

**EPIGENETIC MARKERS FOR DETECTING OXIDATIVE STRESS****FIELD OF THE INVENTION**

The present invention relates to a method for detecting oxidative stress (OS) in a cell. In particular, the method is capable of identifying OS in cell by using a gene panel with at least two genes that  
5 are differentially methylated in a cell with OS relative to a cell without OS. More in particular, the differential methylation takes place on in the gene body and the regulatory region of the genes in the gene panel.

**BACKGROUND OF THE INVENTION**

Living organisms are subjected continuously to a variety of stresses from the outside environment.  
10 In order to resist such stresses, they maintain their homeostasis by various regulatory systems. Oxidative stress refers to a serious imbalance between the levels of reactive oxygen species (ROS) in a cell and its antioxidant defense mechanism. In order to survive this stress, living organisms have a system called redox regulation to cope with the stress to maintain their homeostasis by regulating the redox state. This system functions to adapt to many external stress  
15 agents such as radiation, ultraviolet (UV) light rays, environmental pollutants, high fever, low temperature, hypoxic condition, and infectious diseases as well as to oxidative stress from lifestyle-related diseases such as cancer, diabetes, arteriosclerosis, hypertension and obesity. However, if this regulation mechanism is broken for some reason or other, oxidative stress (OS) occurs. OS can lead to cellular damage, DNA fragmentation, apoptosis and cell death. Early detection of OS  
20 can prevent further damage in the living organism causing the organism to receive early treatment or start using protection.

There are some methods known in the art for early detection of OS. However, none of these methods known in the art have been officially used to detect OS in a cell by using a genomic sample of the body.

25 The human skin is constantly exposed to oxidative stress and free radicals, such as to high quantities of ROS, derived not only from ordinary metabolic reactions but also continuous exposure to air, radiation and UV light rays, environmental pollutants, as well as physical and/or chemical agents (e.g., cosmetics). Under some conditions, the production of ROS may become so great that it may contribute to the pathogenesis of, for example, psoriasis or skin cancer. Oxidative damage  
30 caused by free radicals such as ROS is also a main cause of physical ageing in general, and of the skin in particular. Accordingly, there is a need in the art for detection of OS in cells, for example skin cells to prevent further damage to the cells.

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to air, radiation and UV light rays, environmental pollutants, as well as physical and/or chemical agents (e.g., cosmetics). Under some conditions, the production of ROS may become so great that it may contribute to the pathogenesis of, for example, psoriasis or skin cancer. Oxidative damage caused by free radicals such as ROS is also a main cause of physical ageing in general, and of the skin in particular. Accordingly, there is a need in the art for early detection of OS in cells, for example skin cells to prevent further damage to the cells.

## DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing a method of using a gene panel with at least two genes that are differentially methylated in a cell with OS. In particular, at least two genes including Protein Tyrosine Phosphatase Receptor Type N2 (*PTPRN2*), differential methylation of which, is capable of being used for detecting OS in a cell.

Since environmental factors/ agents such as, UV light exposure, ageing, diet and the like, may trigger OS which can further induce an alteration in the promoter CpG methylation status of the gene by recruiting DNA methyltransferases (DNMTs) and TET enzymes to various promoters, biomarkers that result in differential methylation in a cell with OS is essential to overcome the problems mentioned above. In particular, genes which can be used as biomarkers for detecting OS in a cell include *PTPRN2* and other specific genes. *PTPRN2* and other specific genes in a cell with OS are differentially methylated (i.e. hypomethylated or hypermethylated) compared to the corresponding genes in a cell without OS. Accordingly, *PTPRN2* and other specific genes may be effectively used to determine if a cell has OS. Similarly, a gene panel comprising at least *PTPRN2* and other specific genes may be used to detect OS in a cell as these genes will be differentially methylated compared to a cell without OS. This is particularly advantageous as using epigenetics provides a means of predicting the onset of OS in a cell, thus allowing OS to be treated earlier before causing even more damage to the cell. Further, an epigenetic marker is a long-term biomarker, that is to say it is inheritable and can be used to detect OS in the next generation as well if need be.

According to one aspect of the present invention, there is provided a method of identifying oxidative stress (OS) in a test cell, the method comprising:

- (a) determining the methylation status of at least five genes in a DNA sample obtained from the test cell;
- (b) comparing the methylation status of the genes from step (a) to the methylation status of the corresponding genes in a control without OS,

wherein a difference in the methylation status of the genes in the test cell compared to the corresponding genes in the control is indicative of the cell having OS; and wherein the genes in step (a) are selected from the group consisting of *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, *CACNA1C*, and the regulatory regions of the same.

As used herein, the term "cell" refers to an intact live cell, naturally occurring or modified. The cell may be isolated from other cells, mixed with other cells in a culture, or within a tissue (partial or intact), or an organism. In particular, the cell may be a eukaryote cell. More in particular, the cell may be mammalian cell. The term "mammalian cell" refers to any cell derived from a mammalian subject. The cell may also be a cell derived from the culture and expansion of a cell obtained from a subject. The cell may also have been genetically modified to express a recombinant protein and/or nucleic acid. The mammalian cell may be from humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; rodents such as mice, rats, rabbits, hamsters, and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. In particular, the subject is a mammal. More in particular, the mammal is selected from the group consisting of a mouse, a rat, a guinea pig, a dog, a mini-pig, a human being, a cow, a sheep, a pig, a goat, a horse, a donkey, and a mule. In particular, the mammalian cell may be a skin cell, a stem cell or a cell derived therefrom. More in particular, the mammalian cell may be a skin cell.

As used herein, a "CpG site" or "methylation site" is a nucleotide within a nucleic acid (DNA or RNA) that is susceptible to methylation either by natural occurring events *in vivo* or by an event instituted to chemically methylate the nucleotide *in vitro*. Some of these sites may be hypermethylated and some may be hypomethylated in a cell with OS compared to a cell with no OS.

As used herein, a "methylated nucleic acid molecule" refers to a nucleic acid molecule that contains one or more nucleotides that is/are methylated.

A "CpG island" as used herein describes a segment of DNA sequence that comprises a functionally or structurally deviated CpG density. For example, Yamada et al. have described a set of standards for determining a CpG island: it must be at least 400 nucleotides in length, has a greater than 50% GC content, and an OCF/ECF ratio greater than 0.6 (Yamada et al., 2004, *Genome Research*, 14, 247-266). Others have defined a CpG island less stringently as a sequence at least 200 nucleotides in length, having a greater than 50% GC content, and an OCF/ECF ratio greater than 0.6 (Takai et al., 2002, *Proc. Natl. Acad. Sci. USA*, 99, 3740-3745). In context of the present invention, the terms "methylation profile", "methylation pattern", "methylation state" or "methylation status," are used herein to describe the state, situation or condition of methylation of a genomic sequence, and such terms refer to the characteristics of a DNA segment at a particular genomic locus in relation to methylation. Such characteristics include, but are not limited to, whether any of the cytosine (C) residues within this DNA sequence are methylated, location of methylated C residue(s), percentage of methylated C at any particular stretch of residues, and allelic differences in methylation due to, e.g., difference in the origin of the alleles.

The term "methylation status" refers to the status of a specific methylation site (i.e. methylated vs. non-methylated) which means a residue or methylation site is methylated or not methylated. Then, based on the methylation status of one or more methylation sites, a methylation profile may be determined. Accordingly, the term "methylation profile" or also "methylation pattern" refers to the relative or absolute concentration of methylated C residues or unmethylated C residues at any particular stretch of residues in the genomic material of a biological sample. For example, if cytosine (C) residue(s) not typically methylated within a DNA sequence are methylated, it may be referred to as "hypermethylated"; whereas if cytosine (C) residue(s) typically methylated within a DNA sequence are not methylated, it may be referred to as "hypomethylated". Likewise, if the cytosine (C) residue(s) within a DNA sequence (e.g., the DNA from a sample nucleic acid from a test subject) are methylated as compared to another sequence from a different region or from a different individual (e.g., relative to normal nucleic acid or to the standard nucleic acid of the reference sequence), that sequence is considered hypermethylated compared to the other sequence. Alternatively, if the cytosine (C) residue(s) within a DNA sequence are not methylated as compared to another sequence from a different region or from a different individual, that sequence is considered hypomethylated compared to the other sequence. These sequences are said to be "differentially methylated". Measurement of the levels of differential methylation may be done by a variety of ways known to those skilled in the art. One method is to measure the methylation level of individual interrogated CpG sites determined by the bisulfite sequencing method, as a non-limiting example.

As used herein, a "methylated nucleotide" or a "methylated nucleotide base" refers to the presence of a methyl moiety on a nucleotide base, where the methyl moiety is usually not present in a recognized typical nucleotide base. For example, cytosine in its usual form does not contain a methyl moiety on its pyrimidine ring, but 5-methylcytosine contains a methyl moiety at position 5 of its pyrimidine ring. Therefore, cytosine in its usual form may not be considered a methylated nucleotide and 5-methylcytosine may be considered a methylated nucleotide. In another example, thymine may contain a methyl moiety at position 5 of its pyrimidine ring, however, for purposes herein, thymine may not be considered a methylated nucleotide when present in DNA. Typical nucleotide bases for DNA are thymine, adenine, cytosine and guanine. Typical bases for RNA are uracil, adenine, cytosine and guanine. Correspondingly a "methylation site" is the location in the target gene nucleic acid region where methylation has the possibility of occurring. For example, a location containing CpG is a methylation site wherein the cytosine may or may not be methylated. In particular, the term "methylated nucleotide" refers to nucleotides that carry a methyl group attached to a position of a nucleotide that is accessible for methylation. These methylated nucleotides are usually found in nature and to date, methylated cytosine that occurs mostly in the context of the dinucleotide CpG, but also in the context of CpNpG- and CpNpN-sequences may be considered the most common. In principle, other naturally occurring nucleotides may also be methylated but they will not be taken into consideration with regard to any aspect of the present invention.

