

USE OF NW_006882456.1 IN CHO CELL GENOME FOR STABLE EXPRESSION OF PROTEINS

TECHNICAL FIELD

5 [0001] The present invention relates to the technical field of genes, and particularly to a CHO cell recombinant gene for stable expression of proteins.

BACKGROUND

[0002] Chinese hamster ovary cell (CHO), as a main cell line in the field of biopharmaceuticals, has been developed into many different types of CHO cell lines, including a cell line that can be used for expanding the copy number of genes. However, there is not a specific positive correlation relationship between the increase of transgenic copy number and the increase of target protein yield. Even if the protein expression is increased, the expression level of most CHO cells is not stable. At present, the commonly used methods to construct stable transfected cells are time-consuming and labor-consuming. This is mainly because a large number of monoclonal screening processes are repeated. Therefore, it is generally expected to develop a method, which can obtain high expressed and stably expressed cells in a short time and can ensure that the constructed recombinant cell lines have the same quality level compared with those constructed by the traditional method so as to guarantee the approval of supervision organizations, in the field of cell line construction.

[0003] The traditional method for constructing a cell line expressing foreign protein is to randomly integrate foreign genes into cell genome, and then carry out

monoclonal cell screening via a series of high expression to obtain high a foreign protein high expression cell line. Due to the diversity of site effect differences, the expression levels of recombinant cells generated by random integration are different, so it takes a long time and many steps to select high expression monoclonal cells.

- 5 The monoclonal cells obtained by random integration can not guarantee the stable expression of polypeptide/protein in the cell passage. Construction of recombinant cells each time needs repeated monoclonal screening, which increases the research and development cost of biopharmaceutical.

[0004] Site effect hinders the efficiency of constructing the recombinant cell line
10 via traditional random integration. Repeated high-expression monoclonal screening is time-consuming, labor-consuming and expensive in cost. How to overcome the site effect and rapidly and efficiently obtain stably expressed monoclonal cells by utilizing a site-specific integration technology has been discussed for many years, but no breakthrough has been made.

15 SUMMARY

[0005] Aiming at the above problems in the prior art, the applicant provides a use of NW_006882456.1 in a CHO cell genome for stable expression of proteins. According to the present application, different protein genes can be introduced at fixed positions in the CHO cell genome and are stably expressed; in addition, in the
20 process of realizing the site-specific integration, there is no need to select monoclonal cells for many times to obtain higher expressed cell lines, thereby saving a lot of time.

[0006] The technical solution of the present invention is as follows:

- [0007] Provided is a use for stable expression of proteins at a site within a CHO cell genome, wherein the site for stable expression of proteins within the CHO cell genome is located at the 1020651th base of CHO cell gene NW_006882456.1;
- 5 [0008] 5'NNNNNNNNNNNNNNNNNNNNNNNGG3' within a range of 1020585-1020690 near the site, which can be identified by CRISPR/Cas9 technology, is a target sequence.
- [0009] The protein is a protein having a molecular weight of less than 160KDa.
- [0010] The protein is one of a polypeptide, a functional protein, an antibody and a fused protein.
- 10 [0011] The target sequence is located at 1020640-1020662th bases near the 1020651th base of CHO cell gene NW_006882456.1;
- [0012] Further, the target sequence is 5'-ACCCTTGTGCCCCAAAGACAGGG-3'.
- [0013] The target sequence is 5'-ACCCTGTCTTTGGGGCACAAGGG-3'.
- 15 [0014] The target sequence is 5'-GTCCTTGGGGCACAAGGGTGAGG-3'.
- [0015] The target sequence is 5'-TTTGGGGCACAAGGGTGAGGTGG-3'.
- [0016] The target sequence is 5'-TTGGGGCACAAGGGTGAGGTGGG-3'.
- [0017] The target sequence is 5'-CAAGGGTGAGGTGGGGTAAATGG-3'.
- [0018] The target sequence is 5'-GGTGAGGTGGGGTAAATGGATGG-3'.
- 20 [0019] The target sequence is 5'-AGGTGGGGTAAATGGATGGTTGG-3'.
- [0020] The target sequence is 5'-GGGGTAAATGGATGGTTGGTTGG-3'.
- [0021] The target sequence is 5'-TGGGGCACAAGGGTGAGGTGGGG-3'.
- [0022] The target sequence is 5'-ACAGAGAAACCCTGTCTTTGGGG-3'.

