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(54) Title: METHOD FOR INCREASING THE PLASTICITY LEVEL OF A CELL

(57) Abstract: This application relates to reprogramming of cells, as the present invention discloses a method for increasing the plasticity level in a donor cell by introducing one or more plasticity modifying factor(s), it is an aspect of the present invention to provide a composition for mediating an increase in the plasticity level of a donor cell, said composition comprising one or more plasticity modifying factors.

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METHOD FOR INCREASING THE PLASTICITY LEVEL OF A CELL

Technical field of the invention

The present invention relates to the reprogramming of partly or fully differentiated mammalian cells, so that they can act like stem cells. The present invention
5 provides a method for such reprogramming by increasing the plasticity level of a cell through changes in the pattern of gene expression in the partly or fully differentiated cell. In particular, the present invention relates to plasticity modifying factor(s), said plasticity modifying factor(s) being selected from the group consisting of plasticity modifying factors increasing DNA demethylation,
10 plasticity modifying factors inhibiting maintenance DNA methylation and plasticity modifying factors mediating hyperacetylation of histones.

Background of the invention

The nature and function of any type of cell depends on the specific set of genes expressed (e.g. transcribed and translated). Accordingly, development of cells
15 exhibiting a high plasticity level (i.e. pluripotent or totipotent cells) into more specialized phenotypes exhibiting a decreased plasticity level (for example end-stage-committed cells) is determined by the type of genes expressed during development. Gene expression is directly mediated by sequence specific binding of gene regulatory proteins that can effect positive or repressive regulation. The
20 ability of any of these regulatory proteins to directly mediate gene expression however, appear to depend on the accessibility of their binding site within the cellular DNA.

In mammals, the culmination of oogenesis is the creation of a mature metaphase
25 II (MII) oocyte capable of sustaining fertilization, reprogramming of the parental genomes, and supporting early embryonic development. The oocyte cytoplasm rapidly starts to modify the sperm genome upon fertilization resulting in decondensation of paternal chromatin, substitution of protamines with highly acetylated histones, and active demethylation of DNA (Santos et al. 2002; Spinaci
30 et al. 2004). By contrast, the oocyte-derived maternal chromatin seems more protected from this extensive reprogramming activity undergoing DNA demethylation at a more passive level during subsequent cleavages. The observed

asymmetry in parental genome reprogramming is believed to result from marked differences in the original nucleosomal structure of the maternal and paternal chromatin, respectively.

- 5 Several studies have approached the underlying molecular mechanisms that take place in connection with reprogramming suggesting that the three-dimensional structure of chromatin is highly important in the regulation of gene expression.

Generally, a condensed chromatin structure is associated with an inaccessible and
10 gene repressive state whereas more decondensed and open chromatin is more amenable to gene activation (Turner 2001). Notably, transcription levels may be affected by covalent modifications of the DNA helix and core histones comprising the nucleosome, the core unit of chromatin.

- 15 The complex mechanisms involved in the modifications of chromatin are mediated by a group of highly conserved enzymes, including DNA methyltransferases (DNMTs), methyl-CpG-binding proteins (MeCPs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases/deiminases (HDMs), and chromatin remodeling complexes
20 (ChRs). Covalent DNA methylation, which is the most abundant form of epigenetic modification, occurs at cytosines in the pyrimidine ring of CpG dinucleotides dispersed throughout the genome and is generally associated with gene silencing.

The repressive effect of DNA methylation is mediated by the MeCPs, which again
25 recruit a wide range of co-repressors, including HDACs. Nucleosome core histones are covalently modified at their N-terminal tails, which are highly charged and tightly associated with the DNA helix. In particular, acetylation and methylation have been associated with regulation of gene expression and it has been suggested that the vast number of combinations may actually form a specific
30 histone code, which could determine the binding patterns of regulatory proteins.

By contrast, ChRs do not interact covalently with DNA in a sequence specific manner but are rather recruited to promoters of target genes by sequence specific transcription factors. Once recruited, the catalytic sub-units of the ChRs (ChR-
35 ATPases) cause an altered conformation and position of nucleosomes by ATP-

hydrolysis. This leads to weakened DNA-histone contacts resulting in histone octamers changing positions along the DNA helix, thus either increasing or reducing the binding of transcription factors to promoter regions. The groups of proteins important to epigenetic modifications and chromatin remodeling do not
5 act independently, but are closely coordinated, often by forming large multiprotein complexes.

Remarkably, the highly specialized ability of the MII oocyte cytoplasm to reprogram a sperm genome extends to a comparable level of reprogramming of a
10 diploid somatic nucleus from adult tissue, i.e. somatic cell nuclear transfer (SCNT) or cloning. By contrast, fully grown germinal vesicle (GV) oocytes are not considered capable of adequate reprogramming. For example, when murine GV oocytes are enucleated and the remaining ooplasm subsequently is used for SCNT, nuclear reprogramming is severely reduced without formation of pseudo-pronuclei
15 or cleavage. The factors responsible for gamete and somatic reprogramming are likely to become available during the final stages of oogenesis during the transition from GV to MII stage, thus highlighting the MII oocyte cytoplasm as the key player in the reprogramming of a specialized genome into a totipotent state. However, the exact mechanisms by which the MII oocyte cytoplasm erases the
20 established patterns of gene expression imposed on the sperm genome or a somatic nucleus remain unknown.

Even though successful cloning of frogs and several mammalian species by SCNT has been reported, the therapeutic application of this method has been hindered
25 by technical complications as well as ethical objections. Accordingly, there exists a need to either increase the efficiency of the SCNT method, or alternatively to invent new methods for increasing the level of plasticity of cells.

Takahashi et al. 2006 demonstrated induction of pluripotent stem cells from
30 mouse embryonic or adult fibroblasts by introducing four transcription factors Oct3/4, Sox2, c-Myc and Klf4 under ES cell culture conditions. However, the use of retroviral expression systems resulted in only a small fraction of cells expressing the four transcription factors to become induced pluripotent stem cells (iPS).

Later Wernig et al. 2007 reported that reprogramming of fibroblasts to a pluripotent state may be induced *in vitro* through ectopic expression of the four previously mentioned transcription factors (Oct4 (Oct3/4 or Pou5f1), Sox2, c-Myc and Klf4). By employing said method, the authors showed that the DNA

5 methylation, gene expression and chromatin state in induced reprogrammed cells were similar to those of ES-cells.

In the present application, the inventors have investigated further factors involved in early embryonic reprogramming. They discovered and identified a number of
10 genes involved in epigenetic reprogramming.

Accordingly, a method for epigenetic reprogramming is disclosed in the present application.

Summary of the invention

15 The present invention relates to the construction of a cell having an increased level of plasticity. Such a cell is termed a "supraplastic cell" and is capable of differentiating into a variety of end-stage-committed cell types, including the cell types similar to the cell type of the donor cell from which the supraplastic cell was derived and cell types different from the cell type of the donor cell from which the
20 supraplastic cell was derived.

In a first aspect, the present invention describes a method for increasing the plasticity level of a donor cell, said method comprising providing a mammalian, normoploid somatic cell as the donor cell and increasing the plasticity level in said
25 cell by contacting said donor cell with, or introducing into said donor cell, one or more plasticity modifying factor(s), said plasticity modifying factor(s) being selected from the group consisting of plasticity modifying factors increasing DNA demethylation, plasticity modifying factors inhibiting maintenance DNA methylation and plasticity modifying factors mediating hyperacetylation of
30 histones, thereby obtaining a supraplastic cell with the ability to develop into at least two different end-stage-committed cell types.

In another aspect the invention relates to a supraplastic cell obtained by any of the methods described herein. Such supraplastic cell may be used in several

different aspects. In one aspect the supraplastic cell may be used for various kinds of cell therapy. One type of cell therapy comprises using the supraplastic cell or cells derived from a supraplastic cell for cell replacement by transplantation, for example replacement of bone marrow in patients suffering from diseases and conditions affecting hematopoietic and mesenchymal stem cells in disorders such as anemia, hypovolemic blood loss, hemoglobinopathies, and platelet disorders. Cell therapy may as described in the present invention also comprise delivery of natively and non-natively produced compounds in situ in patients, said compounds being produced by the transplanted supraplastic cell or cells derived therefrom.

As the present invention discloses a method for increasing the plasticity level in a donor cell by introducing one or more plasticity modifying factor(s), it is an aspect of the present invention to provide a composition for mediating an increase in the plasticity level of a donor cell, said composition comprising one or more plasticity modifying factors.

In a further aspect of the present invention, the supraplastic cell is used for the production of target cells exhibiting the phenotype of end-stage-committed cell types including unipotent pre-cursor cells.

Detailed description of the invention

General

Each of these applications, patents, and each document cited in this text, and each of the documents cited in each of these applications, patents, and documents ("application cited documents"), and each document referenced or cited in the application cited documents, either in the text or during the prosecution of the applications and patents thereof, as well as all arguments in support of patentability advanced during prosecution thereof, are hereby incorporated herein by reference.

It should be understood that any feature and/or aspect discussed above in connection with the methods according to the invention apply by analogy to the uses.

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention. Thus, preferred features and characteristics of one aspect of the invention may be applicable to other aspects of the invention.

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Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer step, or group of elements, integers or

10 steps.

Increasing the plasticity level

Stem cells are the master cells that replenish all the body's tissues, from skin and blood to the brain and heart. In children and adults, particular types of stem cells

15 are distributed throughout the body, and are specialized to create particular types of tissues. However, in the early embryo, inner cell mass cells can make derivatives of all three primary germ layers including germ cells, a quality known as pluripotency. The "gold standard" test for pluripotency is the ability of a cell to contribute extensively to all adult cell types, including the germ line.

20

It is an object of the present invention to test the supraplastic cells described herein by injecting these cells into early embryos and observe that these cells can differentiate into all normal adult cell types. The goal for epigenetic reprogramming or production of stem cells or stem-like cells is mainly for

25 regenerative medicine purposes and for understanding the molecular mechanisms behind cellular differentiation and de-differentiation. It is of course preferred to do this in e.g. murine cells for ethical reasons.

The method to assess pluripotency includes as explained injecting these cells into

30 early mouse embryos and observe that these cells differentiate into all normal adult cell types. In human cells, formation of teratomas in severe combined immunodeficient (SCID) mice and/or embryoid body formation *in vitro* is considered proof of pluripotency (Thomson et al. 1998).

In this application, the present inventors describe various plasticity modifying factors that enable reprogramming of partly or fully differentiated cells so they can act like stem cells or stem-like cells. Others have disclosed the principle that pluripotency can be induced in mouse embryonic or adult fibroblasts by
5 introducing four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4, under embryonic stem (ES) cell culture conditions (Takahashi et al. 2006).

The present invention provides an alternative method for such reprogramming by increasing the plasticity level of a cell through changes in the pattern of gene
10 expression by use of new plasticity modifying factors.

The transcriptional profiles, the structure of chromatin (complexes of DNA and histone proteins) and the DNA methylation status of ES cells are very different from those of adult cells, indicating that pluripotency is probably under complex
15 layers of control. It is therefore surprising that contacting partly or fully differentiated cells with, or introducing into partly or fully differentiated cells, a limited number of plasticity modifying factors as described in the present application one can produce stem cell with varying degree of developmental potency.

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The present application describes methods for increasing the plasticity level of a donor cell, said method comprising, providing a donor cell and increasing the plasticity level in said cell by contacting said cell with, or introducing into said cell, one or more plasticity modifying factor(s), said plasticity modifying factor(s) being
25 selected from the group consisting of plasticity modifying factors increasing DNA demethylation, plasticity modifying factors inhibiting maintenance DNA methylation and plasticity modifying factors mediating hyperacetylation of histones, thereby obtaining a supraplastic cell.

30 In one embodiment, the one or more plasticity modifying factor(s) is selected from the group consisting of plasticity modifying factors increasing DNA demethylation and plasticity modifying factors inhibiting maintenance DNA methylation.

In another embodiment, the one or more plasticity modifying factor(s) is selected from the group consisting of plasticity modifying factors increasing DNA demethylation and plasticity modifying factors mediating hyperacetylation of histones.

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In another embodiment, the one or more plasticity modifying factor(s) is selected from the group consisting of plasticity modifying factors inhibiting maintenance DNA methylation and plasticity modifying factors mediating hyperacetylation of histones.

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In one embodiment, the plasticity modifying factor is a factor increasing DNA demethylation.

In one embodiment, the plasticity modifying factor is a factor inhibiting

15 maintenance DNA methylation.

In one embodiment, the plasticity modifying factor is a factor mediating hyperacetylation of histones.

20 The cells of the present invention are useful for cell-based therapy including but not limited to autologous treatment schemes. Thus in one embodiment the present invention relates to a method for creating a stem cell comprising, obtaining a partly or fully differentiated donor cell from an individual, inducing in said cell epigenetic reprogramming by altering the level of expression of
25 mediators of covalent modifications of DNA and/or histones and/or mediators of chromatin remodelling, and introducing the resulting stem cell into an individual by transplantation.

Furthermore, the invention thus also relates to use of a transplanted supraplastic
30 cell or a cell derived herefrom for *in vivo* delivery of a therapeutic compound, said cell either natively producing the compound or being engineered by gene technology to produce the compound.

Plasticity

The term "plasticity" refers to the ability of a cell to develop into at least two end-stage-committed cell types. It is the object of the present invention to provide a donor cell and increasing in said donor cell the level of plasticity to obtain a
5 supraplastic cell. Depending on the increase in the level of plasticity, the supraplastic cell may develop into a variety of target cell types.

Accordingly, in one embodiment of the present invention, the supraplastic cell exhibits the ability of differentiating into target cells, said target cells being
10 derivatives from one of the three germ layers including germ cells.

Accordingly, in one embodiment of the present invention, the supraplastic cell exhibits the ability of differentiating into target cells, said target cells being derivatives from one of the three germ layers.

15

Accordingly, in one embodiment of the present invention, the supraplastic cell exhibits the ability of differentiating into target cells, said target cells being derivatives from one of the representing two germ layers.

20 In a further embodiment, the supraplastic cell exhibits the ability of differentiating into target cells, said target cells being derivatives of the three or two germs layers.

In the case wherein the target cells represent derivatives of two germ layers, one
25 of said germ layers may be of the same developmental origin as the donor cell from which the supraplastic cell was obtained.

In yet another embodiment of the present invention, the supraplastic cell has the ability of differentiating into target cells derived from one germ layer, said germ
30 layer may be of the same developmental origin as the donor cell from which the supraplastic cell was obtained.

Specifically, as used herein "plasticity" refers to the ability of a cell to differentiate into at least one end-stage-committed cell type different from the cell type from
35 which said cell originated. Thus, in the present context the term "plasticity level"

of a cell, refers to the number of end-stage-committed cell types into which said cell is capable of differentiating.

Accordingly, a totipotent stem cell comprises a higher plasticity level than a pluripotent stem cell, as a totipotent stem cell in the present context refers to a cell capable of dividing and producing all types of differentiated cells in an organism, but also the strict extra-embryonic tissue (i.e. outer chorion) whereas a pluripotent stem cell in the present context refers to a cell capable of differentiating into all cell types comprising the adult human body including some tissues of the placenta (inner chorion, yolk sac, amnion, allantois), but not the strict extra-embryonic tissues derived from trophoblast cells (i.e. outer chorion).

The pluripotent stem cell thus comprises a higher plasticity level than a multipotent stem cell, which in the present context refers to a cell capable of dividing and producing only cell types representing the same germ layer, e.g. the hematopoietic stem cell within the mesoderm.

Accordingly, a method for increasing the plasticity level of a donor cell provides the potential of epigenetic reprogramming. Specifically as used herein the term "epigenetic reprogramming" refers to the erasure and remodeling of epigenetic marks, such as, but not limited to, covalent DNA methylation, covalent histone modifications and non-covalent chromatin remodeling. The term "reprogram" refers to the phenomenon, in which a partly or fully differentiated donor cell acquires an increase in the level of plasticity, hence becomes multipotent, pluripotent or totipotent. Donor cells may be reprogrammed to varying degrees as it is possible that an individual donor cell is reprogrammed to be multipotent or pluripotent or even totipotent. Thus, the increased level of plasticity obtained by the method disclosed in the present invention may vary and may thus be increased into different levels.

30

The term "reprogrammed cell" refers to a cell that had formerly attained a particular degree of differentiation i.e. a particular level of plasticity, but has subsequently regained the ability to differentiate into at least two types of specialized cells (e.g., has become multipotent, pluripotent or totipotent), it has been de-differentiated. It is in general highly unlikely that differentiated cells (i.e.

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cells comprising a decreased level of plasticity) will revert into their precursor cells (i.e., reprogram) *in vivo*. However, using the method of the present invention, differentiated cells may be reprogrammed or de-differentiated into multipotent, pluripotent or totipotent cells both *in vitro* and *in vivo*. The terms "differentiated cell" and "cell comprising a decreased level of plasticity" are used interchangeably to refer to a cell comprising a poorer ability of differentiating into different specialized cell types when compared to its counterparts termed "undifferentiated cell", "supraplastic cell" and "cell exhibiting an increased level of plasticity" (the latter terms also being used herein interchangeably).

10

In order to determine whether the plasticity level is increased in a supraplastic cell, the plasticity level of said cell is compared to the plasticity level of the donor cell by subjecting said supraplastic and donor cell to one or more differentiation protocols and determining the number and/or type of target cells into which the supraplastic cell and donor cell as well as cells derived herefrom are able to differentiate.

A cell is determined as having an increased level of plasticity (i.e. to be a supraplastic cell), if the number of target cell types into which said cell may develop is larger than the number of target cell types into which the donor cell may develop; or if a supraplastic cell derived from a donor cell is capable of developing into at least one type of target cell into which the donor cell cannot develop.

25 The term "differentiated cell" refers to a cell that has developed from a relatively unspecialized phenotype (e.g. multipotent stem cell) to a more specialized phenotype (e.g. nerve cell, pancreatic beta-cell, cardiomyocyte).

According to the present invention an increase in the plasticity level is desirable, as a cell exhibiting a high level of plasticity has the potential to develop into a number of different cell types, i.e. shows an increased differentiation potential or developmental potential.

30

Plasticity modifying factors

As described herein, the present inventors have investigated several factors involved in early embryonic reprogramming, and thus in one embodiment relates to one or more plasticity modifying factor(s) increasing DNA demethylation.

- 5 Examples given herein comprises *MBD2* factor(s) and *MBD4* factor(s)

The *MBD2* factor is selected from the group consisting of DNA sequences encoded by the *MBD2* gene, proteins encoded by the *MBD2* DNA sequence, compounds increasing the transcription of the native *MBD2* gene, compounds increasing the
10 translation of *MBD2* mRNA species and compounds increasing the activity of the proteins encoded by the *MBD2* gene. As will be apparent to the skilled person, the term "*MBD2*" also covers any well known variants (e.g. *MBD2a*, *MBD2b* and *MBD2t*) as well as any other putative splice variant isoforms hereof.

- 15 The *MBD4* factor is selected from the group consisting of DNA sequences encoded by the *MBD4* gene, proteins encoded by said DNA sequence, compounds increasing the transcription of the native *MBD4* gene, compounds increasing the translation of *MB42* mRNA species and compounds increasing the activity of the proteins encoded by the *MBD4* gene. Again the term "*MBD4*" covers any well
20 known variants as well as any other putative splice variant isoforms hereof.

In another embodiment, the one or more plasticity modifying factor(s) inhibiting maintenance DNA methylation, comprise *DNMT1* factor(s).