In context of the present invention, the terms "methylation profile", "methylation pattern", "methylation state" or "methylation status," are used herein to describe the state, situation or

condition of methylation of a genomic sequence, and such terms refer to the characteristics of a DNA segment at a particular genomic locus in relation to methylation. Such characteristics include, but are not limited to, whether any of the cytosine (C) residues within this DNA sequence are methylated, location of methylated C residue(s), percentage of methylated C at any particular stretch of residues, and allelic differences in methylation due to, e.g., difference in the origin of the alleles.

The term "hypermethylation" refers to the average methylation state corresponding to an increased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample. In particular, control refers to a cell with no indication of OS.

The term "hypomethylation" refers to the average methylation state corresponding to a decreased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample. In particular, control refers to a cell with no indication of OS.

As used herein, the term "gene" refers to the respective genomic DNA sequence, including any promoter and regulatory sequences of the gene (e.g., enhancers and other gene sequences involved in regulating expression of the gene), and/or the body of the gene in itself. A gene sequence may be an expressed sequence (e.g., expressed RNA, mRNA, cDNA). Further, where SNPs are known within genes the term shall be taken to include all sequence variants thereof.

As used herein, the term "genomic material" refers to nucleic acid molecules or fragments of the genome of the subject or group of subjects. In particular, such nucleic acid molecules or fragments are DNA or RNA or hybrids thereof, and most preferably are molecules of the DNA genome of a subject or group of subjects.

As used herein, the "promoter" or "gene promoter" used interchangeably with the term 'regulatory region' or 'regulatory sequence' refers to the respective contiguous gene DNA sequence extending from 1.5 kb upstream to 1.5 kb downstream relative to the transcription start site (TSS), or contiguous portions thereof. In particular, 'regulatory region' refers to the respective contiguous gene DNA sequence extending from 1.5 kb upstream to 0.5 kb downstream relative to the TSS. In some examples, 'regulatory region' refers to the respective contiguous gene DNA sequence extending from 1.5 kb upstream to the downstream edge of a CpG island that overlaps with the region from 1.5 kb upstream to 1.5 kb downstream from TSS (and in such cases, may thus extend even further beyond 1.5 kb downstream), and contiguous portions thereof. In particular, with respect to *PTPRN2*, any CpG dinucleotide of the gene that is coordinately methylated with the 'regulatory region' of the gene, has substantial diagnostic/classification utility as disclosed herein.

As used herein, the "DNA sample" refers to the DNA extracted from the cell according to any aspect of the present invention using known methods in the art.

In particular, when there is differential methylation detected in a test cell, that is to say that the cell displays hypermethylation or hypomethylation at, at least one CpG site in comparison to the control (i.e., a cell without indication of OS), then the test cell has OS.

In particular, in the method according to any aspect of the present invention, in step (a) the  
5 methylation status of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17, 18, 19, 20, 21, 22, 23 or 24 genes are determined. More in particular, in step (a) the methylation status of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17, 18, 19, 20, 21, 22, 23, 24 or 25 genes are determined.

In one example, the methylation status of at least 5 genes are determined in step (a). The 5 genes  
10 are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, and *HDAC4*.

In another example, the methylation of at least 6 genes are determined in step (a). The 6 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, and *ADARB2*.

In another example, the methylation of at least 7 genes are determined in step (a). The 7 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, and *CDH4*.

15 In another example, the methylation of at least 8 genes are determined in step (a). The 8 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, and *DIP2C*.

In another example, the methylation of at least 9 genes are determined in step (a). The 9 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, and *SHANK2*.

20 In another example, the methylation of at least 10 genes are determined in step (a). The 10 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, *SHANK2*, and *CAMTA1*.

In another example, the methylation of at least 11 genes are determined in step (a). The 11 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, *SHANK2*, *CAMTA1*, and *RPTOR*.

25 In another example, the methylation of at least 12 genes are determined in step (a). The 12 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, *SHANK2*, *CAMTA1*, *RPTOR* and *RASA3*.

In a further example, the methylation of at least 13 genes are determined in step (a). The 13 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3* and *SDK1*.

30 In a further example, the methylation of at least 14 genes are determined in step (a). The 14 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1* and *AGAP1*.

In a further example, the methylation of at least 15 genes are determined in step (a). The 15 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*,  
35 *RPTOR*, *RASA3*, *SDK1*, *AGAP1* and *TBCD*.

In a further example, the methylation of at least 16 genes are determined in step (a). The 16 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD* and *SEPT9*.

In a further example, the methylation of at least 17 genes are determined in step (a). The 17 genes  
5 are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9* and *FRMD4A*.

In a further example, the methylation of at least 18 genes are determined in step (a). The 18 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A* and *MCF2L*.

10 In a further example, the methylation of at least 19 genes are determined in step (a). The 19 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L* and *FOXP1*.

In a further example, the methylation of at least 20 genes are determined in step (a). The 20 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*,  
15 *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1* and *RPS6KA2*.

In a further example, the methylation of at least 21 genes are determined in step (a). The 21 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2* and *SORCS2*.

20 In another example, the methylation of at least 22 genes are determined in step (a). The 22 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2* and *NXN*.

In another example, the methylation of at least 23 genes are determined in step (a). The 23 genes  
25 are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN* and *TRAPPC9*.

In another example, the methylation of at least 24 genes are determined in step (a). The 24 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*,  
30 *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9* and *AUTS2*.

In another example, the methylation of at least 25 genes are determined in step (a). The 25 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*,  
35 *NXN*, *TRAPPC9*, *AUTS2* and *CACNA1C*. More in particular, one of the genes in step (a) is the gene Protein Tyrosine Phosphatase Receptor Type N2 (*PTPRN2*), and/or the regulatory region of *PTPRN2*. *PTPRN2* is phosphatidylinositol phosphatase with the ability to dephosphorylate phosphatidylinositol 3-phosphate and phosphatidylinositol 4,5-diphosphate which plays important

roles in lipid signaling, cell signaling and membrane trafficking. The differential methylation of the gene body and/or regulatory region of *PTPRN2* is indicative of OS in the cell relative to a cell without OS. That is to say, the hypomethylation or hypermethylation of the *PTPRN2*, and/or the regulatory region of *PTPRN2* is indicative of OS.

- 5 The genes in step (a) are selected from the group consisting of Adenosine Deaminase RNA Specific B2 (*ADARB2*), Nucleoredoxin (*NXM*), Sidekick Cell Adhesion Molecule 1 (*SDK1*), Calmodulin Binding Transcription Activator 1 (*CAMTA1*), MCF.2 Cell Line Derived Transforming Sequence Like (*MCF2L*), Sortilin Related VPS10 Domain Containing Receptor 2 (*SORCS2*), Disco Interacting Protein 2 Homolog C (*DIP2C*), FERM Domain Containing 4A (*FRMD4A*), histone
- 10 deacetylase 4 (*HDAC4*), Mitotic spindle assembly checkpoint protein (*MAD1L1*), PR/SET Domain 16 (*PRDM16*), Ras GTPase-activating protein 3 (*RASA3*), Ribosomal Protein S6 Kinase A2 (*RPS6KA2*), Tubulin Folding Cofactor D (*TBCD*), tenascin-X (*TNXB*), Trafficking Protein Particle Complex Subunit 9 (*TRAPPC9*), and), ArfGAP With GTPase Domain (*AGAP1*), Calcium channel, voltage-dependent, L type, alpha 1C subunit (*CACNA1C*), Regulatory Associated Protein Of MTOR
- 15 Complex 1 (*RPTOR*), Cadherin 4 (*CDH4*), SEPTIN9 (*SEPT9*), Forkhead Box P1 (*FOXP1*), Activator Of Transcription And Developmental Regulator (*AUTS2*) and SH3 And Multiple Ankyrin Repeat Domains 2 (*SHANK2*).

In one example, the genes in step (a) may also include the genes provided in Table 3.

The method according to any aspect of the present invention, further comprises the step of:

- 20 (i) performing bisulfite modification to the DNA sample before step (a).

- 'Bisulfite treatment' of genomic DNA used interchangeably with the term 'bisulfite modification', refers to the treatment of the genomic DNA with a deaminating agent such as a bisulfite that may be used to treat all DNA, methylated or not. In particular, the term "bisulfite" as used herein encompasses any suitable type of bisulfite, such as sodium bisulfite, or other chemical agents that
- 25 are capable of chemically converting a cytosine (C) to an uracil (U) without chemically modifying a methylated cytosine and therefore can be used to differentially modify a DNA sequence based on the methylation status of the DNA, e.g., U.S. Pat. Pub. US 2010/0112595. As used herein, a reagent that "differentially modifies" methylated or non-methylated DNA encompasses any reagent that modifies methylated and/or unmethylated DNA in a process through which distinguishable
  - 30 products result from methylated and non-methylated DNA, thereby allowing the identification of the DNA methylation status. Such processes may include, but are not limited to, chemical reactions (such as a C to U conversion by bisulfite) and enzymatic treatment (such as cleavage by a methylation-dependent endonuclease). Thus, an enzyme that preferentially cleaves or digests methylated DNA is one capable of cleaving or digesting a DNA molecule at a much higher
  - 35 efficiency when the DNA is methylated, whereas an enzyme that preferentially cleaves or digests unmethylated DNA exhibits a significantly higher efficiency when the DNA is not methylated.