- [0023] The target sequence is 5'-CACAGAGAAACCCTGTCTTTGGG-3'.
- [0024] The target sequence is 5'-AGTGAGTTCTAAGACAGCCAGGG-3'.
- [0025] The target sequence is 5'-ACACAGAGAAACCCTGTCTTTGG-3'.
- [0026] The target sequence is 5'-GTTTCTCTGTGTAACAGCCCTGG-3'.
- 5 [0027] The target sequence is 5'-ACTCACTCTGTAGACCAGGCTGG-3'.
- [0028] Provided is a targeting vector. The targeting vector comprises the target sequence of 5' NNNNNNNNNNNNNNNNNNNNNNGG3', which can be used for site editing and can be identified by CRISPR/Cas9 technology.
- [0029] The targeting vector is prepared by performing BbsI digestion on
- 10 PSK-u6-gRNA plasmids and then connecting with a target sequence DNA fragment containing a BbsI sticky end.
- [0030] Provided is a recombinant donor vector for expressing foreign genes in CHO cells. The recombinant donor vector contains a homologous arm within a range of 500 bp-800 bp upstream and downstream of the 1020651th base of the site
- 15 NW_006882456.1.
- [0031] The recombinant donor vector is prepared by inserting protein genes into a region between 5' arm and 3' arm of a plasmid, so that a nucleotide sequence is located at the downstream of a promoter and is regulated by the promoter to obtain a recombinant CHO cell expression plasmid.
- 20 [0032] The promoter is one of CMV (Human cytomegalovirus-derived strong mammalian expression promoter), EF-1 α (Human elongation factor 1 α -derived strong mammalian expression promoter), SV40 (Simian vacuolar virus 40-derived mammalian expression promoter), PGK1 (Phosphoglycerate kinase gene-derived

mammalian promoter), UBC (Human ubiquitin C gene-derived mammalian promoter), human beta actin (β -actin gene-derived mammalian promoter) and CAG (strong hybrid mammalian promoter).

5 [0033] Provided is a CHO recombinant cell line for stably expressing foreign proteins. The vector or plasmid is used in the construction process of the recombinant cell line.

[0034] Provided is a method for expressing proteins with CHO cell genes, comprising the following steps:

10 [0035] (1) co-transforming CHO cells using Cas9, the targeting vector and the recombinant donor vector to obtain recombinant CHO cells;

[0036] (2) culturing the recombinant CHO cells on a plate, collecting the supernatant, detecting the expression level, and carrying out suspension domestication on adherent recombinant CHO cells;

15 [0037] (3) culturing the recombinant CHO cells subjected to suspension domestication in a shaking flask, and detecting the expression level of proteins.

[0038] The present invention further provides a method of selecting a stable expression site in CHO cell genome:

20 [0039] 1) Lentiviruses with fluorescent tags are constructed and the titers are deduced. The igk-luc genes are integrated into multiple cloning sites of the pLVX-CMV-MCS-T2A-ZsGreen vector, and then transfected into HEK-293T cells together with two plasmids pSPAX2 and pMD2G. The cell supernatants are collected twice at 48 h and 72 h, and then undergo ultracentrifugation to obtain lentivirus.

[0040] 2) CHO cells are coated on a 6-well plate and cultured overnight. On the

next day, the lentivirus is diluted and CHO cells are infected at low MOI (MOI<1) (virus particles corresponding to each cell). After infection for 96 hrs, the CHO cells are sorted by a flow cytometer, and the cells with the brightest fluorescence intensity are directly inoculated into a 96-well plate. One week later, when the cells have
5 grown into monoclonal colonies, cells are observed under a fluorescence microscope, and the brightest cell colony with normal morphology and growth is selected and transferred into a 24-well plate for amplification culture. When the cells are cultured to a convergence degree near 90%, they are transferred into a 6-well plate for culture, and finally amplified into a culture dish of 10 cm for culture. A part of cells are
10 cryopreserved and the remaining cells continue to undergo amplification culture.

