82
77_2M17_day28_Gent	0.76	0.88	0.59
78_2M18_day28_Gent	1.22	0.58	0.84
19_3F119_day1_Cis	0.82	0.88	0
20_3F120_day1_Cis	1.11	0.87	0.44
21_3F121_day1_Cis	1.1	1.02	0.52
48_3F122_day5_Cis	1.37	1.71	1.96
49_3F123_day5_Cis	1.53	1.92	2.32
50_3F124_day5_Cis	1.53	1.99	1.14
94_3F125_day28_Cis	1.33	0.61	8.1
95_3F126_day28_Cis	1.27	0.65	6.58
96_3F127_day28_Cis	1.21	0.89	5.53
4_3M19_day1_Cis	0.73	0.65	0.35
5_3M20_day1_Cis	0.84	0.66	0.19
6_3M21_day1_Cis	0.98	1.11	0.5
34_3M22_day5_Cis	1.8	1.53	2.07
35_3M23_day5_Cis	1.03	1.2	0.57
36_3M24_day5_Cis	1.77	1.28	3.87
79_3M25_day28_Cis	1.08	0.66	8.05
80_3M26_day28_Cis	0.95	0.76	2.96
81_3M27_day28_Cis	1.28	0.65	5.45

Sample	RATRAB13X_DB81_seg 15-17	RATRAB13X_DB81 seg22	MMU09507_DB81 seg15
22_4F128_day1_Tob	1.02	0.41	0.35
23_4F129_day1_Tob	0.77	0.71	0.3
24_4F130_day1_Tob	0.87	0.78	0.22
51_4F131_day5_Tob	1.71	2.49	1.04
52_4F132_day5_Tob	1.44	2.11	0.99
53_4F133_day5_Tob	1.23	1.57	0.92
97_4F134_day28_Tob	1.28	0.88	0.84
98_4F135_day28_Tob	1.01	0.63	0.52
99_4F136_day28_Tob	0.73	0.88	0.85
7_4M28_day1_Tob	0.78	0.82	0.34
8_4M29_day1_Tob	0.98	0.95	0.7
9_4M30_day1_Tob	0.91	0.82	0.56
37_4M31_day5_Tob	0.75	0.68	0.17
38_4M32_day5_Tob	1.04	0.81	0.28
39_4M33_day5_Tob	1.32	1.26	0.27
82_4M34_day28_Tob	0.93	0.67	0.66
83_4M35_day28_Tob	1.05	0.8	0.97
84_4M36_day28_Tob	0.94	0.62	0.68
25_5F137_day1_CadCl	1.63	1.73	0.81
26_5F138_day1_CadCl	1.8	1.83	0.42
27_5F139_day1_CadCl	1.55	1.16	1.1
54_5F141_day5_CadCl	1.81	1.56	0.87
55_5F142_day5_CadCl	0.97	1.1	0.56
100_5F143_day28_CadCl	0.83	0.7	0.33
101_5F144_day28_CadCl	0.89	0.9	0.66
102_5F145_day28_CadCl	0.82	0.46	1.58
10_5M37_day1_CadCl	0.73	0.92	0.15
11_5M38_day1_CadCl	0.92	1.29	0.32
12_5M39_day1_CadCl	1.01	0.67	0.33
40_5M40_day5_CadCl	1.18	1.65	0.33
41_5M41_day5_CadCl	0.88	1.58	0.49
42_5M42_day5_CadCl	1.07	1.21	0.38
85_5M43_day28_CadCl	1.43	0.63	0.42
86_5M44_day28_CadCl	0.8	0.87	0.45
87_5M45_day28_CadCl	0.64	0.49	0.42
28_6F146_day1_Dox	0.99	2.07	0.48
29_6F147_day1_Dox	1.04	0.89	0.52
30_6F148_day1_Dox	1.32	1.66	0.93
56_6F149_day5_Dox	1.85	1.95	2.07
57_6F150_day5_Dox	1.51	1.89	1.46
58_6F151_day5_Dox	1.9	3.3	1.82
103_6F171_day28_Dox	0.66	0.54	4.47
104_6F172_day28_Dox	0.69	1.05	2.99

Sample	RATRAB13X_DB81_seg 15-17	RATRAB13X_DB81 seg22	MMU09507_DB81 seg15
105_6F173_day28_Dox	0.91	0.63	2.89
13_6M46_day1_Dox	0.88	0.69	0.74
14_6M47_day1_Dox	1.15	0.58	0.71
15_6M48_day1_Dox	0.75	0.77	0.59
43_6M49_day5_Dox	0.96	1.06	1.74
44_6M50_day5_Dox	1.2	1.32	1.67
88_6M71_day28_Dox	0.54	0.37	3.04
89_6M72_day28_Dox	0.78	0.64	2.93
90_6M73_day28_Dox	1.02	0.45	2.13
115_7F155_day1_ValpA	1.28	1.05	0.48
116_7F156_day1_ValpA	0.7	0.56	0.34
117_7F157_day1_ValpA	0.82	0.87	0.27
121_7F158_day5_ValpA	0.7	1.07	0.62
122_7F159_day5_ValpA	0.91	0.78	0.31
123_7F160_day5_ValpA	1.47	1.13	0.42
127_7F161_day28_ValpA	0.73	0.71	0.23
128_7F162_day28_ValpA	0.82	0.85	0.45
129_7F163_day28_ValpA	0.44	0.74	0.24
112_7M55_day1_ValpA	0.79	1.45	0.2
113_7M56_day1_ValpA	0.7	0.86	0.2
114_7M57_day1_ValpA	0.75	0.63	0.29
118_7M58_day5_ValpA	1.38	0.95	0.26
119_7M59_day5_ValpA	1.14	1.14	0.3
120_7M60_day5_ValpA	1.45	1.1	0.24
124_7M61_day28_ValpA	0.59	0.96	0.12
125_7M62_day28_ValpA	0.77	0.75	0.11
126_7M63_day28_ValpA	0.49	0.62	0.21
62_8F181_day28_Naive	0.68	0.9	0.5
63_8F182_day28_Naive	0.77	0.84	0.6
64_8F183_day28_Naive	0.68	0.88	0.73
59_8M81_day28_Naive	1.77	1.46	0.47
60_8M82_day28_Naive	0.83	0.8	0.8
61_8M83_day28_Naive	0.54	0.53	0.43

EXAMPLE 3: Validation analysis of the selected markers

5 To validate the optimal signature for classification of renal toxicity of a compound after five days of application a test of the signatory polynucleotide/gene expression in rat kidney samples consisting of tissues from rats exposed to three drug compounds and a single control group was carried out (Teva Pharmaceutical Industries, IL). The purpose of this analysis was to test the ability of the optimized

signature to correctly predict the level of toxicity of the three drug compounds prior to the ability to demonstrate renal damage using histopathological examination of the rat kidney samples.

In this Example, the samples with known renal toxic effect (detailed in plates 1-3 described in Table 25 and in Table 9) are referred to as the “labeled samples”, and the samples of the blind test (detailed in plates 4-7 described in Table 25), where no information on the renal toxic effect was available, are referred to as the “un-labeled samples”. The experimental details and the analysis performed initially on the labeled samples, as described in Example 2, are referred to as the “discovery stage” of the study, while the details and analysis performed at the second stage of the study are referred to as the “validation stage”.

The signature disclosed in Table 13 herein was further revised and refined, and then validated by testing the un-labeled rat kidney samples, consisting of tissues from rats exposed to three drug compounds (T1 and T2 presented in Figures 1-2 and compound T3, Riluzole (6-(trifluoromethoxy)benzothiazol-2-amine) and from a single control group.

qRT-PCR was performed on the labeled and un-labeled samples as described in section “RT Preparation and Real-Time qRT-PCR Analysis” hereinabove, using primers for 8 amplicons of 4 genes (a wild-type and a splice-variant amplicon for each gene disclosed in Table 13), as detailed in Example 2 hereinabove. New primers were designed for part of the transcripts, and new conditions for several qRT-PCR reactions were used, as described below.

Two equivalent modified signatures were constructed based on new qRT-PCR results obtained from the test conducted with the labeled samples, which are listed in Tables 26 and 27 below. The modified signatures were then applied to the un-labeled samples and successfully predicted the level of toxicity of the three test compounds as well as of the control.

Table 23 provides the normalized qRT-PCR results for the amplicons used for both classifiers (shown in Tables 26 and 27) on the labeled and un-labeled samples. The measurements were normalized in two steps. First - according to the house-keeping-genes normalization factor, and second - each plate was normalized according to ratio of the intensity measurements from samples appearing in it and in the shuffled (normalization) plate (Table 25 provides the plates details and the section “Inter-Plate Normalization” provides details of the normalization process). The normalized values were further scaled by a factor, for ease of viewing. This data, as well as the additional gender column was used by the classifiers to reach the toxicity calls. Table 23 also provides the true label (Normal/Toxic) for the labeled samples.

Table 23: Normalized qRT-PCR results for amplicons used with labeled and un-labeled samples

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
56_6F149_day5_Dox	0.851	1.377	1.438
48_3F122_day5_Cis	0.705	2.695	1.643
47_2F115_day5_Gent	2.64	1.515	1.644
110_1F108_day28_Saline	0.775	0.741	0.431
34_3M22_day5_Cis	1.262	3.816	2.034
44_6M50_day5_Dox	1.017	2.129	1.321
106_1M7_day28_Saline	0.489	0.728	0.804

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
116 7F156 day1 ValpA	1.134	1.026	2.289
59 8M81 day28 Naive	1.117	0.965	1.232
124 7M61 day28 ValpA	0.543	0.732	0.588
65 1M1 day1 Saline	1.029	0.933	1.176
74 1F104 day5 Saline	0.801	0.911	1.516
57 6F150 day5 Dox	1.053	0.905	1.723
43 6M49 day5 Dox	1.551	2.586	1.536
128 7F162 day28 ValpA	0.95	0.774	1.731
120 7M60 day5 ValpA	0.631	0.846	1.019
114 7M57 day1 ValpA	0.939	1.488	1.581
37 4M31 day5 Tob	0.559	0.863	0.99
126 7M63 day28 ValpA	0.459	0.713	0.589
112 7M55 day1 ValpA	1.405	1.506	1.842
73 1M6 day5 Saline	0.413	0.966	0.768
62 8F181 day28 Naive	0.931	1.262	0.933
123 7F160 day5 ValpA	1.428	0.85	1.509
109 1F107 day28 Saline	0.743	0.775	0.942
118 7M58 day5 ValpA	1.002	0.975	1.372
68 1F101 day1 Saline	1.725	1.074	1.424
117 7F157 day1 ValpA	1.225	0.999	1.929
36 3M24 day5 Cis	3.597	5.526	4.156
33 2M15 day5 Gent	0.983	0.948	1.085
127 7F161 day28 ValpA	1.044	0.872	1.171
75 1F105 day5 Saline	0.044	0.838	1.262
52 4F132 day5 Tob	2.483	0.855	1.589
50 3F124 day5 Cis	1.404	3.771	4.947
63 8F182 day28 Naive	0.809	0.95	0.772
38 4M32 day5 Tob	0.802	1.237	0.968
72 1M5 day5 Saline	0.654	0.963	1.245
67 1M3 day1 Saline	0.805	1.2	0.903
35 3M23 day5 Cis	0.843	1.509	0.981
51 4F131 day5 Tob	1.529	0.988	1.6
32 2M14 day5 Gent	0.954	1.09	0.993
122 7F159 day5 ValpA	0.617	0.791	0.94
121 7F158 day5 ValpA	1.258	0.694	1.425
119 7M59 day5 ValpA	0.997	1.082	1.585
71 1M4 day5 Saline	0.605	0.731	0.975
111 1F109 day28 Saline	0.996	0.904	1.014
60 8M82 day28 Naive	0.631	1.255	1.006
129 7F163 day28 ValpA	0.587	0.57	1.037
69 1F102 day1 Saline	0.573	0.793	1.449
108 1M9 day28 Saline	0.831	0.812	0.634
46 2F114 day5 Gent	1.104	0.78	0.955

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
125 7M62 day28 ValpA	1.011	0.979	0.52
113 7M56 day1 ValpA	0.547	0.721	0.637
45 2F113 day5 Gent	1.699	1.142	1.442
61 8M83 day28 Naive	0.411	0.637	1.209
53 4F133 day5 Tob	2.317	0.858	1.424
107 1M8 day28 Saline	0.562	0.719	0.642
115 7F155 day1 ValpA	1.544	1.041	0.9
58 6F151 day5 Dox	1.791	1.227	2.913
31 2M13 day5 Gent	0.82	1.402	1.842
49 3F123 day5 Cis	2.113	1.642	3.172
39 4M33 day5 Tob	0.946	1.646	2.062
70 1F103 day1 Saline	1.021	1.173	1.629
66 1M2 day1 Saline	0.753	0.883	0.705
64 8F183 day28 Naive	0.843	0.943	1.282
54 UK5F126 T1 Ds2 Day1	1.265	1.153	0.609
123 UK4F119 T1 Ds1 Day5	0.657	1.01	0.816
60 UK7F139 T2 Ds1 Day1	3.38	0.7	0.126
17 UK5M29 T1 Ds2 Day1	0.653	1.19	0.835
117 UK2F107 control Day5	1.229	2.274	0.68
109 UK12M69 T3 Ds1 Day5	0.482	1.3	0.62
18 UK5M30 T1 Ds2 Day1	0.383	0.623	0.696
57 UK5F129 T1 Ds2 Day1	0.472	0.798	0.804
151 UK14F180 T3 Ds2 Day5	0.363	0.705	1.004
7 UK3M13 T1 Ds1 Day1	0.734	0.923	0.299
113 UK14M80 T3 Ds2 Day5	0.605	0.846	0.658
72 UK11F164 T3 Ds1 Day1	0.282	0.582	0.114
104 UK10M56 T2 Ds2 Day5	0.668	1.256	0.655
91 UK6M31 T1 Ds2 Day5	1.164	1.663	1.018
124 UK4F120 T1 Ds1 Day5	0.703	1.013	1.499
114 UK14M81 T3 Ds2 Day5	0.43	0.949	1.003
69 UK9F154 T2 Ds2 Day1	0.92	0.762	0.111
46 UK1F105 control Day1	0.349	0.692	0.164
34 UK11M64 T3 Ds1 Day1	0.649	1.074	0.726
130 UK6F132 T1 Ds2 Day5	1	1.554	0.919
3 UK1M3 control Day1	0.398	0.756	0.171
75 UK13F173 T3 Ds2 Day1	0.897	0.662	0.798
101 UK8M47 T2 Ds1 Day5	0.397	1.149	0.172
102 UK8M48 T2 Ds1 Day5	0.337	2.147	0.179
96 UK6M36 T1 Ds2 Day5	0.71	0.996	0.756
39 UK13M76 T3 Ds2 Day1	0.359	0.643	0.158
19 UK7M37 T2 Ds1 Day1	0.623	0.7	0.51
144 UK12F167 T3 Ds1 Day5	1.957	1.301	0.638
2 UK1M2 control Day1	0.342	0.812	0.222