Accordingly, before step (a) according to any aspect of the present invention is carried out, the genomic DNA contained/ obtained or extracted from the cell, is first bisulfite treated.

An alternative method available in the art may be used instead of bisulfite treatment. A skilled person will understand which other methods to use. In one example, TET-assisted pyridine borane sequencing (TAPS) may be used for detection of 5mC and 5hmC (Yibin Liu, et al., *Nature Biotechnology*, 37: 424–429 (2019)).

- 5 The cell used according to any aspect of the present invention is obtained from a biological sample selected from the group consisting of blood, brain, sperm and any other tissue or sample that provides genomic DNA to be used in the method according to any aspect of the present invention. In particular, the biological sample may comprise any biological material obtained from the subject that contains DNA, and may be liquid, solid or both, may be tissue or bone, or a body fluid such as
- 10 blood, lymph, etc. In particular, the biological sample useful for the present invention may comprise biological cells or fragments thereof.

- The term “test” used in conjunction with the term cell herein refers to a cell that is subjected to the method according to any aspect of the present invention and is the basis for an analysis application of the present invention. A ‘test cell’ is therefore a cell or a group of cells being tested according to
- 15 any aspect of the present invention or a profile being obtained or generated in this context. Conversely, the term “reference” or ‘control’ shall denote, mostly predetermined, entities which are used for a comparison with the test entity. In particular, a ‘test cell’ refers to a cell being tested for OS where the methylation status has to be determined and a ‘control’ refers to a cell without OS where the methylation status is already known and used as a reference.

- 20 The term ‘epigenetic change’ as used herein refers to a chemical (e.g., methylation) change or protein (e.g., histones) change that takes place to a gene body or a promoter thereof. Through epigenetic changes, environmental factors like diet, stress and prenatal nutrition can make an imprint on genes passed from one generation to the next. More in particular, the epigenetic marker for OS in a cell is the gene body and/or regulatory region of *PTPRN2* and at least one other gene.
- 25 The differential methylation of these genes in a test cell relative to a cell with no OS (i.e. the control cell) is indicative of OS in the test cell. In particular, promoter-specific hyper/hypomethylation leads to changes in the expression of different genes. More in particular, the differential methylation of *PTPRN2* and the other genes result in the differential expression of *PTPRN2*. This differential expression of the gene relative to a control, where there is no differential methylation, is indicative
- 30 of OS in the cell tested.

According to a further aspect of the present invention, there is provided a gene panel with at least five genes for detecting the presence of OS in a cell, wherein one of the five genes is *PTPRN2* and the other four genes are selected from the group consisting of

- MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*,
- 35 *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, and *CACNA1C*.

According to yet another aspect of the present invention, there is provided a use of a gene panel with at least five genes for indicating the presence of OS in a cell, wherein one of the five genes is *PTPRN2* and the other four genes are selected from the group consisting of

*MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, and *CACNA1C*

and wherein differential methylation of at least the five genes indicates the presence of OS in the cell.

In particular, the differential methylation refers to the hypermethylation or hypomethylation of the genes.

#### BRIEF DESCRIPTION OF FIGURES

Figure 1 is a graph showing the number of probes that were differentially methylated in different gene bodies and promoter regions of cells where artificial OS (high UV for 24hrs) was induced according to Example 1. As can be seen, genes, *MAD1L1* (meiotic spindle arrest component) and *PTPRN2* (similar to receptor-like protein tyrosine phosphatases) have the highest differentially methylated probe distribution. The other genes which are also differentially methylated in a cell with OS are shown.

Figure 2 is a graph showing the number of probes that were differentially methylated in different gene bodies and promoter regions of cells artificial OS (low UV for 72hrs) was induced according to Example 1. As can be seen, genes, *MAD1L1* (meiotic spindle arrest component) and *PTPRN2* (similar to receptor-like protein tyrosine phosphatases) have the highest differentially methylated probe distribution. The other genes which are also differentially methylated in a cell with OS are shown.

Figure 3 is a graph showing the number of probes that were differentially methylated in different gene bodies and promoter regions of cells where artificial OS (high H<sub>2</sub>O<sub>2</sub> for 24hrs) was induced according to Example 2. As can be seen, genes, *MAD1L1* (meiotic spindle arrest component) and *PTPRN2* (similar to receptor-like protein tyrosine phosphatases) have the highest differentially methylated probe distribution. The other genes which are also differentially methylated in a cell with OS are shown.

Figure 4 is a graph showing the number of probes that were differentially methylated in different gene bodies and promoter regions of cells artificial OS (low H<sub>2</sub>O<sub>2</sub> for 24hrs) was induced according to Example 2. As can be seen, genes, *MAD1L1* (meiotic spindle arrest component) and *PTPRN2* (similar to receptor-like protein tyrosine phosphatases) have the highest differentially methylated probe distribution. The other genes which are also differentially methylated in a cell with OS are shown.

Figure 5 is a graph showing the number of probes that were differentially methylated in different gene bodies and promoter regions of cells where artificial OS according to Example 3 with Medox® in cells was induced. As can be seen, genes, *MAD1L1* (meiotic spindle arrest component) and *PTPRN2* (similar to receptor-like protein tyrosine phosphatases) have the highest differentially

methylyated probe distribution. The other genes which are also differentially methylyated in a cell with OS are shown.

Figure 6 is a graph showing the top 20 overlapping genes at different treatments.

### EXAMPLES

- 5 The foregoing describes preferred embodiments, which, as will be understood by those skilled in the art, may be subject to variations or modifications in design, construction or operation without departing from the scope of the claims. These variations, for instance, are intended to be covered by the scope of the claims.

#### Example 1

##### 10 *Oxidative Stress on Human Tissue with UV light*

Artificial oxidative stress was induced in the cell culture system and skin tissue model to analyze the methylation status of promoters.

- T-Skin models were obtained from Episkin SA, France which is composed of reconstructed human skin. Each skin model consists of a dermal equivalent overlaid by a stratified, well-differentiated  
15 epidermis derived from normal human keratinocytes. Upon receiving the skin models, it was recovered by incubating in T-Skin culture medium overnight at 37°C in a 5% CO<sub>2</sub> incubator.

To induce oxidative stress on a human tissue system, skin models (5x replicates) were exposed to UV radiation (UVA 24 J/cm<sup>2</sup> + UVB 50mJ/cm<sup>2</sup>) (high UV) daily for 24 hrs.

- In another group, skin models (5x replicates) were exposed to UV radiation (UVA 12 J/cm<sup>2</sup> + UVB  
20 25mJ/cm<sup>2</sup> daily) (low UV) daily for 72 hrs.

- Exposure to UV radiation leads to the generation of ROS which finally results in the development of oxidative stress within the cells. A control set of skin models (5x replicates) were maintained for 72hrs without any exposure to UV radiation. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood &  
25 Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

- The genomic DNA (500ng) from tissue samples were subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites  
30 quantitatively across the genome at single-nucleotide resolution.

#### *Quality control and data processing*

Methylation EPIC array data processing was performed in R version 4.1.2 (2021-11-01) using the minfi version 1.40.0. The raw intensity data (IDAT) were imported into the R (4.1.2), processed using the minfi (1.4.0) Bioconductor package,<sup>18</sup>. Quality check on samples was performed to keep

probes that have a detection P-value < 0.01 in one or more samples or have a mean detection P-value < 0.05 in all samples. Then samples were normalized using functional normalization (implemented by preprocessFunnorm function in minfi) for type-bias correction and background correction.

- 5 Prior to differential methylation analysis, the probes with non-specific binding, cross reactive probes, probes affected by common SNPs, and probes annotated to the X,Y chromosomes were also filtered out. Beta-value and M-value of normalized and filtered samples were calculated using getBeta and getM function respectively, the samples were then subjected to further downstream analysis.

10 *Differential methylation analysis*

Differential methylation analysis was performed using packages limma version 3.50.1 and DMRcate version 2.8.5. Contrast matrix was set up by comparing each corresponding treatment and control group and empirical Bayesian algorithm was used to fit the M-values based on the design and contrast model. Probes with adjusted P-value lower than 0.05 were considered as  
15 differentially methylation positions (DMPs). Annotation was performed using IlluminaHumanMethylationEPICanno.ilmn12.hg19 and annotatr package (1.20.0).

As seen in Figures 1 and 2, one of the top genes that were differentially methylated in the promoter region is *PTPRN2* (Protein Tyrosine Phosphatase Receptor Type N2). The other genes that were also significantly differentially methylated is shown in Figures 1 and 2.

20 **Example 2**

*Oxidative Stress on Human Tissue with H<sub>2</sub>O<sub>2</sub>*

- Another way to induce oxidative stress on a human tissue system is through Hydrogen peroxide treatment which leads to the generation of ROS within the cells. The skin models (5x replicates) were treated with two different concentrations of Hydrogen peroxide [100µM (low) and 200µM  
25 (high)] for 2hrs and were maintained for 24hrs. A control set of skin models (5x replicates) were maintained for 24hrs without any treatment with Hydrogen peroxide. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

- 30 Same method of quality control and data processing as that disclosed in Example 1 was carried out on the samples here. Further, the same differential methylation analysis as disclosed in Example 1 was carried out on the data obtained from Example 2.

The results are shown in Figures 3 and 4.

**Example 3**

- 35 *Oxidative Stress on cell culture system with Medox®*

To investigate oxidative stress in the cell culture system, Mesenchymal Stem Cells (MSCs) were treated with Medox® (Evonik, Batch:H-080719) which contains a lot of natural anthocyanins, associated with antioxidative and anti-inflammatory properties. Bone marrow derived MSCs were cultured for 1 week in Mesencult ACF Plus Medium with two doses of Medox® (4x replicates): 25  
5 µg/ml (low) and 100 µg/ml (high). The media with Medox® was replaced every second day for 1 week. As a control (4x replicates), MSCs were cultured for 1 week in Mesencult ACF Plus Medium without any Medox® treatment. Medox® treatment is expected to produce the opposite reaction to OS.

