

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 reducing SIRT-1 expression
5 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
 - 10 b) recovering the heterologous polypeptide from the cell or the cultivation medium,
wherein the expression of the endogenous SIRT-1 gene(s) has been reduced.
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:
15
 - 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
 - b) selecting a mammalian cell wherein the activity of the endogenous SIRT-1
20 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 mammalian cell cultivated under the same conditions that has the identical genotype but endogenous SIRT-1 gene expression.
- 25 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.

- 68 -

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.