- 25 The *DNMT1* factor is selected from the group consisting of compounds decreasing the transcription of the native *DNMT1* gene, compounds decreasing the translation degradation of *DNMT1* mRNA species, compounds increasing the translation degradation of *DNMT1* mRNA species and compounds inhibiting the DNA methyltransferase activity of the proteins encoded by *DNMT1*. As will be apparent
30 to the skilled person, the term "*DNMT1*" also covers any well known variants (e.g. *DMNT1s* and *DMNT1o*) as well as any other putative splice variant isoforms hereof. The compounds decreasing the translational degradation of *DNMT1* mRNA species does according to the present invention also include siRNA and microRNA.

As will be apparent from the literature, *DNMT1*, *DNMT3a*, *DNMT3b* (DNA methyltransferase 1, 3a, and 3b) carry out the process of DNA methylation wherein *DNMT1* is the proposed maintenance DNA methyltransferase that is responsible for replicating DNA methylation patterns to the daughter strands
 5 during DNA replication.

The present invention relates to epigenetic reprogramming or de-differentiation by increasing the plasticity level of a donor cell, such as inducing pluripotency through plasticity modifying factors. Others have shown that the transcription
 10 factor(s) selected from the group consisting of Oct4, Sox2, c-Myc, and Klf4 can induce de-differentiation. Thus, in one embodiment, the plasticity modifying factors of the present invention such as but not limited to *MBD2* factor(s), *MBD4* factor(s) and *DNMT1* factor(s) is used in combination with any of the factors Oct4, Sox2, c-Myc, and Klf4 for the purpose of de-differentiation.

15

In one embodiment, the present invention relates to epigenetic reprogramming by increasing the plasticity level of a donor cell contacting said donor cell with, or introducing into said donor cell, one or more *DNMT1* factor(s) and/ or *MBD2* factor(s) and/or *MBD4* factor(s) combined with the induction of expression of any
 20 of the transcription factors selected from the group consisting of *OCT4*, *SOX2*, *C-MYC* and *KLF4*.

In a further embodiment the one or more plasticity factors mediating hyperacetylation of histones (HATs) is encoded by one or more of the genes
 25 selected from the group of HATs consisting of but not limited to *ATF2*, *CDYL*, *CREBBP*, *ELP3*, *EP300*, *GCN5L2*, *GTF3C1*, *HAT1*, *HTATIP*, *MYST1*, *MYST2*, *MYST3*, *MYST4*, *NCOA1*, *NCOA2*, *NCOA3*, *OGT*, *PCAF*, and *TAF1*. Like in the above this group also includes splice variants and isoforms, of the genes mentioned herein.

30 In general the one or more plasticity modifying factor(s) disclosed in the present invention may be selected from the group consisting of small chemical entities, peptides, proteins, RNA species including but not limited to siRNA, microRNA, shRNA, peptide aptamers, modified nucleic acids and DNA species.

In order to obtain an decrease in DNA methylation, an inhibition of maintenance DNA methylation or mediate hyperacetylation of histones, the level of gene expression of the genes encoding DNA demethylation, DNA methylation, or hyperacetylation of histones may be altered at the DNA or mRNA level.

5

In an embodiment of the present invention, the plasticity modifying factor is directed towards the mRNA of the mediators of covalent modifications of DNA and histones and mediators of chromatin remodeling, the plasticity modifying factor being selected from the group consisting of an antisense oligonucleotide, a siRNA, 10 shRNA and microRNA. Thus, instead of interfering directly with the mediators of covalent modifications of DNA and histones and mediators of chromatin remodelling production at the protein level, in this embodiment the production of the mediators of covalent modifications of DNA and histones and mediators of chromatin remodeling is targeted.

15

Accordingly, said plasticity modifying factor may either increase or decrease the expression of the genes encoding the mediators of covalent modifications of DNA and histones and mediators of chromatin remodelling. It is an object of the present invention to increase the level of gene expression of *MBD2* and/or *MBD4* 20 whereas it is an object to decrease the level of gene expression of *DNMT1*.

A preferred plasticity modifying factor is an antisense oligonucleotide that may be specifically directed to the mRNA encoded by the gene of interest by way of complementarity. Preferably, the antisense oligonucleotide is capable of mediating 25 RNase H cleavage of the target mRNA. Thus, the antisense oligonucleotide comprises a stretch of DNA residue and/or DNA analogue residues to make the mRNA/DNA hybrid substrate for RNase H. In a preferred embodiment, the antisense oligonucleotide is of the so-called gapmer structure, which is well known to a person skilled in the art in the field of antisense oligonucleotides.

30

Another preferred plasticity modifying factor is a siRNA (short interfering RNA). siRNAs are short double stranded RNA complexes of typically 20-22 nucleotides with a overhang of 1-2 nucleotides at the 3'end. siRNAs are capable of activating a sophisticated cellular machinery ultimately leading to degradation or 35 translational inhibition of mRNAs that are complementary to the guide strand of

the siRNA complex. siRNAs are typically not produced in the cell, but are introduced into the cell to mediate RNAi (RNA interference).

Yet another preferred plasticity modifying factor is shRNAs (short hairpin RNAs).

- 5 shRNA may be produced from a gene introduced into a cell and may thus be viewed as vector mediated RNAi.

MicroRNAs (miRNA) are small (typically 21-23 nucleotides in length) and noncoding RNAs which regulates gene expression by mediating sequence-specific
10 repression of mRNA translation. miRNA may be overexpressed in a cell using transfection of synthetic miRNAs or miRNA-expressing plasmids or by down-regulation using transfection of miRNA inhibitors.

In a preferred embodiment, the plasticity modifying factor interferes with the
15 mediators of covalent modifications of DNA and histones and mediators of chromatin remodelling production at the protein level. Accordingly, said plasticity modifying factor is selected from the group consisting of a protein such as but not limited to a monoclonal antibody and a polyclonal antibody, an aptamer, a peptide, and a small chemical entity acting as inhibitor or activator of protein
20 function. It is an object of the present invention to increase the level of activity of products encoded by *MBD2* and/or *MBD4* whereas it is an object to decrease the level of activity of products encoded of *DNMT1*.

The mediator of covalent modification of DNA, covalent modification of histones
25 and/or modification of chromatin remodelling may be encoded by the genes selected from the group consisting of *DNMT1*, *DNMT3A*, *DNMT3B*, *MBD1*, *MBD2*, *MBD4*, *MECP2*, *ZBTB33*, *ZBTB38*, *ZBTB4*, *ATF2*, *CDYL*, *CREBBP*, *ELP3*, *EP300*, *GCN5L2*, *GTF3C1*, *HAT1*, *HTATIP*, *MYST1*, *MYST2*, *MYST3*, *MYST4*, *NCOA1*, *NCOA2*, *NCOA3*, *OGT*, *PCAF*, *TAF1*, *HDAC1*, *HDAC2*, *HDAC3*, *HDAC4*, *HDAC5*,
30 *HDAC6*, *HDAC7A*, *HDAC8*, *HDAC9*, *HDAC10*, *HDAC11*, *SIRT1*, *SIRT2*, *SIRT3*, *SIRT4*, *SIRT5*, *SIRT6*, *SIRT7*, *DOT1L*, *EHMT1*, *EHMT2*, *EZH2*, *MLL1*, *NSD1*, *PRDM2*, *PRDM9*, *PRMT1*, *PRMT2*, *PRMT3*, *PRMT4*, *PRMT5*, *PRMT6*, *PRMT7*, *SETD1A*, *SETD2*, *SETD7*, *SETD8*, *SETDB1*, *SMYD3*, *SUV39H1*, *SUV39H2*, *SUV420H1*, *SUV420H2*, *AOF2*, *FBXL11*, *JMJD2A*, *JMJD2B*, *JMJD2C*, *PADI4*, *CHD1*, *CHD2*, *CHD3*, *CHD4*,
35 *SMARCA1*, *SMARCA2*, *SMARCA4*, *SMARCA5*.

According to the inventors of the present invention, an embodiment of the present invention discloses that the level of expression of at least one of the genes *MBD2* and/or *MBD4* or any well known variants (*MBD2a*, *MBD2b*, *MBD2t*), as well as
5 putative splice variant isoforms hereof is increased.

In yet another embodiment of the present invention, the level of expression of the genes selected from the group consisting of *DNMT3A*, *DNMT3B*, *MBD2*, *MBD4*, *ZBTB33*, *ATF2*, *CDYL*, *EP300*, *NCOA1*, *NCOA2*, *OGT*, *TAF1*, *HDAC2*, *HDAC3*,
10 *HDAC9*, *SIRT1*, *SIRT4*, *DOT1L*, *EHMT1*, *PRMT1*, *PRMT3*, *SETD2*, *SETD8* and *SUV39H2* are increased.

In a preferred embodiment of the present invention the level of expression of the genes selected from the group consisting of *MBD2*, *MBD4*, *ZBTB33* and *SIRT4* are
15 increased.

In yet another embodiment of the present invention, the level of expression of the gene *DNMT1* or any well known variants (*DNMT1s* and *DNMT1o*), as well as putative splice variant isoforms hereof, is repressed.
20

In a further embodiment, the level of expression of the genes selected from the group consisting of *DNMT1*, *MBD1*, *CREBBP*, *ELP3*, *GCN5L2*, *GTF3C1*, *HAT1*, *HTATIP*, *MYST1*, *MYST2*, *MYST3*, *MYST4*, *NCOA3*, *HDAC1*, *HDAC6*, *SIRT3*, *SIRT5*, *SIRT6*, *SIRT7*, *EZH2*, *PRDM2*, *PRMT2*, *PRMT7*, *SETDB1*, *SMYD3*, *SUV39H1*,
25 *SUV420H1*, *SUV420H2*, *AOF2*, *CHD4*, *SMARCA2*, *SMARCA4* and *SMARCA5* are repressed.

In yet an embodiment the level of expression of the genes selected from the group consisting *DNMT1*, *MBD1*, *ELP3*, *GCN5L2*, *HTATIP*, *MYST1*, *HDAC6*, *SIRT5*,
30 *SIRT7*, *PRMT7*, *SMYD3*, *SUV39H1*, *SMARCA2*, *SMARCA4* are repressed.

Donor cell

In the present context, the term "donor cell" refers to a normoploid somatic cell from the group consisting of end-stage-committed cell types including unipotent

pre-cursor cells, and multipotent stem cells, e.g. isolated from adults, newly born and foetuses.

In a preferred embodiment of the present invention, an end-stage-committed
5 somatic cell belonging to any of the three germ layers may be isolated using techniques known in the art, and is selected from the group comprising of keratinizing epithelial cells, wet stratified barrier epithelial cells, exocrine secretory epithelial cells, hormone secreting cells, gut-, exocrine-, glands- and urogenital tract cells, metabolism and storage cells, barrier function cells (lung, gut, exocrine
10 glands and urogenital tract), epithelial cells lining closed internal body cavities, ciliated cells with propulsive function, extracellular matrix secretion cells, contractile cells, blood and immune system cells, sensory transducer cells, autonomic neuron cells, sense organ and peripheral neuron supporting cells, central nervous system neurons and glial cells, lens cells, pigment cells and nurse
15 cells. Presently preferred cells are cells readily obtainable i.e. cell which may be obtained without penetration of the body. Such cell may be but is not limited to epithelial cells, e.g. buccal mucosa cells.

An adult stem cell on the other hand refers to a partly differentiated cell
20 characterized by its ability to divide or *self-renew* indefinitely and generate a number of cell types of the same germ layer from which it originates. Adult stem cells may be found throughout the foetal or adult body and may thus be obtained from already developed tissue using techniques known in the art, said tissues being selected from the group consisting of, but not limited to, brain tissue, heart
25 tissue, adipose tissue, skin, liver and bone marrow tissue. Thus, in a preferred embodiment of the present invention, the adult stem cells of the present invention are obtained from adipose tissue, skin and bone marrow tissue.

The source of the donor cell of the present invention comprises all mammalian
30 species. In one embodiment, the source of the donor cell is human.

It must be emphasized that the isolated donor cell itself may exhibit a range of plasticity levels. Accordingly, the donor cell of the present invention may be selected from the group consisting of end-stage-committed cell types including

unipotent pre-cursor cells, and multipotent stem cells, e.g. isolated from adults, newly born and fetuses.

Accordingly the donor cell of the present invention is a cell, in which the plasticity
5 level is to be increased.

Opposite the term "supraplastic cell" which refers to a donor cell in which the plasticity level has been increased.

10 Target cell

The term "target cell" relates in the present context to a cell developed by differentiation from a supraplastic cell. Target cells may for instance exhibit a phenotype different from the phenotype exhibited by the donor cell from which the supraplastic cell was derived. In an embodiment of the present invention the
15 target cell may be selected from the group consisting of end-stage-committed cells including unipotent pre-cursor cells, oligopotent cells, multipotent cells, and pluripotent cells.

End-stage-committed cell

20 The term "end-stage-committed-cell" refers to all differentiated cell types including tissue-specific unipotent pre-cursor cells with the ability to generate by proliferation and differentiation the specific type of tissue.

Introducing the plasticity modifying factor(s) into a cell

25 In the present application, the introduction of one or more plasticity mediating factor(s) into a cell and the induction of epigenetic reprogramming may be performed employing various methods. Accordingly, the terms introduced herein will be used interchangeably.

30 Expression of the plasticity modifying factors presented in the present application may be mediated through any means for regulating gene expression, such as, but not limited to, transfection of naked DNA, viral vectors (both episomal and integrative), antisense oligonucleotides, siRNA, shRNA, microRNA, antibodies and

aptamers into the cell. Said transfection may either increase or repress the level of selected factors.

In the present invention the viral vector and/or plasmid vector are employed to
5 deliver gene constructs such as a promoter/enhancer, a reporter gene and/or selection marker into the cell.

Transfection may also be performed by various methods all well known to the person skilled in the art. Said methods include but are not limited to, the use of
10 calcium phosphate transfection, DEAE-dextran-mediated transfection, polybrene, protoplast fusion, electroporation, lipid-mediated delivery (e.g., liposomes), microinjection, particle bombardment for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into the cell, which is to be reprogrammed. The transfection may be either transient or stable.

15 Conventional viral and non-viral based gene transfer methods may be used to introduce the mediators into donor cells. Such methods may be used to administer e.g. nucleic acids encoding reprogramming or plasticity increasing polypeptides. Preferably, nucleic acids are administered for *in vivo* or *ex vivo* gene
20 therapy uses. Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vesicle such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery into the cell.

25 Methods of non-viral delivery of nucleic acids include lipofection, microinjection, ballistics, virosomes, liposomes, immunoliposomes, polycation or lipid nucleic acid conjugates, naked DNA, artificial viroplasmids and agent enhanced uptake of DNA.

The level of reprogramming or increase in plasticity may be assessed by various
30 methods all of which are known to the skilled person. The methods includes but are not limited to the measurement of global DNA methylation patterns (e.g. using methylation chips), down regulation of cell type-specific markers and up-regulation of pluripotency markers. The pluripotent cells of the present invention may e.g. be characterised by biological markers for cellular multipotency,
35 pluripotency or totipotency, e.g. any of these pluripotency markers such as, but

not limited to, *NANOG*, Oct-4 (*POU5F1*), *SOX2*, or *REX1*. Thus, cells having an increased OCT4 expression would generally be considered to possess an increased plasticity and this suggests that cells that have been exposed to the gene products of *MBD2* and *MBD4* could have increased plasticity. Example 17

5 demonstrates that altering the expression profiles of one or more of the genes (*MBD2*, *MBD4* and *DNMT1*) may be a valid method of increasing the plasticity of cells through an upregulation of Oct-4 (*POU5F1*),

10 The cells and uses thereof

The cells disclosed in the present invention are mammalian cells. However, in a presently preferred embodiment the cells are human cells.

Before the era of human ES-cell-based therapy can be realized, the potential

15 usefulness of these cells must be confirmed in animal models. Nevertheless, once verified, the present invention describes a method for cell therapy based upon transplantation of supraplastic cells or derivatives of the supraplastic cells for cell or tissue replacement or for delivery of compounds produced by the transplanted cells following transplantation into an individual.

20

It has been contemplated to use differentiated derivatives of human embryonic stem cell lines for cell-based therapy involving transplantation of the cells to an individual with the aim of either replacing lost cells or using the transplanted cells to deliver biologically active compounds to an individual.

25

The pluripotency of embryonic stem cell represents a risk of the recipient developing tumours originating from the embryonic stem cells, so-called teratomas. It is therefore desirable that the cells to be used in such transplantation-based treatment have a desired differentiation potential, which precludes the risk

30 of teratoma formation. Importantly, the present invention allows for therapeutic use of supraplastic cells or derivatives thereof with various degrees of differentiation potential compared to embryonic cells.

The supraplastic cells of the present invention may be used for the production of

35 derivatives of the supraplastic cell, such derivatives comprising a target cell with a

desired phenotype as well as a partly differentiated supraplastic cell. Such derivatives of supraplastic cells may be used as described above in transplantation therapy for cell replacement and/or tissue regeneration or for delivery of compounds into an individual of compounds produced by the transplanted cells.

5

An additional problem in cell-based therapy relates to the need for histocompatibility between the individual receiving a transplantation and the cells being transplanted to the individual. For example, the proposed therapeutic uses of non-histocompatible fetal stem cells in cell replacement treatment of

- 10 Parkinson's disease or in cell delivery of brain cell growth factors to cure amyotrophic lateral sclerosis is only feasible due to the fact that the target tissue (brain) is immunologically sequestered. Most importantly, the invention allows for therapeutic use of fully histocompatible, i.e. autologous, supraplastic cells and cells derived from the supraplastic cells exhibiting various degrees of
- 15 differentiation potential compared to embryonic cells thus broadening the range of diseases and conditions amenable to cell-based therapy.

Epigenetic reprogramming of somatic cells into stem cells attracts attention because of the potential for autologous transplantation therapy, as cellular

- 20 derivatives of the reprogrammed cells will not be rejected by the recipient. Although autologous stem cells may be isolated from tissues such as bone marrow from an adult individual, the number of such cells is low, and the adult stem cells are difficult to isolate for expansion. Importantly, as described in this application, this quantitative hurdle may be overcome by employing the strategy of epigenetic
- 25 reprogramming of partly or fully differentiated cells from the individual to be treated.

Another source of autologous stem cells is umbilical cord blood, umbilical cord tissues as well as amniotic fluid, amnion epithelium, and placenta sampled at the

30 time of birth or by means of ante-natal diagnostic invasive procedures and kept in storage under suitable conditions until the individual in question requires cell-based therapy. The use of such cells depends upon their suitability for therapy after prolonged storage, and the use is limited to individuals who have had samples of umbilical blood, umbilical cord tissues or amniotic fluid taken at the

- 35 time of birth or ante-natal diagnostic procedures. Importantly, as described in this

application, these hurdles with regard to stem cell quality and availability may be overcome by employing the strategy of epigenetic reprogramming of partly or fully differentiated cells from any individual to be treated, said reprogramming being carried out at the time that cell-based therapy is desired.

5

Thus far, in one embodiment the present invention relates to a method for cell therapy in which the transplantation of supraplastic cells or derivatives thereof is done to restore a tissue such as, but not limited to, bone marrow, liver, skin or cartilage. It is contemplated that the cells for replacement therapy may be
10 transplanted as such by intravenous injection or by depositing the cells into a specific tissue, or the cells may be transplanted as a partly or fully developed tissue. Furthermore, transplantation of target cells as described herein for delivery of compounds produced natively by the transplanted target cells or
transplantation of target cells for delivery of compounds not natively produced by
15 the transplanted target cells is envisaged.