This was followed by collection of cell pellet and genomic DNA was purified from the cell pellet  
10 using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

The genomic DNA (500ng) from the cell pellet was subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites  
15 quantitatively across the genome at single-nucleotide resolution.

DNA methylation profiling has been proven to be a powerful analytical tool to accurately identify the origin of tissue and the effect of environmental factors. It has several advantages as a biomarker classifier as it is a stable marker, and it can facilitate quantitative analysis at single-nucleotide resolution.

20 Same method of quality control and data processing as that disclosed in Example 1 was carried out on the samples here. Further, the same differential methylation analysis as disclosed in Example 1 was carried out on the data obtained from Example 3.

With the low treatment to Medox® on MSCs, 35,532 differentially methylated probes ( $p < 0.05$ ) were identified, out of which 15,368 probes were hypermethylated and 20,164 probes were  
25 hypomethylated. These probes were associated with 13,656 unique genes which are generally hypomethylated across promoter and gene body with up to 10-15% methylation difference as compared to the control group. One of the top 50 genes that was identified from the 13,656 unique genes was *PTPRN2* (Protein Tyrosine Phosphatase Receptor Type N2) amongst other genes involved in Rho Kinase-mediated signalling, cell to cell junction, cell polarity and vesicle transport.  
30 This is shown in Figure 5. In particular, the top candidates were associated with the Rho-GTPase pathways, the disc-large complex and histone-methyltransferase. The Rho pathway is required for stress fibre formation and focal adhesion (where the cytoskeleton/cell connects integrins with the ECM).

*Results of all examples (i.e. Examples 1, 2 and 3)*

35 The genes that were significantly differentially methylated from Example 1 were juxtaposed with the genes that were significantly differentially methylated from Examples 2 and 3. The list of genes are shown in Table 1 below.

| Genes differentially methylated in 5 categories                                                                         | Genes differentially methylated in 4 categories                                                                   | Genes differentially methylated in 3 categories                                                         | Genes differentially methylated in 2 categories                                                                                          | Genes differentially methylated in 1 category                                                                                                                                                                        | Genes differentially methylated in 1 category                                                                                                                                                               | Genes differentially methylated in 1 category                                                                                                                                                            |
|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ADARB2<br>ATP11A<br>CAMTA1<br>DIP2C<br>FRMD4A<br>HDAC4<br>MAD1L1<br>PRDM16<br>PTPRN2<br>TBCD<br>TNXB<br>TRAPPC9<br>TRIO | AGAP1<br>ARHGAP26<br>ATXN1<br>CACNA1C<br>COL23A1<br>DAB1<br>INPP5A<br>MGMT<br>RASA3<br>RPS6KA2<br>RPTOR<br>SHANK2 | ANKRD11<br>KLHL29<br>MCF2L<br>MSI2<br>NXN<br>PRKCE<br>PRKCH<br>RORA<br>SDK1<br>SMOC2<br>SMYD3<br>SORCS2 | ARHGEF10<br>C7orf50<br>CDKAL1<br>COL4A2<br>EHMT2<br>FHIT<br>GNG7<br>KIF26B<br>LPP<br>PRKCA<br>PTPRG<br>SNX29<br>TSPAN9<br>TSSC1<br>WVVOX | ADAMTS17<br>ADCY2<br>ALX4<br>ARHGAP15<br>ARHGEF10L<br>ARHGEF4<br>BAIAP2<br>BCAS3<br>BCR<br>C6orf10<br>CCDC88C<br>CDH4<br>CDK6<br>CELSR1<br>CMIP<br>COL1A1<br>PKNOX2<br>PRKAR1B<br>RAD51B<br>RERE<br>TSNARE1<br>TTC7B | COL22A1<br>DLGAP2<br>ERC2<br>ERGIC1<br>FMNL2<br>FNDC3B<br>FOXK1<br>FSTL4<br>GABBR1<br>GAK<br>GPR133<br>GRID1<br>GRIK4<br>IGF1R<br>ISPD<br>JAZF1<br>SHROOM3<br>SLC12A7<br>SRGAP3<br>SSBP3<br>UTRN<br>ZDHHC14 | MACF1<br>MAEA<br>MAGI1<br>MAML3<br>MECOM<br>MEGF11<br>MEIS1<br>MYT1L<br>NCKAP5<br>NOTCH4<br>NPAS3<br>OSBPL5<br>PARK2<br>PAX6<br>PCNT<br>PEX14<br>SYNGAP1<br>TEAD1<br>THSD4<br>TMEM132D<br>ZFXH3<br>ZMIZ1 |

Table 1. Genes differentially methylated in the 5 categories: (i) high UV (Example 1), (ii) low UV (Example 1), (iii) high H<sub>2</sub>O<sub>2</sub> (Example 2), (iv) low H<sub>2</sub>O<sub>2</sub> (Example 2) and (v) high Medox® (Example 3).

- 5 The three independent experiment setups to analyze the methylation status of promoters during oxidative stress have shown differential methylation of *PTPRN2* promoter and other genes, suggesting these genes to be a potential biomarker for oxidative stress.

#### Example 4

##### *Oxidative Stress on Human Tissue with UV light rays*

- 10 Artificial oxidative stress was induced in the cell culture system and skin tissue model to analyze the methylation status of promoters.

T-Skin models were obtained from Episkin SA, France which is composed of reconstructed human skin. Each skin model consists of a dermal equivalent overlaid by a stratified, well-differentiated epidermis derived from normal human keratinocytes. Upon receiving the skin models, it was recovered by incubating in T-Skin culture medium overnight at 37°C in a 5% CO<sub>2</sub> incubator.

- 15 To induce oxidative stress on a human tissue system, skin models (5x replicates) were exposed to UV radiation (UVA 24 J/cm<sup>2</sup> + UVB 50mJ/cm<sup>2</sup>) (high UV) and cultured for 24 hrs.

In another group, skin models (5x replicates) were exposed to UV radiation (UVA 12 J/cm<sup>2</sup> + UVB 25mJ/cm<sup>2</sup> daily) (low UV) and cultured for 24 hrs.

Exposure to UV radiation leads to the generation of ROS which finally results in the development of oxidative stress within the cells. A control set of skin models (5x replicates) were maintained for 24hrs without any exposure to UV radiation. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

The genomic DNA (500ng) from tissue samples were subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution.

#### *Oxidative Stress on Human Tissue with H<sub>2</sub>O<sub>2</sub>*

Another way to induce oxidative stress on a human tissue system is through Hydrogen peroxide treatment which leads to the generation of ROS within the cells. The skin models (5x replicates) were treated with two different concentrations of Hydrogen peroxide [100µM (low) and 200µM (high)] for 2hrs and were maintained for 24hrs. A control set of skin models (5x replicates) were maintained for 24hrs without any treatment with Hydrogen peroxide. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

The genomic DNA (500ng) from tissue samples were subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution.

#### *Oxidative Stress on Human Tissue with Particulate Matter 2.5*

Another way to induce oxidative stress on a human tissue system is through Particulate Matter 2.5 (PM<sub>2.5</sub>) treatment which leads to the generation of ROS within the cells by its chemical components and metals. The skin models (5x replicates) were treated with two different concentrations of PM<sub>2.5</sub> [15 µg/cm<sup>2</sup> (low) and 30 µg/cm<sup>2</sup> (high)] and were maintained for 24hrs. A control set of skin models (5x replicates) were maintained for 24hrs without any treatment with PM<sub>2.5</sub>. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

The genomic DNA (500ng) from tissue samples were subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution.

#### *Oxidative Stress on Human Tissue with Glyoxal*

Another way to induce oxidative stress on a human tissue system is through Glyoxal treatment which provokes oxidative stress by increasing the level of ROS within the cells by producing advanced glycation end-products. The skin models (5x replicates) were treated with two different concentrations of glyoxal [0.5 mM (low) and 1 mM (high)] and were maintained for 24hrs. A control set of skin models (5x replicates) were maintained for 24hrs without any treatment with glyoxal. Followed by the treatment, skin models were collected, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

The genomic DNA (500ng) from tissue samples were subjected to bisulfite conversion using the EZ DNA Methylation-Gold™ Kit (Zymo Research). The methylation levels were quantified using Infinium MethylationEPIC v2.0 Kit (Illumina) which can analyze over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution.

#### *Oxidative Stress on Human Tissue with ageing*

Another way to induce oxidative stress on a human tissue system is through ageing which leads to the generation of Reactive oxygen and nitrogen species (RONS) within the cells.

The skin models were maintained in the deep well plate with media for 7 days (4x replicates), 14 days (4x replicates) and 21 days (4x replicates) respectively to induce ageing in the skin tissue. Skin models were collected after 7 days, 14 days and 21 days respectively, and genomic DNA was purified from the tissue samples using the DNeasy® Blood & Tissue Kit (Qiagen). The genomic DNA was quantified using the PicroGreen® or NanoDrop™ 2000.

#### *Quality control and data processing*

A total of 103 samples from 16 different treatments and their respective controls were analyzed. The treatments were grouped into two batches. Batch 1 consisted of all 24 hour treatments, at high and low concentrations. All treatments in batch 1 shared the same control group. Table 2 shows the sample information for batch 1.