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
52 UK3F118 T1 Ds1 Day1	0.566	0.706	2.31
31 UK11M61 T3 Ds1 Day1	0.64	0.919	0.96
129 UK6F131 T1 Ds2 Day5	0.009	1.36	0.359
23 UK7M41 T2 Ds1 Day1	0.481	0.88	0.938
43 UK1F102 control Day1	0.406	2.051	0.328
81 UK2M8 control Day5	0.686	1.318	0.252
80 UK2M7 control Day5	0.788	1.981	0.273
70 UK11F161 T3 Ds1 Day1	0.457	0.764	0.232
16 UK5M28 T1 Ds2 Day1	0.78	0.517	0.385
79 UK13F178 T3 Ds2 Day1	0.49	0.802	0.92
50 UK3F116 T1 Ds1 Day1	0.66	0.913	1.49
112 UK12M72 T3 Ds1 Day5	0.722	1.65	0.649
146 UK12F169 T3 Ds1 Day5	1.807	1.003	0.716
140 UK10F156 T2 Ds2 Day5	1.186	1.386	0.345
143 UK10F160 T2 Ds2 Day5	0.308	0.676	0.169
10 UK3M16 T1 Ds1 Day1	0.181	0.757	0.291
94 UK6M34 T1 Ds2 Day5	0.391	1.037	0.871
147 UK12F170 T3 Ds1 Day5	0.483	1.338	1.035
71 UK11F163 T3 Ds1 Day1	0.575	0.611	0.183
122 UK2F112 control Day5	0.592	1.127	0.159
47 UK1F106 control Day1	0.405	0.823	0.196
15 UK5M27 T1 Ds2 Day1	0.755	0.906	0.76
97 UK8M43 T2 Ds1 Day5	0.233	1.884	0.107
106 UK10M59 T2 Ds2 Day5	0.497	1.01	0.513
118 UK2F108 control Day5	0.911	2.171	0.279
148 UK12F171 T3 Ds1 Day5	0.899	0.576	1.226
35 UK11M65 T3 Ds1 Day1	0.374	0.663	1.044
21 UK7M39 T2 Ds1 Day1	0.662	0.67	0.555
38 UK13M75 T3 Ds2 Day1	0.347	0.969	0.203
55 UK5F127 T1 Ds2 Day1	0.885	0.849	0.739
100 UK8M46 T2 Ds1 Day5	0.493	1.539	0.113
67 UK9F152 T2 Ds2 Day1	1.747	0.832	0.211
131 UK6F133 T1 Ds2 Day5	0.964	1.84	0.852
142 UK10F159 T2 Ds2 Day5	0.711	1.128	0.148
128 UK4F124 T1 Ds1 Day5	1.527	1.373	1.598
95 UK6M35 T1 Ds2 Day5	0.713	1.348	0.761
8 UK3M14 T1 Ds1 Day1	0.396	0.615	0.271
138 UK8F147 T2 Ds1 Day5	0.626	1.163	1.399
41 UK13M78 T3 Ds2 Day1	0.174	0.632	0.275
76 UK13F175 T3 Ds2 Day1	0.912	1.119	2.153
152 UK14F181 T3 Ds2 Day5	0.762	0.942	1.567
86 UK4M20 T1 Ds1 Day5	0.473	1.353	0.183
59 UK7F137 T2 Ds1 Day1	0.473	0.613	0.115

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
111 UK12M71 T3 Ds1 Day5	0.832	1.082	0.857
145 UK12F168 T3 Ds1 Day5	1.015	1.358	0.625
28 UK9M52 T2 Ds2 Day1	1.016	0.773	0.531
108 UK12M67 T3 Ds1 Day5	0.699	1.585	0.525
11 UK3M17 T1 Ds1 Day1	0.198	0.73	0.376
24 UK7M42 T2 Ds1 Day1	0.76	0.695	0.859
42 UK1F101 control Day1	0.415	0.976	0.276
29 UK9M53 T2 Ds2 Day1	0.424	0.635	0.505
61 UK7F140 T2 Ds1 Day1	0.474	0.464	0.124
20 UK7M38 T2 Ds1 Day1	0.876	0.951	0.888
141 UK10F158 T2 Ds2 Day5	1.237	1.161	0.185
87 UK4M21 T1 Ds1 Day5	0.702	1.266	0.149
150 UK14F179 T3 Ds2 Day5	1.066	0.718	0.875
136 UK8F144 T2 Ds1 Day5	1.149	1.329	1.006
93 UK6M33 T1 Ds2 Day5	1.825	2.423	1.197
85 UK4M19 T1 Ds1 Day5	0.356	1.783	0.214
4 UK1M4 control Day1	0.617	0.668	0.192
126 UK4F122 T1 Ds1 Day5	1.325	1.477	3.532
36 UK11M66 T3 Ds1 Day1	0.468	0.569	1.137
74 UK11F166 T3 Ds1 Day1	0.465	1.366	0.132
14 UK5M26 T1 Ds2 Day1	0.462	0.696	0.478
119 UK2F109 control Day5	0.994	1.083	0.162
25 UK9M49 T2 Ds2 Day1	0.589	0.722	0.585
103 UK10M55 T2 Ds2 Day5	0.857	1.146	0.997
27 UK9M51 T2 Ds2 Day1	0.545	0.618	0.475
26 UK9M50 T2 Ds2 Day1	0.528	0.999	0.661
44 UK1F103 control Day1	0.563	0.692	0.161
62 UK7F141 T2 Ds1 Day1	0.959	0.536	0.267
92 UK6M32 T1 Ds2 Day5	0.453	1.13	0.631
12 UK3M18 T1 Ds1 Day1	0.231	0.524	0.135
5 UK1M5 control Day1	0.3	1.146	0.184
153 UK14F182 T3 Ds2 Day5	1.119	1.058	1.708
56 UK5F128 T1 Ds2 Day1	0.413	0.611	0.738
58 UK5F130 T1 Ds2 Day1	0.762	0.707	0.652
132 UK6F134 T1 Ds2 Day5	1.254	2.468	1.238
49 UK3F114 T1 Ds1 Day1	0.758	0.783	1.436
90 UK4M24 T1 Ds1 Day5	0.657	1.097	0.296
64 UK9F149 T2 Ds2 Day1	0.384	0.602	0.197
110 UK12M70 T3 Ds1 Day5	0.495	0.946	0.398
127 UK4F123 T1 Ds1 Day5	1.014	2.104	3.397
121 UK2F111 control Day5	0.394	0.882	0.222
83 UK2M10 control Day5	0.719	1.033	0.103
98 UK8M44 T2 Ds1 Day5	0.321	1.66	0.148

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
99 UK8M45 T2 Ds1 Day5	0.477	1.079	0.127
107 UK10M60 T2 Ds2 Day5	0.788	1.057	0.746
73 UK11F165 T3 Ds1 Day1	0.344	0.637	0.134
40 UK13M77 T3 Ds2 Day1	0.418	0.923	0.29
89 UK4M23 T1 Ds1 Day5	1.11	1.249	0.186
134 UK6F136 T1 Ds2 Day5	1.111	1.684	0.528
133 UK6F135 T1 Ds2 Day5	1.126	1.672	1.583
1 UK1M1 control Day1	0.578	0.554	0.148
30 UK9M54 T2 Ds2 Day1	0.577	0.522	0.458
82 UK2M9 control Day5	0.269	1.398	0.157
37 UK13M73 T3 Ds2 Day1	0.359	0.767	0.152
45 UK1F104 control Day1	0.754	0.709	0.197
105 UK10M57 T2 Ds2 Day5	0.457	0.942	0.686
135 UK8F143 T2 Ds1 Day5	0.83	0.936	0.736
33 UK11M63 T3 Ds1 Day1	0.568	0.949	0.767
77 UK13F176 T3 Ds2 Day1	0.667	1.13	1.45
63 UK7F142 T2 Ds1 Day1	0.518	0.646	0.157
48 UK3F113 T1 Ds1 Day1	0.646	0.912	1.195
65 UK9F150 T2 Ds2 Day1	1.729	1.006	0.167
84 UK2M11 control Day5	0.152	0.819	0.093
125 UK4F121 T1 Ds1 Day5	1.337	1.458	1.317
139 UK8F148 T2 Ds1 Day5	1.524	0.978	1.386
9 UK3M15 T1 Ds1 Day1	0.47	0.87	0.249
115 UK14M82 T3 Ds2 Day5	0.472	1.087	1.024
137 UK8F145 T2 Ds1 Day5	1.111	1.439	1.293
13 UK5M25 T1 Ds2 Day1	0.644	0.517	0.654
66 UK9F151 T2 Ds2 Day1	1.125	1.079	0.192
88 UK4M22 T1 Ds1 Day5	0.638	1.23	0.189
149 UK12F172 T3 Ds1 Day5	1.093	1.094	1.413
32 UK11M62 T3 Ds1 Day1	0.551	0.7	0.441
68 UK9F153 T2 Ds2 Day1	0.594	0.84	0.14
22 UK7M40 T2 Ds1 Day1	0.823	0.629	0.47
6 UK1M6 control Day1	0.245	0.66	0.402
120 UK2F110 control Day5	0.456	1.399	0.233
78 UK13F177 T3 Ds2 Day1	0.956	1.47	2.313
53 UK5F125 T1 Ds2 Day1	1.034	0.867	1.261
116 UK14M83 T3 Ds2 Day5	0.766	2.34	1.119
51 UK3F117 T1 Ds1 Day1	1.082	0.957	1.891
154 UK14F183 T3 Ds2 Day5	0.944	1.259	1.475
16 2F110 day1 Gent	1.361	0.772	0.842
42 5M42 day5 CadCl	1.067	1.057	1.036
102 5F145 day28 CadCl	1.138	0.785	0.769
9 4M30 day1 Tob	1.601	1.137	1.582

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
97 4F134 day28 Tob	1.189	0.624	0.713
105 6F173 day28 Dox	1.263	1.328	0.985
79 3M25 day28 Cis	4.741	4.41	3.509
78 2M18 day28 Gent	1.614	0.853	0.863
54 5F141 day5 CadCl	2.101	1.154	2.018
5 3M20 day1 Cis	0.679	0.668	0.806
77 2M17 day28 Gent	1.342	0.861	1.072
82 4M34 day28 Tob	1.217	0.324	0.76
12 5M39 day1 CadCl	0.727	0.919	1.224
26 5F138 day1 CadCl	1.183	1.005	1.097
94 3F125 day28 Cis	4.104	2.839	3.443
93 2F118 day28 Gent	1.315	0.553	0.617
17 2F111 day1 Gent	1.037	1.118	1.155
92 2F117 day28 Gent	1.194	0.817	0.865
89 6M72 day28 Dox	0.73	1.54	2.342
55 5F142 day5 CadCl	1.109	0.786	1.292
15 6M48 day1 Dox	0.824	0.993	1.387
3 2M12 day1 Gent	0.904	0.987	1.361
84 4M36 day28 Tob	1.099	0.69	1.087
87 5M45 day28 CadCl	0.529	0.652	1.356
25 5F137 day1 CadCl	1.265	1.446	2.129
98 4F135 day28 Tob	1.267	0.528	0.889
41 5M41 day5 CadCl	0.769	1.132	3.613
83 4M35 day28 Tob	1.373	0.551	1.268
2 2M11 day1 Gent	1.08	1.115	2.011
13 6M46 day1 Dox	1.286	1.283	1.761
24 4F130 day1 Tob	1.084	1.104	0.967
101 5F144 day28 CadCl	0.772	0.712	1.068
21 3F121 day1 Cis	0.759	0.853	1.514
88 6M71 day28 Dox	2.381	1.434	1.437
100 5F143 day28 CadCl	0.578	0.681	0.68
10 5M37 day1 CadCl	0.993	1.239	2.276
7 4M28 day1 Tob	0.577	1.132	1.506
18 2F112 day1 Gent	2.078	0.84	1.387
28 6F146 day1 Dox	1.43	1.41	2.256
8 4M29 day1 Tob	0.949	0.912	1.173
91 2F116 day28 Gent	1.188	0.554	0.729
22 4F128 day1 Tob	0.809	0.563	1.025
86 5M44 day28 CadCl	0.654	0.849	2.128
14 6M47 day1 Dox	1.148	1.042	1.516
40 5M40 day5 CadCl	1.211	1.051	2.866
96 3F127 day28 Cis	2.053	2.398	1.813
30 6F148 day1 Dox	1.501	1.46	1.627

Sample	W41270_DB81_seg11	H31883_DB71_seg13	MUSCYCG1R_DB81_seg19-20
103_6F171_day28_Dox	1.44	1.642	1.991
19_3F119_day1_Cis	1.331	1.416	1.492
85_5M43_day28_CadCl	0.522	0.578	0.932
80_3M26_day28_Cis	1.904	2.653	3.094
27_5F139_day1_CadCl	0.983	1.632	1.856
76_2M16_day28_Gent	1.941	0.842	1.661
90_6M73_day28_Dox	0.652	1.611	2.105
29_6F147_day1_Dox	0.935	1.116	1.551
23_4F129_day1_Tob	0.983	0.661	0.987
1_2M10_day1_Gent	0.85	1.053	2.861
20_3F120_day1_Cis	1.029	2.092	3.259
104_6F172_day28_Dox	0.787	1.408	1.779
6_3M21_day1_Cis	1.224	2.559	2.003
95_3F126_day28_Cis	4.516	5.592	7.02
81_3M27_day28_Cis	3.866	3.563	3.473
99_4F136_day28_Tob	1.798	0.807	0.903
4_3M19_day1_Cis	0.496	1.134	1.277
11_5M38_day1_CadCl	1	1.143	3.981

Sample	W64472_DB81_seg2	W83813_DB81_seg27	AI045075_DB71_seg6	Gender	Label
56_6F149_day5_Dox	2.03	1.442	1.248	F	Toxic
48_3F122_day5_Cis	1.795	1.716	1.804	F	Toxic
47_2F115_day5_Gent	1.733	1.688	1.337	F	Toxic
110_1F108_day28_Saline	0.865	1.019	0.724	F	Normal
34_3M22_day5_Cis	3.64	0.857	1.683	M	Toxic
44_6M50_day5_Dox	2.924	0.986	1.348	M	Toxic
106_1M7_day28_Saline	0.775	0.535	0.544	M	Normal
116_7F156_day1_ValpA	1.082	1.179	1.006	F	Normal
59_8M81_day28_Naive	2.184	1.679	1.27	M	Normal
124_7M61_day28_ValpA	1.085	0.493	0.696	M	Normal
65_1M1_day1_Saline	1.57	1.166	1.202	M	Normal
74_1F104_day5_Saline	1.013	0.889	0.535	F	Normal
57_6F150_day5_Dox	1.441	1.177	1.319	F	Toxic
43_6M49_day5_Dox	3.4	1.095	1.844	M	Toxic
128_7F162_day28_ValpA	0.788	1.254	0.906	F	Normal
120_7M60_day5_ValpA	0.92	0.778	1.017	M	Normal
114_7M57_day1_ValpA	1.123	0.912	1.436	M	Normal
37_4M31_day5_Tob	1.193	0.589	1.327	M	Toxic
126_7M63_day28_ValpA	0.965	0.46	0.669	M	Normal
112_7M55_day1_ValpA	1.887	1.495	2.189	M	Normal