[0041] 3) All the CHO cell gene integration sites of lentivirus are searched by a genome walking technology Lenti-X Integration Site Analysis Kit (Clontech: 631263).

[0042] Several cell lines with the brightest fluorescence intensity, normal cell
15 morphology and growth rate are used as materials. Genomic DNA is subjected to overnight digestion by utilizing three restriction enzymes namely ADraI, SspI and HpaI. Among them, genomic DNA is 2.5 µg and the restriction enzyme is 80U. 100 µL of reaction system is prepared and subjected to overnight digestion at 37 °C (16-18 hours).

20 **[0043]** The enzyme digestion product is purified and recovered by DNA recovery kit. 4.8 µL of genomic DNA and 1.9 µL genomic Walker adaptor (25 µM) and 0.5 µL of T4 ligase are prepared into 8 µL of system for ligation. The system is subjected to overnight ligation at 16 °C. The ligation system is heated at 70°C for 5 min to

inactivate a ligase. 32 μ L of TE buffer is added into each system to prepare a corresponding library.

[0044] Two rounds of nested PCR are carried out on the library to amplify an LTR region and an adjacent genomic region. The relevant PCR reaction operation steps can refer to the specification of Lenti-X Integration Site Analysis Kit (Clontech: 631263).

[0045] Finally, the PCR product is subjected to electrophoresis, and the main bands are cut and recovered, and then sent for sequencing. After all the lentiviral integration information of each cell line is obtained, relevant information about CHO cell line with only single copy lentivirus integration is selected, and alignment is made between its sequence information and CHO-K1 genome information on BLAST to find high-expression integration sites.

[0046] As the database is updated, after sequence alignment, the original site information is changed from the 1020651th base of the CHO cell gene NW_006882456.1 to 148091th base of CHO cell gene NW_003614241.1. The range is changed from 1020585-1020690 to 148052-148157.

[0047] The beneficial technical effects of the present invention are as follows:

[0048] The present invention adopts the site-specific integration method. Site-specific integration of the target gene into the stable expression region can well overcome the problem of uncertainty of integration sites caused by random integration, thereby effectively avoiding the repeated multiple rounds of high expression monoclonal screening, and effectively reducing the research and development time of stable expression of cell lines for biological drug production, and reducing the cost.

[0049] According to the present invention, the protein genes are introduced into the site-specific position of the CHO cell gene and stably expressed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] Fig. 1 is a diagram of the present invention.

5 [0051] Fig.2 is a diagram showing an identification results of CHO cell genes with NGGH 75KDa genes.

[0052] Fig.3 shows sequencing of OoPCR_fwd and OoPCR_rev on CHO cells genes with NGGH 75KDa genes.

[0053] Fig. 4 shows expression of HAS in cells at different passages.

10 [0054] Fig. 5 shows expression of NGGH in cells at different passages.

[0055] Fig.6 shows a mass profile of antibody proteins secreted from each recombinant CHO cell every day.

DESCRIPTION OF THE EMBODIMENTS

[0056] The present invention is described in detail in combination with
15 accompanying drawings and embodiments.

[0057] Fig.1 is a diagram of a donor plasmid used to be integrated into the site and a simulation diagram of how to be site-specifically integrated into the site through homologous recombination. Among them, GOI is a target gene which is integrated into a target site through two homologous recombination arms of 5' arm and 3' arm
20 under the screening pressure of 4 µg/ml puromycin. In addition, the upstream sequence of 5'arm is a negative screening tag which can be used to remove randomly integrated monoclonal cells to ensure that the final obtained cell line is a recombinant CHO cell line subjected to site-specific integration.

[0058] Example 1

[0059] Selection of high expression sites

[0060] NW_006882456.1 (NW_003614241.1) (6# chromosome) is integrated at 1020651 (148091) base and Zsgreen1 gene is integrated to the position. This
5 fluorescent cell is subcultured for no less than 50 generations, and the fluorescence expression level is detected by a flow cytometry. The 50th generation of fluorescence cell still has good green fluorescence protein expression level, and the fluorescence signal can be stably retained during the passage of cells.