Table 2. Batch 1 samples

| Group                                   | Treatment                                                                                          | Replicates |
|-----------------------------------------|----------------------------------------------------------------------------------------------------|------------|
| Control                                 | Untreated, 24h                                                                                     | 5          |
| Pollution Treatment                     | Treated with 30 µg/cm <sup>2</sup> particulate matter 2.5 for 24h                                  | 5          |
|                                         | Treated with 15 µg/cm <sup>2</sup> particulate matter 2.5 for 24h                                  | 5          |
| H <sub>2</sub> O <sub>2</sub> Treatment | Treated with systemic exposure to 200 µM H <sub>2</sub> O <sub>2</sub> for 2h and cultured for 24h | 5          |
|                                         | Treated with systemic exposure to 100 µM H <sub>2</sub> O <sub>2</sub> for 2h and cultured for 24h | 5          |
| Glyoxal Treatment                       | Treated with systemic exposure to 1 mM glyoxal 1 mM for 24h                                        | 5          |
|                                         | Treated with systemic exposure to 0.5 mM glyoxal 1 mM for 24h                                      | 5          |
| UV-radiation Treatment                  | Exposed to UVA 24 J/cm <sup>2</sup> + UVB 50mJ/cm <sup>2</sup> and cultured for 24h                | 5          |
|                                         | Exposed to UVA 12 J/cm <sup>2</sup> + UVB 25mJ/cm <sup>2</sup> and cultured for 24h                | 5          |

|      |                                          |   |
|------|------------------------------------------|---|
| Aged | Untreated, cultured for 2 weeks in media | 6 |
|------|------------------------------------------|---|

Methylation EPIC array data processing was performed in R version 4.2.2 (2021-11-10 r83330) using the minfi version 1.42.0. The raw intensity data (IDAT) were imported into R (4.2.2) and processed using the minfi (1.42.0) Bioconductor package. Quality check on samples were

5 performed to keep probes that had a detection P-value <0.01 in one or more samples or had a mean detection P-value <0.05 in all samples. The samples were then normalized using functional normalization (implemented by preprocessFunnorm function in minfi) for type-bias correction and background correction.

Prior to differential methylation analysis, the probes with non-specific binding, cross reactive

10 probes, probes affected by common SNPs, and probes annotated to the X,Y chromosomes were also filtered out. Beta-value and M-value of normalized and filtered samples were calculated using getBeta and getM function respectively, the samples were then subjected to further downstream analysis.

*Differential methylation analysis*

15 Pair-wise differential methylation analysis (total of 16 pairs) was performed using the limma package version 3.52.4 . The batch 1 samples were analyzed together, while for the batch 2 samples, the UV light and aged samples were analyzed separately. Contrast matrix was set up by comparing each corresponding treatment and control group and empirical Bayesian algorithm was used to fit the M-values based on the design and contrast model. Probes with adjusted P-value

20 lower than 0.05 were considered as differentially methylation positions (DMPs). Annotation was performed using IlluminaHumanMethylationEPICanno.ilm10b2.hg19. Genes that overlapped at all treatments were then identified. As seen in Table 3, there are a total of 1250 genes in common between the 16 comparisons. The rows indicate the total number of DMPs that appear for that gene in each comparison. Figure 6 shows the top 20 genes seen in all 16 comparisons.

Table 3. List of 1250 genes that are differentially methylated in all treatments

|             |              |              |               |               |                |               |                |              |                   |               |                 |
|-------------|--------------|--------------|---------------|---------------|----------------|---------------|----------------|--------------|-------------------|---------------|-----------------|
| 1. PTPRN2   | 41. NAV2     | 81. NFATC1   | 121. SDK2     | 161. CTNNA2   | 201. KDM2B     | 241. BCL2     | 281. HK1       | 321. MEIS2   | 361. VPS13B       | 401. PTPRE    | 441. CACNB4     |
| 2. MAD1L1   | 42. LPP      | 82. FOXN3    | 122. EBF3     | 162. KCNAB2   | 202. PRDM2     | 242. GPR133   | 282. PTPN14    | 322. MTUS1   | 362. VT11A        | 402. TOX2     | 442. FMN2       |
| 3. PRDM16   | 43. ZMIZ1    | 83. SLIT3    | 123. PCDHGA4  | 163. MAGI2    | 203. SH3BP4    | 243. SIM1     | 283. FAM38A    | 323. PTK2B   | 363. DGKZ         | 403. COL27A1  | 443. GATAD2A    |
| 4. TNXB     | 44. CUX1     | 84. ADAMTS2  | 124. LRP1     | 164. PACRG    | 204. COL5A1    | 244. LHFPL2   | 284. PLXNA4    | 324. TBC1D1  | 364. LOC105376360 | 404. DLG5     | 444. RYR1       |
| 5. HDAC4    | 45. CASZ1    | 85. ZFH3     | 125. MACROD2  | 165. PDE4DIP  | 205. GPC6      | 245. VAC14    | 285. CLEC16A   | 325. CDK14   | 365. PDE11A       | 405. FANCC    | 445. SRPK2      |
| 6. ADARB2   | 46. KLHL29   | 86. MBP      | 126. TSSC1    | 166. H0XC4    | 206. PTPRG     | 246. AFF3     | 286. ERG       | 326. GABRG3  | 366. PFKFB3       | 406. ZNF407   | 446. CDC57      |
| 7. CDH4     | 47. PITX2    | 87. TSNARE1  | 127. EXT1     | 167. RNF220   | 207. ESRRG     | 247. ARHGEF3  | 287. GRM4      | 327. PCGF3   | 367. POU6F2       | 407. DDAH1    | 447. COL9A3     |
| 8. DIP2C    | 48. HIVEP3   | 88. CCDC88C  | 128. MAST4    | 168. NRXN1    | 208. JARID2    | 248. BAHCC1   | 288. LINC00299 | 328. PDE2A   | 368. FHL2         | 408. KIAA1949 | 448. DOT1L      |
| 9. SHANK2   | 49. PARD3    | 89. CDH23    | 129. SKI      | 169. GRK5     | 209. CORO2B    | 249. FHOD3    | 289. PTPRS     | 329. TCERG1L | 369. JAKMIP3      | 409. MTHFD1L  | 449. TMEM132C   |
| 10. CAMTA1  | 50. RORA     | 90. TCF4     | 130. KIRREL3  | 170. HECW1    | 210. MAN1C1    | 250. NCALD    | 290. RAP1GAP2  | 330. TRAK1   | 370. MIB2         | 410. PTPRK    | 450. TTC7A      |
| 11. RPTOR   | 51. PRKAR1B  | 91. SMG6     | 131. MACROD1  | 171. AHR      | 211. ATP2B2    | 251. SFMBT2   | 291. SPTBN1    | 331. MYO10   | 371. RABGAP1L     | 411. ACOX3    | 451. CABIN1     |
| 12. RASA3   | 52. RERE     | 92. PIEC1    | 132. HRNBP3   | 172. CAPZB    | 212. MAML2     | 252. INPP4B   | 292. STK10     | 332. ZFPM1   | 372. AHNAK        | 412. BNC2     | 452. VEPH1      |
| 13. SDK1    | 53. HCCA2    | 93. THSD4    | 133. MEGF11   | 173. FAM53B   | 213. LRBA      | 253. PLEKHA6  | 293. ADAP1     | 333. ACSF3   | 373. BAT2         | 413. DLX5     | 453. ARID3A     |
| 14. AGAP1   | 54. GALTNT9  | 94. PKNOX2   | 134. NRG1     | 174. KNDCC1   | 214. MACF1     | 254. CDK6     | 294. PLCH2     | 334. CADM1   | 374. CTNND2       | 414. NHP4     | 454. CD82       |
| 15. TBDC    | 55. FSTL4    | 95. KDM4B    | 135. CACNA1H  | 175. SND1     | 215. PACS2     | 255. COBL     | 295. SLC9A3    | 335. MEF2C   | 375. DENND3       | 415. BRD2     | 455. MAPK4      |
| 16. SEPT9   | 56. MECOM    | 96. MGMT     | 136. TIAM1    | 176. GLIS1    | 216. ASAP2     | 256. GLIS3    | 296. COL13A1   | 336. MYO1E   | 376. EHMT2        | 416. CNTNAP2  | 456. PLXNB2     |
| 17. FRMDA4  | 57. PRKCA    | 97. SRGAP3   | 137. SHROOM3  | 177. ODZ4     | 217. DLGAP4    | 257. IRRFIP1  | 297. PITPNC1   | 337. PLCG2   | 377. RGS7         | 417. CRYL1    | 457. SGK1       |
| 18. MCF2L   | 58. TBC1D22A | 98. C9orf3   | 138. THADA    | 178. PCCA     | 218. PLEKHG5   | 258. KCNN3    | 298. RIN3      | 338. JPH3    | 378. RUNX2        | 418. DNAJB6   | 458. DGKD       |
| 19. FOXP1   | 59. DPP6     | 99. MICAL3   | 139. ERGIC1   | 179. ITPK1    | 219. ARHGEF10L | 259. TNRC18   | 299. TRPM1     | 339. SORBS1  | 379. TOLLIP       | 419. HSPG2    | 459. WNT3       |
| 20. RP56KA2 | 60. PPP2R2C  | 100. GMD5    | 140. EXOC4    | 180. LDIRAD4  | 220. ARHGAP22  | 260. ATP10A   | 300. FMN1      | 340. AAC3    | 380. VOPP1        | 420. NCOA2    | 460. CSGALNACT1 |
| 21. SORCS2  | 61. LRP5     | 101. SORBS2  | 141. PARK2    | 181. VAV2     | 221. MICAL2    | 261. FAM19A5  | 301. MRC2      | 341. EIF2C2  | 381. EYA2         | 421. FAM178B  | 461. CYFIP1     |
| 22. NXN     | 62. SH3RF3   | 102. PCSK6   | 142. VGLL4    | 182. ZC3H3    | 222. SIPA1L3   | 262. GTF2IRD1 | 302. ZFVFE28   | 342. RAB40C  | 382. HIPK2        | 422. ARHGEF17 | 462. ETS1       |
| 23. TRAPPC9 | 63. SMYD3    | 103. ANKS1B  | 143. DNAH17   | 183. TSPAN9   | 223. COL18A1   | 263. KSR1     | 303. OCA2      | 343. MTSS1   | 383. LIMS1        | 423. FAM65C   | 463. PKP3       |
| 24. AUTS2   | 64. SH3PXD2A | 104. IGF1R   | 144. NFIX     | 184. CDKAL1   | 224. EPHB2     | 264. BTBD9    | 304. OSBP2     | 344. SVIL    | 384. PVRL1        | 424. KIF26A   | 464. WWC1       |
| 25. CACNA1C | 65. SYN3     | 105. CELSR1  | 145. PHACTR1  | 185. BACH2    | 225. GAK       | 265. CDC42BPB | 305. SBNO2     | 345. ADGRG1  | 385. RASGRF1      | 425. RBM19    | 465. ZNF365     |
| 26. PDE4D   | 66. MAGI1    | 106. NOTCH4  | 146. ROBO1    | 186. OBSN     | 226. PAX5      | 266. RTNMR1L  | 306. STK24     | 346. CLMN    | 386. VIPR2        | 426. RIN2     | 466. ZNF827     |
| 27. PRKCZ   | 67. TACC2    | 107. ZBTB20  | 147. ASAP1    | 187. SLC38A10 | 227. CTBP1     | 267. MAPK8IP3 | 307. MPRIIP    | 347. INTS1   | 387. FAM189A1     | 427. CHCHD6   | 467. A2B1       |
| 28. ANKRD11 | 68. RGS12    | 108. NEDD4L  | 148. GLI2     | 188. TTC7B    | 228. PTPRF     | 268. SPEG     | 308. PARVB     | 348. PFKP    | 388. LDB2         | 428. KCNQ5    | 468. GNAO1      |
| 29. CMIP    | 69. ARID1B   | 109. SYNE2   | 149. RIMBP2   | 189. CREB5    | 229. RXRA      | 269. BRSK2    | 309. PLEKHA7   | 349. RBMS1   | 389. CARD14       | 429. TNK2     | 469. MGIL       |
| 30. NCOR2   | 70. DAB1     | 110. ANO1    | 150. DLGAP1   | 190. CTIF     | 230. TBC1D16   | 270. LARGE    | 310. ANK2      | 350. NOS1AP  | 390. ITGB4        | 430. MAEA     | 470. SCARB1     |
| 31. INPP5A  | 71. ATXN1    | 111. KIF26B  | 151. ERC2     | 191. TRIM26   | 231. BLCAP     | 271. PRKG1    | 311. BRE       | 351. SEMA6A  | 391. NR3C1        | 431. MCTP1    | 471. SH3BP2     |
| 32. KCNQ1   | 72. PRKAG2   | 112. RUNX1   | 152. LTBP1    | 192. CDH22    | 232. DENND1A   | 272. AP2A2    | 312. FAM20C    | 352. AATK    | 392. PITPNM2      | 432. MGRN1    | 472. GRID2      |
| 33. COL23A1 | 73. DLGAP2   | 113. RAI1    | 153. IFT140   | 193. PDZD2    | 233. MSRA      | 273. ARHGAP10 | 313. FAM83A    | 353. AGAP3   | 393. C6orf10      | 433. RNF213   | 473. PPM1H      |
| 34. C7orf50 | 74. GNG7     | 114. SLC12A7 | 154. SNTG2    | 194. C10orf11 | 234. NPAS3     | 274. ARHGEF7  | 314. GNAS      | 354. PDE1C   | 394. COLEC11      | 434. DIP2B    | 474. SRCIN1     |
| 35. ABR     | 75. ARHGEF10 | 115. B3GNTL1 | 155. EHMT1    | 195. COL22A1  | 235. IGSF21    | 275. FHAD1    | 315. NRG2      | 355. CHD7    | 395. KLIF2        | 435. FAM160A1 | 475. ST7        |
| 36. NTM     | 76. RPH3AL   | 116. SLC03A1 | 156. KIAA0182 | 196. ABTB2    | 236. PC        | 276. SAMD12   | 316. BCAR1     | 356. HPCAL1  | 396. NOTCH1       | 436. GPCD1    | 476. TFDP1      |
| 37. ATP11A  | 77. TP73     | 117. MEGF6   | 197. CCNY     | 237. RGMA     | 277. SETBP1    | 317. PAX7     | 357. LGR6      | 397. PEMT    | 437. PKD1         | 477. ADARB1   |                 |
| 38. SNX29   | 78. GRID1    | 118. TEAD1   | 198. MYO18A   | 238. RGS6     | 278. GALTNT2   | 318. PDZRN3   | 358. SPIR      | 398. LTBP3   | 438. ARNT2        | 478. DAPK1    |                 |
| 39. TRIO    | 79. KCNMA1   | 119. LMIF1   | 199. ACOT7    | 239. BCAR3    | 279. PDXN      | 319. SLC43A2  | 359. CELF2     | 399. MARK2   | 439. RBFOX3       | 479. FUJ2536  |                 |
| 40. CBAZT3  | 80. CTBP2    | 120. IQSEC1  | 200. DDR1     | 240. SYNJ2    | 280. GTDC1     | 320. ZEB2     | 360. KAZN      | 400. NTN1    | 440. SYNGAP1      | 480. ROR2     |                 |