Stem cells are known to be able to remain in a given tissue and to produce growth factors, signal molecules etc., which may effect proliferation and differentiation of neighbouring cells. By providing such conditioning factors to a tissue it is believed
20 that the naturally occurring adult stem cells may be stimulated so that new tissue is produced. In another embodiment, the transplantation of supraplastic cells or cells derived herefrom is aimed at depositing the cells in a given tissue such as, but not limited to, cardiac muscle or brain tissue in order to attain delivery to the surrounding tissue of compounds such as growth factors, signal molecules etc.
25 produced natively by the supraplastic cells or cells derived herefrom of the present invention.

The possibility of making cell lines with all of the properties of ES cells directly from non-conventional adult sources such as but not limited to the skin holds
30 obvious appeal. It could be a powerful way of making patient specific stem cells to provide tissue-matched cells for therapy, and a source of cells for research into the pathogenesis of complex diseases.

The ability of stem cells to remain in a given tissue may also be used to deliver
35 compounds that are not normally produced by the stem cells by genetically

engineering the stem cells prior to transplantation to produce such desired compounds as growth factors, signal molecules, enzymes etc. In yet another embodiment, the supraplastic cells are genetically engineered prior to transplantation to produce compounds not natively produced by the supraplastic
5 cells or cells derived herefrom, and the transplantation of the genetically engineered supraplastic cells or cells derived herefrom is aimed at depositing the cells in a given tissue such as, but not limited to, cardiac muscle or brain tissue in order to attain delivery to the surrounding tissue of compounds such as growth factors, signal molecules etc. produced by the genetically engineered supraplastic
10 cells or cells derived herefrom of the present invention.

Gene expression

In the present context the term "altering the level of expression of the one or more plasticity modifying factor(s) of covalent modifications of DNA, histones
15 and/or chromatin remodelling" covers a situation wherein the level of gene expression is either activated/increased (up-regulated) and/or repressed/decreased (down-regulated).

"Gene expression" refers to the conversion of the information, contained in a
20 gene, into a gene product. A gene product may be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for
25 example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristilation, and glycosylation.

"Gene activation" refers to any process which results in an increase in production of a gene product. A gene product may be either RNA (including, but not limited
30 to, mRNA, rRNA, tRNA, and structural RNA) or protein. Accordingly, gene activation includes those processes which increase transcription of a gene and/or translation of a mRNA. Examples of gene activation processes which increase transcription include, but are not limited to, those which facilitate formation of a transcription initiation complex, those which increase transcription initiation rate,
35 those which increase transcription elongation rate, those which increase

processivity of transcription and those which relieve transcriptional repression (by, for example, blocking the binding of a transcriptional repressor). Gene activation can constitute, for example, inhibition of repression as well as stimulation of expression above an existing level. Examples of gene activation processes which increase translation include those which increase translational initiation, those which increase translational elongation and those which increase mRNA stability. In general, gene activation comprises any detectable increase in the production of a gene product. In a preferred embodiment of the invention, the production of a gene product is increased by at least 2-fold, e.g 5-fold, such as 10- fold, e.g 20- fold, such as 30- fold, e.g 40-fold, such as 50- fold, e.g 60-fold, such as 70- fold, e.g 80-fold, such as 90- fold, e.g 100-fold.

In the present context the term "increased level of expression" refers to a situation wherein the level of expression of a specific gene in a cell is increased when compared to the level of gene expression in a similar cell comprising the same genotype.

Determining whether the level of expression of a specific gene in a cell is increased may be measured by the following steps:

20

- providing a first cell having a genotype which comprises the specific gene of interest
- providing a second cell with the same genotype as the first cell and further adding in said cell at least one modification increasing the level of expression of the specific gene,
- measuring the level of gene expression of the specific gene in both the first and second cell,
- identifying a cell as having an increased level of expression of a specific gene of interest if the second cell compared to the first cell show an increased level of expression of at least 1%, e.g. 5%, 10%, 20%, 50% or 75%.

35

The level of gene expression may be measured by several different methods all which are known to the skilled person. The methods include e.g. Northern Blotting and quantative RT-PCR. Alternatively, if the level of expression of one or more genes is of interest DNA microarray technology or "tag based" technologies like
5 SAGE or SuperSAGE can provide a relative measure of the cellular concentration of the mRNAs of interest.

In the present context the term "repressed level of expression" refers to a situation wherein the level of expression of a specific gene in a cell is down-regulated when compared to the level of gene expression in a similar cell
10 comprising the same genotype.

Determining whether the level of expression of a specific gene in a cell is repressed may be measured by the following steps:

- 15 - providing a first cell having a genotype which comprises the specific gene of interest
- providing a second cell with the same genotype as the first cell and further adding in said cell at least one modification repressing the level
20 of expression of the specific gene,
- measuring the level of gene expression of the specific gene in both the first and second cell,
- 25 - identifying a cell as having an repressed level of expression of the specific gene of interest if the second cell compared to the first cell show an repressed level of expression of at least least 1%, e.g. 5%, 10%, 20%, 50% or 75%.

30 Several methods of modifying gene-expression are known to the skilled person.

"Gene repression" refers to any process which results in a decrease in production of a gene product. A gene product may be either RNA (including, but not limited to, mRNA, rRNA, tRNA, and structural RNA) or protein. Accordingly, gene
35 repression includes those processes which decrease transcription of a gene and/or

translation of a mRNA. Examples of gene repression processes which decrease transcription include, but are not limited to, those which inhibit formation of a transcription initiation complex, those which decrease transcription initiation rate, those which decrease transcription elongation rate, those which decrease processivity of transcription and those which antagonize transcriptional activation (by, for example, blocking the binding of a transcriptional activator). Gene repression can constitute, for example, prevention of activation as well as inhibition of expression below an existing level. Examples of gene repression processes which decrease translation include those which decrease translational initiation, those which decrease translational elongation and those which decrease mRNA stability. Transcriptional repression includes both reversible and irreversible inactivation of gene transcription. In general, gene repression comprises any detectable decrease in the production of a gene product. In general, gene activation comprises any detectable increase in the production of a gene product.

15 In a preferred embodiment of the invention, the production of a gene product is decreased by at least 2-fold, such as 10- fold, such as 30- fold, such as 50- fold, such as 90- fold. Most preferably, gene repression results in complete inhibition of gene expression, such that no gene product is detectable.

20 Sequence identity

Whenever mentioned in the present application, the nucleotide sequence encoding *MBD2* is selected from the group consisting of a) a nucleotide sequence set forth in SEQ ID NO: 1, and b) nucleotide sequences having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO: 1.

25

The same applies to the nucleotide sequence encoding *MBD4*. Said nucleotide sequence is selected from the group consisting of a) a nucleotide sequence set forth in SEQ ID NO: 4, and b) nucleotide sequences having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO: 4.

30

In accordance with the above the nucleotide sequence encoding *DNMT1* is selected from the group consisting of a) a nucleotide sequence set forth in SEQ ID NO: 7, and b) nucleotide sequences having at least 75% identity to the nucleotide sequence set forth in SEQ ID NO: 7.

35

As commonly defined "identity" is here defined as sequence identity between genes or proteins at the nucleotide or amino acid level, respectively.

Thus, in the present context "sequence identity" is a measure of identity between proteins at the amino acid level and a measure of identity between nucleic acids

5 at nucleotide level. The protein sequence identity may be determined by comparing the amino acid sequence in a given position in each sequence when the sequences are aligned. Similarly, the nucleic acid sequence identity may be determined by comparing the nucleotide sequence in a given position in each sequence when the sequences are aligned.

10

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps may be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid
15 residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical
20 positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). In one embodiment the two sequences are the same length.

One may manually align the sequences and count the number of identical amino
25 acids. Alternatively, alignment of two sequences for the determination of percent identity may be accomplished using a mathematical algorithm. Such an algorithm is incorporated into the NBLAST and XBLAST programs of (Altschul et al. 1990). BLAST nucleotide searches may be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic
30 acid molecules of the invention. BLAST protein searches may be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST may be utilised. Alternatively, PSI-Blast may be used to perform an iterated search which detects distant relationships
35 between molecules. When utilising the NBLAST, XBLAST, and Gapped BLAST

programs, the default parameters of the respective programs may be used. See <http://www.ncbi.nlm.nih.gov>. Alternatively, sequence identity may be calculated after the sequences have been aligned e.g. by the BLAST program in the EMBL database (www.ncbi.nlm.nih.gov/cgi-bin/BLAST). Generally, the default settings with
5 respect to e.g. "scoring matrix" and "gap penalty" may be used for alignment. In the context of the present invention, the BLASTN and PSI BLAST default settings may be advantageous.

The percent identity between two sequences may be determined using techniques
10 similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

Compositions

An inherent feature of the supraplastic cells of the present invention is their
15 capacity to renew themselves and to differentiate into a broad spectrum of derivatives of all three embryonic germ layers: ectoderm, mesoderm, and endoderm. Human embryonic stem cell lines have already been isolated, and their ability for multilineage differentiation, including neural lineage, has been demonstrated both *in vivo* and *in vitro*. This ability to develop has drawn clinical
20 attention to stem cells as a novel source for new cell-based therapeutic strategies such as tissue regeneration.

It would obviously be desirable to generate medicaments that enabled the creation of the supraplastic cells of the present invention by simply adding a
25 composition comprising any of the plasticity modifying factors described herein either *in vivo* or *in vitro* to partly or fully differentiated cells. Thus, in one embodiment the present invention relates to a composition for mediating an increase in the plasticity level of a donor cell, said composition comprises one or more of the plasticity modifying factors described herein.

30

In one embodiment said composition for mediating an increase in the plasticity level of a donor cell comprises one or more of the plasticity modifying factors selected from the group consisting of *MBD2* factor(s), *MBD4* factor(s) and *DNMT1* factor(s).

35

In a preferred embodiment said composition for mediating an increase in the plasticity level of a donor cell, said composition comprises *MBD2* factor(s).

In a preferred embodiment said composition for mediating an increase in the plasticity level of a donor cell, said composition comprises *MBD4* factor(s).

In a preferred embodiment said composition for mediating an increase in the plasticity level of a donor cell, said composition comprises *DNMT1* factor(s).

10 In one embodiment, the composition may comprise *MBD2* factor(s) in combination with *MBD4* factor(s).

In one embodiment, the composition may be any of the above mentioned compositions further combined with *DNMT1* factor(s).

15

In one embodiment the compositions described herein may further comprising one or more histone acetyltransferases (HATs).

Vector

20 Reprogramming of fibroblasts to a pluripotent state can be induced through ectopic expression of transcription factors such as *OCT4*, *SOX2*, *C-MYC* and *KLF4*. Expression of these four transcription factors proved to be a robust method to induce reprogramming of somatic cells to a pluripotent state. Thus in one embodiment the present invention relates to a vector comprising at least one of

25 the genes selected from the group of *AOF2*, *DNMT1*, *DNMT3A*, *DNMT3B*, *MBD1*, *MBD2*, *MBD4*, *MECP2*, *ZBTB33*, *ZBTB38*, *ZBTB4*, *ATF2*, *CDYL*, *CREBBP*, *ELP3*, *EP300*, *GCN5L2*, *GTF3C1*, *HAT1*, *HTATIP*, *MYST1*, *MYST2*, *MYST3*, *MYST4*, *NCOA1*, *NCOA2*, *NCOA3*, *OGT*, *PCAF*, *TAF1*, *HDAC1*, *HDAC2*, *HDAC3*, *HDAC4*, *HDAC5*, *HDAC6*, *HDAC7A*, *HDAC8*, *HDAC9*, *HDAC10*, *HDAC11*, *SIRT1*, *SIRT2*, *SIRT3*,

30 *SIRT4*, *SIRT5*, *SIRT6*, *SIRT7*, *DOT1L*, *EHMT1*, *EHMT2*, *EZH2*, *MLL1*, *NSD1*, *PRDM2*, *PRDM9*, *PRMT1*, *PRMT2*, *PRMT3*, *PRMT4*, *PRMT5*, *PRMT6*, *PRMT7*, *SETD1A*, *SETD2*, *SETD7*, *SETD8*, *SETDB1*, *SMYD3*, *SUV39H1*, *SUV39H2*, *SUV420H1*, *SUV420H2*, *AOF2*, *FBXL11*, *JMJD2A*, *JMJD2B*, *JMJD2C*, *PADI4*, *CHD1*, *CHD2*, *CHD3*, *CHD4*, *SMARCA1*, *SMARCA2*, *SMARCA4*, *SMARCA5*, *BMP15*, *CCNB1* and *DPPA3*.

35

Another embodiment relate to a vector according to the present invention, wherein said vector comprising at least one of the genes selected from *DMNT1*, *MBD1*, *ELP3*, *GCN5L2*, *HTATIP*, *MYST1*, *HDAC6*, *SIRT5*, *SIRT7*, *PRMT7*, *SMYD3*, *SUV39H1*, *SMARCA2*, *SMARCA4*, *DNMT3A*, *DNMT3B*, *MBD2*, *MBD4*, *ZBTB33*, *ATF2*,
 5 *CDYL*, *EP300*, *NCOA1*, *NCOA2*, *OGT*, *TAF1*, *HDAC2*, *HDAC3*, *HDAC9*, *SIRT1*, *SIRT4*, *DOT1L*, *EHMT1*, *PRMT1*, *PRMT3*, *SETD2*, *SETD8* and *SUV39H2*.

In one embodiment the vector comprises at least one of the genes selected from the group consisting of *DMNT1*, *MBD2* and *MBD4*.

10

In a presently preferred embodiment, the vector comprises at least one of the genes *OCT4*, *SOX2*, *C-MYC* and *KLF4* in combination with *MBD2* and/or *MBD4*.

The invention will hereinafter be described by way of the following non-limiting
 15 Figures and Examples.

Examples

MATERIALS AND METHODS

Collection of mouse oocytes

Oocytes were obtained from 3-week-old B6D2F1 mice (Taconic Europe, Ejby,
 20 Denmark) and were carefully processed to avoid human contamination. Mice were handled under standard laboratory conditions and experiments were conducted following national guidelines on animal care (Danish Ministry of Justice; approval 2003/561-713).

25 For the collection of GV oocytes, mice were each injected with 15 IU menotropin (Menopur®; Ferring, Copenhagen, Denmark) and killed by cervical dislocation 42 hours later. Ovaries were isolated and antral follicles were punctured by 27-ga needles. Cumulus-enclosed GV oocytes were released into alpha MEM medium (Invitrogen, Carlsbad, CA) supplemented with 4 mmol/l hypoxanthine (Sigma, St.
 30 Louis, MO) to inhibit GV breakdown during handling. Only spherical GV oocytes with a distinct GV and attached cumulus cells were collected and immediately underwent mechanical denudation with a fine bore glass pipette.

For the collection of MII oocytes, mice were first primed with 15 IU menotropin (Menopur®; Ferring) followed 44 hr later by injection of 5 IU of choriongonadotropin (Pregnyl®; Organon, Oss, The Netherlands) and were then killed 15 hr later. Collected oviducts were punctured in alpha-MEM to yield the 5 cumulus oocyte complexes (COCs). COCs were briefly exposed to 80 IU/ml hyaluronidase (SynVibro® Hyadase; MediCult, Jyllinge, Denmark) at 37°C to remove attached cumulus granulosa cells. Only spherical MII oocytes with a distinct first polar body were selected.

- 10 After denudation, all oocytes were briefly exposed to 10 mg/ml protease (Sigma) at 37°C to remove the zona pellucida and remaining attached cumulus cell debris. Oocytes were washed several times and collected for immediate RNA extraction.

RNA extraction and quality assessment

- 15 Oocyte RNA was obtained using the RNeasy® Micro Kit (Qiagen, Valencia, CA), which enriches mRNAs from small samples by selectively excluding shorter RNAs such as rRNAs and tRNAs. Briefly, pools of oocytes were lysed with guanidine-isothiocyanate-containing buffer and were applied to spin columns for adsorption of the RNA. Carrier was included to improve the yield of low-abundance mRNAs 20 further. Pellets were washed with 80% ethanol, centrifuged and air-dried. Subsequently, the RNA was eluted in nuclease-free water and immediately stored at -80°C until use.

- To ensure that high quality RNA was hybridized to the gene expression arrays, 25 isolated RNA was assessed on a 2100 Bioanalyzer using a RNA 6000 Pico LabChip (Agilent Technologies, Palo Alto, CA).

- The protocol used for microarray chip hybridization required at least 100 ng of total RNA for each biological replicate. Due to limited sample quantities of 30 oocytes, pooling of total RNA from repeated experiments was necessary, so with an expected average amount of 0.3 to 0.5 ng of total RNA in a murine oocyte, each replicate was constructed from pooled RNA of oocytes from approximately 20 mice. Sufficient oocyte RNA was isolated for the generation of three and five biological replicates of GV and MII oocytes, respectively.

Amplification and hybridization to microarray chips

Due to the small amounts of initial RNA, the process required an amplifying two cycle target labeling assay step to obtain sufficient amounts of labeled cRNA target for analysis with arrays. A minimum of 100 ng of total RNA was used to synthesize double stranded cDNA with the Superscript Choice system (Invitrogen) with an oligo(dT) primer containing a T7 RNA polymerase promoter (GenSet). The cDNA was used as the template for an *in vitro* transcription reaction to synthesize antisense cRNA (BioArray high yield RNA transcript labeling kit; Enzo, Farmingdale, NY). The signal was then amplified in a second cDNA synthesis step using random primers for first strand synthesis and T/ oligo(dT) primer for the second strand synthesis. The amplified cDNA was used as template for a second *in vitro* transcription to synthesize biotin labeled antisense cRNA (BioArray high yield RNA transcript labeling kit; Enzo).

The labeled cRNA was fragmented at 94°C for 35 min in fragmentation buffer (40 mM Tris, 30 mM magnesium acetate, 10 mM potassium acetate) and was hybridized for 16 hr to the Affymetrix GeneChip® Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA), which covers transcripts and variants from 34,000 well characterized mouse genes. Probe sets on this array are derived from sequences from GeneBank, dbEST, and RefSeq. The arrays were washed and stained with phycoerythrin streptavidin using a Fluidics Station 450 (Affymetrix), and the arrays were scanned in a GeneArray 3000 scanner (Affymetrix). Data were digitalized and converted into CEL format (v. 3) for data analysis.

Microarray data analyses

To adjust the overall chip brightness of the arrays to a similar level and thus make the individual chips comparable to each other, the quantile normalization method was used (Bolstad et al. 2003). To summarize the probe level data for each probe set, an expression index was calculated by the guanine cytosine corrected robust multi array analysis (GCRMA) method (Irizarry et al. 2003). Expression values were estimated on logarithmic scale (base 2) instead of raw intensities because of the variance-stabilizing effect of this transformation.