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	AI045075_ DB71_seg6	Gender	Label
73_1M6_day5_Saline	0.801	0.629	0.518	M	Normal
62_8F181_day28_Naive	1.493	1.594	1.044	F	Normal
123_7F160_day5_ValpA	1.226	1.17	1.145	F	Normal
109_1F107_day28_Saline	0.718	0.642	0.982	F	Normal
118_7M58_day5_ValpA	1.169	0.876	0.997	M	Normal
68_1F101_day1_Saline	1.041	1.508	0.905	F	Normal
117_7F157_day1_ValpA	0.995	1.13	0.983	F	Normal
36_3M24_day5_Cis	6.414	1.461	4.298	M	Toxic
33_2M15_day5_Gent	2.048	1.042	1.374	M	Toxic
127_7F161_day28_ValpA	1.51	0.875	0.822	F	Normal
75_1F105_day5_Saline	1.154	1.395	0.845	F	Normal
52_4F132_day5_Tob	1.871	1.262	1.644	F	Toxic
50_3F124_day5_Cis	1.976	1.366	1.503	F	Toxic
63_8F182_day28_Naive	0.899	1.367	0.915	F	Normal
38_4M32_day5_Tob	1.449	0.892	1.614	M	Toxic
72_1M5_day5_Saline	0.943	1.059	0.763	M	Normal
67_1M3_day1_Saline	3.263	1.107	1.751	M	Normal
35_3M23_day5_Cis	1.448	0.758	0.93	M	Toxic
51_4F131_day5_Tob	1.063	1.058	1.367	F	Toxic
32_2M14_day5_Gent	1.962	0.702	1.402	M	Toxic
122_7F159_day5_ValpA	0.702	0.926	0.883	F	Normal
121_7F158_day5_ValpA	0.812	1.146	1.052	F	Normal
119_7M59_day5_ValpA	0.894	0.992	0.877	M	Normal
71_1M4_day5_Saline	1.141	0.796	0.887	M	Normal
111_1F109_day28_Saline	0.864	1.01	1.061	F	Normal
60_8M82_day28_Naive	2.082	1.127	1.021	M	Normal
129_7F163_day28_ValpA	0.478	0.761	0.77	F	Normal
69_1F102_day1_Saline	0.5	0.819	0.476	F	Normal
108_1M9_day28_Saline	1.037	0.889	0.829	M	Normal
46_2F114_day5_Gent	1.427	1.441	1.778	F	Toxic
125_7M62_day28_ValpA	0.991	1.208	0.719	M	Normal
113_7M56_day1_ValpA	1.092	0.818	1.098	M	Normal
45_2F113_day5_Gent	1.403	1.395	1.636	F	Toxic
61_8M83_day28_Naive	0.965	0.736	0.548	M	Normal
53_4F133_day5_Tob	1.785	0.942	2.402	F	Toxic
107_1M8_day28_Saline	0.596	0.284	0.393	M	Normal
115_7F155_day1_ValpA	1.596	1.366	1.522	F	Normal
58_6F151_day5_Dox	2.561	1.399	1.18	F	Toxic
31_2M13_day5_Gent	1.53	0.76	1.256	M	Toxic
49_3F123_day5_Cis	1.265	1.265	1.09	F	Toxic
39_4M33_day5_Tob	1.447	1.117	1.224	M	Toxic
70_1F103_day1_Saline	0.876	1.169	0.753	F	Normal
66_1M2_day1_Saline	0.897	0.674	0.582	M	Normal

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	AI045075_ DB71_seg6	Gender	Label
64_8F183_day28_Naive	0.683	1.224	0.868	F	Normal
54_UK5F126_T1_Ds2_Day1	0.944	1.186	0.483	F	
123_UK4F119_T1_Ds1_Day5	0.571	0.795	0.692	F	
60_UK7F139_T2_Ds1_Day1	1.863	0.305	1.308	F	
17_UK5M29_T1_Ds2_Day1	1.213	0.984	0.759	M	
117_UK2F107_control_Day5	4.213	0.933	3.974	F	
109_UK12M69_T3_Ds1_Day5	0.852	0.754	0.615	M	
18_UK5M30_T1_Ds2_Day1	0.661	0.34	0.448	M	
57_UK5F129_T1_Ds2_Day1	0.559	0.689	0.547	F	
151_UK14F180_T3_Ds2_Day5	0.368	0.729	0.37	F	
7_UK3M13_T1_Ds1_Day1	1.118	0.216	0.843	M	
113_UK14M80_T3_Ds2_Day5	0.779	0.728	1.451	M	
72_UK11F164_T3_Ds1_Day1	0.483	0.322	0.354	F	
104_UK10M56_T2_Ds2_Day5	0.731	0.601	0.609	M	
91_UK6M31_T1_Ds2_Day5	0.803	1.092	0.797	M	
124_UK4F120_T1_Ds1_Day5	0.528	0.885	0.627	F	
114_UK14M81_T3_Ds2_Day5	0.656	0.933	0.53	M	
69_UK9F154_T2_Ds2_Day1	0.804	0.201	1.43	F	
46_UK1F105_control_Day1	0.734	0.542	0.825	F	
34_UK11M64_T3_Ds1_Day1	1.31	0.663	1.103	M	
130_UK6F132_T1_Ds2_Day5	0.863	1.125	0.766	F	
3_UK1M3_control_Day1	0.883	0.267	1.049	M	
75_UK13F173_T3_Ds2_Day1	0.659	0.67	0.444	F	
101_UK8M47_T2_Ds1_Day5	0.929	0.248	1.016	M	
102_UK8M48_T2_Ds1_Day5	0.996	0.203	1.11	M	
96_UK6M36_T1_Ds2_Day5	0.982	0.885	1.01	M	
39_UK13M76_T3_Ds2_Day1	1.566	0.137	1.146	M	
19_UK7M37_T2_Ds1_Day1	0.632	0.666	0.744	M	
144_UK12F167_T3_Ds1_Day5	1.176	1.146	1.082	F	
2_UK1M2_control_Day1	0.91	0.216	0.885	M	
52_UK3F118_T1_Ds1_Day1	0.683	0.843	0.636	F	
31_UK11M61_T3_Ds1_Day1	1.296	0.497	0.678	M	
129_UK6F131_T1_Ds2_Day5	0.554	0.55	0.629	F	
23_UK7M41_T2_Ds1_Day1	1.305	0.84	1.159	M	
43_UK1F102_control_Day1	1.43	0.612	0.851	F	
81_UK2M8_control_Day5	1.317	0.25	2.054	M	
80_UK2M7_control_Day5	2.363	0.112	1.078	M	
70_UK11F161_T3_Ds1_Day1	0.52	0.402	0.596	F	
16_UK5M28_T1_Ds2_Day1	0.462	0.56	0.396	M	
79_UK13F178_T3_Ds2_Day1	0.602	0.761	0.527	F	
50_UK3F116_T1_Ds1_Day1	0.49	0.926	0.572	F	
112_UK12M72_T3_Ds1_Day5	1.034	1.388	1.102	M	
146_UK12F169_T3_Ds1_Day5	0.822	0.659	1.032	F	

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	A1045075_ DB71_seg6	Gender	Label
140_UK10F156_T2_Ds2_Day5	1.178	0.555	1.842	F	
143_UK10F160_T2_Ds2_Day5	0.485	0.354	0.637	F	
10_UK3M16_T1_Ds1_Day1	0.778	0.155	0.574	M	
94_UK6M34_T1_Ds2_Day5	0.527	0.654	0.539	M	
147_UK12F170_T3_Ds1_Day5	0.479	0.701	0.501	F	
71_UK11F163_T3_Ds1_Day1	0.859	0.394	0.85	F	
122_UK2F112_control_Day5	1.05	0.522	1.214	F	
47_UK1F106_control_Day1	0.495	0.276	0.605	F	
15_UK5M27_T1_Ds2_Day1	1.326	0.889	0.932	M	
97_UK8M43_T2_Ds1_Day5	1.445	0.11	1.566	M	
106_UK10M59_T2_Ds2_Day5	0.899	0.615	1.338	M	
118_UK2F108_control_Day5	1.325	0.362	1.597	F	
148_UK12F171_T3_Ds1_Day5	0.546	1.155	0.727	F	
35_UK11M65_T3_Ds1_Day1	0.567	0.898	0.918	M	
21_UK7M39_T2_Ds1_Day1	0.801	0.955	0.98	M	
38_UK13M75_T3_Ds2_Day1	2.291	0.276	1.479	M	
55_UK5F127_T1_Ds2_Day1	0.898	0.861	1.067	F	
100_UK8M46_T2_Ds1_Day5	1.42	0.209	1.449	M	
67_UK9F152_T2_Ds2_Day1	1.104	0.289	2.008	F	
131_UK6F133_T1_Ds2_Day5	0.989	1.025	0.935	F	
142_UK10F159_T2_Ds2_Day5	0.554	0.269	0.799	F	
128_UK4F124_T1_Ds1_Day5	0.745	1.511	1.162	F	
95_UK6M35_T1_Ds2_Day5	1.181	1.077	1.161	M	
8_UK3M14_T1_Ds1_Day1	1.501	0.178	1.13	M	
138_UK8F147_T2_Ds1_Day5	0.464	0.865	0.771	F	
41_UK13M78_T3_Ds2_Day1	1.042	0.164	0.566	M	
76_UK13F175_T3_Ds2_Day1	0.847	0.874	1.132	F	
152_UK14F181_T3_Ds2_Day5	0.587	0.81	0.666	F	
86_UK4M20_T1_Ds1_Day5	1.835	0.125	2.031	M	
59_UK7F137_T2_Ds1_Day1	0.492	0.183	0.54	F	
111_UK12M71_T3_Ds1_Day5	0.88	1.054	1.285	M	
145_UK12F168_T3_Ds1_Day5	0.601	0.791	0.928	F	
28_UK9M52_T2_Ds2_Day1	1.462	0.773	1.006	M	
108_UK12M67_T3_Ds1_Day5	0.8	0.818	1.133	M	
11_UK3M17_T1_Ds1_Day1	0.981	0.143	0.805	M	
24_UK7M42_T2_Ds1_Day1	0.791	0.798	0.709	M	
42_UK1F101_control_Day1	0.569	0.323	0.674	F	
29_UK9M53_T2_Ds2_Day1	0.692	0.595	0.729	M	
61_UK7F140_T2_Ds1_Day1	0.468	0.213	0.419	F	
20_UK7M38_T2_Ds1_Day1	1.076	0.931	1.059	M	
141_UK10F158_T2_Ds2_Day5	1.029	0.354	3.003	F	
87_UK4M21_T1_Ds1_Day5	0.991	0.208	0.904	M	
150_UK14F179_T3_Ds2_Day5	0.623	0.711	0.977	F	

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	AI045075_ DB71_seg6	Gender	Label
136_UK8F144_T2_Ds1_Day5	0.532	0.922	1.02	F	
93_UK6M33_T1_Ds2_Day5	2.203	1.236	1.793	M	
85_UK4M19_T1_Ds1_Day5	0.687	0.165	0.937	M	
4_UK1M4_control_Day1	1.352	0.235	1.318	M	
126_UK4F122_T1_Ds1_Day5	0.945	1.328	1.367	F	
36_UK11M66_T3_Ds1_Day1	1.106	0.512	0.845	M	
74_UK11F166_T3_Ds1_Day1	1.064	0.356	1.252	F	
14_UK5M26_T1_Ds2_Day1	0.785	0.656	0.544	M	
119_UK2F109_control_Day5	0.907	0.515	1.067	F	
25_UK9M49_T2_Ds2_Day1	0.656	0.741	0.541	M	
103_UK10M55_T2_Ds2_Day5	1.446	1.022	0.831	M	
27_UK9M51_T2_Ds2_Day1	1.23	0.777	1.015	M	
26_UK9M50_T2_Ds2_Day1	0.866	0.725	0.588	M	
44_UK1F103_control_Day1	0.524	0.2	0.503	F	
62_UK7F141_T2_Ds1_Day1	1.072	0.29	0.836	F	
92_UK6M32_T1_Ds2_Day5	0.784	0.596	0.73	M	
12_UK3M18_T1_Ds1_Day1	0.439	0.169	0.4	M	
5_UK1M5_control_Day1	1.411	0.299	1.289	M	
153_UK14F182_T3_Ds2_Day5	1.24	1.599	1.436	F	
56_UK5F128_T1_Ds2_Day1	0.415	0.549	0.514	F	
58_UK5F130_T1_Ds2_Day1	0.502	0.725	0.639	F	
132_UK6F134_T1_Ds2_Day5	1.223	0.454	1.478	F	
49_UK3F114_T1_Ds1_Day1	0.752	0.825	0.49	F	
90_UK4M24_T1_Ds1_Day5	0.902	0.207	0.783	M	
64_UK9F149_T2_Ds2_Day1	0.741	0.365	0.857	F	
110_UK12M70_T3_Ds1_Day5	0.499	0.72	1.175	M	
127_UK4F123_T1_Ds1_Day5	0.938	1.593	1.089	F	
121_UK2F111_control_Day5	0.609	0.276	0.702	F	
83_UK2M10_control_Day5	1.034	0.412	1.069	M	
98_UK8M44_T2_Ds1_Day5	1.258	0.27	1.516	M	
99_UK8M45_T2_Ds1_Day5	1.447	0.299	1.237	M	
107_UK10M60_T2_Ds2_Day5	0.623	0.962	0.83	M	
73_UK11F165_T3_Ds1_Day1	0.507	0.205	0.37	F	
40_UK13M77_T3_Ds2_Day1	1.597	0.218	1.063	M	
89_UK4M23_T1_Ds1_Day5	1.35	0.2	1.364	M	
134_UK6F136_T1_Ds2_Day5	0.652	1.074	0.868	F	
133_UK6F135_T1_Ds2_Day5	1.056	1.674	1.487	F	
1_UK1M1_control_Day1	0.783	0.17	0.633	M	
30_UK9M54_T2_Ds2_Day1	0.837	0.614	0.471	M	
82_UK2M9_control_Day5	2.621	0.25	1.879	M	
37_UK13M73_T3_Ds2_Day1	1.35	0.211	1.117	M	
45_UK1F104_control_Day1	0.897	0.381	1.062	F	
105_UK10M57_T2_Ds2_Day5	1.049	0.626	0.878	M	

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	AI045075_ DB71_seg6	Gender	Label
135_UK8F143_T2_Ds1_Day5	0.616	0.961	1.923	F	
33_UK11M63_T3_Ds1_Day1	1.844	0.989	1.025	M	
77_UK13F176_T3_Ds2_Day1	1.043	0.929	1.211	F	
63_UK7F142_T2_Ds1_Day1	0.969	0.387	0.49	F	
48_UK3F113_T1_Ds1_Day1	0.548	0.917	0.762	F	
65_UK9F150_T2_Ds2_Day1	1.73	0.32	3.044	F	
84_UK2M11_control_Day5	0.629	0.162	0.645	M	
125_UK4F121_T1_Ds1_Day5	0.606	0.893	0.831	F	
139_UK8F148_T2_Ds1_Day5	0.572	0.824	1.232	F	
9_UK3M15_T1_Ds1_Day1	1.502	0.245	1.416	M	
115_UK14M82_T3_Ds2_Day5	0.494	1.103	0.765	M	
137_UK8F145_T2_Ds1_Day5	0.773	1.303	0.94	F	
13_UK5M25_T1_Ds2_Day1	0.592	0.473	0.541	M	
66_UK9F151_T2_Ds2_Day1	1.211	0.444	2.152	F	
88_UK4M22_T1_Ds1_Day5	0.684	0.323	0.717	M	
149_UK12F172_T3_Ds1_Day5	0.677	1.096	1.021	F	
32_UK11M62_T3_Ds1_Day1	0.808	0.789	0.713	M	
68_UK9F153_T2_Ds2_Day1	0.666	0.254	0.71	F	
22_UK7M40_T2_Ds1_Day1	1.477	1.182	1.253	M	
6_UK1M6_control_Day1	0.749	0.127	0.476	M	
120_UK2F110_control_Day5	0.898	0.34	0.976	F	
78_UK13F177_T3_Ds2_Day1	1.227	1.454	0.575	F	
53_UK5F125_T1_Ds2_Day1	1.028	1.045	0.965	F	
116_UK14M83_T3_Ds2_Day5	3.181	0.911	1.037	M	
51_UK3F117_T1_Ds1_Day1	1.047	1.186	0.981	F	
154_UK14F183_T3_Ds2_Day5	0.704	1.018	0.58	F	
16_2F110_day1_Gent	0.763	0.963	1.251	F	
42_5M42_day5_CadCl	1.337	1.104	1.083	M	
102_5F145_day28_CadCl	0.414	0.259	0.728	F	
9_4M30_day1_Tob	1.43	1.126	2.04	M	
97_4F134_day28_Tob	1.073	0.876	0.959	F	
105_6F173_day28_Dox	2.157	0.734	0.964	F	
79_3M25_day28_Cis	5.917	1.587	4.504	M	
78_2M18_day28_Gent	1.55	0.852	1.734	M	
54_5F141_day5_CadCl	2.282	1.782	1.866	F	
5_3M20_day1_Cis	0.815	0.799	0.495	M	
77_2M17_day28_Gent	1.353	0.857	3.06	M	
82_4M34_day28_Tob	0.61	0.39	0.679	M	
12_5M39_day1_CadCl	1.499	1.034	0.951	M	
26_5F138_day1_CadCl	1.059	1.194	1.133	F	
94_3F125_day28_Cis	2.504	1.749	2.301	F	
93_2F118_day28_Gent	0.806	0.421	0.967	F	
17_2F111_day1_Gent	0.963	0.98	0.889	F	