|               |               |               |               |               |               |                |                |               |               |               |
|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------------|---------------|---------------|---------------|
| 481. VAX2     | 521. MAP2     | 561. PLCL2    | 601. ITGB5    | 641. CHD9     | 681. ATG7     | 721. GABBR2    | 761. SMTN      | 801. LAMB3    | 841. DNAJC6   | 881. TK2      |
| 482. CACNA2D4 | 522. PLXND1   | 562. RAP1GAP  | 602. LTBP4    | 642. ITGA6    | 682. EDL3     | 722. PAPLN     | 762. SULT2B1   | 802. MYO7B    | 842. FAM171A1 | 882. ASB2     |
| 483. CACNB2   | 523. STK39    | 563. WDR27    | 603. MIR5095  | 643. KIAA1530 | 683. EPS8     | 723. PNP1A6    | 763. EDNRB     | 803. NXP1     | 843. H19      | 883. FLJ12825 |
| 484. CDYL     | 524. ARS8     | 564. XKR4     | 604. MKLN1    | 644. MIR99AHG | 684. MED27    | 724. SH2D3C    | 764. ERBB2     | 804. PSMB8    | 844. HLA-E    | 884. GNA11    |
| 485. AFAP1L2  | 525. HOXA3    | 565. ATP2C1   | 605. ZNRF1    | 645. SMAD6    | 685. MIR3134  | 725. ST6GAL2   | 765. INS-IGF2  | 805. SNTG1    | 845. KCNK9    | 885. RBM38    |
| 486. ELL      | 526. HMEPA1   | 566. CUP2     | 606. BAI1     | 646. TNNI3    | 686. RFX2     | 726. ABL1      | 766. MLLT3     | 806. TIOBP    | 846. NKAIN3   | 886. SRRM4    |
| 487. OXR1     | 527. SPTBN4   | 567. DGKI     | 607. DPP9     | 647. TXNRD2   | 687. TBX2     | 727. EVI5L     | 767. SLAIN1    | 807. UBE2D3   | 847. SLC25A13 | 887. ZBTB7B   |
| 488. RBPM5    | 528. CACNA1A  | 568. GALNTL6  | 608. EML6     | 648. UBR4     | 688. TNFRSF19 | 728. PPIIA4    | 768. TBC1D8    | 808. AMBRA1   | 848. UNC13A   | 888. ARHGEF18 |
| 489. SPOCK1   | 529. DNAH10   | 569. GRIK3    | 609. ERBB4    | 649. WDR90    | 689. TRIM27   | 729. SACS      | 769. TCF25     | 809. FMNL3    | 849. USP47    | 889. BEGAIN   |
| 490. EXO3     | 530. FAT3     | 570. JAK1     | 610. PTPRD    | 650. ZBTB22   | 690. CAMK4    | 730. TBX1      | 770. TMC8      | 810. HDAC7    | 850. ZNF598   | 890. CXC5     |
| 491. KIAA1522 | 531. INSR     | 571. RGL2     | 611. RNFI50   | 651. ZNF710   | 691. FURIN    | 731. TGFBI     | 771. AKT2      | 811. HSPA12B  | 851. ADCY3    | 891. DAB2     |
| 492. KIFC3    | 532. PHF21B   | 572. SCFD2    | 612. SERGEF   | 652. BAIAP3   | 692. IGF2R    | 732. TPCN2     | 772. C9orf171  | 812. KIAA1688 | 852. COL15A1  | 892. DLG1     |
| 493. OSBP13   | 533. SLC16A3  | 573. TAPBP    | 613. TJP2     | 653. KIAA1549 | 693. PTPN3    | 733. ADSSL1    | 773. CASR      | 813. RAB2A    | 853. DAZAP1   | 893. FAM110B  |
| 494. SPG7     | 534. SLC24A4  | 574. VARS     | 614. ACTN1    | 654. LSP1     | 694. WNT5A    | 734. LOC645323 | 774. FGFR3     | 814. RELL1    | 854. DEDD2    | 894. SLC17A7  |
| 495. UNC5C    | 535. ST3GAL2  | 575. GHR      | 615. BRUNOL4  | 655. USP6NL   | 695. LRRC8D   | 735. NEURL4    | 775. FRMD6-AS2 | 815. RHBD2    | 855. ESPNP    | 895. TRABD    |
| 496. ADCY5    | 536. TLN2     | 576. ALPK2    | 616. EMX205   | 656. FUT8     | 696. PLXDC1   | 736. PPARG     | 776. MICA      | 816. SNX24    | 856. FAM129B  | 896. UBR3     |
| 497. FAT1     | 537. TNFAIP8  | 577. CHID1    | 617. KLHDC4   | 657. SNCAIP   | 697. PPP1R13L | 737. TTBK1     | 777. MICALL2   | 817. SOLH     | 857. LAMB1    | 897. WDTG1    |
| 498. GFRA1    | 538. BMP1B    | 578. TGIF1    | 618. MYO9B    | 658. DHRS3    | 698. RCAN1    | 738. ZEB1      | 778. MIPOL1    | 818. ZMYM2    | 858. LHX2     | 898. CDC88B   |
| 499. NRXN2    | 539. CLSTN1   | 579. ISPD     | 619. TMEM51   | 659. RIMS1    | 699. SORCS1   | 739. GULP1     | 779. SMAD7     | 819. FLII     | 859. NDRG1    | 899. FGR      |
| 500. PLEKHG3  | 540. DNAH2    | 580. NINJ2    | 620. TTC15    | 660. TECPR2   | 700. ADAMTS9  | 740. KCNQ2     | 780. TESC      | 820. HIVEP1   | 860. NKAIN2   | 900. HLA-DOA  |
| 501. SEC14L1  | 541. GNAQ     | 581. SLC6A6   | 621. ABHD2    | 661. PTCN1    | 701. FBUM1    | 741. LRP8      | 781. TMEM117   | 821. KANSL1   | 861. PI4KA    | 901. MAPK15   |
| 502. CGNL1    | 542. GREB1    | 582. SLC9A3R2 | 622. CHD3     | 662. ATM      | 702. FOXP4    | 742. STK19     | 782. ZFP41     | 822. LRTOMT   | 862. PROM1    | 902. NXPH4    |
| 503. CLASP1   | 543. PRRT1    | 583. SPTBN2   | 623. EIF4G1   | 663. NR5A2    | 703. GFPT2    | 743. CDH5      | 783. BDH1      | 823. TFEB     | 863. SLC27A1  | 903. PRR16    |
| 504. PXN      | 544. ZNF385B  | 584. CUGBP2   | 624. P4HB     | 664. NRP1     | 704. IRAK2    | 744. CHN1      | 784. BLK       | 824. TSPAN5   | 864. CDK19    | 904. SARDH    |
| 505. SLC7A5   | 545. HMGGA2   | 585. OLFM2    | 625. PLA2G6   | 665. ROBO2    | 705. NKD2     | 745. D2HGDH    | 785. GRK6      | 825. DNAJC17  | 865. LAMC1    | 905. STK40    |
| 506. MYO7A    | 546. MEST     | 586. RARA     | 626. AHDC1    | 666. SHANK1   | 706. SRGAP1   | 746. DCC       | 786. GTF2F2    | 826. HTRA1    | 866. LRPAP1   | 906. ACSL6    |
| 507. SBF2     | 547. ODZ2     | 587. RBM33    | 627. ANO6     | 667. CACHD1   | 707. TAOK3    | 747. IRF2      | 787. HM13      | 827. QSOX1    | 867. NCS1     | 907. APC2     |
| 508. TLL2     | 548. PIGG     | 588. TULP4    | 628. ATP9B    | 668. FSIPI    | 708. TSC22D1  | 748. KIAA1462  | 788. ISLR2     | 828. SP5B4    | 868. PDE10A   | 908. C10orf90 |
| 509. ASPH     | 549. ACTN4    | 589. AGPAT3   | 629. CTNBNB1  | 669. GRIA4    | 709. TSPAN4   | 749. PRDM8     | 789. NCAM2     | 829. TJP1     | 869. PPM1L    | 909. FOXF1    |
| 510. CABLES1  | 550. ADPRHL1  | 590. CACNA2D1 | 630. IFFO2    | 670. HCN4     | 710. ISM1     | 750. RTN1      | 790. PAK1      | 830. TPST2    | 870. WNT3A    | 910. KCNH8    |
| 511. CERS6    | 551. C15orf27 | 591. MEG3     | 631. INF2     | 671. MAP3K9   | 711. PHRF1    | 751. SLC10A7   | 791. SCTR      | 831. TLLP10   | 871. ZBTB4    | 911. LEF1     |
| 512. DYNC1H1  | 552. FAM83F   | 592. PLB1     | 632. NFATC4   | 672. CTNNA1   | 712. RHBD1    | 752. TEF       | 792. THBS2     | 832. AGPAT1   | 872. ADAMTS18 | 912. PIK3R5   |
| 513. LSAMP    | 553. HMCN1    | 593. PRR5L    | 633. SLC22A18 | 673. PGS1     | 713. TTC38    | 753. ZSWIM4    | 793. DNAH1     | 833. CDKN1C   | 873. APLP2    | 913. PITPNA   |
| 514. NLGN1    | 554. HRH1     | 594. ZNF385A  | 634. BCR      | 674. RFX4     | 714. ZBTB46   | 754. CDC40     | 794. DPYS      | 834. ASPG     | 874. ASPG     | 914. TELO2    |
| 515. PIK3AP1  | 555. NRCAM    | 595. AKAP12   | 635. EPHA4    | 675. STAG1    | 715. FBXL18   | 755. COL12A1   | 795. FAM167A   | 835. GRASP    | 875. BAZ2B    | 915. YPEL2    |
| 516. SULF2    | 556. DPYD     | 596. HIMHA1   | 636. GGT1     | 676. UBE2E1   | 716. MAP2K5   | 756. EDARADD   | 796. GSK3B     | 836. MAP1B    | 876. BSG      | 916. ANO7     |
| 517. TERT     | 557. FRMD4B   | 597. LMBTL4   | 637. SLC41A3  | 677. WT1      | 717. PLAGL1   | 757. ENTPD1    | 797. LINC01599 | 837. UBE2J2   | 877. SDC1     | 917. FBXW8    |
| 518. ADAMTS16 | 558. KIAA0513 | 598. SYNPO    | 638. SYT7     | 678. CDH2     | 718. PPIA3    | 758. EVL       | 798. PDLIM1    | 838. USP2     | 878. SEMA4A   | 918. CYB561   |
| 519. AGRN     | 559. KIF5C    | 599. BBX      | 639. UXS1     | 679. CUEDC1   | 719. TRAM2    | 759. KCNC1     | 799. PPARD     | 839. ADAM22   | 879. SGM52    | 919. ENG      |
| 520. LARP1    | 560. PITPNM3  | 600. HSF4     | 640. WDFY2    | 680. RAMP1    | 720. FBXO34   | 760. LOXL3     | 800. SSU72     | 840. ATOH8    | 880. SPON1    | 920. FBLN5    |