To remove the ambiguity of multiple and less specific probe sets, only transcripts with an Affymetrix oligonucleotide probe set corresponding to a single gene ("_at"

- suffices) or with anticipated oocyte specific splice variants ("_a_at" suffices) was retained, thus only allowing less specific probe sets if a unique probe set for a gene was missing on the gene chip at the time of manufacturing. Probe sets were cross examined in the ADAPT database (Leong et al. 2005) to ensure that only
- 5 probe sets representing mRNAs remained, thus avoiding regions outside exons and 3' untranslated region (UTR). Eventually, if a gene was represented by more than one unique probe set, the probe set with most present calls or positioned in the most distal 3' end of the transcript was selected.
- 10 Hierarchical clustering was performed only on genes showing statistically significant up- or down-regulation as identified by t test and with a P value corrected for multiple testing with the Benjamini-Hochberg method to avoid erroneous result by less variant or absent genes. Significance level was 0.05, distance metric was 1 - r, and linkage method was centroid. To validate the
- 15 overall quality of the experiment, hierarchical clustering was also performed on sample level, since biological replicates representing the same condition should to be grouped together because of identical global expression pattern. Accordingly, they should be separated from samples representing other conditions.
- 20 Normalization and calculation of gene expression indices were performed with the gcrma package (Wu et al. 2004), comparisons of samples were performed with the simpleaffy package (Wilson & Miller 2005), and Benjamini-Hochberg corrected P-values were calculated using the multtest package (Pollard et al. 2004). All packages were run in the R statistical software (<http://www.r-project.org>).
- 25 Hierarchical clustering was performed in DNA Chip Analyzer (dChip) 2005 (Li & Wong 2003). Microarray analyses are reported according to the MIAME statement (Brazma et al. 2001).

Real-time quantitative RT-PCR

- 30 Oocytes were collected and total RNA was extracted as described above. Complementary DNA (cDNA) was synthesized with SuperScript III (Invitrogen) or iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) using random hexamers or a random hexamer/oligo(dT) blend as first strand primer. Equal amounts of total RNA (24 ng) were converted into cDNA for GV and MII samples,

respectively. Subsequently, the cDNA elute was diluted 1:20 before down-stream application.

For PCR amplification, the gene specific primers were constructed using Primer3
5 (Rozen & Skaletsky 2000) or PrimerBank (Wang & Seed 2003). Primers were carefully chosen or designed to eliminate the risk of amplifying unspecific targets, such as primer dimers or genomic DNA. Primer sequences, exon complementarity, melting temperature, GenBank accession number, and size of the amplified product are presented in table 1. Expression levels were quantified in real-time on
10 a LightCycler® Instrument (Roche Diagnostics, Germany) using LightCycler® FastStart DNA Master PLUS SYBR Green I (Roche Applied Science) or SYBR® Premix Ex Taq™ kit (Takara, Madison, WI) as detection chemistry. The PCR reaction mixture consisted of 2 µl of diluted cDNA, 1 µl of gene specific primers, 2 µl or 5 µl of SYBR® Green I master mix (depending on manufacturer), and added
15 water to a total volume of 10 µl. First, cDNA was denatured and pre-incubated by heating for 10 sec at 95°C. Template was then amplified by 45 cycles consisting of denaturation for 5 sec at 95°C, annealing 20 sec at 55°C, and extension for 10 sec at 72°C. At least three replicates were used for each reaction, and a minus RT and a minus template served as negative controls. To verify RT-PCR product
20 identity, melting point curves were generated following amplification. Samples were heated to 95°C followed by immediate cooling for 15 sec at 65°C and then slowly heating by 0.1°C/sec up to 95°C while monitoring fluorescence continuously.

To determine the effect of meiotic maturation on the expression of a candidate
25 internal reference gene, a range of housekeeping genes was evaluated. Briefly, identical amounts of input RNA from GV and MII oocytes were used for each cDNA reaction. Putative candidate reference genes were amplified and the gene showing the lowest variability was chosen as internal reference using Norm-Finder (Andersen et al. 2004).

30

All target genes were normalized against the chosen internal reference gene within the log-linear phase of the amplification curve, and relative quantification was assessed using the $2^{-\Delta\Delta CT}$ method, where $\Delta\Delta CT = (CT_{\text{-target}} - CT_{\text{-reference}})_{\text{MII}} - (CT_{\text{-target}} - CT_{\text{-reference}})_{\text{GV}}$ (Pfaffl 2001). All fold changes are presented as means
35 with 95% confidence intervals.

EXAMPLE 1

Oocyte collection and validation of RNA

- On average, 320 and 421 oocytes were pooled for each biological replicate of GV
5 and MII oocytes, respectively. An examination of total RNA on a 2100 Bioanalyzer
confirmed the extraction of high-quality RNA (data not shown). Correlations
between biological replicates were substantially high, showing identical global
transcription patterns and thus high reliability of the microarray gene chip
procedure performed on minute amounts of oocyte RNA (Figure 1 and Table 2).
- 10 An unsupervised hierarchical cluster analysis using all detected probe sets showed
that biological replicates representing the same developmental stage were
independently grouped together as expected. Genes known to be preferentially
expressed in oocytes such as the zona pellucida glycoproteins (*ZP1*, *ZP2*, and
15 *ZP3*), growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15
(*BMP15*), moloney sarcoma oncogene (*MOS*), cyclin B1 (*CCNB1*), spindlin (*SPIN*),
MATER (*NALP5*), zygote arrest 1 (*ZAR1*), STELLA (*DPPA3*), and OCT4 (*POU5F1*)
were abundantly detected on the chip set (Table 3). Although oocytes underwent
careful denudation, potential contamination of heterologue RNA from cumulus-
granulosa cells were investigated. The absence of cumulus transcripts such as
20 hyaluronic synthase 2 (*HAS2*), pentraxin 3 (*PTX3*), and gremlin 1 (*GREM1*)
confirmed the oocyte homogeneity of the collected samples.

EXAMPLE 2

Global oocyte gene expression and inclusion of genes for further analyses

- 25 After excluding probe sets that were 100% absent in both groups of replicates,
the data were reduced from 45,101 to 24,919 probe sets. To minimize false
positives, a probe set was then included for further analysis provided that a 100%
present call was found in either one of the two groups of replicates, irrespective of
its call percentage in the other replicate group. Accordingly, a total of 14,697
30 probe sets were expressed exclusively in GV oocytes, in MII oocytes, or both. A t-
test for difference in expression level between GV and MII oocytes Benjamini-
Hochberg corrected for multiple testing yielded that 5,169 (35%) probe sets were
significantly down-regulated, 609 (4%) probe sets were up-regulated, and 8,919

(61%) probe sets were equally expressed. A complete list of up- and down-regulated genes will be available at <http://www.ebi.ac.uk/arrayexpress/>.

EXAMPLE 3

5 General characteristics of reprogramming genes

Table 3 shows the average expression indices, fold changes and corrected P-values supplemented with values for a number of transcripts preferentially expressed in the oocyte. In total, 57 genes (70 %) were detected in GV- and/or MII-oocytes on the chip set. Within the 33 genes detected as down-regulated, 14
 10 had fold changes from -1 to -2, eight had fold changes from -2 to -5, three had fold changes from -5 to -10, and eight had fold changes less than -10. A total of 24 genes were detected as up-regulated with 16 genes having fold changes from 1 to 2 and eight genes having fold changes ranging from 2 to 5. However, when only statistically significant fold changes were considered, four genes were up-
 15 regulated and 18 genes were down-regulated, whereas 35 genes indicated an unchanged level of gene expression. The dendrogram in figure 2 illustrates the final clustering tree, where significantly regulated genes close to each other have high similarity in their standardized expression values across all eight samples.

20 EXAMPLE 4

DNA methyltransferases and methyl-CpG-binding proteins

The gene of the oocyte variant form of the maintenance DNA methyltransferase, *Dnmt1o*, was abundantly expressed in GV oocytes and then significantly down-regulated in MII whereas the two de novo DNA methyltransferases *DNMT3A* and
 25 *DNMT3B* both showed unchanged level of expression. Of the seven known MeCPs that bind to methylated DNA, two were significantly up-regulated (*MBD2*, *MBD4*), one was down-regulated (*MBD1*), one (*Kaiso/ZBTB33*) showed no change while *MECP2*, *ZBTB4* and *ZBTB38* were not detected on the chip.

EXAMPLE 5

Histone acetyltransferases

HATs may be classified into five subgroups according to sequence homology and internal protein domains. All five members of the MYST-subgroup were detected in oocytes. TIP60 (*HTATIP*), MOF (*MYST1*) and MORF (*MYST4*) showed significant down-regulation, while HBO1 (*MYST2*) and MOZ (*MYST3*) were unchanged. Of the members belonging to the GNAT-subgroup, elongation protein 3 homolog (ELP3) and GCN5 (*GCN5L2*) were significantly down-regulated, histone aminotransferase 1 (*HAT1*) was unchanged and p300/CBP-associated factor (*PCAF*) was absent. The two transcriptional 'coactivators' E1A binding protein p300/p300 (*EP300*) and CREB binding protein/CBP (*CREBBP*), which are almost functionally equivalent (P300/CBP-subgroup), were both expressed in oocytes at an unchanged level as were the three nuclear hormone-receptor cofactors (SRC-subgroup) SRC-1 (*NCOA1*), GRIP1 (*NCOA2*), and ACTR (*NCOA3*). The basal transcription factors TAFII250 (*TAF1*) and TFIIC (*GTF3C*) were also expressed at an unchanged level. The more recent identified HATs, *CDYL*, *ATF2*, and *OGT*, were all expressed in oocytes at an unchanged level.

EXAMPLE 6

Histone deacetylases

HDACs are subdivided into four classes according to phylogenesis. Class I members *HDAC1*, *HDAC2*, and *HDAC3* were expressed in oocytes, whereas *HDAC8* was absent. *HDAC1* was down-regulated and *HDAC2* and *HDAC3* were unchanged. No class II HDAC was detected, except *HDAC6* and *HDAC9*, which were down-regulated and unaffected, respectively. Except *SIRT2*, all sirtuins or class III HDACs were expressed in oocytes of which *SIRT4* was up-regulated, *SIRT3*, *SIRT5* and *SIRT7* were down-regulated, and *SIRT1* and *SIRT6* showed no change. The single class IV member, *HDAC11*, was not detected.

EXAMPLE 7

Histone methyltransferases and histone demethylases/deiminases

HMTs either activate or repress gene function depending on the residue of the modified amino acid. Histone methylation at positions H3K4, H3K36, or H3K79 is associated with gene activation. Of the activating HMTs, Dot1L (*DOT1L*) was undifferentially expressed, SMYD3 (*SMYD3*) was down-regulated, and SET1 (*SETD1A*), MLL (*MLL1*), SET7/9 (*SETD7*), and Meisetz (*PRDM9*) were absent. Of the HMTs associated with repression (H3K9, H3K27 and H4K20), SUV39h1 (*SUV39H1*) and ESET (*SETDB1*) were down-regulated, SUV39h2 (*SUV39H2*), Eu-HMTase1/GLP1 (*EHMT1*), ENX-1 (*EZH2*), PR-SET7/SET8 (*SETD8*), SUV4-20h1 (*SUV420H1*), SUV4-20h2 (*SUV420H2*), and RIZ (*PRDM2*) were unchanged and G9a (*EHMT2*) was absent. NSD1 (*NSD1*), which can act both as activator and repressor, was not detected.

Of the arginine HMTs, one was slightly up-regulated (*PRMT3*), one was down-regulated (*PRMT7*), two were undifferentially expressed (*PRMT1*, *PRMT2*), and three were absent (*PRMT4*, *PRMT5*, *PRMT6*).

All currently known HDMs were absent in oocytes, except LSD1 (*AOF2*), which was expressed at an unchanged level.

EXAMPLE 8

Chromatin remodeling ATPases

The ChR-ATPases are classified into subfamilies according to the presence of conserved domains outside their catalytic region. The two bromodomain SNF2-subfamily ATPases hBRM (*SMARCA2*) and BRG1 (*SMARCA4*) were both expressed in oocytes and down-regulated. The SANT domain ISWI-subfamily members SNF2L (*SMARCA1*) and SNF2H (*SMARCA5*) were absent or unchanged, respectively. Among the four chromodomain CHD-subfamily ATPases, only Mi-2 β (*CHD4*) was expressed at unchanged level while *CHD1*, *CHD2* and Mi-2 α (*CHD3*) were absent.

EXAMPLE 9

Real time quantitative RT-PCR

The four transcripts having statistically significant up-regulation and the 18 transcripts being down-regulated as shown by the present microarray data were
5 selected for further analysis by real-time quantitative RT-PCR. To select an appropriate internal reference gene, the expression of the three housekeeping genes β -actin (*ACTB*), porphobilinogen deaminase (*HMBS*) and TATA box binding protein (*TBP*) using equivalent amounts of total RNA converted into cDNA were tested. A statistically insignificant relationship existed between oocyte
10 developmental stage and expression of *ACTB*, which was confirmed by using NormFinder. Accordingly, *ACTB* was used as internal reference gene for further analyses.

The RT-PCR expression data for the 22 reprogramming gene transcripts are
15 presented in figure 3. The absence of non-specific amplified products was confirmed by melting curve analysis. When comparing RT-PCR fold changes with the microarray data, some discrepancy regarding direction and amplitude was observed. To test whether such differences could be explained by the use of random hexamer primers during cDNA synthesis, the RT-PCR using oligo(dT)
20 primers (Fermentas, Helsingborg, Sweden) was repeated. In this case, there was a significantly higher correlation between array data and qRT-PCR data regarding direction and amplitude, suggesting the occurrence of selective deadenylation during meiotic maturation.

25 Example 10

Cloning and expression of *MBD2*

Sequence information for the human *MBD2* gene is obtained by use of the Swiss Protein database (www.expasy.org). Swiss-Prot entry Q9UBB5 is used to access
DNA sequence information regarding the human *MBD2* gene and its splicing
30 isoforms. Human *MBD2* is a 411 amino acid protein with a molecular weight of 43255 Da.

A synthetic *MBD2* gene is purchased from commercial companies like GENEART (www.geneart.com). Here a DNA fragment is manufactured according to the human *MBD2* gene sequence as specified by the Swiss-Prot Q9UBB5 entry. Both DNA fragments coding for the standard human *MBD2* protein as well as DNA
5 fragments coding for splicing and sequence variants is obtained in this way.

Synthetic genes are normally delivered cloned into a standard bacterial vector. This bacterial plasmid with a *MBD2* gene insert allows for multiplication of the *MBD2* gene material according to the needs for subsequent use.

10

Restriction enzyme sites useful for DNA fragment recloning is inserted before and after the *MBD2* gene reading frame. Such restriction enzyme sites is designed into the purchased *MBD2* construct, or a PCR reaction with PCR primers containing such restriction enzyme sites is used to amplify a *MBD2* gene fragment with these
15 these sites attached at proper positions.

The human *MBD2* gene fragment is recloned into a commercially available expression system using cloning sites as described by the manufacturer. The RheoSwitch Mammalian Expression System from New England BioLabs
20 (www.neb.com) allows for induction and adjustable control of gene expression for the inserted reading frame. The T-REx System from Invitrogen (www.invitrogen.com) utilizes a CMV promoter to deliver higher levels of induced expression than other regulated mammalian expression systems.

25 Primary human fibroblast cells are cultivated by use of hospital derived material from neonatal circumcisions. Alternatively primary human fibroblasts are obtained from commercial suppliers like Cascade Biologics (www.cascadebio.com), a part of Invitrogen (www.invitrogen.com), and primary human fibroblast cells are grown using media and protocols supplied by the cell line manufacturer. Expression
30 system vectors containing a *MBD2* gene fragment are transformed into primary human fibroblasts by use of methods described by the expression vector manufacturer. Transformation is performed in the form of transient expression experiments, or with the purpose of generating a stable cell line with a chromosomally integrated *MBD2* gene construct.

35

The level of *MBD2* expression in transformed primary human fibroblast cells with or without expression vector *MBD2* expression is analysed by use of *MBD2* specific antibodies in an ELISA assay. Abcam (www.abcam.com) is an example of a commercial supplier of *MBD2* antibodies, and the codes ab26848, ab33931, 5 ab38646, ab49136 and ab11985 are examples of such Abcam products.

A significantly increased *MBD2* protein expression level is observed for transformed primary human fibroblast cells relative to control cells without *MBD2* overexpression.

10

Example 11

Cloning and expression of *MBD4*

Sequence information for the human *MBD4* gene is obtained by use of the Swiss Protein database (www.expasy.org). Swiss-Prot entry O95243 is used to access 15 DNA sequence information regarding the human *MBD4* gene and its splicing isoforms. Human *MBD4* is a 580 amino acid protein (unprocessed precursor) with a molecular weight of 66051 Da.

A synthetic *MBD4* gene is purchased from commercial companies like GENEART 20 (www.geneart.com). Here a DNA fragment is manufactured according to the human *MBD4* gene sequence as specified by the Swiss-Prot O95243 entry. Both DNA fragments coding for the standard human *MBD4* protein as well as DNA fragments coding for splicing and sequence variants is obtained in this way.

25 Normally, a synthetic gene is either delivered already cloned into a standard bacterial vector or it is cloned into such a vector when received from the commercial supplier. This bacterial plasmid with a *MBD4* gene insert allows for multiplication of the *MBD4* gene material according to the needs for subsequent use.

30

Restriction enzyme sites useful for DNA fragment recloning is inserted before and after the *MBD4* gene reading frame. Such restriction enzyme sites is designed into the purchased *MBD4* gene fragment, or a PCR reaction with PCR primers

containing such restriction enzyme sites is used to amplify a *MBD4* gene fragment with these these sites attached at proper positions.

The human *MBD4* gene fragment is recloned into a commercially available
5 expression system using cloning sites as described by the manufacturer. The RheoSwitch Mammalian Expression System from New England BioLabs (www.neb.com) allows for induction and adjustable control of gene expression for the inserted reading frame. The T-REx System from Invitrogen (www.invitrogen.com) utilizes a CMW promoter to deliver higher levels of induced
10 expression than other regulated mammalian expression systems.

Primary human fibroblast cells are cultivated by use of hospitably derived material from neonatal circumcisions. Alternatively primary human fibroblasts are obtained from commercial suppliers like Cascade Biologics (www.cascadebio.com), a part of
15 Invitrogen (www.invitrogen.com), and primary human fibroblast cells are grown using media and protocols supplied by the cell line manufacturer. Expression system vectors containing a *MBD4* gene fragment are transformed into primary human fibroblasts by use of methods described by the expression vector manufacturer. Transformation is performed in the form of transient expression
20 experiments, or with the purpose of generating a stable cell line with a chromosomally integrated *MBD4* gene construct.

The level of *MBD4* expression in transformed primary human fibroblast cells with or without expression vector *MBD4* expression is analysed by use of *MBD4* specific
25 antibodies in an ELISA assay. Abcam (www.abcam.com) is an example of a commercial supplier of *MBD4* antibodies, and the codes ab3756, ab13793 and ab12187 are examples of such Abcam products.

A significantly increased *MBD4* protein expression level is observed for
30 transformed primary human fibroblast cells relative to control cells without *MBD4* overexpression.

Example 12

Simultaneous expression of *MBD2* and *MBD4*

A transient overexpression of both *MBD2* and *MBD4* is established by co-
5 transfection of the *MBD2* expression plasmid described in Example 5 and the
MBD4 expression plasmid described in Example 6 into primary human fibroblast
cells as previously described.

A stable cell line that overexpresses both *MBD2* and *MBD4* is established by
10 constructing a DNA fragment, where both genes are placed in line behind separate
regulated promoters. The resulting dual gene construct inserted into the matching
expression plasmid system is transformed into human primary fibroblast cells for
stable transformant isolation as previously described in example 5 and 6.

15 The level of *MBD2* and *MBD4* protein expression is measured using specific
antibodies as described in example 5 and 6.

At significantly increased expression of both *MBD2* and *MBD4* is observed in dual
gene overexpression experiments compared to the background level found for
20 human primary fibroblast cells.