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	AI045075_ DB71_seg6	Gender	Label
92_2F117_day28_Gent	1.079	0.441	2.632	F	
89_6M72_day28_Dox	1.613	0.686	0.669	M	
55_5F142_day5_CadCl	0.854	0.906	0.805	F	
15_6M48_day1_Dox	1.26	0.956	1.257	M	
3_2M12_day1_Gent	1.567	1.02	1.072	M	
84_4M36_day28_Tob	1.379	0.832	1.273	M	
87_5M45_day28_CadCl	0.781	0.531	0.672	M	
25_5F137_day1_CadCl	1.155	1.772	1.64	F	
98_4F135_day28_Tob	1.144	0.621	1.021	F	
41_5M41_day5_CadCl	1.662	1.157	1.409	M	
83_4M35_day28_Tob	1.281	0.803	1.397	M	
2_2M11_day1_Gent	1.869	1.409	1.848	M	
13_6M46_day1_Dox	2.478	0.936	1.622	M	
24_4F130_day1_Tob	1.5	1.153	5.307	F	
101_5F144_day28_CadCl	0.827	0.783	0.975	F	
21_3F121_day1_Cis	0.81	0.844	0.631	F	
88_6M71_day28_Dox	3.226	0.442	1.434	M	
100_5F143_day28_CadCl	1.098	1.033	1.58	F	
10_5M37_day1_CadCl	1.622	1.027	1.108	M	
7_4M28_day1_Tob	1.083	0.999	0.825	M	
18_2F112_day1_Gent	1.408	0.898	2.053	F	
28_6F146_day1_Dox	1.548	2.054	1.631	F	
8_4M29_day1_Tob	1.619	1.049	1.223	M	
91_2F116_day28_Gent	1.164	0.434	1.78	F	
22_4F128_day1_Tob	0.73	0.799	1.195	F	
86_5M44_day28_CadCl	1.107	0.817	0.836	M	
14_6M47_day1_Dox	1.45	0.964	1.454	M	
40_5M40_day5_CadCl	1.675	1.671	2.393	M	
96_3F127_day28_Cis	2.643	0.786	1.555	F	
30_6F148_day1_Dox	1.367	1.752	1.268	F	
103_6F171_day28_Dox	1.462	0.719	0.653	F	
19_3F119_day1_Cis	1.261	1.209	0.788	F	
85_5M43_day28_CadCl	0.807	0.633	0.333	M	
80_3M26_day28_Cis	3.371	0.786	1.428	M	
27_5F139_day1_CadCl	1.372	1.894	1.263	F	
76_2M16_day28_Gent	1.245	0.756	1.469	M	
90_6M73_day28_Dox	1.604	0.586	0.785	M	
29_6F147_day1_Dox	0.977	1.003	0.741	F	
23_4F129_day1_Tob	1.166	0.982	1.623	F	
1_2M10_day1_Gent	1.48	1.161	1.221	M	
20_3F120_day1_Cis	1.096	1.345	0.899	F	
104_6F172_day28_Dox	1.023	0.955	0.54	F	
6_3M21_day1_Cis	2.455	1.09	1.64	M	

Sample	W64472_ DB81_seg2	W83813_ DB81_seg27	A1045075_ DB71_seg6	Gender	Label
95_3F126_day28_Cis	5.783	1.864	3.336	F	
81_3M27_day28_Cis	3.008	1.371	1.753	M	
99_4F136_day28_Tob	1.118	0.987	1.478	F	
4_3M19_day1_Cis	1.176	0.902	0.811	M	
11_5M38_day1_CadCl	1.512	0.957	1.069	M	

Experimental Design

- 5 Three compounds (T1, T2, T3) and a control were administered to male and female rats at various time points by oral gavage: 3 treatments (T1, T2, T3) were given in 2 doses (Ds1, Ds2) for two periods (one day and 5 days). Each group consisted of 6 male and 6 female rats. The additional groups consisted of 6 males and 6 female rats treated with control compound for a single day and for five days, as described in Table 24.

10 Table 24: Un-labeled samples groups and doses

Groups	Test Items	Dose (mg/kg)	Route of administration	Days of treatment	Animals per group
1M,F	Control – 1% methylcellulose	-	Oral gavage	1	6
2M,F		-	Oral gavage	5	6
3M,F	T1	10	Oral gavage	1	6
4M,F		10	Oral gavage	5	6
5M,F		40	Oral gavage	1	6
6M,F		40	Oral gavage	5	6
7M,F	T2	25	Oral gavage	1	6
8M,F		25	Oral gavage	5	6
9M,F		50	Oral gavage	1	6
10M,F		50	Oral gavage	5	6
11M,F	T3	5	Oral gavage	1	6
12M,F		5	Oral gavage	5	6
13M,F		10	Oral gavage	1	6
14M,F		10	Oral gavage	5	6

- 15 T2 is a drug known to cause renal damage after 7 days of treatment at the 50 mg/kg dose. The chemical structure of T2 is shown in Figure 2. T2 "no observed effect level" (NOEL, i.e., the maximal dose at which no toxic effect is visible) is 15 mg/kg. Kidneys obtained from rats receiving 50 mg/kg of T2, demonstrated direct cortical tubular toxicity. Figures 3-5 demonstrate the results of histopathological examination of the treated kidneys after 7 days (Figures 3B, 4B and 5B), or of the control animals (Figures 3A, 4A and 5A). As demonstrated in Figures 3B, 4B and 5B the changes were multifocal, consisting of tubular basophilia, indicating regeneration following necrosis.

- 20 Figure 3 demonstrates histological section of the kidney, magnified x 200. Figure 3A represents a histological section of the kidney of a control animal. Normal aspects of cortical tubules (eosinophilic

cytoplasm) are marked by arrows. Figure 3B represents a histological section of the kidney of a 50 mg/kg treated animal. Basophilic tubules in the Cortex, indicating post-necrotic regeneration are marked by arrows.

Figure 4 demonstrates histological section of the kidney, magnified x 400. Figure 4A represents a histological section of the kidney of a control animal. Normal aspects of cortical tubules (eosinophilic cytoplasm) are marked by arrows. Figure 4B represents a histological section of the kidney of a 50 mg/kg treated animal. Basophilic tubules in the Cortex, indicating post-necrotic regeneration are marked by arrows.

Figure 5 demonstrates histological section of the kidney, magnified x 600. Figure 5A represents a histological section of the kidney of a control animal. Normal aspects of cortical tubules (eosinophilic cytoplasm) are marked by arrows. Figure 5B represents a histological section of the kidney of a 50 mg/kg treated animal. Basophilic tubules in the Cortex, indicating post-necrotic regeneration are marked by arrows.

T1 is another drug which shows renal damage, mainly in females, after 28 days of treatment at the 30 mg/kg dose. The chemical structure of T1 is shown in Figure 1.

T3 is Riluzole (6-(trifluoromethoxy)benzothiazol-2-amine), which shows no renal damage after up to 35 days of treatment, and therefore was used as a negative control.

At autopsy, both kidneys of each animal were removed and each kidney was cross sectioned. Half of each kidney was sent to RNA extraction and analysis.

RNA Isolation from Kidney Tissues

RNA was isolated as described previously. RNA samples which did not meet the conventional RNA quality criteria including A260: A280 ratio, A260:A230 ratio; and RNA integrity as detected in agarose gel, were omitted from further processing.

RT Preparation and Real-Time qRT-PCR Analysis

RT was prepared and qRT-PCR performed and analyzed as described hereinabove. qRT-PCR was performed for the four genes comprising the signature of the discovery stage described in Table 13. Two amplicons were tested for each gene – one representing the wild-type transcript, and another representing an alternative splice variant. Each amplicon was measured using the labeled samples described in Table 9 herein, as well as the un-labeled samples. Samples from both groups (labeled and un-labeled) were tested in seven 96 well plates as described in Table 25. In order to correct possible run to run variations, the following controls were added: 1. several samples were repeated in all 96 wells plates; 2. control shuffled plate was designed which combined samples from all seven test plates.

Table 25 presents plates set up in a validation stage. The names of the un-labeled samples comprise of the rat's group, the rats ID number starts with UK (stand for unknown), the toxicant it was exposed to (T1, T2 or T3), the dose given Ds1 or Ds2 and day of treatment.

Table 25: Plates set up in the validation stage

Plate 1

1	Gentamycin	1	day 1 toxicants M	1_2M10_day1_Gent
2	Gentamycin	1		2_2M11_day1_Gent
3	Gentamycin	1		3_2M12_day1_Gent
4	Cisplatin	1		4_3M19_day1_Cis
5	Cisplatin	1		5_3M20_day1_Cis
6	Cisplatin	1		6_3M21_day1_Cis
7	Tobramycin	1		7_4M28_day1_Tob
8	Tobramycin	1		8_4M29_day1_Tob
9	Tobramycin	1		9_4M30_day1_Tob
10	Cadmium chloride	1		10_5M37_day1_CadCl
11	Cadmium chloride	1		11_5M38_day1_CadCl
12	Cadmium chloride	1		12_5M39_day1_CadCl
13	Doxorubicin	1		13_6M46_day1_Dox
14	Doxorubicin	1		14_6M47_day1_Dox
15	Doxorubicin	1		15_6M48_day1_Dox
16	Valproic acid	1		112_7M55_day1_ValpA
17	Valproic acid	1		113_7M56_day1_ValpA
18	Valproic acid	1		114_7M57_day1_ValpA
19	Gentamycin	1	day 1 toxicants F	16_2F110_day1_Gent
20	Gentamycin	1		17_2F111_day1_Gent
21	Gentamycin	1		18_2F112_day1_Gent
22	Cisplatin	1		19_3F119_day1_Cis
23	Cisplatin	1		20_3F120_day1_Cis
24	Cisplatin	1		21_3F121_day1_Cis
25	Tobramycin	1		22_4F128_day1_Tob
26	Tobramycin	1		23_4F129_day1_Tob
27	Tobramycin	1		24_4F130_day1_Tob
28	Cadmium chloride	1		25_5F137_day1_CadCl
29	Cadmium chloride	1		26_5F138_day1_CadCl
30	Cadmium chloride	1		27_5F139_day1_CadCl
31	Doxorubicin	1		28_6F146_day1_Dox

32	Doxorubicin	1		29_6F147_day1_Dox
33	Doxorubicin	1		30_6F148_day1_Dox
34	Valproic acid	1		115_7F155_day1_ValpA
35	Valproic acid	1		116_7F156_day1_ValpA
36	Valproic acid	1		117_7F157_day1_ValpA
37	Naïve	28	Controls All days	59_8M81_day28_Naïve
38	Naïve	28		60_8M82_day28_Naïve
39	Naïve	28		61_8M83_day28_Naïve
40	Naïve	28		62_8F181_day28_Naïve
41	Naïve	28		63_8F182_day28_Naïve
42	Naïve	28		64_8F183_day28_Naïve
43	Control/Saline	1		65_1M1_day1_Saline
44	Control/Saline	1		66_1M2_day1_Saline
45	Control/Saline	1		67_1M3_day1_Saline
46	Control/Saline	1		68_1F101_day1_Saline
47	Control/Saline	1		69_1F102_day1_Saline
48	Control/Saline	1		70_1F103_day1_Saline

Plate 2

1	Gentamycin	5	day 5 toxicants M	31_2M13_day5_Gent
2	Gentamycin	5		32_2M14_day5_Gent
3	Gentamycin	5		33_2M15_day5_Gent
4	Cisplatin	5		34_3M22_day5_Cis
5	Cisplatin	5		35_3M23_day5_Cis
6	Cisplatin	5		36_3M24_day5_Cis
7	Tobramycin	5		37_4M31_day5_Tob
8	Tobramycin	5		38_4M32_day5_Tob
9	Tobramycin	5		39_4M33_day5_Tob
10	Cadmium chloride	5		40_5M40_day5_CadCl
11	Cadmium chloride	5		41_5M41_day5_CadCl
12	Cadmium chloride	5		42_5M42_day5_CadCl
13	Doxorubicin	5		43_6M49_day5_Dox
14	Doxorubicin	5		44_6M50_day5_Dox
15	Valproic acid	5		118_7M58_day5_ValpA
16	Valproic acid	5		119_7M59_day5_ValpA

17	Valproic acid	5		120_7M60_day5_ValpA
18	Gentamycin	5	day 5 toxicants F	45_2F113_day5_Gent
19	Gentamycin	5		46_2F114_day5_Gent
20	Gentamycin	5		47_2F115_day5_Gent
21	Cisplatin	5		48_3F122_day5_Cis
22	Cisplatin	5		49_3F123_day5_Cis
23	Cisplatin	5		50_3F124_day5_Cis
24	Tobramycin	5		51_4F131_day5_Tob
25	Tobramycin	5		52_4F132_day5_Tob
26	Tobramycin	5		53_4F133_day5_Tob
27	Cadmium chloride	5		54_5F141_day5_CadCl
28	Cadmium chloride	5		55_5F142_day5_CadCl
29	Doxorubicin	5		56_6F149_day5_Dox
30	Doxorubicin	5		57_6F150_day5_Dox
31	Doxorubicin	5		58_6F151_day5_Dox
32	Valproic acid	5		121_7F158_day5_ValpA
33	Valproic acid	5		122_7F159_day5_ValpA
34	Valproic acid	5		123_7F160_day5_ValpA
35	Control/Saline	5		71_1M4_day5_Saline
36	Control/Saline	5		72_1M5_day5_Saline
37	Control/Saline	5		73_1M6_day5_Saline
38	Control/Saline	5		74_1F104_day5_Saline
39	Control/Saline	5		75_1F105_day5_Saline
40	Amph. Control	1	Control	130_1M6a_day1_Ctrl
41	Amph. Control	5		131_1M13a_day5_Ctrl
42	Amph. Control	28		132_1M20a_day28_Ctrl
43	Amph. Control	1		133_1F106a_day1_Ctrl
44	Amph. Control	5		134_1F113a_day5_Ctrl
45	Amph. Control	28		135_1F120a_day28_Ctrl
46	Amphotericin	1	Amphotericin	136_6M61a_day1_Amp
47	Amphotericin	1		137_6M62a_day1_Amp
48	Amphotericin	1		138_6M63a_day1_Amp
49	Amphotericin	5		139_6M67a_day5_Amp
50	Amphotericin	5		140_6M68a_day5_Amp
51	Amphotericin	5		141_6M69a_day5_Amp
52	Amphotericin	28		142_6M73a_day28_Amp
53	Amphotericin	28		143_6M74a_day28_Amp

