

at the end of a fed-batch cultivation is reduced for recombinant cells according to the current disclosure, i.e. the timespan with viability above a certain threshold value is increased, compared to cells with fully functional SIRT-1 gene.

5 The present disclosure provides a mammalian cell wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

10 The present disclosure provides a mammalian cell wherein the expression of the endogenous SIRT-1 gene has been reduced and wherein said mammalian cell has at least one of increased productivity for heterologous polypeptides and/or reduced lactate production during cultivation and/or extended high viability levels during cultivation and/or extended cultivation time compared to a cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

15 The present disclosure provides a method for at least one of increasing heterologous polypeptide titer and/or reducing lactate production and/or extended high viability levels during cultivation and/or extension of cultivation time of a recombinant mammalian cell having reduced (endogenous) SIRT-1 expression comprising an exogenous nucleic acid encoding said heterologous polypeptide compared to a cell cultivated under the same conditions that has the identical genotype but endogenous
20 SIRT-1 gene expression. In one aspect, the present disclosure provides a method for increasing heterologous polypeptide titer and/or reducing lactate production of a recombinant mammalian cell comprising an exogenous nucleic acid encoding a heterologous polypeptide by knocking out SIRT-1 expression compared to a mammalian cell cultivated under the same conditions that has the identical genotype
25 but endogenous SIRT-1 gene expression.

The present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous SIRT-1 genes in a mammalian cell to reduce the activity of the endogenous SIRT-1 gene, and

5 b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1 gene has been reduced,

thereby producing a recombinant mammalian cell with increased recombinant productivity and/or reduced lactate production compared to a compared to a cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

10 In another aspect, the present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

15 a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous SIRT-1 genes in a mammalian cell to knock out the activity of the endogenous SIRT-1 gene, and

b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1 gene has been knocked out,

20 thereby producing a recombinant mammalian cell with increased recombinant productivity and/or reduced lactate production compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.

The present disclosure provides a method for producing a heterologous polypeptide comprising the steps of

- 5 a) cultivating a mammalian cell comprising an exogenous deoxyribonucleic acid encoding the heterologous polypeptide optionally under conditions suitable for the expression of the heterologous polypeptide, and
- b) recovering the heterologous polypeptide from the cell or the cultivation medium,

10 wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

In another aspect, the present disclosure provides a method for producing a heterologous polypeptide comprising the steps of

- a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the heterologous polypeptide, and
- 15 b) recovering the heterologous polypeptide from the cell or the cultivation medium,

wherein the expression of the endogenous SIRT-1 gene(s) has been knocked out.

The present disclosure provides a method for producing a recombinant mammalian cell having/with improved and/or increased recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:

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- a) applying a nucleic acid or an enzyme or a nuclease-assisted gene targeting system targeting the endogenous SIRT-1 genes to a mammalian cell to reduce or eliminate or diminish or (completely) knock-out the activity or/and function or/and expression of the endogenous SIRT-1 gene, and
- 25 b) selecting a mammalian cell wherein the activity or/and function or/and expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out,

Patent Claims

1. A method for increasing heterologous polypeptide titer and/or reducing lactate production of a recombinant mammalian cell comprising an exogenous nucleic acid encoding a heterologous polypeptide by knocking out SIRT-1 expression compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.
2. A method for producing a heterologous polypeptide comprising the steps of
 - a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the heterologous polypeptide, and
 - b) recovering the heterologous polypeptide from the cell or the cultivation medium,wherein the expression of the endogenous SIRT-1 gene(s) has been knocked out.
3. A method for producing a recombinant mammalian cell with improved recombinant productivity and/or reduced lactate production, wherein the method comprises the following steps:
 - a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous SIRT-1 genes in a mammalian cell to knock out the activity of the endogenous SIRT-1 gene, and
 - b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1 gene has been knocked out,thereby producing a recombinant mammalian cell with increased recombinant productivity and/or reduced lactate production compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.
4. The method according to any one of claims 1 to 3, wherein the SIRT-1 gene knockout is a heterozygous knockout or a homozygous knockout.
5. The method according to any one of claims 1 to 4, wherein the productivity of the SIRT-1 modified cell is at least 10 % increased compared to a SIRT-1 competent parent mammalian cell.

6. The method according to any one of claims 1 to 5, wherein the reduction of SIRT-1 gene expression is mediated by a nuclease-assisted gene targeting system.
- 5 7. The method according to claim 6, wherein the nuclease-assisted gene targeting system is selected from the group consisting of CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease and TALEN.
8. The method according to any one of claims 1 to 5, wherein the reduction of SIRT-1 gene expression is mediated by RNA silencing.
- 10 9. The method according to claim 8, wherein RNA silencing is selected from the group consisting of siRNA gene targeting and knock-down, shRNA gene targeting and knock-down, and miRNA gene targeting and knock-down.
10. The method according to any one of claims 1 to 9, wherein the heterologous polypeptide is an antibody.
- 15 11. The method according to any one of claims 1 to 10, wherein the SIRT-1 knockout is performed before the introduction of the exogenous nucleic acid encoding the heterologous polypeptide or after the introduction of the exogenous nucleic acid encoding the heterologous polypeptide.
12. The method according to any one of claims 1 to 11, wherein the mammalian cell is a targeted integration host cell.
- 20 13. The method according to any one of claims 1 to 12 wherein the mammalian cell is a CHO cell.