Example 13

Induction of plasticity by *MBD2* and/or *MBD4* overexpression

25 Detection of surface markers, that characteristically are associated with stem cell
or progenitor cell phenotypes, may be used as a measure for cell plasticity
(developmental potential). Primary human fibroblast cells are so-called end-stage-
committed cells, and detection of surface markers for non-end-stage-committed
cells upon overexpression of *MBD2* and/or *MBD4* signals a possible induction of
30 plasticity.

Companies like Abcam (www.abcam.com), BD Biosciences
(www.bdbiosciences.com) and eBioscience (www.ebioscience.com) market a large

number of different antibodies, that are fluorescently labeled, and that each specifically recognize a particular cell surface marker. Primary human fibroblast cells overexpressing *MBD2* and/or *MBD4* protein is probed with fluorescence labeled antibodies recognizing selected surface marker proteins according to the
5 procedure described by the antibody manufacturer. Fluorescence activated cell sorting equipment like a BD FACSAArray from BD Biosciences (www.bdbiosciences.com) is used to count the number of cells expressing a particular cell surface protein marker, and quantitative data for light signal emissions is obtained for characteristic cell type surface markers.

10

Primary human fibroblast cells overexpressing *MBD2* and/or *MBD4* protein are found to display characteristic cell surface proteins, that identifies them as being different from control primary human fibroblast cells without the *MBD2* and/or *MBD4* overexpression.

15

DNA methylation of cytosine in specific CpG regions is known to be associated with cell differentiation. As induction of plasticity is a process opposite to differentiation, demethylation of CpG sites may also be considered indicative for an increased plasticity, exemplified by the transition from an end-stage committed
20 cell to a non-end-stage committed cell. The methylSEQr Bisulfite Conversion Kit from Applied Biosystems (www.appliedbiosystems.com) is used to determine the methylation status of a CpG motif. Sodium bisulfite deaminates cytosine to produce uracil, and methylated cytosines are protected from this conversion. Hence DNA sequence determination of chromosomal DNA regions with and
25 without the use of bisulfite conversion will identify methylated cytosines.

Primary human fibroblast cells overexpressing *MBD2* and/or *MBD4* protein are analysed for DNA methylation of particular chromosomal regions using a bisulfite conversion kit as described by the kit manufacturer. DNA sequence determination
30 is performed using an ABI PRISM 310 Genetic Analyzer from Applied Biosystems (www.appliedbiosystems.com) as described by the manufacturer.

The CpG methylation patterns for primary human fibroblast cells with overexpression of *MBD2* and/or *MBD4* protein is found to show signs of DNA

demethylation compared to primary human fibroblast cells without the *MBD2* and/or *MBD4* overexpression.

Example 14

5 Enhancement of plasticity induction

DNA methyltransferase 1 (*DNMT1*) is responsible for the methylation of the newly formed DNA strand during DNA replication. Only hemi-methylated CpG sites are recognized, and in this way the pre-existing methylation pattern is copied onto the two daughter cells. Demethylation induced by overexpression of *MBD2* and/or

- 10 *MBD4* protein may thus be counterpoised by *DNMT1* activity, and *DNMT1* inhibition may be required for efficient plasticity induction.

A listing of DNA methylation inhibitors is found in an article by C.B. Yoo and P.A. Jones (Nature Reviews Drug Discovery, volume 5, january 2006, page 37-50).

15

Treatment of primary human fibroblast cells with and without overexpression of *MBD2* and/or *MBD4* protein with known DNA methylation inhibitors like for example 5-azacytidine, hydralazine or procainamide is used to determine the enhancement of plasticity induction measured as described in example 13.

20

Reduction of cellular *DNMT1* activity can also be obtained by use of RNA interference (RNAi) technology. Loss of function for specific genes is induced by way of short RNAi oligonucleotides complementary to the target mRNA transcript. Commercially available RNAi systems like DNMT1 Stealth Select RNAi (RNAi
25 Catalog Nr. HSS102859; HSS102860; HSS102861) from Invitrogen (www.invitrogen.com) (www.rnadesigner.invitrogen.com) is transfected into primary human fibroblast cells as described by the manufacturer and used for plasticity induction analysis as previously described.

- 30 Chromatin structure constitutes another obstacle for an efficient DNA demethylation reaction, where heterochromatin is known to be structurally inaccessible for protein factors. Condensation of chromosomal DNA into heterochromatin is associated with histone deacetylation, whereas DNA wrapped around acetylated histones are accessible to protein factors. Histone acetylases

(HAT) and histone deacetylases (HDAC) perform opposite reactions, and hyperacetylation is generated by use of histone deacetylase inhibitors.

A listing of histone deacetylase inhibitors is found in an article by C.B. Yoo and
5 P.A. Jones (Nature Reviews Drug Discovery, volume 5, January 2006, page 37-50).

Treatment of primary human fibroblast cells with and without overexpression of *MBD2* and/or *MBD4* protein with known histone deacetylase inhibitors like for
10 example butyrate, valproic acid, oxamflatin and apicidin is used to determine the enhancement of plasticity induction measured as described in example 8.

DNMT1 inhibition and/or histone hyperacetylation is observed to result in similar or improved plasticity induction, when primary human fibroblast cells
15 overexpressing *MBD2* and/or *MBD4* protein are analysed as described in example 8.

Example 15

Re-differentiation of de-differentiated donor cells

20 A number of protocols have been tested for the differentiation of human pluripotent stem cells into a wide range of specialized cells. Thus, a donor cell that has been de-differentiated and reprogrammed into a higher level of plasticity may be considered pluripotent if it demonstrates capability of being re-differentiated into one or more end-stage committed cell from each the three primary germ
25 layers (ectoderm, mesoderm, and endoderm).

Ectoderm

To evaluate the differentiation-potential of de-differentiated donor cells into a target cell of ectodermal origin, a protocol for the derivation of dopaminergic
30 neurons using a serum-free suspension culture system is implemented:

Cells are harvested as per any standard procedure, washed with DMEM, and placed in suspension culture in differentiation medium consisting of either MedII/FGF2 medium (DMEM/F12, 1 × N2 [Invitrogen], 20 mM L-glutamine, 0.5

U/ml penicillin, 0.5 U/ml streptomycin, 4 ng/ml FGF-2, and 50% serum-free MedII) or DMEM/N2 medium (DMEM, 1 × N2, 20 mM L-glutamine, 0.5 U/ml penicillin, 0.5 U/ml streptomycin). Cultures are differentiated for 2–6 weeks in suspension, and the medium is changed every 5–7 days. Characteristic folds and
5 rosettes of neural precursors is observed after 5–10 days of culture. Cell aggregates are plated on dishes or Permax slides coated with 20 µg/ml polyornithine (Sigma) and 1 µg/ml laminin (Sigma) in MedII/FGF2 medium or Neurobasal medium (Invitrogen) containing 1 × B27 (Invitrogen), 5% FCS (Hyclone), 2 ng/ml glial-derived neurotrophic factor (*GDNF*) (R&D Systems), 10
10 ng/ml brain-derived neurotrophic factor (*BDNF*) (R&D Systems), 20 mM L-glutamine, 0.5 U/ml penicillin, and 0.5 U/ml streptomycin. These cultures are highly enriched for nestin+ neural precursor rosettes and large networks of βIII tubulin+ neurons. In addition, most of these neurons will also express tyrosine hydroxylase, which is the enzyme responsible for converting L-tyrosine to
15 dihydroxyphenylalanine (*DOPA*), the precursor for dopamine.

Mesoderm

To assess the differentiation-potential of de-differentiated donor cells into a target cell of mesodermal origin, a protocol for the derivation of fully mature primitive
20 erythroid cells is implemented:

First, de-differentiated donor cells are co-cultured with immortalized fetal human liver clone B (FH-B-hTERT) cells (Wege et al. Gastroenterology 2003; 124:432–444). CD34+ cells are subsequently purified and subjected to serum-free liquid
25 culture, allowing for the expansion of de-differentiated donor cell-derived hematopoietic stem cells (HSCs). These cells are then cultured in the presence of insulin growth factor-1 to induce commitment of de-differentiated donor cell - derived HSCs to the erythroid fate. Finally, terminal erythroid differentiation is induced via co-culture of putative erythroid progenitors with MS-5 feeder cells,
30 erythropoietin and hemin. Using this stepwise protocol, upwards of 5×10⁶ terminally differentiated erythrocytes may be generated from 50 000 de-differentiated donor cells in less than one month.

Endoderm

To evaluate the differentiation-potential of de-differentiated donor cells into a target cell of endodermal origin, a protocol for the derivation of pancreatic β -cells is implemented:

- 5 De-differentiated donor cells are passaged onto 1% Matrigel (B&D Biosciences)-coated tissue culture dishes. Then, the culture medium is changed to modified CDM: 50% IMDM (Gibco) plus 50% F12 NUT-MIX (Gibco), supplemented with insulin-transferrin-selenium-A (1:100, Gibco) and 450 μ M monothioglycerol (Sigma), and 5 mg/ml albumin fraction V (Sigma) or X-Vivo10 (Cambrex)
- 10 supplemented with 55 μ M 2-Mercaptoethanol (Gibco) and 0.1% albumin fraction V (Sigma).

- Two days later, the cells are induced into definitive endoderm differentiation with CDM containing 50 ng/ml activin A (Sigma) for 4 days, as detected by the
- 15 expression of the definitive endoderm markers *SOX17* and *Brachyury*. After 4 days of activin A induction, the cells are transferred into CDM with 10⁻⁶ M all-trans retinoic acid (Sigma) for another 4 days to promote pancreatic differentiation, as indicated by the expression of the early pancreatic transcription factors *PDX1* and *HLXB9*. Culture medium is then changed from CDM to modified
- 20 islet maturation medium: DMEM/F12 (Gibco), insulin-transferrin-selenium-A (1:100, Gibco) and 2 mg/ml albumin fraction V (Sigma) with 10 ng/ml bFGF (Invitrogen) for the first 3 days and with 10 mM nicotinamide (Sigma) for the next 5 days. The cells are digested by 0.5 mg/ml dispase (Gibco) and transferred into Ultra Low Attachment culture dishes (Costar) for 5 days to achieve islet
- 25 maturation in suspension culture. The differentiated cells may be evaluated by the expression of islet specific markers such as C-peptide, insulin, glucagon and *GLUT2*.

Example 16

- 30 Reprogramming of human buccal mucosa epithelial cells (ectoderm) into pluripotency followed by re-differentiation into fully mature primitive erythroid cells (mesoderm)

Donor cells in the form of human epithelial cells can easily be obtained non-invasively by gentle scraping from the buccal mucosa. Subsequently, the cells are induced to active DNA demethylation by means of increasing activity of DNA demethylating factors and/or inhibition of maintenance methylation in accordance
5 with the principles outlined in examples 10-14.

The level of reprogramming and putative pluripotency is then tested by the concomitant up-regulation of classical stem cell markers (Oct-4, SSEA3, SSEA4, TRA-1-60, TRA-1-81) and down-regulation of epithelia cell markers (e.g.
10 cytokeratins, lamin A/C).

Final level of differentiation capacity is assessed by exposing the de-differentiated donor cells to the protocol of erythroid cell differentiation as described in example
15.

Example 17

Characterisation of stem cell gene expression on mRNA-level in human dermal fibroblasts transfected with MBD2 and/or MBD4 with/without DNA
20 methyltransferase inhibitor (5-aza)

Human foetal (foreskin) fibroblast cells (hFFs) were cultivated in IMDM medium (Invitrogen) containing 10% FBS and 1% pen/strep.

25 Synthetic DNA fragments with the MBD2 and MBD4 protein reading frames were obtained from GENEART. The DNA fragments were inserted into the pcDNA 3-1(+) expression vektor (Invitrogen). Both the MBD2 and the MBD4 reading frames were sequenced in the final plasmid vectors prior to use for transfection eksperiments.

30

Lipofectamine 2000 (Invitrogen) was used for plasmid transfection of hFF cells according to the manufacturers description (use of standard ratio of DNA to Lipofectamine 2000 reagent). Test of the above transfection protocol using a GFP

reporter plasmid detected a green fluorescence signal in almost half of the transfected hFF cells.

Human foetal fibroblasts (hFFs) were transfected and grown under the following 3
5 conditions (plasmid use combinations):

1. hFFs transfected with the *MBD2* construct,
2. hFFs transfected with the *MBD4* construct, and
3. hFFs transfected with both the *MBD2* and *MBD4* construct.

10

For each transfection, cells were harvested after a period of one, two or three days of culture. In addition, cells from the different transfections were also grown in the presence of 5 μ M 5-azacytidine (5aza) in order to inactive the DNMT1 enzyme activity.

15

Total RNA from hFFs was obtained using the RNeasy[®] Micro Kit (Qiagen, Valencia, CA), which enriches mRNAs from small samples by selectively excluding shorter RNAs such as rRNAs and tRNAs. Cells were deattached from the bottom of the culture dish by trypsination (Trypsin-EDTA treatment followed by resuspension in
20 IMDM medium containing 10% FBS for trypsin inactivation) and isolated by centrifugation. Tubes with cell pellets were rapidly snap frozen by dipping into liquid nitrogen and subsequently stored at -80°C. The pelleted cells were lyzed with guanidine-isothiocyanate-containing buffer and were applied to spin columns for adsorption of the RNA. Carrier RNA was included to improve the yield of low-
25 abundance mRNAs further. Pellets were washed with 80% ethanol, centrifuged and air-dried. Subsequently, the RNA was eluted in nuclease-free water and immediately stored at -80°C until use.

Quantity and integrity were assessed on a NanoDrop N-1000 device (NanoDrop
30 Technologies, Wilmington, DE, USA) and showed to be of good quality according to absorbances at 260 and 280 nm.

RNA was converted into cDNA using SuperScript III reverse transcriptase (Invitrogen) with a blend of oligo-d(T) and random hexamer primers (both
35 Fermentas). Briefly, mixtures containing dNTP mix, water, primer blend and RNA

template were heated at 70°C for 5 min and placed immediately on ice for 3 min to unfold secondary RNA structures. Next, the mixture was added to a blend of first-strand buffer, DTT, RNase inhibitor and SuperScript III reverse transcriptase. Samples were incubated in a thermocycler at 55°C for 1 hour followed by
5 inactivation of reverse transcriptase at 70°C for 15 min. The synthesized cDNA was stored immediately at -20°C.

cDNA quality was evaluated on a NanoDrop N-1000 device (NanoDrop Technologies, Wilmington, DE, USA) and was shown to be of good quality by the
10 outline of the absorbance curve around in relation to 260 and 280 nm.

To assess the level of induced plasticity, six transcripts which has been shown to be characteristic for pluripotent human embryonic stem cells (hESCs) (i.e. *DNMT3B*, *GABBR3*, *GDF3*, *NANOG*, *POU5F1*, *TDGF1*) were designed according to
15 refs. (Adewumi et al., 2007 and Awan et al., 2008). In addition, seven transcripts expressed in multipotent human mesenchymal stem cells (hMSCs) (Abdallah et al., 2008) were designed (i.e. *ALCAM*, *ANPEP*, *CD44*, *CD55*, *DLK1*, *ENG*, *ICAM1*). Primer sequences were designed using either PrimerBank (Wang & Seed 2003) or PerlPrimer (Marshall O., 2007). Three house-keeping genes were used as internal
20 reference genes (i.e. *ACTB*, *GAPDH*, *TBP*).

Fold changes in gene expression were evaluated by real-time qPCR using a LightCycler device (Roche). SYBR Green I dye was used as detection chemistry (Takara). Briefly, SYBR Premix Ex Taq mix, template cDNA, gene specific primers,
25 and RNase-free water were mixed inversion and brief spin. Samples (10 µl) were placed in capillaries and rotor (Genaxxon). PCR conditions were as follows: cDNA was denatured and preincubated by heating for 10 s at 95°C. Template was then amplified by 37 cycles consisting of denaturation for 5 s at 95°C, annealing for 20 s at 55°C, and extension for 10 s at 72°C. To verify RT-PCR product identity,
30 melting point curves were generated following amplification. Samples were heated to 95°C followed by immediate cooling for 15 s at 65°C and then slowly heating by 0.1°C/s to 95°C, while monitoring fluorescence continuously.

All target genes were evaluated against the geometric mean of the three reference house-keeping genes within the log-linear phase of the amplification curve, and ΔC_T values were assessed using the $\Delta\Delta C_T$ method (Pfaffl, 2001).

- 5 Expression levels of the genes specific to undifferentiated human embryonic stem cells as compared to control population (i.e. non-transfected hFFs) were generally modified only to a modest degree and there were no consistent trend for either up- or down-regulation. However, with one noticeable exception, the classical gene for characterizing undifferentiated hESC is the *POU5F1* (*OCT4*) gene, which
- 10 consistently in all transfections shows a relatively strong up regulation. The present data actually indicate a 9 to 14 fold up-regulation of the *POU5F1* (*OCT4*) gene.

Figure legends

15 Figure 1

Shows a correlation plot illustrating the concordance in probe set expression values ($n = 45,101$) between two of the biological replicates.

The high correlation coefficient (r) confirms the reliability of Affymetrix gene chip technology performed on minute amounts of mRNA extracted from

20 mammalian oocytes.

Figure 2

Shows a dendrogram illustrating the final hierarchical clustering tree of 22 significantly up- or down-regulated reprogramming genes (for gene symbols, see

25 Table 3) in MII oocytes when compared with GV oocytes (five and three biological replicates respectively). The expression values for a gene across all eight samples are standardized to have mean 0 and S.D. 1 by linear transformation. The

- distance between two genes is defined as $1 - r$, where r is the standard correlation coefficient between the eight standardized values of two genes. Two genes with
- 30 the closest distance are first merged into a supergene, connected by branches with length representing their distance, and deleted for future merging. The expression level of the newly formed supergene set is the average of standardized expression levels of the two genes (average linkage) for each sample. Then, the

next pair of genes (supergene) with the smallest distance is chosen to merge and the process is repeated until all genes are merged into one final cluster. The genes Prmt3, Mbd4, Mbd2, and Sirt indicate significant down-regulation, whereas the remaining genes indicate down-regulation.

5

Figure 3

Shows a bar chart showing fold changes of 22 reprogramming genes measured by real-time quantitative RT-PCR. Mean fold changes are expressed on a log₂ scale as metaphase II oocyte values relative to germinal vesicle stage oocytes.

10 Whiskers represent 95% confidence intervals.

Figure 4

Shows a bar chart showing Δ Ct changes of 13 genes specific to undifferentiated human embryonic stem cells. Cells have been transfected with MBD2 in absence
15 of DNA methyltransferase inhibitor (DNMTi) 5-azadeoxycytidine (5aza).

Expression levels are shown as compared to control population (non-transfected hFFs) at 1, 2 and 3 days post-transfection.

Figure 5

Shows a bar chart showing Δ Ct changes of 13 genes specific to undifferentiated
20 human embryonic stem cells. Cells have been transfected with MBD4 in absence of DNA methyltransferase inhibitor (DNMTi) 5-azadeoxycytidine (5aza).

Expression levels are shown as compared to control population (non-transfected hFFs) at 1, 2 and 3 days post-transfection.

25 Figure 6

Shows a bar chart showing Δ Ct changes of 13 genes specific to undifferentiated human embryonic stem cells. Cells have been transfected with MBD2 with the DNA methyltransferase inhibitor (DNMTi) 5-azadeoxycytidine (5aza) present.

Expression levels are shown as compared to control population (non-transfected
30 hFFs) at 2 and 3 days post-transfection.