[0061] In addition, this fluorescent cell is subjected to suspension domestication,
10 and the expression level of fluorescent protein subjected to suspension domestication is detected again by a flow cytometry. The results show that more than 95% of the recombinant CHO cells which have been suspended for 50 generations still remain the green fluorescent protein expression after suspension. So it can be considered that this site is extremely stable, and does not lose the site-specifically integrated
15 fluorescent protein gene due to cell passage.

[0062] Example 2

[0063] Selection of specific targets

[0064] According to the principle of proximity, the sequence of
5'CCAGCCTGGTCTACAGAGTGAGTTCTAAGACAGCCAGGGCTGTTACACA
20 GAGAAACCCTGTCTTTGGGGCACAAGGGTGAGGTGGGGTAAATGGATGGT
TGGTTGG 3' is input into CRISPRater system to predict and sort out target sequences with low off-target efficiency. Parameters are set as follows: 1) the maximum number of mismatched bases at the beginning 15 bp after NGG is 0; 2) the

number of mismatched bases at 21 bp after NGG is 2.

[0065] According to the above operation, the following sequence with a score of 0.62 is selected as the target sequence according to its score:

[0066] 5'-ACCCTTGTGCCCCAAAGACAGGG-3';

5 [0067] According to CRISPRater system, low efficacy (score < 0.56); medium efficiency (0.56 ≤ score ≤ 0.74); high efficacy (score > 0.74).

[0068] According to CRISPRater evaluation system, all the target sequences in the range of 1020585-1020690 (148052-148157) near the site NW_006882456.1 (NW_003614241.1) have obtained scores of more than 0.56, which are within a range of a
10 medium efficiency or high efficiency, and can be used as 5' NNNNNNNNNNNNNNNNNNNNNNGG 3' target sequence identified by CRISPR/cas9 technology.

[0069] Example 3

[0070] Selection of promoters

15 [0071] The above CMV (human cytomegalovirus-derived strong mammalian expression promoter) at the position of promoter is replaced with different promoters, including common promoters such as EF-1a (Human elongation factor 1 α -derived strong mammalian expression promoter), SV40 (Simian vacuolar virus 40-derived mammalian expression promoter), PGK1 (Phosphoglycerate kinase gene-derived
20 mammalian promoter), UBC (Human ubiquitin C gene-derived mammalian promoter), human beta actin (β -actin gene-derived mammalian promoter) or CAG (strong hybrid mammalian promoter). After detection, the above promoters can regulate the downstream red fluorescent protein gene sequences and express the

corresponding red fluorescent proteins.

[0072] Example 4

[0073] The human serum albumin gene (HAS, 68KDa) is site-specifically integrated into the specific site: in order to construct CRISPR/cas9 mediated homologous recombination at a later stage, sgRNA and Donor Plasmid need to be constructed, and the method is as follows:

[0074] 1. To construct sgRNA, the following sequence is synthesized first

[0075] sgRNA-1fwd 5' TTTGACCCTTGTGCCCCAAAGACAGT 3'

[0076] sgRNA-1rev 5' TAAAACTGTCTTTGGGGCACAAGGGT 3'

10 [0077] 1) PSK-u6-gRNA plasmid is subjected to BbsI digestion and the digested vector is recovered;

[0078] 2) The synthesized fragment is annealed into double strands with sticky ends.

[0079] sgRNA-1fwd (100 μ M) 4 μ L

15 [0080] sgRNA-1rev (100 μ M) 4 μ L

[0081] 10 $\times$ NEB buffer2 2 μ L

[0082] H₂O 10 μ L

[0083] -----

[0084] 20 μ L

20 [0085] Water bath at 95 °C for 5min, naturally cooling to room temperature in the water;