54	Amphotericin	28		144_6M75a_day28_Amp
55	Amphotericin	1		145_6F161a_day1_Amp
56	Amphotericin	1		146_6F162a_day1_Amp
57	Amphotericin	1		147_6F163a_day1_Amp
58	Amphotericin	5		148_6F167a_day5_Amp
59	Amphotericin	5		149_6F168a_day5_Amp
60	Amphotericin	5		150_6F169a_day5_Amp
61	Amphotericin	28		151_6F173a_day28_Amp
62	Amphotericin	28		152_6F174a_day28_Amp
63	Amphotericin	28		153_6F175a_day28_Amp

Plate 3

1	Gentamycin	28	day 28 toxicants M	76_2M16_day28_Gent
2	Gentamycin	28		77_2M17_day28_Gent
3	Gentamycin	28		78_2M18_day28_Gent
4	Cisplatin	28		79_3M25_day28_Cis
5	Cisplatin	28		80_3M26_day28_Cis
6	Cisplatin	28		81_3M27_day28_Cis
7	Tobramycin	28		82_4M34_day28_Tob
8	Tobramycin	28		83_4M35_day28_Tob
9	Tobramycin	28		84_4M36_day28_Tob
10	Cadmium chloride	28		85_5M43_day28_CadCl
11	Cadmium chloride	28		86_5M44_day28_CadCl
12	Cadmium chloride	28		87_5M45_day28_CadCl
13	Doxorubicin	28		88_6M71_day28_Dox
14	Doxorubicin	28		89_6M72_day28_Dox
15	Doxorubicin	28		90_6M73_day28_Dox
16	Valproic acid	28		124_7M61_day28_ValpA
17	Valproic acid	28		125_7M62_day28_ValpA
18	Valproic acid	28		126_7M63_day28_ValpA
19	Gentamycin	28	day 28 toxicants F	91_2F116_day28_Gent
20	Gentamycin	28		92_2F117_day28_Gent
21	Gentamycin	28		93_2F118_day28_Gent
22	Cisplatin	28		94_3F125_day28_Cis
23	Cisplatin	28		95_3F126_day28_Cis

24	Cisplatin	28		96_3F127_day28_Cis
25	Tobramycin	28		97_4F134_day28_Tob
26	Tobramycin	28		98_4F135_day28_Tob
27	Tobramycin	28		99_4F136_day28_Tob
28	Cadmium chloride	28		100_5F143_day28_CadCl
29	Cadmium chloride	28		101_5F144_day28_CadCl
30	Cadmium chloride	28		102_5F145_day28_CadCl
31	Doxorubicin	28		103_6F171_day28_Dox
32	Doxorubicin	28		104_6F172_day28_Dox
33	Doxorubicin	28		105_6F173_day28_Dox
34	Valproic acid	28		127_7F161_day28_ValpA
35	Valproic acid	28		128_7F162_day28_ValpA
36	Valproic acid	28		129_7F163_day28_ValpA
37	Control/Saline	28	Day 28 control	106_1M7_day28_Saline
38	Control/Saline	28		107_1M8_day28_Saline
39	Control/Saline	28		108_1M9_day28_Saline
40	Control/Saline	28		109_1F107_day28_Saline
41	Control/Saline	28		110_1F108_day28_Saline
42	Control/Saline	28		111_1F109_day28_Saline

Plate 4

1	control	1	1_UK1M1_control_Day1	TT4: Male Day 1
2	control	1	2_UK1M2_control_Day1	
3	control	1	3_UK1M3_control_Day1	
4	control	1	4_UK1M4_control_Day1	
5	control	1	5_UK1M5_control_Day1	
6	control	1	6_UK1M6_control_Day1	
7	T1 Ds1	1	7_UK3M13_T1 Ds1_Day1	
8	T1 Ds1	1	8_UK3M14_T1 Ds1_Day1	
9	T1 Ds1	1	9_UK3M15_T1 Ds1_Day1	
10	T1 Ds1	1	10_UK3M16_T1 Ds1_Day1	
11	T1 Ds1	1	11_UK3M17_T1 Ds1_Day1	
12	T1 Ds1	1	12_UK3M18_T1 Ds1_Day1	
13	T1 Ds2	1	13_UK5M25_T1 Ds2_Day1	
14	T1 Ds2	1	14_UK5M26_T1 Ds2_Day1	
15	T1 Ds2	1	15_UK5M27_T1 Ds2_Day1	

16	T1 Ds2	1	16_UK5M28_T1 Ds2_Day1
17	T1 Ds2	1	17_UK5M29_T1 Ds2_Day1
18	T1 Ds2	1	18_UK5M30_T1 Ds2_Day1
19	T2 Ds1	1	19_UK7M37_T2 Ds1_Day1
20	T2 Ds1	1	20_UK7M38_T2 Ds1_Day1
21	T2 Ds1	1	21_UK7M39_T2 Ds1_Day1
22	T2 Ds1	1	22_UK7M40_T2 Ds1_Day1
23	T2 Ds1	1	23_UK7M41_T2 Ds1_Day1
24	T2 Ds1	1	24_UK7M42_T2 Ds1_Day1
25	T2 Ds2	1	25_UK9M49_T2 Ds2_Day1
26	T2 Ds2	1	26_UK9M50_T2 Ds2_Day1
27	T2 Ds2	1	27_UK9M51_T2 Ds2_Day1
28	T2 Ds2	1	28_UK9M52_T2 Ds2_Day1
29	T2 Ds2	1	29_UK9M53_T2 Ds2_Day1
30	T2 Ds2	1	30_UK9M54_T2 Ds2_Day1
31	T3 Ds1	1	31_UK11M61_T3 Ds1_Day1
32	T3 Ds1	1	32_UK11M62_T3 Ds1_Day1
33	T3 Ds1	1	33_UK11M63_T3 Ds1_Day1
34	T3 Ds1	1	34_UK11M64_T3 Ds1_Day1
35	T3 Ds1	1	35_UK11M65_T3 Ds1_Day1
36	T3 Ds1	1	36_UK11M66_T3 Ds1_Day1
37	T3 Ds2	1	37_UK13M73_T3 Ds2_Day1
38	T3 Ds2	1	38_UK13M75_T3 Ds2_Day1
39	T3 Ds2	1	39_UK13M76_T3 Ds2_Day1
40	T3 Ds2	1	40_UK13M77_T3 Ds2_Day1
41	T3 Ds2	1	41_UK13M78_T3 Ds2_Day1

Plate 5

42	control	1	42_UK1F101_control_Day1
43	control	1	43_UK1F102_control_Day1
44	control	1	44_UK1F103_control_Day1
45	control	1	45_UK1F104_control_Day1
46	control	1	46_UK1F105_control_Day1
47	control	1	47_UK1F106_control_Day1
48	T1 Ds1	1	48_UK3F113_T1 Ds1_Day1
49	T1 Ds1	1	49_UK3F114_T1 Ds1_Day1
50	T1 Ds1	1	50_UK3F116_T1 Ds1_Day1
51	T1 Ds1	1	51_UK3F117_T1 Ds1_Day1
52	T1 Ds1	1	52_UK3F118_T1 Ds1_Day1

TTS: Female Day 1

53	T1 Ds2	1	53_UK5F125_T1 Ds2_Day1	
54	T1 Ds2	1	54_UK5F126_T1 Ds2_Day1	
55	T1 Ds2	1	55_UK5F127_T1 Ds2_Day1	
56	T1 Ds2	1	56_UK5F128_T1 Ds2_Day1	
57	T1 Ds2	1	57_UK5F129_T1 Ds2_Day1	
58	T1 Ds2	1	58_UK5F130_T1 Ds2_Day1	
59	T2 Ds1	1	59_UK7F137_T2 Ds1_Day1	
60	T2 Ds1	1	60_UK7F139_T2 Ds1_Day1	
61	T2 Ds1	1	61_UK7F140_T2 Ds1_Day1	
62	T2 Ds1	1	62_UK7F141_T2 Ds1_Day1	
63	T2 Ds1	1	63_UK7F142_T2 Ds1_Day1	
64	T2 Ds2	1	64_UK9F149_T2 Ds2_Day1	
65	T2 Ds2	1	65_UK9F150_T2 Ds2_Day1	
66	T2 Ds2	1	66_UK9F151_T2 Ds2_Day1	
67	T2 Ds2	1	67_UK9F152_T2 Ds2_Day1	
68	T2 Ds2	1	68_UK9F153_T2 Ds2_Day1	
69	T2 Ds2	1	69_UK9F154_T2 Ds2_Day1	
70	T3 Ds1	1	70_UK11F161_T3 Ds1_Day1	
71	T3 Ds1	1	71_UK11F163_T3 Ds1_Day1	
72	T3 Ds1	1	72_UK11F164_T3 Ds1_Day1	
73	T3 Ds1	1	73_UK11F165_T3 Ds1_Day1	
74	T3 Ds1	1	74_UK11F166_T3 Ds1_Day1	
75	T3 Ds2	1	75_UK13F173_T3 Ds2_Day1	
76	T3 Ds2	1	76_UK13F175_T3 Ds2_Day1	
77	T3 Ds2	1	77_UK13F176_T3 Ds2_Day1	
78	T3 Ds2	1	78_UK13F177_T3 Ds2_Day1	
79	T3 Ds2	1	79_UK13F178_T3 Ds2_Day1	

Plate 6

80	control	5	80_UK2M7_control_Day5	TT6 Male Day 5
81	control	5	81_UK2M8_control_Day5	
82	control	5	82_UK2M9_control_Day5	
83	control	5	83_UK2M10_control_Day5	
84	control	5	84_UK2M11_control_Day5	
85	T1 Ds1	5	85_UK4M19_T1 Ds1_Day5	
86	T1 Ds1	5	86_UK4M20_T1 Ds1_Day5	
87	T1 Ds1	5	87_UK4M21_T1 Ds1_Day5	
88	T1 Ds1	5	88_UK4M22_T1 Ds1_Day5	
89	T1 Ds1	5	89_UK4M23_T1 Ds1_Day5	

90	T1 Ds1	5	90_UK4M24_T1 Ds1_Day5
91	T1 Ds2	5	91_UK6M31_T1 Ds2_Day5
92	T1 Ds2	5	92_UK6M32_T1 Ds2_Day5
93	T1 Ds2	5	93_UK6M33_T1 Ds2_Day5
94	T1 Ds2	5	94_UK6M34_T1 Ds2_Day5
95	T1 Ds2	5	95_UK6M35_T1 Ds2_Day5
96	T1 Ds2	5	96_UK6M36_T1 Ds2_Day5
97	T2 Ds1	5	97_UK8M43_T2 Ds1_Day5
98	T2 Ds1	5	98_UK8M44_T2 Ds1_Day5
99	T2 Ds1	5	99_UK8M45_T2 Ds1_Day5
100	T2 Ds1	5	100_UK8M46_T2 Ds1_Day5
101	T2 Ds1	5	101_UK8M47_T2 Ds1_Day5
102	T2 Ds1	5	102_UK8M48_T2 Ds1_Day5
103	T2 Ds2	5	103_UK10M55_T2 Ds2_Day5
104	T2 Ds2	5	104_UK10M56_T2 Ds2_Day5
105	T2 Ds2	5	105_UK10M57_T2 Ds2_Day5
106	T2 Ds2	5	106_UK10M59_T2 Ds2_Day5
107	T2 Ds2	5	107_UK10M60_T2 Ds2_Day5
108	T3 Ds1	5	108_UK12M67_T3 Ds1_Day5
109	T3 Ds1	5	109_UK12M69_T3 Ds1_Day5
110	T3 Ds1	5	110_UK12M70_T3 Ds1_Day5
111	T3 Ds1	5	111_UK12M71_T3 Ds1_Day5
112	T3 Ds1	5	112_UK12M72_T3 Ds1_Day5
113	T3 Ds2	5	113_UK14M80_T3 Ds2_Day5
114	T3 Ds2	5	114_UK14M81_T3 Ds2_Day5
115	T3 Ds2	5	115_UK14M82_T3 Ds2_Day5
116	T3 Ds2	5	116_UK14M83_T3 Ds2_Day5

Plate 7

117	control	5	117_UK2F107_control_Day5
118	control	5	118_UK2F108_control_Day5
119	control	5	119_UK2F109_control_Day5
120	control	5	120_UK2F110_control_Day5
121	control	5	121_UK2F111_control_Day5
122	control	5	122_UK2F112_control_Day5
123	T1 Ds1	5	123_UK4F119_T1 Ds1_Day5
124	T1 Ds1	5	124_UK4F120_T1 Ds1_Day5
125	T1 Ds1	5	125_UK4F121_T1 Ds1_Day5
126	T1 Ds1	5	126_UK4F122_T1 Ds1_Day5

TT7: Female Day 5

127	T1 Ds1	5	127_UK4F123_T1 Ds1_Day5
128	T1 Ds1	5	128_UK4F124_T1 Ds1_Day5
129	T1 Ds2	5	129_UK6F131_T1 Ds2_Day5
130	T1 Ds2	5	130_UK6F132_T1 Ds2_Day5
131	T1 Ds2	5	131_UK6F133_T1 Ds2_Day5
132	T1 Ds2	5	132_UK6F134_T1 Ds2_Day5
133	T1 Ds2	5	133_UK6F135_T1 Ds2_Day5
134	T1 Ds2	5	134_UK6F136_T1 Ds2_Day5
135	T2 Ds1	5	135_UK8F143_T2 Ds1_Day5
136	T2 Ds1	5	136_UK8F144_T2 Ds1_Day5
137	T2 Ds1	5	137_UK8F145_T2 Ds1_Day5
138	T2 Ds1	5	138_UK8F147_T2 Ds1_Day5
139	T2 Ds1	5	139_UK8F148_T2 Ds1_Day5
140	T2 Ds2	5	140_UK10F156_T2 Ds2_Day5
141	T2 Ds2	5	141_UK10F158_T2 Ds2_Day5
142	T2 Ds2	5	142_UK10F159_T2 Ds2_Day5
143	T2 Ds2	5	143_UK10F160_T2 Ds2_Day5
144	T3 Ds1	5	144_UK12F167_T3 Ds1_Day5
145	T3 Ds1	5	145_UK12F168_T3 Ds1_Day5
146	T3 Ds1	5	146_UK12F169_T3 Ds1_Day5
147	T3 Ds1	5	147_UK12F170_T3 Ds1_Day5
148	T3 Ds1	5	148_UK12F171_T3 Ds1_Day5
149	T3 Ds1	5	149_UK12F172_T3 Ds1_Day5
150	T3 Ds2	5	150_UK14F179_T3 Ds2_Day5
151	T3 Ds2	5	151_UK14F180_T3 Ds2_Day5
152	T3 Ds2	5	152_UK14F181_T3 Ds2_Day5
153	T3 Ds2	5	153_UK14F182_T3 Ds2_Day5
154	T3 Ds2	5	154_UK14F183_T3 Ds2_Day5