Figure 7

Shows a bar chart showing ΔCt changes of 13 genes specific to undifferentiated human embryonic stem cells. Cells have been transfected with MBD4 with the DNA methyltransferase inhibitor (DNMTi) 5-azadeoxycytidine (5aza) present.

- 5 Expression levels are shown as compared to control population (non-transfected hFFs) at 2 and 3 days post-transfection.

Figure 8

- 10 Shows a bar chart showing ΔCt changes of 13 genes specific to undifferentiated human embryonic stem cells. Cells have been transfected with MBD2 and MBD4 with the DNA methyltransferase inhibitor (DNMTi) 5-azadeoxycytidine (5aza) present. Expression levels are shown as compared to control population (non-transfected hFFs) at 2 and 3 days post-transfection.

Tables

Table 1.

Primer list comprising a group of selected genes important to to epigenetic modifications and chromatin remodelling. The housekeeping gene (*Actb*) is used as an internal reference.

5 Gene symbols and descriptions have been adopted from the Mouse Genome Informatics database (<http://www.informatics.jax.org/>). Primer pairs were carefully designed so that they were separated by at least one intron sequence to reduce risk of co-amplified genomic DNA. Additionally, some primers span intron-exon flanks. No primer pair had inter-primer complementarity thus avoiding formation of primer-dimers. All primer sequences were blasted for potential alternative binding sites.

Abbreviations: F, forward primer; R, reverse primer; T_m , melting temperature.

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction ^a	Exon Complementarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
<i>Actb</i>	Actin, beta, cytoplasmic	F: AAGTGTGACGTTGACATCCG	5	60.00	NM_007393	250
		R: GATCCACATCTGCTGGAAGG	6	60.00		

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction^a	Exon Comple-mentarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
<i>Aof2</i>	Amine oxidase (flavin containing) domain 2 (LSD1)	F: GACGTTTGAAGCCACTCTCC R: AAGCCAGAAACACCTGAACC	4 6	59.85 59.19	NM_133872	186
<i>Dnmt1</i>	DNA methyltransferase 1	F: ACCCCCATCTTCTTGCTCC R: GGCATTCTCCTTGTCCTCCT	20 21	60.31 60.60	NM_010066	216
<i>Dnmt3a</i>	DNA methyltransferase 3A	F: GAGTCTAACCCCGTGATGATTG R: CTGGTCTTTGCCCTGCTTT	18, 19 21	60.74 60.38	NM_007872	219
<i>Dnmt3b</i>	DNA methyltransferase 3B	F: CGACTTGGTGATTGGTGGA R: CCAGAAGAATGGACGGTTG	19 20	60.52 59.07	NM_001003961	157
<i>Elp3</i>	Elongation protein 3 homolog	F: AACAGGGGCCATACTGTGAA R: AACCATCAGGACGAAAAGCA	8, 9 10	60.38 60.64	NM_028811	169
<i>Gcn5/2</i>	GCN5 general control of amino acid synthesis-like 2	F: AAGCCTAACATTGAACAGGGTG R: TCCAGTAGTTAAGGCAGAGCAG	4, 5 5	60.5 61.2	NM_020004	124

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction^a	Exon Comple-mentarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
	(Yeast)					
<i>Hdac1</i>	Histone deacetylase 1	F: AGTCTGTTACTACTACGACGGG R: TGAGCAGCAAATTGTGAGTCAT	1, 2 2	60.4 60.5	NM_008228	101
<i>Hdac6</i>	Histone deacetylase 6	F: GCTGGTGGCCGTATTATTCTT R: CAAAGTAAGCTGGGGTGTG	23 24	60.4 60.5	NM_010413	117
<i>Htatip</i>	HIV-1 tat interactive protein, homolog (human)	F: TCCCGGTCCAGATCACACTC R: ACCTTCCGTTTCGTTGAGCG	5 5, 6	62.8 63.0	NM_178637	130
<i>Mbd1</i>	Methyl-CpG binding domain protein 1	F: GAGGACGAGCTACAGCCCTA R: TTGCCACCCCCGAATTTGG	10, 11 11	62.3 62.9	NM_013594	131
<i>Mbd2</i>	Methyl-CpG binding domain protein 2, isotype a and b	F: TGGGAGAAGAGGGCTACAAGG R: GCAGAGGGGTTGAGATGTGT	4 5	59.42 60.12	NM_010773	231

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction^a	Exon Comple- mentarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
<i>Mbd4</i>	Methyl-CpG binding domain protein 4	F: TCCACGGACACAAGTAGAA R: AGAAACTCCCACAGCACAGG	4 6	59.13 60.30	NM_010774	235
<i>Myst1</i>	MYST histone acetyltransferase 1	F: ACGAGGCGATCACCAAAGTG R: AAGCGGTAGCTCTTCTCGAAC	4,5 5	62.5 61.8	NM_026370	166
<i>Myst4</i>	MYST histone acetyltransferase monocytic leukemia 4	F: TGAGGAAAGCATTCCCACATC R: ACACCTTGCTGCACCTTTAGAGG	16 17	60.0 61.0	NM_017479	178
<i>Prmt3</i>	Protein arginine N-methyltransferase 3	F: CAACAGGGTCGTGTTCTCTAC R: TTCAAAGTCAGGGTCACAATGA	15, 16 17	60.0 60.7	NM_133740	175
<i>Prmt7</i>	Protein arginine N-methyltransferase 7	F: TACTGCAGGGGCTGACTTCT R: TCACCTCAGTGGAGTGCTTG	4 5	60.0 60.0	NM_145404	131
<i>Setdb1</i>	SET domain, bifurcated 1	F: CAACTTGACAATCACGGAAAC R: CAAAGGTGACCGATATGTCTGG	12 12, 13	60.8 60.4	NM_018877	156

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction^a	Exon Complementarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
<i>Sirt3</i>	Sirtuin 3 (silent mating type information regulation 2, homolog) 3 (S. cerevisiae)	F: ATCCCGGACTTCAGATCCCC R: CAACATGAAAAAGGGCTTGGG	2, 3 3	62.7 60.0	NM_022433	126
<i>Sirt4</i>	Sirtuin 4 (silent mating type information regulation 2 homolog) 4 (S. cerevisiae)	F: AACCGGACGTCGTTTCTTT R: ATGGCTATTGGGAGCTTTTG	2 3	60.1 59.2	AK016400	169
<i>Sirt5</i>	Sirtuin 5 (silent mating type information regulation 2 homolog) 5 (S. cerevisiae)	F: GCGAAGCCCAAGCACATAG R: TGTGATGGGTTTCGAGCAAAG	3 4	61.8 60.8	NM_178848	149

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction ^a	Exon Comple-mentarity	T _m (°C)	GenBank Accession ID	Product Size (bp)
	cerevisiae)					
<i>Sirt7</i>	Sirtuin 7 (silent mating type information regulation 2, homolog) 7 (S. cerevisiae)	F: GTCCAGCTTGAAGGTACTAAAG R: TGAGGAGTTGCATGACATCATC	7, 8 9	60.3 60.1	NM_153056	165
<i>Smarca2</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	F: ATGTCGCCAATCTGACCAAC R: TCTGCATTCTCTTCAGCCTTC	9 9, 10	61.0 59.7	NM_011416	106
<i>Smarca4</i>	SWI/SNF related,	F: CCCACCTAACCTCACCAAGA	28	59.96	NM_011417	232

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction^a	Exon Comple-mentarity	T_m (°C)	GenBank Accession ID	Product Size (bp)
	matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (BRG1)	R: CAGCATCACATCCTTCTCCA	30	59.79		
<i>Smarca5</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 (SNF2H)	F: GGTTTGGTGTCATTCTC R: TTTGTCCATCTTCCCTGCT	9, 10 11	60.45 59.67	NM_053124	173
<i>Smyd3</i>	SET and MYND domain containing 3	F: GCGTTGTTCTCAATGCCGAAT R: CCAGAAGTCTCACGGAGTCG	2 3	61.8 61.6	NM_027188	137

Gene Symbol	Gene Name (Alias)	Primer Sequences 5'-3' Direction ^a	Exon Comple- mentarity	T _m (°C)	GenBank Accession ID	Produc t Size (bp)
<i>Suv39h1</i>	Suppressor of variegation 3-9 homolog 1 (Drosophila)	F: CAGTGTGTGCTGTAAATCTTCT	1	61.0	NM_011514	179
		R: ATACCCACGCCCACTTAACCAG	2	61.8		

Table 2

Characteristics for microarray chip samples. A high correlation coefficient (r) between biological replicates indicates almost identical global transcription patterns and thus high validity and reliability of the microarray procedure per se performed on 5 minute amounts of RNA.

Abbreviations: GV, germinal vesicle; MII, metaphase II.

Developmental stage	Biological replicates (n)	Median (range) r between biological replicates	P-value
GV oocytes	3	0.993 (0.992 - 0.995)	< 0.0001
MI I oocytes	5	0.972 (0.955 - 0.992)	< 0.0001

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Dnmt3a</i>	DNA methyltransferase 3A		NM_153743	1460324_at	6.21	6.55	0.34	1.26	0.667
<i>Dnmt3b</i>	DNA methyltransferase 3B		NM_001003963	1449052_at	8.72	9.90	1.18	2.26	0.074
Methyl-CpG-Binding Proteins									
<i>Mbd1</i>	Methyl-CPG binding domain protein 1		NM_013594	1417968_at	7.97	2.34	-5.63	-49.38	0.000
<i>Mbd2</i>	Methyl-CPG		NM_010773	1417165_a	3.76	5.53	1.77	3.40	0.040

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	binding domain protein 2			t					6	
<i>Mbd4</i>	Methyl-CPG binding domain protein 4		NM_010774	1449490_a t	4.28	5.75	1.47	2.77	0.006	*
<i>Mecp2</i>	Methyl CPG binding protein 2		NM_010788	1449266_a t	Absent	Absent				
<i>Zbtb33</i>	Zinc finger and BTB domain containing 33	Kaiso	NM_020256	1421094_a t	6.77	8.45	1.68	3.21	0.133	
<i>Zbtb38</i>	Zinc finger and BTB domain	ZBTB38	NM_175537	1438269_a t	Absent	Absent				

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
	containing 38								
<i>Zbtb4</i>	Zinc finger and BTB domain containing 4	ZBTB4		1457293_at	Absent	Absent			
Histone Acetyltransferases									
<i>Atf2</i>	Activating transcription factor 2	CREBP1	NM_009715	1426582_at	9.17	9.35	0.19	1.14	0.922
				t					4
<i>Cdy1</i>	Chromodomain protein, Y chromosome-		NM_009881	1418070_at	10.42	11.62	1.20	2.30	0.155
				t					2

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	like									
<i>Crebbp</i>	CREB binding protein	CBP, Expressed sequence AW558298	NM_001025432	1459804_at	7.64	7.52	-0.12	-1.09	0.8669	
<i>Elp3</i>	Elongation protein 3 homolog (S. Cerevisiae)	AAH01240	NM_028811	1426643_at	11.24	3.61	-7.63	-197.84	0.0005	*
<i>Ep300</i>	E1A binding protein p300	P300	NM_177821	1434765_at	9.13	9.62	0.49	1.40	0.1231	
<i>Gcn5/2</i>	GCN5 general control of amino	GCN5, gcl2	NM_001038010	1426783_at	10.65	8.63	-2.02	-4.06	0.0159	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	acid synthesis-like 2 (yeast)									
<i>Gtf3c1</i>	General transcription factor III C 1	TFIIIC	NM_207239	1434066_at	9.48	8.79	-0.68	-1.60	0.2328	
<i>Hat1</i>	Histone aminotransferase 1		NM_026115	1428061_at	12.07	11.80	-0.26	-1.20	0.3784	
<i>Htatip</i>	HIV-1 tat interactive protein, homolog (human)	Tip60, Esa1	NM_178637	1433980_at	8.64	6.38	-2.26	-4.78	0.0090	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Myst1</i>	MYST histone acetyltransferase 1	MOF	NM_026370	1432080_s_at	12.17	5.65	-6.53	-92.28	0.000
<i>Myst2</i>	MYST histone acetyltransferase 2	HBO1	NM_177619	1433433_at	10.13	9.97	-0.16	-1.11	0.444
<i>Myst3</i>	MYST histone acetyltransferase (monocytic leukemia) 3	MOZ	XM_982685	1436315_at	5.48	4.73	-0.75	-1.69	0.506
<i>Myst4</i>	MYST histone acetyltransferase monocytic	MORF		1423508_at	9.56	7.97	-1.59	-3.01	0.000

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
	leukemia 4								
<i>Ncoa1</i>	Nuclear receptor coactivator 1	SCR-1	NM_010881	1418594_at	9.09	9.59	0.51	1.42	0.6695
<i>Ncoa2</i>	Nuclear receptor coactivator 2	SRC-2, GRIP1, TIF2		1435234_at	11.85	12.12	0.27	1.20	0.8636
<i>Ncoa3</i>	Nuclear receptor coactivator 3	ACTR, AIB1, pCIP, TRAM1, F9/5B1, RAC3, SRC3	NM_008679	1422737_at	9.36	8.79	-0.57	-1.49	0.0792
<i>Ogt</i>	O-linked N-acetylglucosamine (GlcNAc)		NM_139144	1460631_at	8.89	9.54	0.66	1.58	0.1850

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)									
<i>Pcaf</i>	p300/CBP-associated factor		NM_020005	1450821_at	Absent	Absent				
<i>Taf1</i>	TAF1 RNA polymerase II, TATA box	TAFII250, TFIID	XM_886564	1455513_at	7.45	8.41	0.96	1.95	0.1406	

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	binding protein (TBP)-associated factor									
Histone Deacetylases										
<i>Hdac1</i>	Histone deacetylase 1	HD1, RPD3L1	NM_008228	1448246_at	7.17	5.98	-1.19	-2.28	0.0143	*
<i>Hdac2</i>	Histone deacetylase 2	HD2	NM_008229	1449080_at	11.01	11.37	0.36	1.28	0.7878	
<i>Hdac3</i>	Histone deacetylase 3	HD3, RPD3-2	NM_010411	1426437_s_at	5.93	6.46	0.53	1.44	0.4840	
<i>Hdac4</i>	Histone			1415743_a	Absent	Absent				

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	deacetylase 4			t						
<i>Hdac5</i>	Histone deacetylases 5		NM_010412	1415743_a t	Absent	Absent				
<i>Hdac6</i>	Histone deacetylases 6		NM_010413	1448928_a t	8.58	5.96	-2.61	-6.11	0.006	*
<i>Hdac7a</i>	Histone deacetylases 7A		NM_019572	1420812_a t	Absent	Absent				1
<i>Hdac8</i>	Histone deacetylases 8		NM_027382	1428933_a t	Absent	Absent				
<i>Hdac9</i>	Histone deacetylases 9		NM_024124	1434572_a t	8.07	9.07	0.99	1.99	0.055	
<i>Hdac10</i>	Histone deacetylase 10		NM_199198	1454775_a t	Absent	Absent				

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Hdac11</i>	Histone deacetylase 11		NM_144919	1451229_at	Absent	Absent				
<i>Sirt1</i>	Sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae)		NM_019812	1418640_at	10.42	10.80	0.38	1.30	0.6409	
<i>Sirt2</i>	Sirtuin 1 (silent mating type information regulation 2, homolog) 2 (S.		NM_022432	1423507_at	Absent	Absent				

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	cerevisiae)									
<i>Sirt3</i>	Sirtuin 3 (silent mating type information regulation 2, homolog) 3 (S. cerevisiae)		NM_022433	1417892_at	6.84	5.55	-1.29	-2.44	0.0391	*
<i>Sirt4</i>	Sirtuin 2 (silent mating type information regulation 2, homolog) 4 (S. cerevisiae)		XM_991632	1426847_at	6.28	8.26	1.98	3.95	0.0247	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Sirt5</i>	Sirtuin 2 (silent mating type information regulation 2, homolog) 5 (S. cerevisiae)		NM_178848	1428915_a	9.31	3.98	-5.33	-40.31	0.0001	*
<i>Sirt6</i>	Sirtuin 2 (silent mating type information regulation 2, homolog) 6 (S. cerevisiae)		NM_181586	1429631_a	3.76	3.35	-0.40	-1.32	0.4467	
<i>Sirt7</i>	Sirtuin 2 (silent		NM_153056	1424238_a	9.91	5.59	-4.32	-19.92	0.002	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	mating type information regulation 2, homolog) 7 (S. cerevisiae)		t	t					4	
Histone Methyltransferases										
<i>Dot1l</i>	DOT1-like, histone H3 methyltransferase (S. cerevisiae)		NM_199322	1457268_at	7.19	8.08	0.89	1.86	0.132	5

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Ehmt1</i>	Euchromatic histone methyltransferase 1	H3-K9-HMTase 5, Eu-HMTase 1, GLP1, KIAA1876	NM_172545	1454776_at	9.43	9.79	0.36	1.29	0.430	
<i>Ehmt2</i>	Euchromatic histone lysine N-methyltransferase 2		NM_145830	1460692_at	Absent	Absent				
<i>Ezh2</i>	Enhancer of zeste homolog 2 (Drosophila)	ENX-1	NM_007971	1416544_at	14.09	13.74	-0.35	-1.27	0.319	

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Mll1</i>	Myeloid/lymphoid or mixed-lineage leukemia 1		XM_990865	1427283_at	Absent	Absent			
<i>Nsd1</i>	Nuclear receptor-binding SET-domain protein 1		NM_008739	1440972_at	Absent	Absent			
<i>Prdm2</i>	PR domain containing 2, with ZNF domain	RIZ1	XM_984811	1453068_at	11.41	11.08	-0.33	-1.26	0.418
<i>Prdm9</i>	VPR domain	Meisetz	XM_977404	1426107_at	Absent	Absent			

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
	containing 9			t					
<i>Prmt1</i>	Protein arginine N-methyltransferase 1	Hrmt112	NM_019830	1452787_at	7.58	7.83	0.25	1.19	0.7958
<i>Prmt2</i>	Protein arginine N-methyltransferase 2	Hrmt111	NM_133182	1416844_at	12.07	11.61	-0.46	-1.38	0.2357
<i>Prmt3</i>	Protein arginine N-methyltransferase 3	Hrmt113	NM_133740	1426749_at	7.37	8.75	1.38	2.60	0.0405*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Prmt4</i>	Protein arginine N-methyltransferase 4	CARM1	NM_021531	1420668_at	Absent	Absent			
<i>Prmt5</i>	Protein arginine N-methyltransferase 5	SKB1 homolog	NM_013768	1421343_at	Absent	Absent			
<i>Prmt6</i>	Protein arginine N-methyltransferase 6	HMT1 hnRNP methyltransferase-like 6, Hrmt116	NM_178891	1434209_at	Absent	Absent			
<i>Prmt7</i>	Protein arginine		NM_145404	1451248_at	10.43	4.55	-5.89	-59.16	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
	N-methyltransferase 7			t				6	
<i>Setd1a</i>	SET domain containing 1A	SET1	XM_985966	1427116_at	Absent	Absent			
<i>Setd2</i>	SET domain containing 2	HIF1, HYPB	XM_988006	1428555_at	9.64	9.76	0.12	1.09	0.9219
<i>Setd7</i>	SET domain containing (lysine methyltransferase) 7	SET7/9 KIAA1717	NM_080793	1423003_at	Absent	Absent			
<i>Setd8</i>	SET domain	PR-Set7,	NM_030241	1460687_at	9.46	10.49	1.03	2.04	0.120