|                |                |                |                 |                 |                |                |                |                   |
|----------------|----------------|----------------|-----------------|-----------------|----------------|----------------|----------------|-------------------|
| 921. NFAT5     | 961. TBX4      | 1001. VAT1L    | 1041. VWA1      | 1081. UBE2L6    | 1121. BMP6     | 1161. CEP68    | 1201. C8orf76  | 1241. ZDHC24      |
| 922. POLR3E    | 962. ANP32A    | 1002. ZDHC20   | 1042. ATP8B3    | 1082. EVC2      | 1122. CDC64B   | 1162. CYHR1    | 1202. LSM14A   | 1242. HSPA5       |
| 923. SCN8A     | 963. C1orf94   | 1003. CDC42BPG | 1043. CYGB      | 1083. OLFM3     | 1123. FAM69A   | 1163. GABRR2   | 1203. LSM4     | 1243. DBP         |
| 924. SPATS2    | 964. CDG3      | 1004. CPEB4    | 1044. EF2F5     | 1084. SLC17A9   | 1124. MATN1    | 1164. SCARB2   | 1204. NOL12    | 1244. LRRC75A-AS1 |
| 925. TYK2      | 965. CXCR5     | 1005. CRAT     | 1045. MFAP2     | 1085. SLC5A2    | 1125. TMEM190  | 1165. SH3      | 1205. PRNP     | 1245. NDUFA7      |
| 926. ZNF787    | 966. GN81L     | 1006. ETV1     | 1046. RHOT2     | 1086. CABP1     | 1126. ALDH16A1 | 1166. YWHAZ    | 1206. S100PBP  | 1246. COPB2       |
| 927. CCND3     | 967. GRIK5     | 1007. EYA3     | 1047. STAMBP1L1 | 1087. DTD1      | 1127. MIR548G  | 1167. B2M      | 1207. HCK      | 1247. WDR69       |
| 928. CDC42BPA  | 968. UBXN11    | 1008. IGFBP7   | 1048. HS3ST4    | 1088. PDE6A     | 1128. MUPCDH   | 1168. DDX41    | 1208. MUC1     | 1248. OSTM1       |
| 929. FBXO42    | 969. UGP2      | 1009. LINGO3   | 1049. MAP3K4    | 1089. ACAN      | 1129. RABEP1   | 1169. HIC2     | 1209. ZNF248   | 1249. LGALS17A    |
| 930. KCNN1     | 970. ADIPOR2   | 1010. PLEKHG4B | 1050. PEAR1     | 1090. B4GALT5   | 1130. SLC29A3  | 1170. HYAL1    | 1210. PTGS1    | 1250. C10orf82    |
| 931. LMO3      | 971. AK8       | 1011. RAB7A    | 1051. RHOD      | 1091. C6orf132  | 1131. ARHGAP20 | 1171. NCL      | 1211. SLC2A10  |                   |
| 932. LPCAT3    | 972. CBLC      | 1012. REEP6    | 1052. 8-Sep     | 1092. CUL9      | 1132. CAND2    | 1172. PANK2    | 1212. SNAP29   |                   |
| 933. MEGF8     | 973. HIP1R     | 1013. SHC1     | 1053. TBX3      | 1093. F7        | 1133. CEACAM16 | 1173. PHKG2    | 1213. ACR      |                   |
| 934. SDCBP2    | 974. HLF       | 1014. TRPC7    | 1054. B4GALT7   | 1094. ITGA3     | 1134. CHDH     | 1174. ELL3     | 1214. ANG      |                   |
| 935. TGFA      | 975. PANX1     | 1015. BRD4     | 1055. BCL7C     | 1095. KNG2      | 1135. FGF3     | 1175. GABRA2   | 1215. MAPK12   |                   |
| 936. ABCA1     | 976. SLC1A4    | 1016. DHX34    | 1056. CDC50     | 1096. PLTP      | 1136. LPAR2    | 1176. PAK2     | 1216. SMC04    |                   |
| 937. ATP9A     | 977. SPNS2     | 1017. DNAH12   | 1057. CDC27     | 1097. SEMA3B    | 1137. ORAI2    | 1177. PRSS8    | 1217. ASB13    |                   |
| 938. EFCA86    | 978. TACC3     | 1018. FAM174B  | 1058. CHD4      | 1098. SNAP91    | 1138. SLC22A15 | 1178. SIRT2    | 1218. BCAT2    |                   |
| 939. IDUA      | 979. TMEM181   | 1019. IGF2BP1  | 1059. FBXL16    | 1099. STXBPSL   | 1139. STXBP1   | 1179. TRNP1    | 1219. C1orf105 |                   |
| 940. KCNN1     | 980. ZNF331    | 1020. SYNGR1   | 1060. NLRCS     | 1100. ZBTB17    | 1140. ZDHC17   | 1180. ANKRD27  | 1220. C5orf38  |                   |
| 941. NTNG2     | 981. CCDC109B  | 1021. APPL2    | 1061. PLA2G4F   | 1101. C10orf105 | 1141. ARL4D    | 1181. DCK      | 1221. DLAT     |                   |
| 942. PARP8     | 982. CLDN10    | 1022. C7orf49  | 1062. PYGB      | 1102. C11orf80  | 1142. FLJ43860 | 1182. GPR157   | 1222. FAM131C  |                   |
| 943. COL19A1   | 983. FAM124A   | 1023. DUSP6    | 1063. CDT1      | 1103. FLYWCH1   | 1143. IQGAP3   | 1183. KCTD15   | 1223. GNB2L1   |                   |
| 944. CPAMD8    | 984. LPPR1     | 1024. MEGF10   | 1064. DPF1      | 1104. HOMER3    | 1144. MST1P2   | 1184. UNC00426 | 1224. ACOT13   |                   |
| 945. FBN3      | 985. LYSMD2    | 1025. PRKAR1A  | 1065. KL        | 1105. PTPN21    | 1145. WFS1     | 1185. MTF1     | 1225. CDKN1A   |                   |
| 946. H2AFY     | 986. SIRPA     | 1026. SPINT1   | 1066. MAPK8IP1  | 1106. RASGRP2   | 1146. ZCCHC2   | 1186. PM20D1   | 1226. CYB5A    |                   |
| 947. LINC00598 | 987. STSIA1    | 1027. TMEM229B | 1067. MF12      | 1107. RER1      | 1147. ABCC10   | 1187. DUPD1    | 1227. PFN2     |                   |
| 948. AMFR      | 988. BAT3      | 1028. YIF1B    | 1068. RARG      | 1108. RNF11     | 1148. ACVR1C   | 1188. TP53I3   | 1228. UBE2Z    |                   |
| 949. AMOTL2    | 989. CASP9     | 1029. ACVR2A   | 1069. WNT11     | 1109. RNP51     | 1149. MIER2    | 1189. BPTF     | 1229. VPS18    |                   |
| 950. FAM120B   | 990. CHSY3     | 1030. AKAP1    | 1070. ZNF638    | 1110. TXNDC5    | 1150. XPC      | 1190. CCDC48   | 1230. DD5      |                   |
| 951. FAM83C    | 991. GPRC5C    | 1031. BZW2     | 1071. CTSD      | 1111. ALDOA     | 1151. ZDHC18   | 1191. ERGIC2   | 1231. G6PC3    |                   |
| 952. GRIN1     | 992. KIAA0895L | 1032. CLIP4    | 1072. MSRB3     | 1112. IFRD1     | 1152. BAD      | 1192. GAS8     | 1232. MIF      |                   |
| 953. MNT       | 993. 4-Sep     | 1033. DAGLB    | 1073. SDCBP     | 1113. KLF10     | 1153. C5orf45  | 1193. NOBOX    | 1233. MTHFD2   |                   |
| 954. AB12      | 994. SETD3     | 1034. ERAP1    | 1074. UBXN6     | 1114. KIK5      | 1154. DNASE1L3 | 1194. PRSS12   | 1234. BRMS1L   |                   |
| 955. BMF       | 995. SLC8A2    | 1035. EXT2     | 1075. DNAJC11   | 1115. LY6G5C    | 1155. 11-Mar   | 1195. SLC3A2   | 1235. CALHM1   |                   |
| 956. HAND1     | 996. TMEM120B  | 1036. KIF3B    | 1076. ELP3      | 1116. MYOCD     | 1156. UBE2Q1   | 1196. ARHGEF26 | 1236. CD200    |                   |
| 957. NECAB3    | 997. ZNF503    | 1037. LRRC8A   | 1077. GBF1      | 1117. OC1AD2    | 1157. WNT2     | 1197. FAAH     | 1237. KCNJ2    |                   |
| 958. RNU5E     | 998. AP2A1     | 1038. STK11    | 1078. PRR3      | 1118. PPP1R12C  | 1158. ZNF507   | 1198. KIAA0564 | 1238. RG9MTD2  |                   |
| 959. SATB1     | 999. CPM       | 1039. SYT9     | 1079. SHC2      | 1119. RNF4      | 1159. ZNF589   | 1199. PCAT29   | 1239. RGPDI    |                   |
| 960. SYT6      | 1000. POLR1A   | 1040. TSHR     | 1080. SLC16A12  | 1120. BAHD1     | 1160. C19orf25 | 1200. VPS26B   | 1240. TTC21A   |                   |