Shuffled plate

<u>Toxin used</u>	<u>Day</u>	<u>Final name</u>
Cadmium chloride	1	26_5F138_day1_CadCl
Cisplatin	1	6_3M21_day1_Cis
Cisplatin	1	5_3M20_day1_Cis
Control/Saline	1	66_1M2_day1_Saline
Doxorubicin	1	30_6F148_day1_Dox
Doxorubicin	1	28_6F146_day1_Dox
Naïve	28	61_8M83_day28_Naïve
Naïve	28	62_8F181_day28_Naïve
Tobramycin	1	8_4M29_day1_Tob
Valproic acid	1	112_7M55_day1_ValpA
Valproic acid	1	117_7F157_day1_ValpA
Cadmium chloride	5	54_5F141_day5_CadCl
Cadmium chloride	5	41_5M41_day5_CadCl
Cisplatin	5	50_3F124_day5_Cis
Cisplatin	5	36_3M24_day5_Cis
Control/Saline	5	73_1M6_day5_Saline
Control/Saline	5	71_1M4_day5_Saline
Doxorubicin	5	43_6M49_day5_Dox
Tobramycin	5	51_4F131_day5_Tob
Valproic acid	5	123_7F160_day5_ValpA
Valproic acid	5	122_7F159_day5_ValpA
Cadmium chloride	28	85_5M43_day28_CadCl
Cadmium chloride	28	86_5M44_day28_CadCl
Cisplatin	28	79_3M25_day28_Cis
Cisplatin	28	94_3F125_day28_Cis
Cisplatin	28	81_3M27_day28_Cis
Control/Saline	28	106_1M7_day28_Saline
Control/Saline	28	107_1M8_day28_Saline
Control/Saline	28	111_1F109_day28_Saline
Doxorubicin	28	104_6F172_day28_Dox
Tobramycin	28	84_4M36_day28_Tob
Valproic acid	28	126_7M63_day28_ValpA

<u>Toxin used</u>	<u>Day</u>	<u>Final name</u>
control	1	2_UK1M2_control_Day1
T1 Ds1	1	7_UK3M13_T1 Ds1_Day1
T1 Ds1	1	12_UK3M18_T1 Ds1_Day1
T1 Ds2	1	16_UK5M28_T1 Ds2_Day1
T2 Ds1	1	24_UK7M42_T2 Ds1_Day1
T2 Ds1	1	20_UK7M38_T2 Ds1_Day1
T3 Ds1	1	31_UK11M61_T3 Ds1_Day1
T3 Ds2	1	38_UK13M75_T3 Ds2_Day1
T3 Ds2	1	41_UK13M78_T3 Ds2_Day1
control	1	43_UK1F102_control_Day1
control	1	46_UK1F105_control_Day1
T1 Ds1	1	51_UK3F117_T1 Ds1_Day1
T1 Ds1	1	52_UK3F118_T1 Ds1_Day1
T1 Ds2	1	56_UK5F128_T1 Ds2_Day1
T2 Ds1	1	63_UK7F142_T2 Ds1_Day1
T2 Ds1	1	61_UK7F140_T2 Ds1_Day1
T2 Ds2	1	68_UK9F153_T2 Ds2_Day1
T2 Ds2	1	66_UK9F151_T2 Ds2_Day1
T3 Ds1	1	72_UK11F164_T3 Ds1_Day1
T3 Ds2	1	78_UK13F177_T3 Ds2_Day1
control	5	80_UK2M7_control_Day5
control	5	83_UK2M10_control_Day5
control	5	84_UK2M11_control_Day5
control	5	82_UK2M9_control_Day5
T1 Ds1	5	86_UK4M20_T1 Ds1_Day5
T1 Ds1	5	88_UK4M22_T1 Ds1_Day5
T1 Ds2	5	93_UK6M33_T1 Ds2_Day5
T1 Ds2	5	94_UK6M34_T1 Ds2_Day5
T2 Ds1	5	97_UK8M43_T2 Ds1_Day5
T3 Ds1	5	108_UK12M67_T3 Ds1_Day5
T3 Ds1	5	110_UK12M70_T3 Ds1_Day5
control	5	118_UK2F108_control_Day5
T1 Ds1	5	125_UK4F121_T1 Ds1_Day5
T1 Ds2	5	129_UK6F131_T1 Ds2_Day5
T1 Ds2	5	131_UK6F133_T1 Ds2_Day5
T2 Ds1	5	139_UK8F148_T2 Ds1_Day5
T2 Ds2	5	140_UK10F156_T2 Ds2_Day5

<u>Toxin used</u>	<u>Day</u>	<u>Final name</u>
T2 Ds2	5	143_UK10F160_T2 Ds2_Day5
T3 Ds1	5	147_UK12F170_T3 Ds1_Day5
T3 Ds1	5	146_UK12F169_T3 Ds1_Day5
T3 Ds2	5	151_UK14F180_T3 Ds2_Day5
T3 Ds2	5	153_UK14F182_T3 Ds2_Day5

Inter-Plate Normalization

To account for possible differences of qRT-PCR measurements between the different plates due to experimental artifacts, a normalization plate was planned. This plate, designated hereinabove 'shuffled plate', contained samples from all other plates. Real Time PCR was performed on this plate using the primers appropriate for all candidates in tables 26 and 27. For each other plate (plates 1-7 above) and each amplicon, the samples appearing also in the shuffled plate were examined – the ratio between the transcript abundance as measured in the plate to the abundance as measured in the shuffled plate was calculated, outliers were manually removed, and the geometric mean of the left ratios was taken as an additional multiplicative normalization factor for the plate and amplicon.

Real Time qPCR analysis of the selected markers

Example 3.1: Expression of tumor necrosis factor receptor superfamily, member 12a, AA686189, transcripts which are detectable by amplicon as depicted in sequence name W41270_DB81_seg11 in kidney tissues of treated or untreated rats

Expression of tumor necrosis factor receptor superfamily, member 12a transcripts detectable by or according to seg11 - W41270_DB81_seg11_F2R2 (SEQ ID NO: 254) amplicon and primers W41270_DB81_seg11_F2 (SEQ ID NO: 252) and W41270_DB81_seg11_R2 (SEQ ID NO: 253) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization". Following optimization, described in Example 3 herein, the experiments were done using primers concentration of 50nM.

The column entitled W41270_DB81_seg11 in Table 23 contains the normalized expression values of the above-indicated tumor necrosis factor receptor superfamily, member 12a transcript in treated or untreated kidney samples.

As is evident from the column entitled W41270_DB81_seg11 in Table 23, the level of expression of the tumor necrosis factor receptor superfamily, member 12a transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers 59-75 and 106-129, in kidney tissue panel described in Table 9

hereinabove). Statistical analysis was applied to verify the significance of these results, with a P-value for day 5 of $3.4E^{-05}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W41270_DB81_seg11_F2 (SEQ ID NO: 252) forward primer; and W41270_DB81_seg11_R2 (SEQ ID NO: 253) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

W41270_DB81_seg11_F2R2 (SEQ ID NO: 254).

Forward primer (W41270_DB81_seg11_F2 (SEQ ID NO: 252)):

GATCTGGGTAGGTGGTTGTTGG

Reverse primer (W41270_DB81_seg11_R2 (SEQ ID NO: 253)):

CGCACACCCTTATAAAAGTCCC

Amplicon (W41270_DB81_seg11_F2R2 (SEQ ID NO: 254)):

GATCTGGGTAGGTGGTTGTTGGGCGAGAAAGGAGGTCGTAGACTTAGGATATAGGAAACCA
GGAAAACTGACTGAGGAAGGGACTTTTATAAGGGTGTGCG

Example 3.2: Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts which are detectable by amplicon as depicted in sequence name AI045075_DB71_seg6 in kidney tissues of treated or untreated rats

Expression of Interferon stimulated exonuclease 20 (ISG20) transcripts detectable by or according to seg6 - AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263) amplicon and primers AI045075_DB71_seg6_F2 (SEQ ID NO: 261) and AI045075_DB71_seg6_R2 (SEQ ID NO: 262) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization".

The column entitled AI045075_DB71_seg6 in Table 23 contains the normalized expression values of the above-indicated Interferon stimulated exonuclease 20 (ISG20) transcript in treated or untreated kidney samples.

As is evident from the column entitled AI045075_DB71_seg6 in Table 23, the level of expression of the Interferon stimulated exonuclease 20 (ISG20) transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove).

Statistical analysis was applied to verify the significance of these results, P-value for day 5: $1.1E^{-06}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: AI045075_DB71_seg6_F2 (SEQ ID NO: 261) forward primer; and AI045075_DB71_seg6_R2 (SEQ ID NO: 262) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263).

Forward primer (AI045075_DB71_seg6_F2 (SEQ ID NO: 261)):

GGGCCACAATGGAGCTCTAC

Reverse primer (AI045075_DB71_seg6_R2 (SEQ ID NO: 262)):

ACAGGTCTCATTTCATGGAAACTATG

Amplicon (AI045075_DB71_seg6_F2R2 (SEQ ID NO: 263)):

GGGCCACAATGGAGCTCTACAAAATCTCTCAGCGACTCAGAGCCCAGCGAGGGCTGC
CCTGCCTGGGAACATCAGCCTGAACTTCATCCTCATCCAGGATCAGAAGCAGCTACTCCTTG
AAGGACCATAGTTTTCCATGAATGAGACCTGT

Example 3.3: Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts which are detectable by amplicon as depicted in sequence name W64472_DB81_seg2 in kidney tissues of treated or untreated rats

Expression of Interferon stimulated exonuclease 20 (ISG20), AI045075, transcripts detectable by or according to seg2 - W64472_DB81_seg2_F6R1 (SEQ ID NO: 329) amplicon and primers W64472_DB81_seg2_F6 (SEQ ID NO: 327) and W64472_DB81_seg2_R1 (SEQ ID NO: 328) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization".

The column entitled W64472_DB81_seg2 in Table 23 contains the normalized expression values of the above-indicated Interferon stimulated exonuclease 20 (ISG20), AI045075, transcript in treated or untreated kidney samples.

As is evident from the column entitled W64472_DB81_seg2 in Table 23, the level of expression of the Interferon stimulated exonuclease 20 (ISG20), AI045075, transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9

hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $1.0E^{-05}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W64472_DB81_seg2_F6 (SEQ ID NO: 327) forward primer; and
 5 W64472_DB81_seg2_R1 (SEQ ID NO:328) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

10 W64472_DB81_seg2_F6R1 (SEQ ID NO: 329).

Following optimization described in Example 3 herein, the following primers were used to amplify W64472_DB81_seg2_F6R1 (SEQ ID NO: 329) amplicon:

Forward primer (W64472_DB81_seg2_F6 (SEQ ID NO: 327)): ACAGCCTGATGCAGACAGC

Reverse primer (W64472_DB81_seg2_R1 (SEQ ID NO:328)):

15 TCTGGTTCATTATCAAGGGAAGTTG

Amplicon (W64472_DB81_seg2_F6R1 (SEQ ID NO: 329)) :

ACAGCCTGATGCAGACAGCCCTGACTCAACCTGCCAGCCCCCTTACCTGGCCAGCCTTGA
 GGAGATGGAACAGCCCAGGCTACAAGGCCTGCCCCCACTCCTCAACTTCCCTTGATAATG
 AACCAGA

20 Example 3.4: Expression of Cyclin-G1 (CCNG1), H31883, transcripts which are detectable by amplicon as depicted in sequence name H31883_DB71_seg13 in kidney tissues of treated or untreated rats

Expression of Cyclin-G1 (CCNG1) transcripts detectable by or according to seg13 - H31883_DB71_seg13_F5R5 (SEQ ID NO: 332) amplicon and primers H31883_DB71_seg13_F5 (SEQ
 25 ID NO: 330) and H31883_DB71_seg13_R5 (SEQ ID NO: 331) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization".

The column entitled H31883_DB71_seg13 in Table 23 contains the normalized expression values
 30 of the above-indicated Cyclin-G1 (CCNG1) transcript in treated or untreated kidney samples.

As is evident from the column entitled H31883_DB71_seg13 in Table 23, the level of expression of the Cyclin-G1 (CCNG1) transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-
 35 129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $2.1E^{-05}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: H31883_DB71_seg13_F5 (SEQ ID NO: 330) forward primer; and H31883_DB71_seg13_R5 (SEQ ID NO: 331) reverse primer.

5 The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon:

H31883_DB71_seg13_F5R5 (SEQ ID NO: 332).

10 Following optimization described in Example 3 herein, the following primers were used to amplify H31883_DB71_seg13_F5R5 (SEQ ID NO: 332) amplicon:

Forward primer (H31883_DB71_seg13_F5 (SEQ ID NO: 330)):

CAGAATTGCTGCCTCAATCTAGTCC

Reverse primer (H31883_DB71_seg13_R5 (SEQ ID NO: 331)): GGTTTTGCAGATGTACTCGGTTCC

Amplicon (H31883_DB71_seg13_F5R5 (SEQ ID NO: 332)):

15 CAGAATTGCTGCCTCAATCTAGTCCCATTTGAGAAAATTTGTTTCTACTGTCTCAATAA
CTGGATGAAATATCACTCTGAAAACCTGCCTATTGCACTAAAGCTAGTTTAGGCTTGATAAA
ACACTCCAGGAGGTTTTTACCACAGACTGTTTCTATTAAAACTGCTGCTTCTCATGTACAATT
TTGTTTTTAAAAGGAACCGAGTACATCTGCAAAACC

20 Example 3.5: Expression of Cyclin-G1 (CCNG1), H31883, transcripts which are detectable by amplicon as depicted in sequence name MUSCYCG1R_DB81_seg19-20 in kidney tissues of treated or untreated rats

Expression of Cyclin-G1 (CCNG1) transcripts detectable by or according to seg19-20 - MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296) amplicon and primers MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294) and MUSCYCG1R_DB81_seg19-20_R1 (SEQ
25 ID NO: 295) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization". Following optimization described in Example 3 herein, the annealing temperature of the PCR was 62°C.

30 The column entitled MUSCYCG1R_DB81_seg19-20 in Table 23 contains the normalized expression values of the above-indicated Cyclin-G1 (CCNG1) transcript in treated or untreated kidney samples.

35 As is evident from the column entitled MUSCYCG1R_DB81_seg19-20 in Table 23, the level of expression of the Cyclin-G1 (CCNG1) transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples

numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical analysis was applied to verify the significance of these results, P-value for day 5: $1.3E^{-04}$.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294) forward primer; and MUSCYCG1R_DB81_seg19-20_R1 (SEQ ID NO: 295) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon: MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296).

Forward primer (MUSCYCG1R_DB81_seg19-20_F1 (SEQ ID NO: 294)):

GAGATCCAAGCACTGAAGTATGTAGAGT

Reverse primer (MUSCYCG1R_DB81_seg19-20_R1 (SEQ ID NO: 295)):

TCAGGAGTACAGTGGATACATTTCTCTT

Amplicon (MUSCYCG1R_DB81_seg19-20_F1R1 (SEQ ID NO: 296)):

GAGATCCAAGCACTGAAGTATGTAGAGTTAACAGAAGGAGTAGAATGTATTCAGAAA
CATTCCAAGGTATGCCAAGGTGATAGCATTGATCCTATTAGCAAGCTACAAGAGAAATGTAT
CCACTGTACTCCTGA

Example 3.6: Expression Etoposide induced 2.4 (EI24) mRNA, H31799, transcripts which are detectable by amplicon as depicted in sequence name W83813_DB81_seg27 in kidney tissues of treated or untreated rats

Expression of Etoposide induced 2.4 mRNA (EI24) transcripts detectable by or according to seg27 - W83813_DB81_seg27_F1R1 (SEQ ID NO: 287) amplicon and primers W83813_DB81_seg27_F1 (SEQ ID NO: 285) and W83813_DB81_seg27_R1 (SEQ ID NO: 286) was measured by real time PCR. The value of the expression was measured by Real-Time PCR and normalized relative to the expression of the house keeping genes, as described in section "RT Preparation and Real-Time RT-PCR Analysis" hereinabove and by the shuffled plate values, as described in section "Inter-Plate Normalization".