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	containing (lysine methyltransferase) 8	SET07, SET8		t					5	
<i>Setdb1</i>	SET domain, bifurcated 1	H3-K9-HMTase	NM_018877	1451833_at	11.93	10.41	-1.52	-2.88	0.022	*
<i>Smyd3</i>	SET and MYND domain containing 3	Zmynd1	NM_027188	1452817_at	8.49	4.37	-4.12	-17.36	0.000	*
<i>Suv39h1</i>	Suppressor of variegation 3-9 homolog 1 (Drosophila)	SUV39H	NM_011514	1432227_at	12.52	5.76	-6.76	-108.01	0.000	*

Gene Symbol	Gene Name	Name	Transcript ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
Suv39h2	Suppressor of variegation 3-9 homolog 2 (Drosophila)		NM_022724	1422979_at	8.84	9.01	0.17	1.13	0.9824	
Suv420h1	Suppressor of variegation 4-20 homolog 1 (Drosophila)		NM_144871	1455171_at	3.19	2.89	-0.30	-1.23	0.3699	
Suv420h2	Suppressor of variegation 4-20 homolog 2 (Drosophila)		NM_146177	1424059_at	3.09	2.24	-0.85	-1.80	0.2875	

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
Histone Demethylases or Deiminases									
<i>Aof2</i>	Amine oxidase (flavin containing) domain 2	LSD1	NM_133872	1426761_at	10.12	10.09	-0.03	-1.02	0.6457
<i>Fbx11</i>	F-box and leucine-rich repeat protein 11	JHDM1	NM_001001984	1454477_at	Absent	Absent			
<i>Jmjd2a</i>	Jumonji domain containing 2A		NM_172382	1446699_at	Absent	Absent			
<i>Jmjd2b</i>	Jumonji domain		NM_172132	1424044_at	Absent	Absent			

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	containing 2B			t						
<i>Jmjd2c</i>	Jumonji domain containing 2C	Gasc1	NM_144787	1424458_at	Absent	Absent				
<i>Padi4</i>	Peptidyl arginine deiminase, type IV	PADI4, PAD4	NM_011061	1422760_at	Absent	Absent				
Chromatin Remodelling ATPases										
<i>Chd1</i>	Chromodomain helicase DNA binding protein 1		NM_007690	1420990_at	Absent	Absent				

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MI Oocyte Mean (Log₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Chd2</i>	Chromodomain helicase DNA binding protein 2		XM_976731	1445843_at	Absent	Absent			
<i>Chd3</i>	Chromodomain helicase DNA binding protein 3	Mi-2 alpha	XM_908691	1428466_at	Absent	Absent			
<i>Chd4</i>	Chromodomain helicase DNA binding protein 4	Mi-2 beta	NM_145979	1436343_at	8.52	7.69	-0.83	-1.78	0.4519
<i>Smarca</i>	SWI/SNF	SNF2L	NM_053123	1460292_at	Absent	Absent			

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MI Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
1	related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1			_at						
<i>Smarca2</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin,	hBRM	NM_011416	1430526_a_at	11.55	9.40	-2.15	-4.44	0.0117	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	subfamily a, member 2									
<i>Smarca4</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	BRG1	NM_011417	1426804_at	10.67	8.09	-2.58	-5.97	0.0055	*
<i>Smarca5</i>	SWI/SNF related, matrix associated, actin	SNF2H	NM_053124	1424207_at	10.18	9.08	-1.09	-2.13	0.1589	

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
	dependent regulator of chromatin, subfamily a, member 5									
Oocyte Specific Transcripts										
<i>Bmp15</i>	Bone morphogenetic protein 15		NM_009757	1450210_at	11.54	9.84	-1.71	-3.26	0.0039	*
<i>Ccnb1</i>	Cyclin B1		NM_172301	1416076_at	13.71	13.25	-0.45	-1.37	0.2513	

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Dppa3</i>	Developmental pluripotency-associated 3	STELLA	NM_139218	1424295_at	13.92	12.86	-1.05	-2.08	0.0764
<i>Gdf9</i>	Growth differentiation factor 9		NM_008110	1448120_at	15.29	14.16	-1.13	-2.18	0.0652
<i>Mos</i>	Moloney sarcoma oncogene		NM_020021	1450775_at	14.69	13.50	-1.19	-2.29	0.0854
<i>Nalp5</i>	NACHT, leucine rich repeat and PYD containing 5	MATER	NM_001039143	1418882_at	15.61	12.63	-2.98	-7.91	0.0124*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log₂)	MII Oocyte Mean (Log₂)	Fold Change	Fold Change (Unlogged)	BH P-value	P < 0.05
<i>Pou5f1</i>	POU domain, class 5, transcription factor 1	OCT4	NM_013633	1417945_at	11.38	10.54	-0.85	-1.80	0.122	
<i>Spin</i>	Spindlin		NM_011462	1460164_at	14.91	14.25	-0.66	-1.58	0.155	
<i>Zar1</i>	Zygote arrest 1		NM_174877	1434494_at	15.47	14.47	-1.00	-2.00	0.071	
<i>Zp1</i>	Zona pellucida glycoprotein 1		XM_986504	1450306_at	14.21	9.88	-4.34	-20.10	0.003	*
<i>Zp2</i>	Zona pellucida glycoprotein 2		NM_011775	1449016_at	14.96	10.24	-4.72	-26.34	0.003	*
<i>Zp3</i>	Zona pellucida		NM_011776	1419007_at	15.33	11.25	-4.08	-16.96	0.005	*

Gene Symbol	Gene Name	Name Alias(es)	Transcript Accession ID	Affymetrix Probe Set ID	GV Oocyte Mean (Log ₂)	MII Oocyte Mean (Log ₂)	Fold Change	Fold Change (Unlogg ed)	BH P- valu e	P < 0.05
	glycoprotein 3			t					8	

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Claims

1. A method for increasing the plasticity level of a donor cell, said method comprising

5 -providing a mammalian, normoploid somatic cell as the donor cell

 -increasing the plasticity level in said cell by contacting said donor cell with,
 or introducing into said cell, at least one plasticity modifying factor selected
 from the group consisting of *DNMT1* factors, *MBD2* factors and *MBD4*

10 factors,

 thereby obtaining a supraplastic cell with the ability to develop into at least two
 different end-stage-committed cell types.

15 2. A method according to claim 1, wherein the *MBD2* factor is selected from the
 group consisting of DNA sequences encoded by the *MBD2* gene, proteins encoded
 by said DNA sequence, compounds increasing the transcription of the native
 MBD2 gene, compounds increasing the translation of *MBD2* mRNA species and
 compounds increasing the activity of the proteins encoded by the *MBD2* gene.

20

 3. A method according to claim 1, wherein the *MBD4* factor is selected from the
 group consisting of DNA sequences encoded by the *MBD4* gene, proteins encoded
 by said DNA sequence, compounds increasing the transcription of the native
 MBD4 gene, compounds increasing the translation of *MBD2* mRNA species and

25 compounds increasing the activity of the proteins encoded by the *MBD4* gene.

 4. A method according to claim 1, wherein the *DNMT1* factor is selected from the
 group consisting of compounds decreasing the transcription of the native *DNMT1*
 gene, compounds increasing the degradation of *DNMT1* mRNA species and

30 compounds inhibiting the DNA methyltransferase activity of the proteins encoded
 by *DNMT1*.

5. A method according to any of the preceeding claims, wherein the at least one plasticity modifying factor is further combined with at least one plasticity modifying factors mediating hyperacetylation of histones
, wherein said mediator of histone acetylation is selected from the group
5 consisting of *ATF2 factor(s)*, *CDYL factor(s)*, *CREBBP factor(s)*, *ELP3 factor(s)*, *EP300 factor(s)*, *GCN5L2 factor(s)*, *GTF3C1 factor(s)*, *HAT1 factor(s)*, *HTATIP factor(s)*, *MYST1 factor(s)*, *MYST2 factor(s)*, *MYST3 factor(s)*, *MYST4 factor(s)*, *NCOA1 factor(s)*, *NCOA2 factor(s)*, *NCOA3 factor(s)*, *OGT factor(s)*, *PCAF factor(s)*, and *TAF1 factor(s)*.
- 10
6. A method according to any of the preceeding claims, wherein the one or more plasticity modifying factor(s) and the mediators of histone acetylation is selected from the group consisting of a small chemical entity, a peptide, a protein, an RNA species including but not limited to siRNA-, microRNA- and, shRNA-molecules,
15 peptide aptamers, modified nucleic acids and DNA species.
7. A supraplastic cell according to any of claims 1-6.
8. A supraplastic cell according to claim 7, said supraplastic cell being partly
20 differentiated.
9. Use of the supraplastic cell of claims 7-8 for the production of target cells, said target cells being derived from the supraplastic cell and exhibiting an end-stage-committed phenotype including a unipotent pre-cursor target cell.
- 25
10. A target cell according to claim 9.

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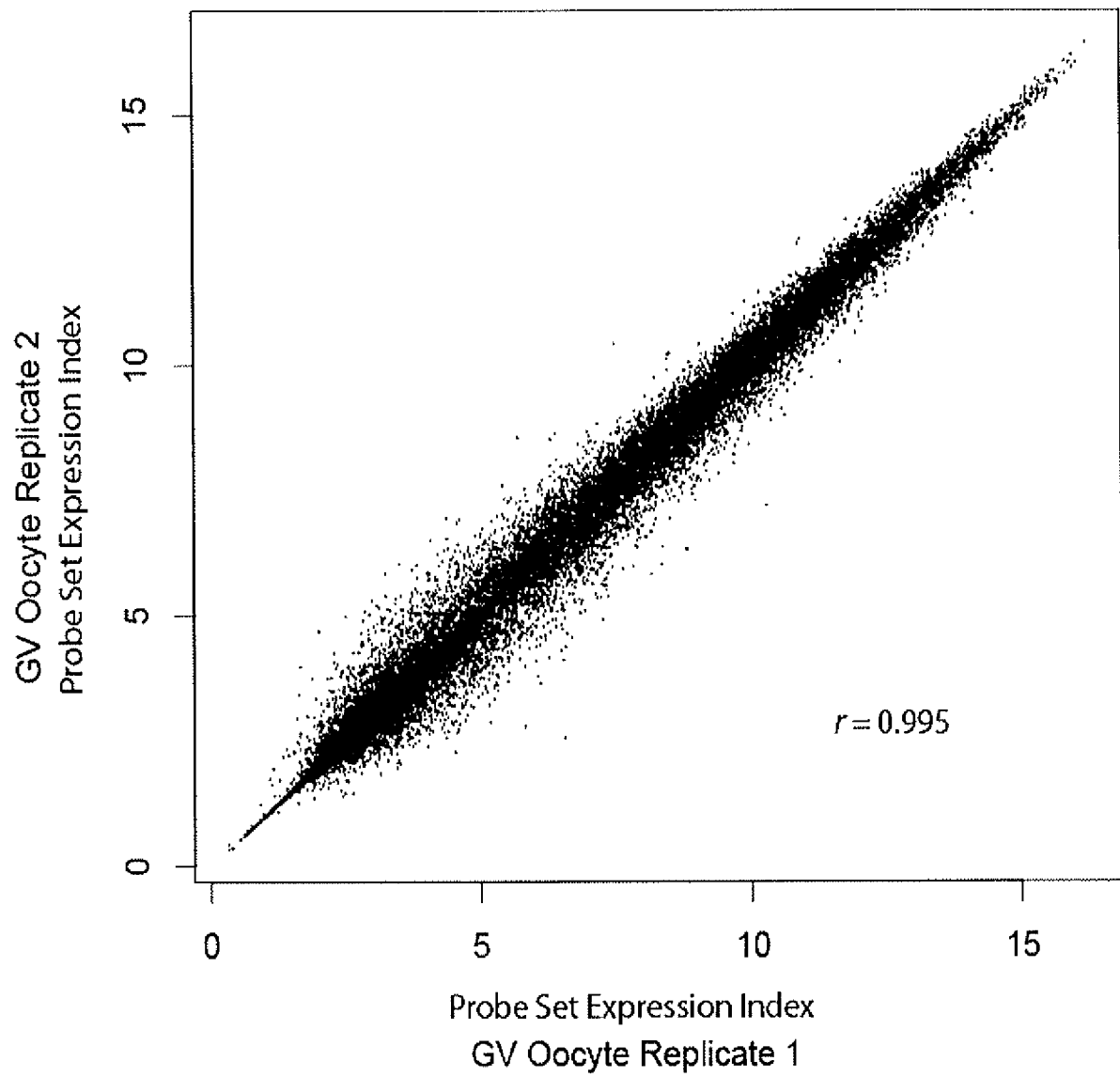


Fig. 1

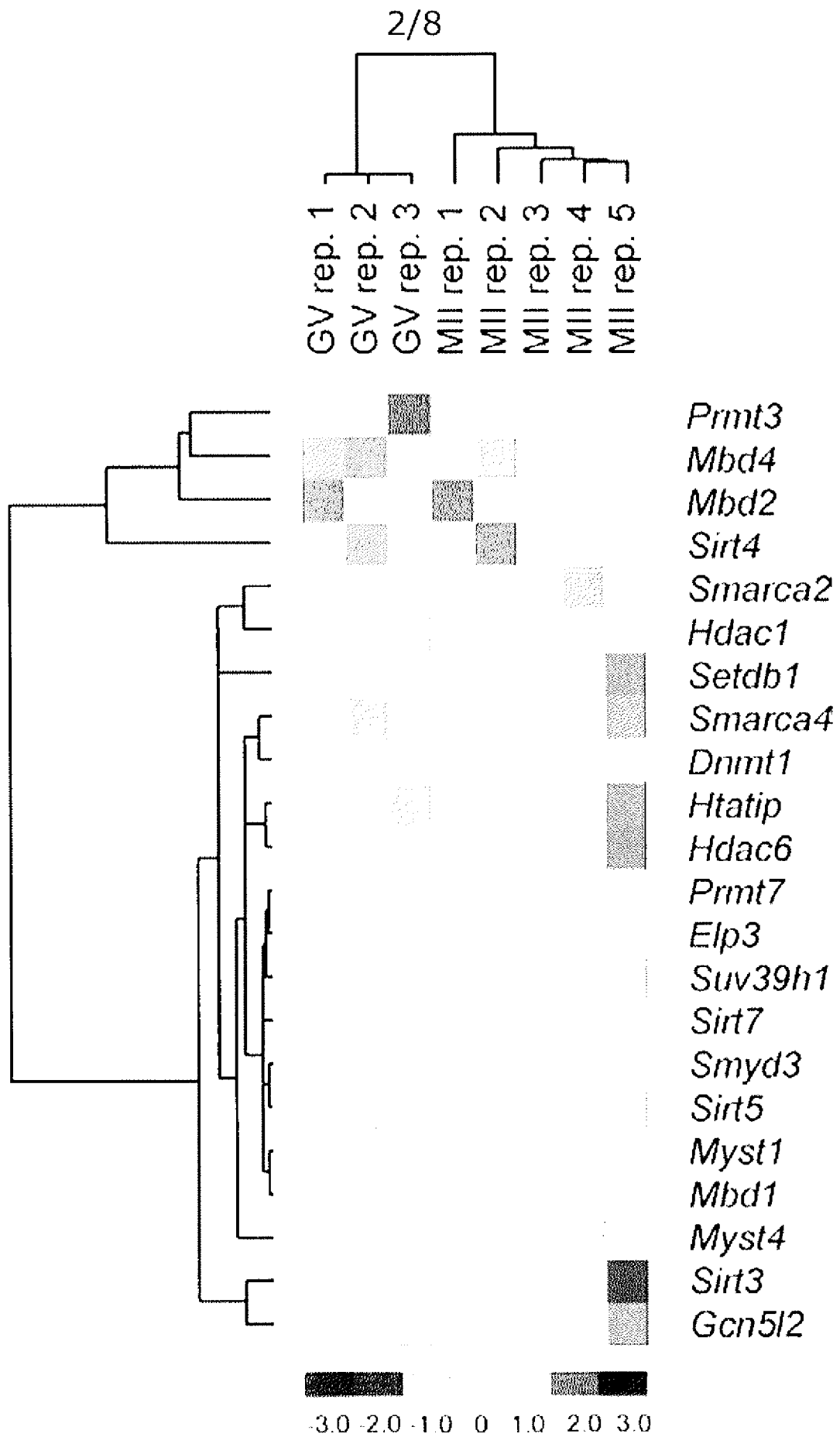


Fig. 2

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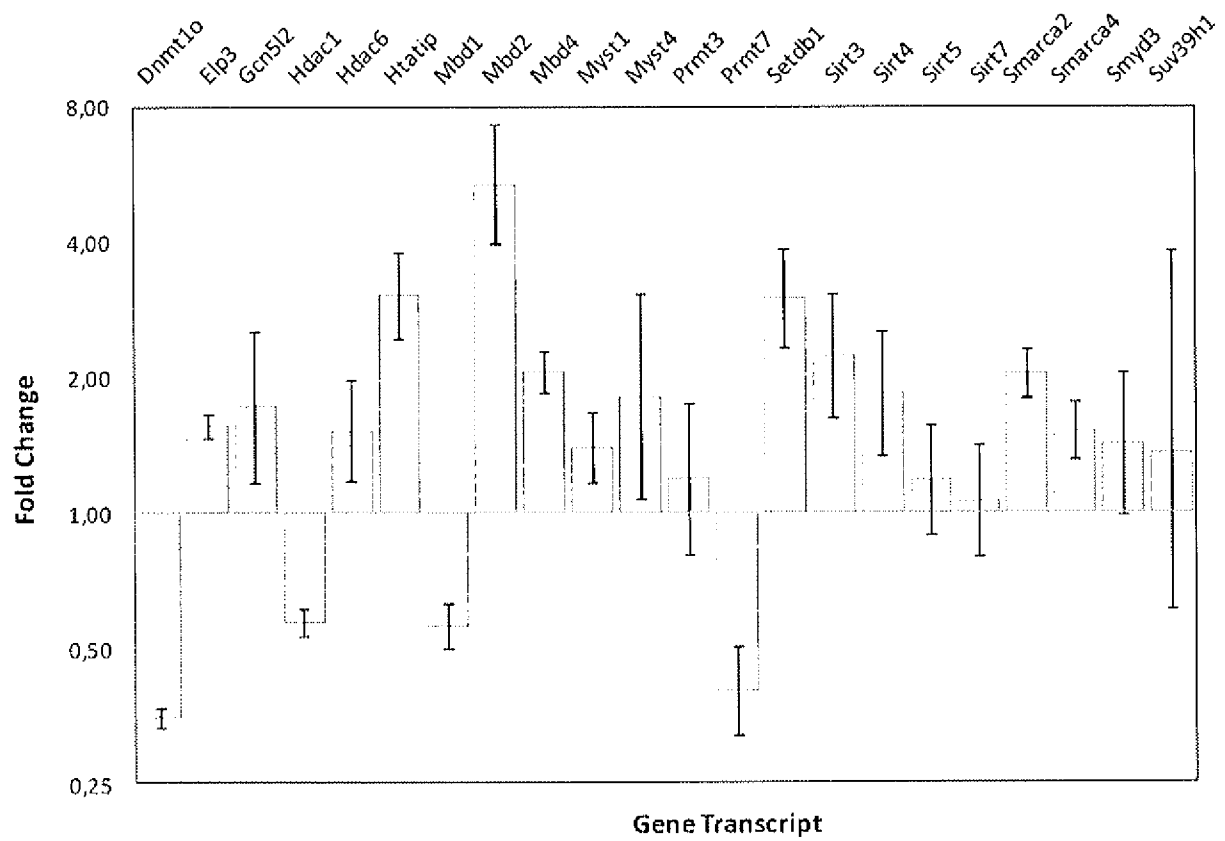


