Claims

1. A method for controlling transcription of a genetic sequence in a cell comprising

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and

b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises said genetic sequence operably linked to an inducible promoter, and said promoter is regulated by the transcriptional regulator protein;

wherein said first and second genetic safe harbour sites are different.

2. A method as claimed in claim 1 wherein the genetic sequence is a transgene.

3. A method as claimed in claim 1 wherein the genetic sequence encodes a non-coding RNA.

4. A method as claimed in any one of claims 1 to 3 wherein the activity of said transcriptional regulator protein is controlled by an exogenously supplied substance.

5. A method as claimed in any one of claims 1 to 4 wherein said transcriptional regulator protein is constitutively expressed.

6. A method as claimed in any preceding claim wherein said transcriptional regulator protein is selected from any one of: tetracycline – responsive transcriptional activator protein (rtTa), Tetracycline repressor (TetR), VgEcR synthetic receptor or the Gene Switch hybrid transcriptional regulator protein, and derivatives of any thereof.

7. A method as claimed in any preceding claim wherein said transcriptional regulator protein is rtTA or any derivative thereof.

8. A method as claimed in claim 5 wherein the activity of tTA is controlled by tetracycline or a derivative thereof, optionally doxycycline.

9. A method as claimed in claim 7 or claim 8 wherein the inducible promoter includes a Tet Responsive Element (TRE).

10. A method as claimed in any preceding claim wherein said first and second genomic safe harbour sites are selected from any two of the hROSA26 locus, the AAVS1 locus, the CLYBL gene, the *CCR5* gene or the *HPRT* gene.

11. A method as claimed in any preceding claim wherein insertion of said gene encoding a transcriptional regulator protein into a first genetic safe harbour site occurs on both chromosomes of the cell and/or insertion of said inducible cassette into a second genetic safe harbour site occurs on both chromosomes of the cell.

12. A method as claimed in any preceding claim wherein additional genetic material is inserted at the first/and or second genomic safe harbour sites, optionally one or more of the following:

a) suicide gene;
b) selectable marker;
c) reporter gene; and/or
d) gene for a non-coding RNA.

13. A method as claimed in any preceding claim wherein said method is performed *ex vivo*.

14. A method as claimed as claimed in any preceding claim wherein said cell is selected from a pluripotent stem cell, a somatic stem cell or a mature cell.

15. A method as claimed in claim 1 or claim 2 wherein said method is for the programming of pluripotent stem cells into defined mature cells.

16. A method as claimed in claim 1 or claim 2 wherein said cell is a pluripotent stem cell and said genetic sequence is a transgene for one or more master regulators, optionally a transcription factor.

17. A method as claimed in claim 16 wherein the transcription of said genetic sequence results in the forward programming of the cell into a defined mature cell type.

18. A method as claimed in claim 17 wherein said mature cell type is selected from any one of the following cell types: nerve cells, myocytes, osteocytes, chondrocytes, epithelial cells, secretory cells, and/or blood cells.

19. A method as claimed in any one of claims 14 to 18 wherein said pluripotent stem cell is selected from an induced pluripotent stem cell (iPSC) or an embryonic stem cell (ESC).

20. A method as claimed in any one of claims 1 to 19 wherein said cell is human.

21. A method as claimed in claim 1 wherein said method is for the insertion of a genetic sequence for the purposes of gene therapy, optionally wherein the genetic sequence encodes a protein including a wild-type protein, a modified protein, an antigen, an enzyme, a selectable marker or a non-coding RNA molecule.

22. A cell made by the method of claim 1.

23. A cell with a modified genome that comprises an inserted gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and an inserted inducible cassette comprising a genetic sequence operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein and said first and second sites are different.

24. Use of a cell as claimed in claim 22 or claim 23 in therapy.

25. Use of a cell as claimed in claim 22 or 23 for *in vitro* diagnostics.

26. Use of a cell as claimed in claim 22 or 23 for tissue engineering, optionally cultured meat.

~~27. A method for the production of myocytes from pluripotent stem cells, comprising the steps of:~~

~~a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and~~

~~b) targeted insertion of the MYOD1 gene operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein; wherein said first and second genetic safe harbour sites are different,~~

~~and culturing said cells in the presence of retinoic acid.~~

~~28. A method for the production of oligodendrocytes from pluripotent stem cells, comprising the steps of:~~

~~a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and~~

~~b) targeted insertion of the SOX-10 gene operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein; wherein said first and second genetic safe harbour sites are different,~~

~~and culturing said cells in the presence of retinoic acid.~~

~~29. A method for reducing the transcription and/or translation of an endogenous gene in a cell, comprising the following steps:~~

~~a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and~~

~~b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises DNA encoding a non-coding RNA sequence operably linked to an inducible promoter and said promoter is regulated by the transcriptional regulator protein and wherein said non-coding RNA sequence suppresses the transcription or translation of an endogenous gene;~~

~~wherein said first and second genetic safe harbour sites are different.~~

~~30. A method for knocking out of an endogenous gene in a cell, comprising the following steps:~~

~~a) targeted insertion of a gene encoding a transcriptional regulator protein and a gene encoding Cas9 into a first genetic safe harbour site; and~~

~~b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises a guide RNA operably linked to an inducible promoter and said promoter is regulated by the transcriptional regulator protein and wherein said gRNA sequence targets the endogenous gene;~~

~~wherein said first and second genetic safe harbour sites are different.~~

~~31. A method as claimed in any one of claims 1 to 21 or 27 to 29 wherein a further inducible cassette or transgene is inserted into a further GSH, which is different to the first and second GSH.~~

~~32. An optimised tetR sequence comprising a sequence with 80%, 85%, 90%, 95% or 99% homology to SEQ ID No. 6.~~

~~33. A method for the production of myocytes from pluripotent stem cells expressing MYOD1, comprising culturing said cells in the presence of retinoic acid.~~

1. An *ex vivo* method for controlling transcription of a genetic sequence in a cell comprising

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and

b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises said genetic sequence operably linked to an inducible promoter, and said promoter is regulated by the transcriptional regulator protein;

wherein said first and second genetic safe harbour sites are different, and

wherein the method does not comprise a process for modifying the germ line genetic identity of a human being.