[0086] 3) Fragment ligation and construction of recombinant plasmid

[0087] The recovered linear vector 50ng

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|--|
| Ligation at room temperature for more than 1 h, or Ligation at 4 °C connection overnight |
|--|
- [0088] Annealed fragment 1 μ L
- [0089] 10 $\times$ T4 ligase buffer 1 μ L
- [0090] T4 ligase 1 μ L
- [0091] H₂O
- 5 [0092] -----
- [0093] to 10 μ L
- [0094] 4) Ligation, transformation;
- [0095] 5) The clones are selected and identified by PCR, the primers for identification are:
- 10 [0096] M13-synthesized R primers, and those clones which can be amplified into bands are positive clones.
- [0097] 2. Construction of donor plasmid
- [0098] The specific information of donor plasmid is shown in Fig.1: except for GOI, remaining parts are all synthesized; the sequence within 600bp upstream and
- 15 downstream of the target is the left and right homologous arm sequence information of donor plasmid, and GOI is a process in which HSA genes are integrated onto the donor plasmid through the existing C115 kit from Vazyme Biotech Co.
- [0099] 3. Three plasmids, Cas9 (Technical University of Denmark, donated by Dr. Helene F Kildegaard), SgRNA and Donor plasmid are co-transfected with CHO cells
- 20 cultured at 37°C and 5% CO₂, wherein the molar ratio of the three plasmids is 1:1:1, the transfection reagent is Lipofectamine 3000 (Thermo Fisher Scientific), and the specific transfection process refers to the instruction. Then, 4 μ g/ml puromycin is added into the cells for screening, the process lasts for 10 days totally; monoclonal

cells are sorted by a MoFloXDP FACS machine (Beckman Coulter), and cells without any fluorescence are selected and inoculated into a 96-well plate.

[00100] 4. After growing for 2 weeks, a part of cells are identified by PCR, such as 5' junction PCR, 3' Junction PCR and out-out PCR. Positive cells are reserved.

5 [00101] Example 5

[00102] The glucagon-like peptide-1-human serum albumin fusion protein genes (NGGH, 75KDa) are site-specifically integrated at specific sites: to construct CRISPR/Cas9-mediated homologous recombination at a later stage, sgRNA and Donor Plasmid need to be constructed, for which the method is as below:

10 [00103] 1. Construction of sgRNA, which is the same as in example 4.

[00104] 2. Construction of Donor plasmid.

[00105] The specific information of donor plasmid is as shown in Fig. 1: except for GOI, the remaining parts are all synthesized; the sequence within 600bp upstream and downstream of the target is the left and right homologous arm sequence information
15 of the donor plasmid, and GOI is a process in which NGGH genes are integrated onto the donor plasmid through the existing C115 kit from Vazyme Biotech Co.

[00106] 3. Three plasmids, Cas9, SgRNA and Donor plasmid are co-transfected with CHO cells cultured at 37°C and 5% CO₂, wherein the molar ratio of the three plasmids is 1:1:1, the transfection reagent is Lipofectamine 3000 (Thermo Fisher
20 Scientific), and the specific transfection process refers to the instruction. After then, 4 µg/ml puromycin is added into the cells for screening, the process lasts for 10 days totally. Monoclonal cells are sorted by a MoFloXDP FACS machine (Beckman Coulter), and cells without any fluorescence are selected and inoculated into a 96-well

plate.

[00107] 4. After growing for 2 weeks, a part of cells are identified by PCR, such as 5' junction PCR, 3' Junction PCR and out-out PCR. Positive cells are reserved.

[00108] Example 6

5 [00109] The antibody genes (Avastin, 160KDa) are site-specifically integrated at specific sites: to construct CRISPR/Cas9-mediated homologous recombination at a later stage, sgRNA and Donor Plasmid need to be constructed, for which the method is as below:

1. Construction of sgRNA, which is the same as in example 4.

10 2. Construction of Donor plasmid

The specific information of donor plasmid is as shown in Fig. 1: except for GOI, the remaining parts are all synthesized; the sequence within 600bp upstream and downstream of the target is the left and right homologous arm sequence information of the donor plasmid, and GOI is a process in which Avastin genes are integrated onto
15 the donor plasmid through the existing C115 kit from Vazyme Biotech Co.

[00110] Three plasmids, Cas9 (Technical University of Denmark, donated by Dr. Helene F Kildegaard), SgRNA and Donor plasmid are co-transfected with CHO cells cultured at 37°C and 5% CO₂, wherein the molar ratio of the three plasmids is 1:1:1, the transfection reagent is Lipofectamine 3000 (Thermo Fisher Scientific), and the
20 specific transfection process refers to the instruction. Then, 4 µg/ml puromycin is added into the cells for screening, the process lasts for 10 days totally; monoclonal cells are sorted by a MoFloXDP FACS machine (Beckman Coulter), and cells without any fluorescence are selected and inoculated into a 96-well plate.