Fig. 2

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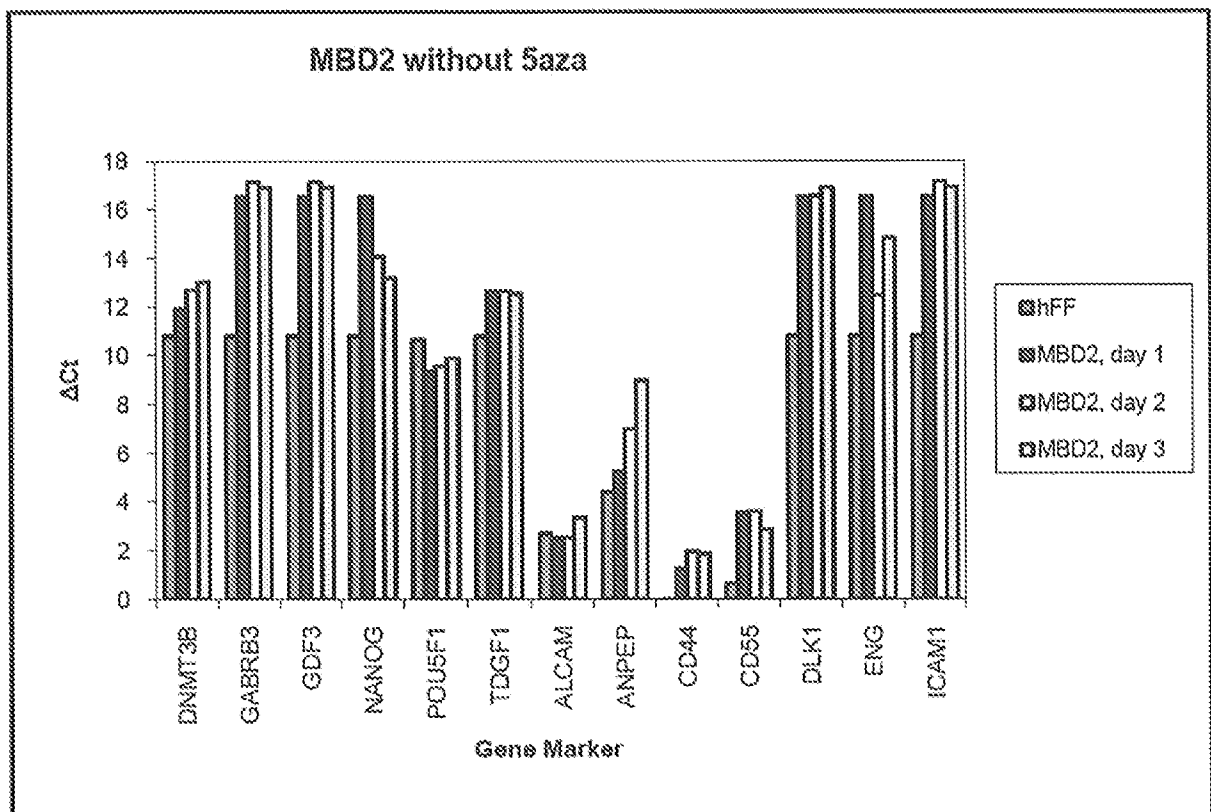


Fig.

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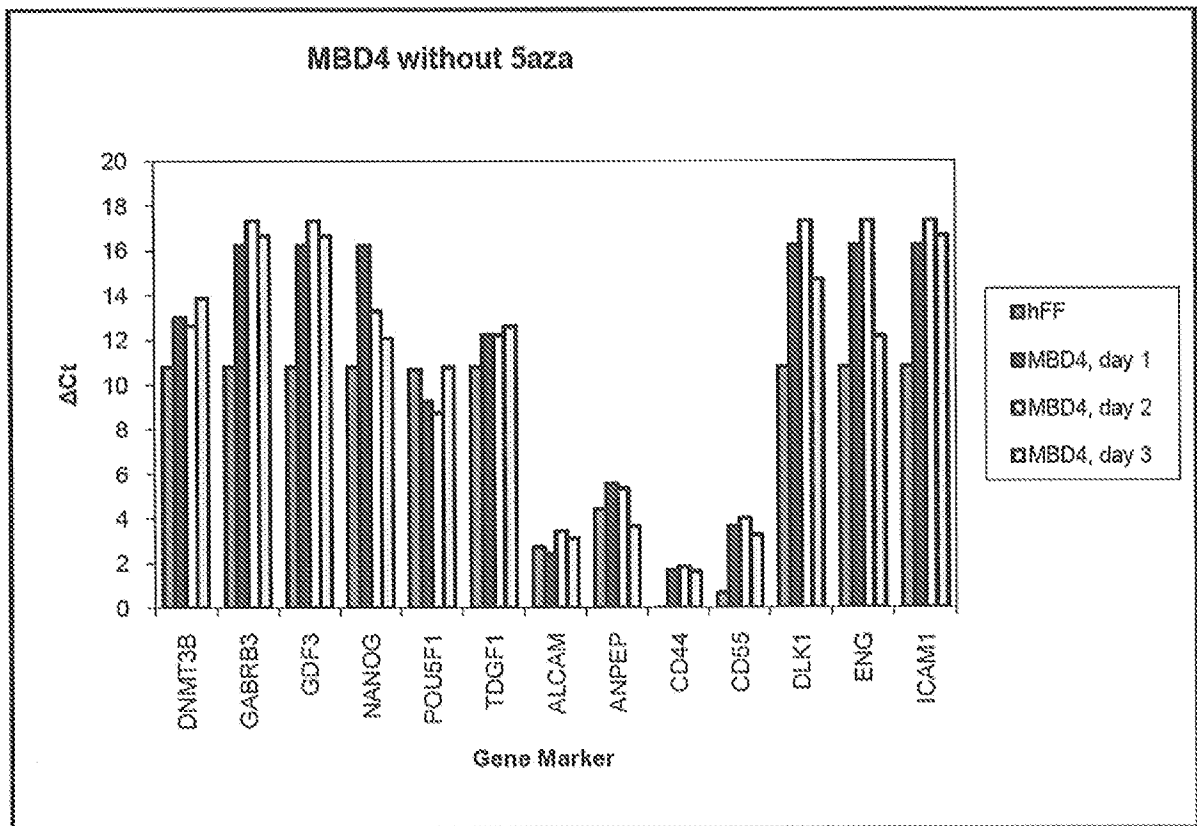


Fig. 5

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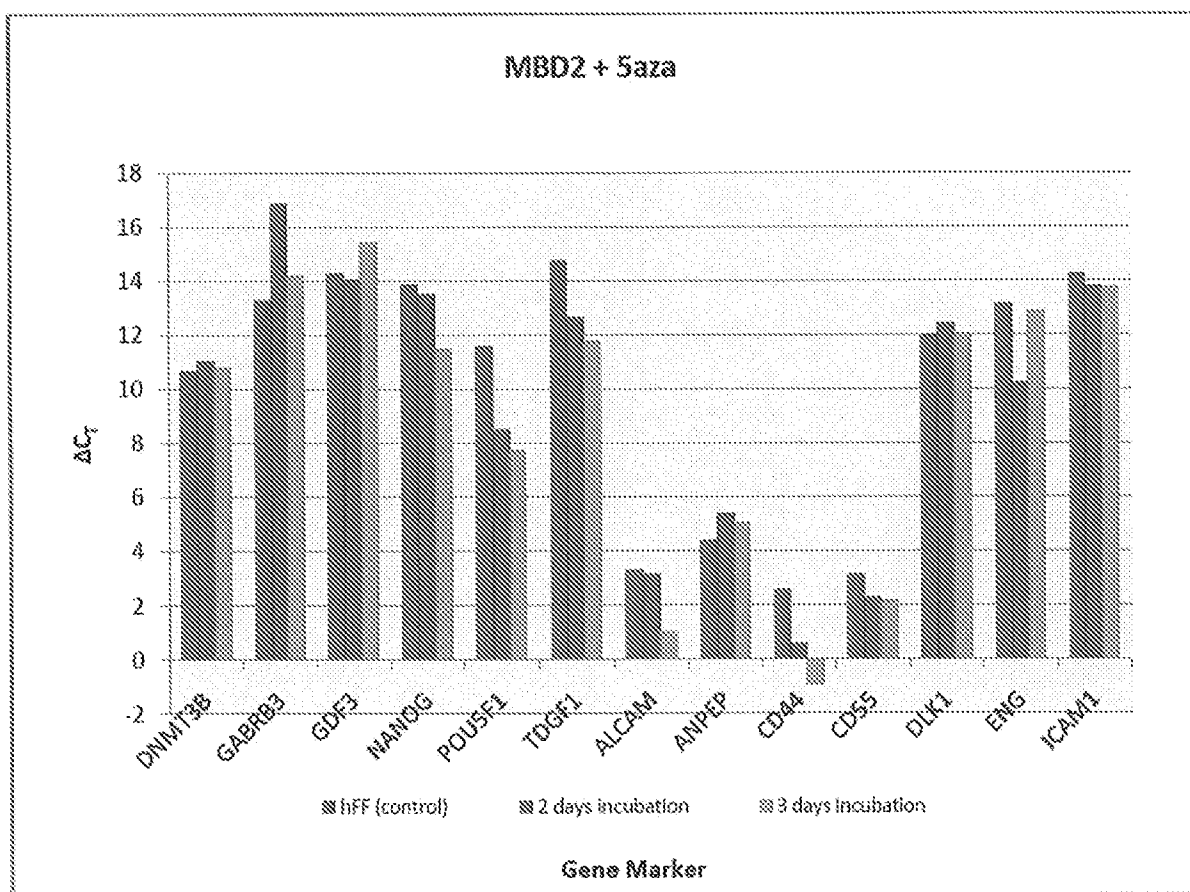


Fig. 6

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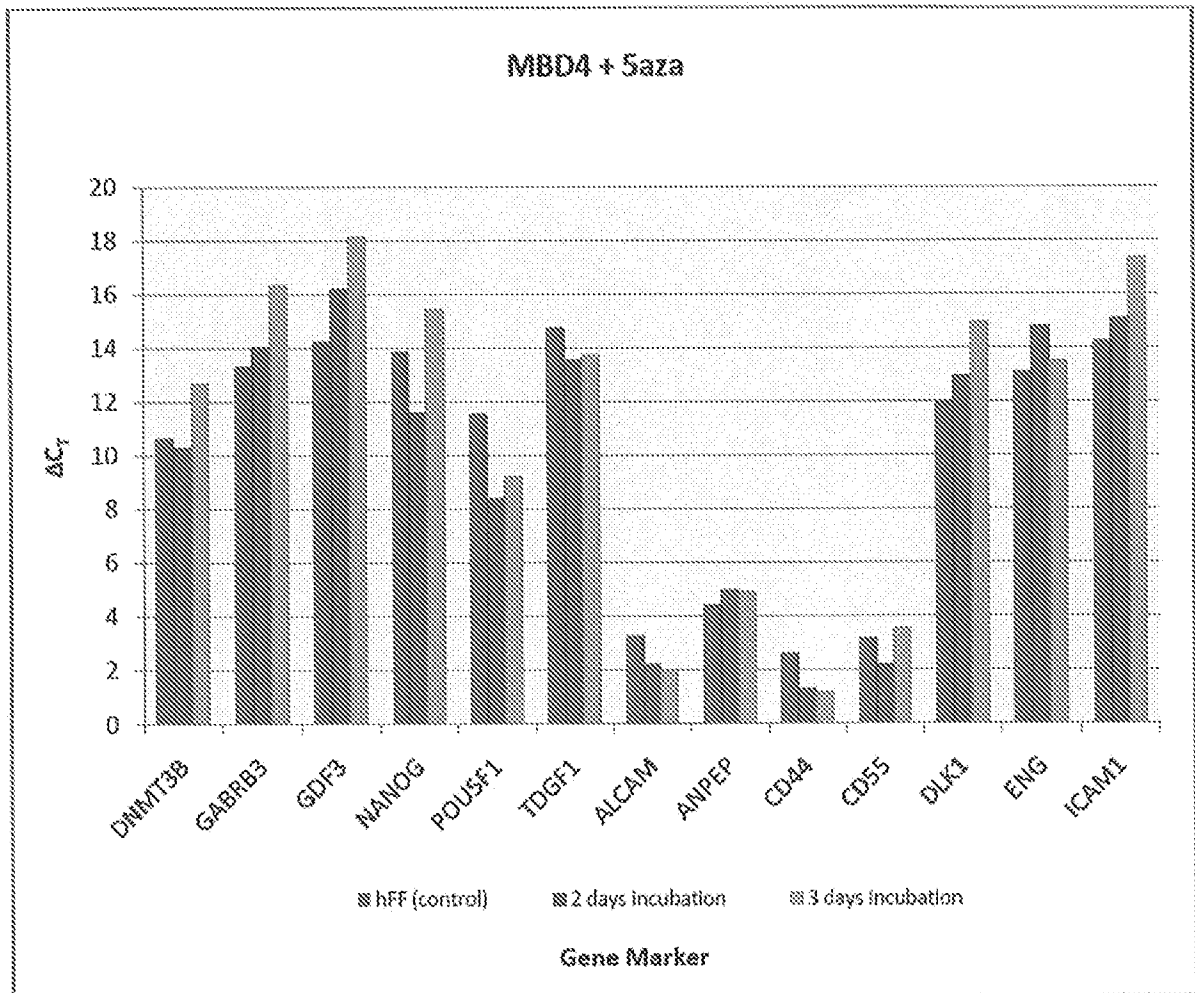


Fig. 7

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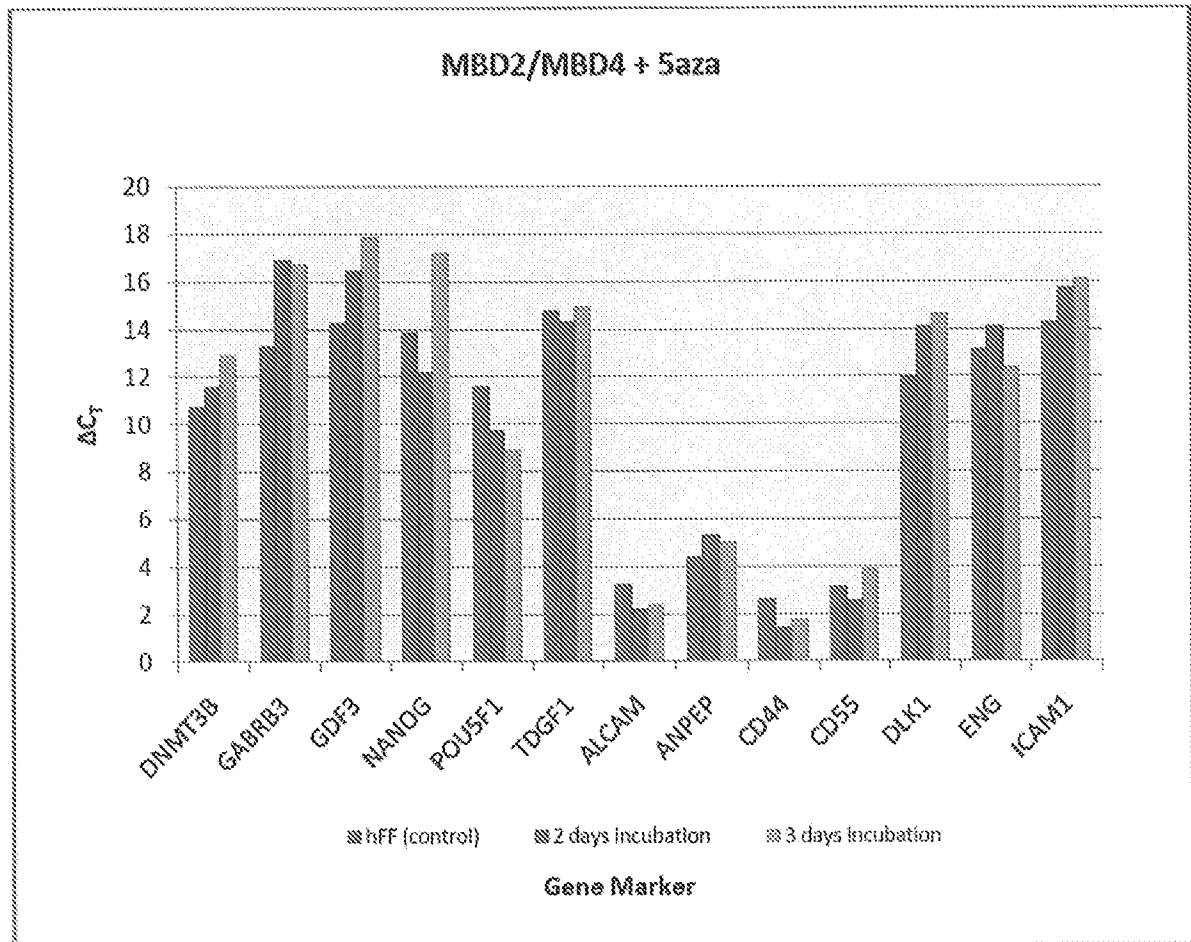


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2008/050197

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HENDRICH B ET AL: "Identification and characterization of a family of mammalian methyl-CpG binding proteins" MOLECULAR AND CELLULAR BIOLOGY, WASHINGTON, DC, US, vol. 18, no. 11, 1 November 1998 (1998-11-01), pages 6538-6547, XP002097747 ISSN: 0270-7306 page 6539, right-hand column, paragraph 3; figure 5 ----- -/--	7

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

27 November 2008

Date of mailing of the international search report

30/12/2008

Name and mailing address of the ISA/

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Authorized officer

Turri, Matteo

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2008/050197

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALADA EVA ET AL: "Transcript overexpression of the MBD2 and MBD4 genes in CD4(+) T cells from systemic lupus erythematosus patients" JOURNAL OF LEUKOCYTE BIOLOGY, vol. 81, no. 6, June 2007 (2007-06), pages 1609-1616, XP009096014 ISSN: 0741-5400 page 1611, last paragraph - page 1612, paragraph 1	7
A	----- WERNIG MARIUS ET AL: "In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state" NATURE (LONDON), vol. 448, no. 7151, July 2007 (2007-07), page 318, XP002468993 ISSN: 0028-0836 cited in the application	
A	----- TAKAHASHI K ET AL: "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 126, no. 4, 25 August 2006 (2006-08-25), pages 663-676, XP003013968 ISSN: 0092-8674 cited in the application	
A	----- ADEWUMI O ET AL: "Characterization of human embryonic stem cell lines by the International Stem Cell Initiative" NATURE BIOTECHNOLOGY 200707 US, vol. 25, no. 7, July 2007 (2007-07), pages 803-816, XP002505626 ISSN: 1087-0156 1546-1696 cited in the application	
P,A	----- ABDALLAH B M ET AL: "Human mesenchymal stem cells: from basic biology to clinical applications." GENE THERAPY JAN 2008, vol. 15, no. 2, January 2008 (2008-01), pages 109-116, XP002505627 ISSN: 1476-5462 cited in the application ----- -/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2008/050197

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THEISE N D: "New principles of cell plasticity" COMPTE RENDUS - BIOLOGIES, ELSEVIER, PARIS, FR, vol. 325, no. 10, 1 October 2002 (2002-10-01), pages 1039-1043, XP004395816 ISSN: 1631-0691 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2008/050197

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.