The column entitled W83813_DB81_seg27 in Table 23 contains the normalized expression values of the above-indicated Etoposide induced 2.4 mRNA (EI24) transcript in treated or untreated kidney samples.

As is evident from the column entitled W83813_DB81_seg27 in Table 23, the level of expression of the Etoposide induced 2.4 mRNA (EI24) transcript detectable by the above amplicon was higher in the samples treated with toxic compounds (samples 1-58 and 76-105, in kidney tissue panel described in Table 9 hereinabove) than in the control samples (naïve, saline and valproic acid treated samples; samples numbers- 59-75 and 106-129, in kidney tissue panel described in Table 9 hereinabove). Statistical

analysis was applied to verify the significance of these results, P-value for day 5: 0.04.

Primer pairs are also optionally and preferably encompassed within the present invention; for example, for the above experiment, the following primer pair was used as a non-limiting illustrative example only of a suitable primer pair: W83813_DB81_seg27_F1 (SEQ ID NO: 285) forward primer; and W83813_DB81_seg27_R1 (SEQ ID NO: 286) reverse primer.

The present invention also preferably encompasses any amplicon obtained through the use of any suitable primer pair; for example, for the above experiment, the following amplicon was obtained as a non-limiting illustrative example only of a suitable amplicon W83813_DB81_seg27_F1R1 (SEQ ID NO: 287):

Forward primer (W83813_DB81_seg27_F1 (SEQ ID NO: 285)):
AATCTAGGCTGCCTCCTGGAG

Reverse primer (W83813_DB81_seg27_R1 (SEQ ID NO: 286)):

AGGTCAATTATACAAGGCATGACTTTAG

Amplicon (W83813_DB81_seg27_F1R1 (SEQ ID NO: 287)):

AATCTAGGCTGCCTCCTGGAGGAAGATACTTAGGAGTTCAGAAGTGAAGAGATGAGG
CTTATAATACTTTTTCCTAAAGTCATGCCTTGTATAATTGACCT

Classification Using qRT-PCR Data

Given the qRT-PCR panel for the selected genes detailed in Table 23, the optimal classifier was built based on the measured expression levels of the labeled samples, as described in Table 9. This was performed again to achieve a more accurate and controlled signature.

Two equivalent optimal signatures for prediction of renal damage at day-5, as derived in the validation stage analysis of the labeled samples, are given in Table 26 (four amplicons of three genes) and Table 27 (six amplicons of four genes). Table 28 summarizes the performance of the 4-genes signature on the various groups of the labeled samples.

Table 26: Optimal Signature Day 5 (Validation Stage Analysis)

Contig	Gene name	Splice Variant / Wild Type	Internal name	Probeset
AA686189	TNFRSF12A	SV	W41270_DB81_seg11	1371785_at
AI045075	ISG20	SV	W64472_DB81_seg2	1390507_at
AI045075	ISG20	WT	AI045075_DB71_seg6	1390507_at
H31883	CCNG1	WT	H31883_DB71_seg13	1367764_at

Table 27: Optimal Signature Day 5 (Validation Stage Analysis)

Contig	Gene name	Splice Variant / Wild Type	Internal name	Probeset
AA686189	TNFRSF12A	SV	W41270_DB81_seg11	1371785_at
AI045075	ISG20	SV	W64472_DB81_seg2	1390507_at
AI045075	ISG20	WT	AI045075_DB71_seg6	1390507_at
H31883	CCNG1	WT	H31883_DB71_seg13	1367764_at
H31883	CCNG1	SV	MUSCYCG1R_DB81_seg19-20	1367764_at
H31799	EI24	SV	W83813_DB81_seg27	1388642_at

Table 28: Performance of the 4-genes signature on the various groups of the labeled samples

Group (Compound, Period, Gender)	No. of samples	Average Toxicity Score	Standard Deviation of Toxicity Score
Doxorubicin Day5 Male	2	0.77	0.07
Gentamycin Day5 Female	3	0.73	0.12
Cisplatin Day5 Female	3	0.69	0.14
Gentamycin Day5 Male	3	0.63	0.03
Cisplatin Day5 Male	3	0.63	0.27
Tobramycin Day5 Female	3	0.61	0.28
Doxorubicin Day5 Female	3	0.61	0.19
Tobramycin Day5 Male	3	0.59	0.18
Valproic-Acid Day1 Male	3	0.57	0.42
Saline Day1 Male	3	0.51	0.45
Naïve Male	3	0.41	0.42
Valproic-Acid Day1 Female	3	0.33	0.51
Valproic-Acid Day5 Female	3	0.18	0.3
Naïve Female	3	0.14	0.24
Saline Day1 Female	3	0.11	0.14
Valproic-Acid Day28 Female	3	0.07	0.12
Valproic-Acid Day5 Male	3	0.02	0.02
Valproic-Acid Day28 Male	3	0.01	0
Saline Day5 Female	2	0.01	0.01
Saline Day28 Female	3	0.01	0.01
Saline Day5 Male	3	0	0
Saline Day28 Male	3	0	0

The two optimal classifiers (signatures described in Tables 26 and 27) were applied to the qRT-PCR reads of the un-labeled samples and their calls was used to assign a level of toxicity to each group and, eventually, each compound.

5 Results

qRT-PCR Data Analysis of Labeled Samples described in Table 9 and plate 1-3 in Table 25.

Transcripts abundance was measured using qRT-PCR for 8 amplicons from the 4 genes disclosed in Table 13 hereinabove. As in Example 1, an optimal signature for day-5 was identified, distinguishing the day-5 “Toxic” samples from all control (and naïve rats) samples. Iterative feature selection and Random-Forest classifier were used as in the discovery stage, described in Example 1 hereinabove. The input data was the new qRT-PCR measurements and the gender of the rat as an extra two-values features. The same parameters as in the discovery stage were used again, performing cross validation by leaving-out randomly selected samples – 3 control samples and 3 toxic samples and repeating 500 times, choosing the bound on ‘toxicity’ as to maximize the accuracy. Two equivalent (but not identical in results) signatures were found, presented in Tables 26 and 27, consisting of 4 and 6 amplicons, coming from 3 and 4 genes, respectively. The cross-validation performance evaluations of these signatures show 80% sensitivity at about 85% specificity. The Out-Of-Bag OOB performance evaluation gave the confusion matrix presented in Table 29 (overall accuracy of 83%) and ROC curve presented in Figure 6.

Table 29: Confusion matrix

	Normal	Toxic
Predicted as Normal	34	4
Predicted as Toxic	7	19

It was possible to find a bound on the Random-Forest call – 30% of the trees in the forests giving a “Toxic” call - such that all “Toxic” samples pass that bound while only one group of control samples (Naïve males) has more than 50% (2 out of 3) of its members pass that bound. See Tables 26 and 27 for details on the signatures and Table 28 for their performance

qRT-PCR Data Analysis of the Un-labeled Samples, described in plates 4-7 in Table 25

The classifiers described in Tables 26 and 27, were used for testing the un-labeled samples. Both classifiers were applied to the measurements of each sample, used the 30% bound from above, and the number of samples pass that bound in each group were summarized. The results are given in the following Table 30

Table 30

Group (Compound, Dose, Period, Gender)	Animals (#)	'Toxic' by 4-genes Classifier (#)	'Toxic' by 6-genes Classifier (#)	'Toxic' by 4- genes Classifier (%)	'Toxic' by 6-genes Classifier (#)	Average 'Toxic' (%)
T2 Ds1 Day5 Male	6	5	5	83	83	0.83
T1 Ds2 Day5 Female	6	5	3	83	50	0.665
control Day5 Male	5	3	3	60	60	0.6
T2 Ds2 Day1 Female	6	4	3	67	50	0.585
T3 Ds1 Day5 Male	5	3	2	60	40	0.5
T2 Ds2 Day5 Female	4	2	2	50	50	0.5
T1 Ds1 Day5 Male	6	3	3	50	50	0.5
T1 Ds1 Day5 Female	6	3	3	50	50	0.5
control Day5 Female	6	3	2	50	33	0.415
T3 Ds2 Day5 Male	4	2	1	50	25	0.375
control Day1 Male	6	2	2	33	33	0.33
T1 Ds2 Day5 Male	6	2	2	33	33	0.33
T3 Ds2 Day1 Male	5	2	1	40	20	0.3
T2 Ds2 Day5 Male	5	1	2	20	40	0.3
T2 Ds1 Day5 Female	5	2	1	40	20	0.3
T2 Ds1 Day1 Male	6	2	1	33	17	0.25
T3 Ds2 Day5 Female	5	1	1	20	20	0.2
T3 Ds2 Day1 Female	5	1	1	20	20	0.2
T3 Ds1 Day1 Female	5	1	1	20	20	0.2
T2 Ds1 Day1 Female	5	1	1	20	20	0.2
control Day1 Female	6	1	1	17	17	0.17
T1 Ds1 Day1 Male	6	1	1	17	17	0.17
T3 Ds1 Day5 Female	6	1	0	17	0	0.085
T3 Ds1 Day1 Male	6	0	0	0	0	0
T2 Ds2 Day1 Male	6	0	0	0	0	0
T1 Ds2 Day1 Male	6	0	0	0	0	0

Group (Compound, Dose, Period, Gender)	Animals (#)	'Toxic' by 4-genes Classifier (#)	'Toxic' by 6-genes Classifier (#)	'Toxic' by 4- genes Classifier (%)	'Toxic' by 6-genes Classifier (#)	Average 'Toxic' (%)
T1 Ds2 Day1 Female	6	0	0	0	0	0
T1 Ds1 Day1 Female	5	0	0	0	0	0

The first two groups – “T2 Ds1 Day5 Male” and “T1 Ds2 Day5 Female” showed a clear nephrotoxic effect (more than 66% of samples were marked as “Toxic” when averaging the two classifiers), while the following six groups – “control Day5 Male”, “T2 Ds2 Day1 Female”, “T3 Ds1 Day5 Male”, “T2 Ds2 Day5 Female”, “T1 Ds1 Day5 Male”, “T1 Ds1 Day5 Female” were also suspicious of showing the effect (at least 50% of samples predicted as ‘Toxic’ on average). Therefore, consistently with the information available for the compounds T1 and T2, the results demonstrated that T1 and T2 are nephrotoxic agents. The T3 and control groups showing ‘possible’ toxic effect are either real false-positive of the classifier, or a result of some technical problem.

Using the classifier the toxicity was established after the rats were treated for 5 days only, while the visible pathological damage was seen only after 28 days for T1 and after 7 days for T2, as shown in Figures 3-5.

Therefore, combinations of biomarkers according to the present invention can be used for early detection of drug-induced nephrotoxicity. These signatures predict the future occurrence of drug induced renal toxicity before it is detected using the traditional endpoint analysis such as histopathology or clinical chemistry.

According to the present invention, an optimized assay based on these signatures can be implemented in pre-clinical studies. The markers of the invention can be used alone or in combination with other known markers for renal damage identification.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

CLAIMS

1. A diagnostic marker comprising a protein or a polynucleotide encoding same, selected from the markers listed in any one of Tables 14 and 17.
2. The diagnostic marker of claim 1, wherein the protein or the polynucleotide is present in a kidney tissue, body fluid or secretion.
3. The diagnostic marker of claim 1, wherein said marker is an isolated polynucleotide encoding a protein having an amino acid sequence as set forth in any one of SEQ ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236.
4. The diagnostic marker of claim 3, wherein the isolated polynucleotide is at least 85% homologous to any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82, 84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207.
5. The diagnostic marker of claim 4, wherein the isolated polynucleotide is at least 95% homologous to any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82,84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207.
6. The diagnostic marker of claim 5, wherein the isolated polynucleotide has a nucleic acid sequence as set forth in any one of SEQ. ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 82,84-90, 92, 94, 95-99, 101- 104, 106-108, 110, 111, 113, 114, 116-119, 133, 135-137, 178, 180-184, 189, 192-194, 196, 198, 199, 203, 206, 207.
7. The diagnostic marker of claim 6, wherein the isolated polynucleotide is consisting of a nucleic acid sequence as set forth in any one of SEQ. ID NOs: 2, 4, 5, 7, 8, 10, 11, 13, 14, 16-19, 21-23, 25, 26, 28, 29, 31, 33, 35, 37, 39, 41, 251, 254, 257, 260, 263, 266, 269, 272, 275, 278, 281, 284, 287, 290, 293, 296, 299, 302, 305, 308, 311, 314, 317, 320, 323, 326.
8. The diagnostic marker of claim 1, wherein said marker is an isolated protein or polypeptide having an amino acid sequence at least 85% homologous to any one of SEQ ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236.
9. The diagnostic marker of claim 8, wherein the isolated protein or polypeptide has an amino acid sequence at least 95% homologous to any one of SEQ ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-228, 233, 235, 236.
10. The diagnostic marker of claim 8, wherein the isolated protein or polypeptide has an amino acid sequence as set forth in any one of SEQ ID NOs: 62-71, 141, 143, 144, 146-153, 155, 156, 158-160, 162, 167-169, 172, 174-176, 210-214, 218, 221-223, 225, 227-

228, 233, 235, 236.

11. A method for screening for renal injury in a subject, comprising detecting in a sample obtained from said subject at least one diagnostic marker according to any one of claims 1-10.
- 5 12. The method of claim 11, wherein screening for renal injury comprises at least one of detecting the presence or severity of the renal injury, early detection of renal injury, prognosis of the subject, treatment selection for said subject, treatment monitoring of said subject and combinations thereof.
- 10 13. A method for predicting renal injury in a subject receiving a treatment with a test compound, comprising (a) administering a dose of the test compound to at least one subject; (b) after a selected time period, obtaining a biological sample from the at least one subject; (c) measuring in the biological sample the expression level of at least two markers selected from those listed in Table 12, fragments thereof, and markers homologous thereto; and (d) determining whether the sample is in the positive class for
15 onset of renal injury using a classifier comprising at least the two markers for which the expression level is measured.
- 20 14. The method of claim 13, wherein the at least two markers are polynucleotides having a nucleic acids sequence selected from the group consisting of SEQ ID NOs: 1, 3, 6, 9, 12, 15, 20, 24, 27, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 81-137 and 177-207, or polypeptide or proteins encoded therefrom.
- 25 15. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected form the group consisting of SEQ ID NOs: 1, 3, 6, 9, 12, 20, 24, 27, 30, 32, 34, 36, 38, 46, 48, 50, 52, 54, 56, 60, or polypeptide or proteins encoded therefrom, corresponding to markers listed in any one of Tables 14, 15, 26 and 27.
- 30 16. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 6, 12, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 13.
17. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 48, 52, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 26.
18. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 6, 12, 48, 52, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 27.
- 35 19. The method of claim 14, wherein the expression level of a set of four markers having a nucleic acid sequence set forth in SEQ ID NOs:1, 3, 6, 12, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 13 is measured, and wherein said expression of the set of four markers is indicative of the onset of renal injury.