2. An *ex vivo* method as claimed in claim 1, wherein the genetic sequence is a transgene or encodes a non-coding RNA.

3. An *ex vivo* method as claimed in claim 1 or claim 2, wherein the activity of said transcriptional regulator protein is controlled by an exogenously supplied substance.

4. An *ex vivo* method as claimed in any one of claims 1 to 3, wherein said transcriptional regulator protein is constitutively expressed.

5. An *ex vivo* method as claimed in any preceding claim, wherein said transcriptional regulator protein is selected from any one of: tetracycline - responsive transcriptional activator protein (rtTa), Tetracycline repressor (TetR), VgEcR synthetic receptor or a hybrid transcriptional regulator protein comprising a DNA binding domain from the yeast GAL4 protein, a truncated ligand binding domain from the human progesterone receptor and an activation domain from the human NF-κB.

6. An *ex vivo* method as claimed in claim 5, wherein the activity of rtTA is controlled by tetracycline or a derivative thereof, optionally doxycycline, and wherein the inducible promoter includes a Tet Responsive Element (TRE).

7. An *ex vivo* method as claimed in any preceding claim, wherein said first and second genomic safe harbour sites are selected from any two of the hROSA26 locus, the AAVS1 locus, the *CLYBL* gene or the *CCR5* gene.

8. An *ex vivo* method as claimed in any preceding claim, wherein additional genetic material is inserted at the first/and or second genomic safe harbour sites, optionally one or more of the following:

a) suicide gene;

b) selectable marker;

c) reporter gene; and/or

d) gene for a non-coding RNA.

9. An *ex vivo* method as claimed in any preceding claim, wherein said cell is selected from a pluripotent stem cell, a somatic stem cell or a mature cell.

10. An *ex vivo* method as claimed in any preceding claim, wherein said cell is a human, marsupial, non-human primate, camelid or livestock animal cell.

11. An *ex vivo* method as claimed in claim 1 or claim 2, wherein said method is for the programming of pluripotent stem cells into defined mature cells.

12. An *ex vivo* method as claimed in claim 1 or claim 2, wherein said cell is a pluripotent stem cell and said genetic sequence is a transgene for one or more master regulators, optionally a transcription factor, and optionally wherein the transcription of said genetic sequence results in the forward programming of the cell into a defined mature cell type.

13. An *ex vivo* method as claimed in claim 1, wherein said method is for the insertion of a genetic sequence for the purposes of gene therapy, optionally wherein the genetic sequence encodes a protein including a wild-type protein, a modified protein, an antigen, an enzyme, a selectable marker or a non-coding RNA molecule.

14. A cell made by the method of claim 1.

15. A cell with a modified genome that comprises an inserted gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and an inserted inducible cassette comprising a genetic sequence operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein and said first and second sites are different.

16. A cell as claimed in claim 14 or claim 15, for use in therapy, or use of a cell as claimed in claim 14 or claim 15 for *in vitro* diagnostics or for tissue engineering.

17. An *ex vivo* method for the production of myocytes from pluripotent stem cells, comprising the steps of:

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and

b) targeted insertion of the *MYOD1* gene operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein; wherein said first and second genetic safe harbour sites are different,

and culturing said cells in the presence of retinoic acid,

wherein the method does not comprise a process for modifying the germ line genetic identity of a human being.

18. An *ex vivo* method for the production of oligodendrocytes from pluripotent stem cells, comprising the steps of:

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and

b) targeted insertion of the *SOX-10* gene operably linked to an inducible promoter into a second genetic safe harbour site, wherein said inducible promoter is regulated by the transcriptional regulator protein; wherein said first and second genetic safe harbour sites are different,

and culturing said cells in the presence of retinoic acid,

wherein the method does not comprise a process for modifying the germ line genetic identity of a human being.

19. An *ex vivo* method for reducing the transcription and/or translation of an endogenous gene in a cell, comprising the following steps:

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site; and

b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises DNA encoding a non-coding RNA sequence operably linked to an inducible promoter and said promoter is regulated by the transcriptional regulator protein and wherein said non-coding RNA sequence suppresses the transcription or translation of an endogenous gene;

wherein said first and second genetic safe harbour sites are different, and

wherein the method does not comprise a process for modifying the germ line genetic identity of a human being.

20. An *ex vivo* method for knocking out an endogenous gene in a cell, comprising the following steps:

a) targeted insertion of a gene encoding a transcriptional regulator protein and a gene encoding Cas9 into a first genetic safe harbour site; and

b) targeted insertion of an inducible cassette into a second genetic safe harbour site, wherein said inducible cassette comprises a guide RNA operably linked to an inducible promoter and said promoter is regulated by the transcriptional regulator protein and wherein said gRNA sequence targets the endogenous gene;

wherein said first and second genetic safe harbour sites are different, and

wherein the method does not comprise a process for modifying the germ line genetic identity of a human being.