## CLAIMS

1. A method of identifying oxidative stress (OS) in a test cell, the method comprising:
  - (a) determining the methylation status of at least five genes in a DNA sample obtained from the test cell;
  - 5 (b) comparing the methylation status of the genes from step (a) to the methylation status of the corresponding genes in a control without OS, wherein a difference in the methylation status of the genes in the test cell compared to the corresponding genes in the control is indicative of the cell having OS; and wherein the genes in step (a) are selected from the group consisting of *PTPRN2*, *MAD1L1*, *PRDM16*,  
10 *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, *CACNA1C*,  
and the regulatory regions of the same.
- 15 2. The method according to claim 1, wherein one of the genes in step (a) is the gene Protein Tyrosine Phosphatase Receptor Type N2 (*PTPRN2*), and/or the regulatory region of *PTPRN2*.
3. The method according to either claim 1 or 2, wherein in step (a), the methylation status of  
20 at least 10 genes is determined.
4. The method according to any one of the preceding claims, wherein the 5 genes are *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, and *HDAC4*.
- 25 5. The method according to any one of the preceding claims, wherein the methylation of at least 20 genes is determined.
6. The method according to claim 5, wherein the 20 genes are *PTPRN2*, *MAD1L1*, *PRDM16*,  
30 *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, and *RPS6KA2*.
7. The method according to any one of the preceding claims, wherein in step (a) the methylation status of all the genes *PTPRN2*, *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*,  
35 *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, and *CACNA1C* is determined.
8. The method according to any one of the preceding claims, wherein the OS is brought about by ageing, Ultraviolet (UV) light exposure and/or H<sub>2</sub>O<sub>2</sub> exposure.
- 40 9. The method according to any one of the preceding claims, further comprising the step of:

(i) performing bisulfite modification to the DNA sample before step (a).

10. The method according to any one of the preceding claims wherein the cell is a eukaryote.

5 11. The method according to any one of the preceding claims, wherein the cell is from a mammal.

12. The method according to claim 11, wherein the mammal is a mouse, a rat, a guinea pig, a dog, a mini-pig, a human being, a cow, a sheep, a pig, a goat, a horse, a donkey, and a  
10 mule.

13. The method according to any one of the preceding claims, wherein the cell is a skin cell, a stem cell or a cell derived therefrom.

15 14. Use of a gene panel with at least five genes for indicating the presence of OS in a cell, wherein one of the five genes is *PTPRN2* and the other four genes are selected from the group consisting of *MAD1L1*, *PRDM16*, *TNXB*, *HDAC4*, *ADARB2*, *CDH4*, *DIP2C*, *SHANK2*, *CAMTA1*, *RPTOR*, *RASA3*, *SDK1*, *AGAP1*, *TBCD*, *SEPT9*, *FRMD4A*, *MCF2L*, *FOXP1*, *RPS6KA2*, *SORCS2*, *NXN*, *TRAPPC9*, *AUTS2*, and *CACNA1C*.

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