20. The method of claim 14, wherein the expression level of a set of four markers having a nucleic acid sequence set forth in SEQ ID NOs:1, 3, 48, 52 or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 26 is measured, and wherein said expression of the set of four markers is indicative of the onset of renal injury.
- 5 21. The method of claim 14, wherein the expression level of a set of six markers having a nucleic acid sequence set forth in SEQ ID NOs:1, 3, 6, 12, 48, 52 or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 27 is measured, and wherein said expression of the set of six markers is indicative of the onset of renal injury.
- 10 22. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 9, 12, 15, 24, 32, 36, 40, 44, 46, 48, 52, 54, 56, 58, 60, or polypeptide or proteins encoded therefrom, corresponding to markers listed in any one of Tables 17 and 18.
- 15 23. The method of claim 14, wherein the at least two markers have a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 12, 46, 48, 56, 58, 60, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 16.
- 20 24. The method of claim 14, wherein the expression level of a set of seven markers having a nucleic acid sequence set forth in SEQ ID NOs:1, 12, 46, 48, 56, 58, 60, or polypeptide or proteins encoded therefrom, corresponding to markers listed in Table 16 is measured, and wherein said expression of the set of seven markers is indicative of the onset of renal injury.
- 25 25. The method of claim 13, wherein the subject is a mammal selected from the group consisting of a human, cat, dog, monkey, mouse, pig, rabbit, and rat.
26. The method of claim 25, wherein the subject is a test subject.
27. The method of claim 13, wherein the test compound is administered to the subject in a form selected from the group consisting of intravenously (IV), orally (PO, per os), intraperitoneally (IP), intranasal, inhalation, eye drops and ointments.
- 30 28. The method of claim 13, wherein the biological sample is selected from the group consisting of kidney tissue, body fluid and body secretion.
29. The method of claim 13, wherein the test compound is nephrotoxic agent selected from the group consisting of aminoglycosides; platinum based chemotherapy agents; heavy metals; DNA interacting drugs; antifungal agents; proximal tubule damaging agents; and vasoconstriction compounds.
- 35 30. The method of claim 13, wherein the renal injury is associated with at least one kidney disease or pathology, selected from the group consisting of nephrotoxicity, renal toxicity, nephritis, kidney necrosis, kidney damage, glomerular and tubular injury, focal segmental glomerulosclerosis, kidney dysfunction, nephritic syndrome, acute renal failure, chronic renal failure, proximal tubal dysfunction, acute kidney transplant rejection and chronic

kidney transplant refection.

31. The method of claim 13, wherein the selected time period after which the sample is obtained from the test subject is prior to or at the onset of the appearance of histopathological or clinical indications of renal injury.
- 5 32. The method of claim 31, wherein the selected time period is selected from the group consisting of about 1 day, about 5 days, about 7 days, about 14 days, about 21 or about 28 days.
33. The method of claim 32, wherein the selected time period is 1 day or less.
- 10 34. The method of claim 31, wherein the time period is 5 days of daily compound administration and wherein the markers are selected from the set of markers listed in any one of Tables 13, 14, 15, 26 or 27.
35. The method of claim 31, wherein the time period is 1 day after compound administration and wherein the markers are selected from the set of markers listed in any one of Tables 16, 17 or 18.
- 15 36. The method of claim 13, wherein the classifier is a non-linear classifier.
37. The method of claim 13, wherein the classifier is a linear classifier.
38. The method of claim 13, wherein the classifier is a Random Forest classifier.
39. The method of claim 14, wherein the marker is a polynucleotide and wherein the level of expression is detected by an amplification or hybridization assay.
- 20 40. The method of claim 39, wherein the amplification assay is selected from the group consisting of quantitative or semiquantitative PCR, Northern blot, dot or slot blot, nuclease protection and microarray assays.
41. The method of claim 14, wherein the marker is a polypeptide or protein and wherein the level of expression is detected by an immunoassay.
- 25 42. The method of claim 41, wherein said immunoassay is selected from the group consisting of an ELISA, an RIA, a slot blot, immunohistochemical assay, FACS, a radio-imaging assay or a Western blot.
43. An isolated polynucleotide probe or primer comprising a nucleic acid sequence that specifically hybridizes to a marker having a nucleic acid sequence as set forth in any one of SEQ ID NOs: 1-61, 81-137, 177-207.
- 30 44. A set of at least two isolated polynucleotide probes or primers, wherein each of the probes or primers comprises a nucleic acid sequence that specifically hybridizes to an amplicon comprising SEQ ID NOs: 251, 254, 257, 260, 263, 266, 269, 272, 275, 278, 281, 284, 287, 290, 293, 296, 299, 302, 305, 308, 311, 314, 317, 320, 323, 326, 329, 332.
- 35 45. The set of claim 44 wherein the probes or primers hybridize to at least a plurality of

markers selected from any one of Tables 13, 14, 15, 26 and 27.

46. The set of claim 44, wherein the plurality of markers comprises all the markers of Table 13.

47. The set of claim 44, wherein the probes or primers hybridize to at least a plurality of markers selected from any one of Tables 16, Table 17 and Table 18.

48. The set of claim 47, wherein the plurality of markers comprises all the markers of Table 16.

49. The set of claim 44, wherein the at least two primers or probes are consisting of a nucleic acid sequence set forth in any one of SEQ ID NO: 249-250, 252-253, 255-256, 258-259, 261-262, 264-265, 267-268, 270-271, 273-274, 276-277, 279-280, 282-283, 285-286, 288-289, 291-292, 294-295, 297-298, 300-301, 303-304, 306-307, 309-310, 312-313, 315-316, 318-319, 321-322, 324-325, 327-328, 330-331.

50. The set of probes of claim 44, wherein the probes are attached to a solid support.

51. The set of probes of claim 50, wherein the solid support is selected from the group consisting of a membrane, a glass support and silicon support.

52. A kit for detecting renal injury, comprising at least two primers or probes and reagents for detecting at least two polynucleotide markers corresponding to those listed in Table 12, fragments thereof, and markers homologous thereto.

53. The kit of claim 52, comprising at least a plurality of primers or probes for detecting a plurality of markers listed in Table 12, fragments thereof, and markers homologous thereto.

54. The kit of claim 52, wherein the primer or probes are consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NOs.: 249-250, 252-253, 255-256, 258-259, 261-262, 264-265, 267-268, 270-271, 273-274, 276-277, 279-280, 282-283, 285-286, 288-289, 291-292, 294-295, 297-298, 300-301, 303-304, 306-307, 309-310, 312-313, 315-316, 318-319, 321-322, 324-325, 327-328, 330-331.

55. The kit of claim 52, wherein the reagents for detecting the at least two marker are reagents for employing a NAT-based technology.

56. The kit of claim 55, wherein the NAT-based assay is selected from the group consisting of a PCR, Real-Time PCR, LCR, Self-Sustained Synthetic Reaction, Q-Beta Replicase, Cycling Probe Reaction, Branched DNA, RFLP analysis, DGGE/TGGE, Single-Strand Conformation Polymorphism, Dideoxy Fingerprinting, Microarrays, Fluorescence In Situ Hybridization or Comparative Genomic Hybridization.

57. A kit for detecting renal injury, comprising at least two antibodies capable of recognizing or interacting with at least to polypeptides or proteins corresponding to the markers listed in Table 12, fragments thereof and markers homologous thereto, further comprising

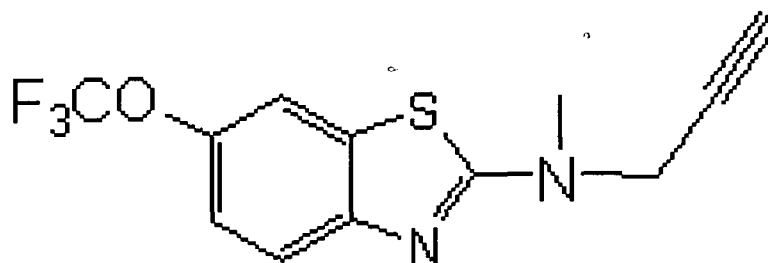
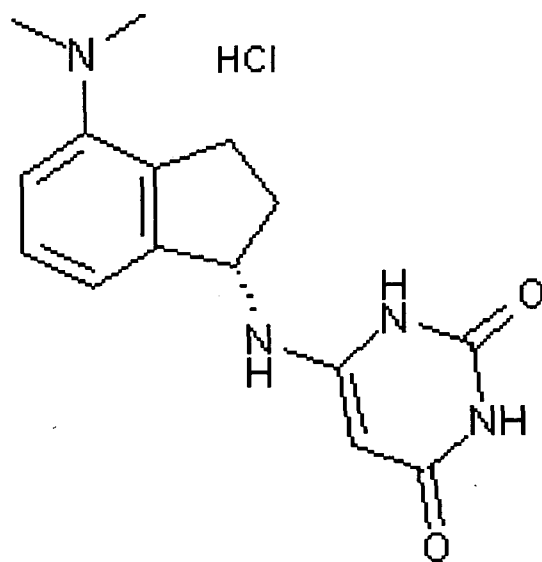
reagents for employing an immunoassay.

58. The kit of claim 57, comprising at least a plurality of antibodies capable of recognizing or interacting with a plurality of polypeptide or proteins corresponding to the markers listed in Table 12, fragments thereof and markers homologous thereto.

5 59. The kit of claim 57 wherein the immunoassays is selected from the group consisting of ELISA, an RIA, a slot blot, an immunohistochemical assay, FACS, in-vivo imaging, a radio-imaging assay, or a Western blot.

10 60. A method for selecting a treatment or monitoring a treatment for renal injury comprising (a) obtaining a first sample from a subject suffering from renal injury; (b) administering the treatment to the subject; (c) obtaining a second sample from said subject; (d) measuring in the first and second samples the expression level of at least two markers selected from those listed in Table 12 or Table 21; and (d) determining a change in the expression and/or level of the polynucleotides or polypeptides in said second sample compared to the expression and/or level in said first sample, wherein relative change in
15 said expression and/or level of the polynucleotide or polypeptide is useful for selecting a treatment and/or monitoring a treatment of the disease.

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**FIGURE 1****FIGURE 2**

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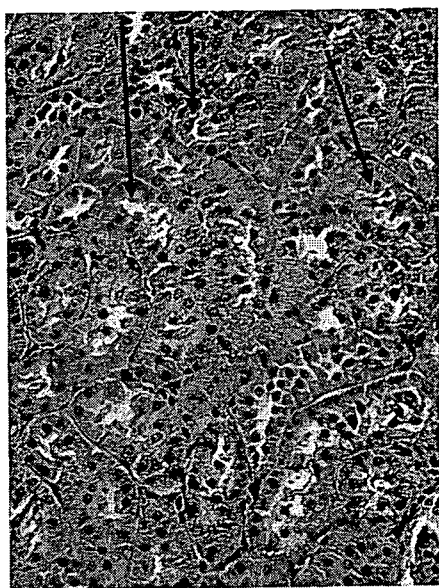


FIGURE 3A



FIGURE 3B

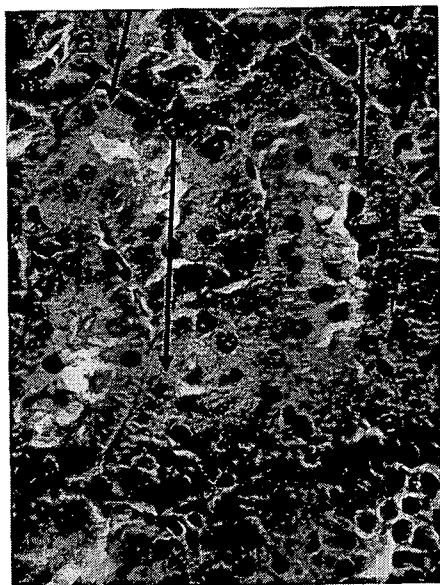


FIGURE 4A



FIGURE 4B

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FIGURE 5A

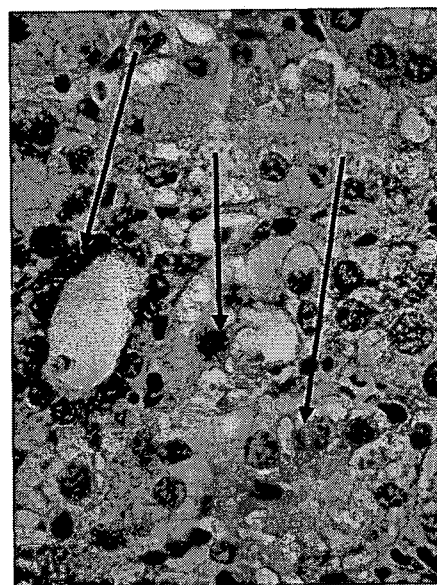
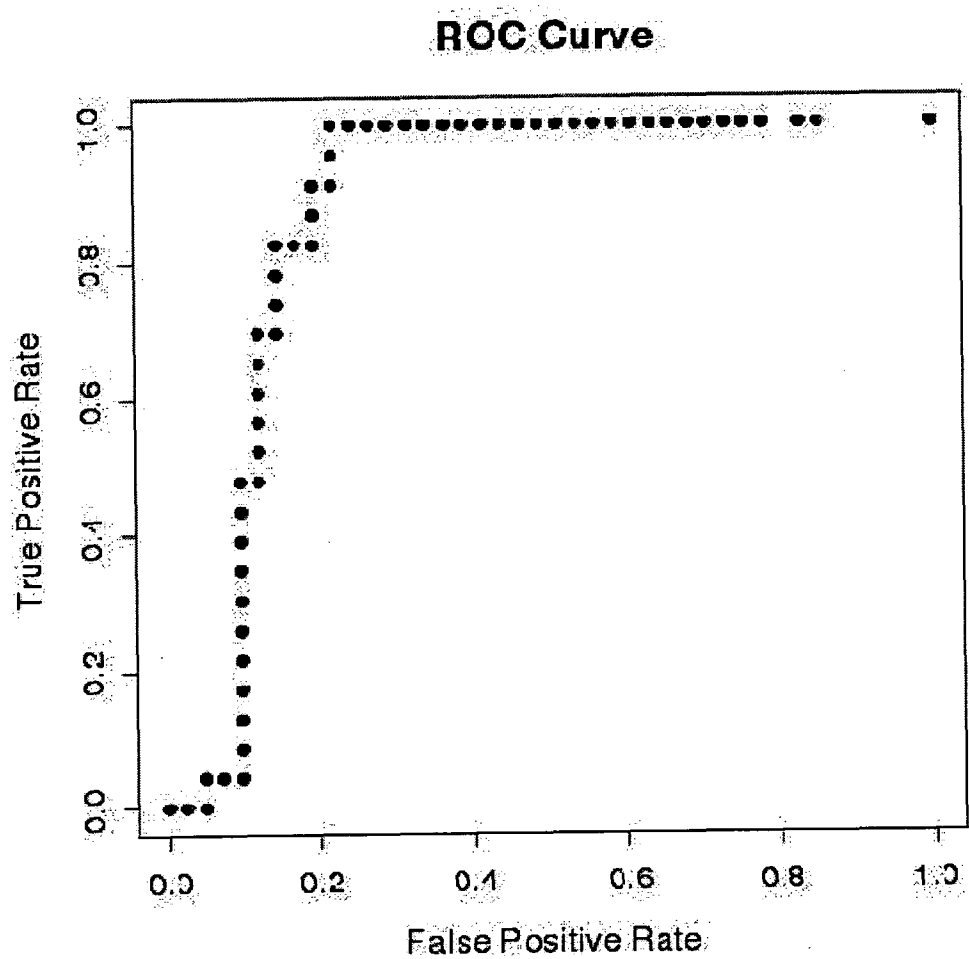


FIGURE 5B

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**FIGURE 6**