[00111] 4. After growing for 2 weeks, a part of cells are identified by PCR, such as 5' junction PCR, 3' Junction PCR and out-out PCR. Positive cells are reserved.

[00112] Fig. 2 is a diagram showing identification results of CHO gene with NGGH 75KDa genes; wherein lanes 1-3 show 5' junction PCR results of three monoclonal cells, and lanes 4-5 show 3' junction PCR results; there are obvious strips in all the lanes, confirming that there is gene knock-in.

[00113] Fig. 3 shows that OoPCR_fwd and OoPCR_rev are utilized for sequencing to determine the sequence accuracy at the junction (for 5' junction and 3' junction, the junction between the 5' upstream and the genome), wherein the sequencing results confirm that GOI is accurately inserted into the target area.

[00114] Test examples

[00115] The three cell lines prepared from examples 4-6 are subjected to ELISA detection to observe whether the targeted protein is expressed and whether it is stably expressed for a long term.

[00116] Detection method: the three detections are all conducted through ELISA. All the selected positive cells are cultured in a 6-well plate to test whether target proteins are stably expressed for a long term. The used kits are human albumin ELISA Kit (RK00157) and human IgG (Total) ELISA Kit (RK00393) from Abclonal Co..

[00117] Figs. 4 and 5 show the cell expression of HAS and NGGH at different passages.

[00118] It can be seen from the figures that NGGH and HSA can stably express corresponding genes within 50 passages in the plate, and the expression levels of the

target protein by the sorted three NGGH site-specific integrated cell lines and two HAS site-specific integrated cell lines are close to each other.

[00119] Fig.6 shows the mass of antibody proteins secreted from each recombinant CHO cell every day. Obviously, cells can express and secrete corresponding proteins
5 under different passages, showing good stability. The results coincide with previous fluorescent cell results. The results show that at this site, CRISPR/cas9 mediated site-specific integration can be carried out and the corresponding protein is stably expressed.

[00120] After the 5'-ACCCTTGTGCCCCAAAGACAGGG-3' sequence is selected
10 for the above test, good results are obtained. Therefore, it is proposed that the target sequences of claims 5-19 can successfully construct site-specifically integrated stable expression cell lines, and can stably express the target proteins.

Claims

WHAT IS CLAIMED IS:

1. A use for stable expression of proteins at a site within a CHO cell genome,
wherein the site for stable expression of proteins within the CHO cell genome is
5 located at the 1020651th base of CHO cell gene NW_006882456.1;

5' NNNNNNNNNNNNNNNNNNNNNNNNGG3' within a range of
1020585-1020690 near the site, which can be identified by CRISPR/Cas9 technology,
is a target sequence.

10 2. The use according to claim 1, wherein the protein is a protein having a
molecular weight of less than 160KDa.

3. The use according to claim 1, wherein the protein is one of a polypeptide, a
functional protein, an antibody and a fused protein.

15 4. The use according to claim 1, wherein the target sequence is located at the
1020640-1020662th bases near the 1020651th base of CHO cell gene
NW_006882456.1; the target sequence is
5'-ACCCTTGTGCCCCAAAGACAGGG-3'.

20 5. The use according to claim 1, wherein the target sequence is
5'-ACCCTGTCTTTGGGGCACAAGGG-3'.

6. The use according to claim 1, wherein the target sequence is
5'-GTCTTTGGGGCACAAGGGTGAGG-3'.

7. The use according to claim 1, wherein the target sequence is
5 5'-TTTGGGGCACAAGGGTGAGGTGG-3'.

8. The use according to claim 1, wherein the target sequence is
5'-TTGGGGCACAAGGGTGAGGTGGG-3'.

10 9. The use according to claim 1, wherein the target sequence is
5'-CAAGGGTGAGGTGGGGTAAATGG-3'.

10. The use according to claim 1, wherein the target sequence is
5'-GGTGAGGTGGGGTAAATGGATGG-3'.

15 11. The use according to claim 1, wherein the target sequence is
5'-AGGTGGGGTAAATGGATGGTTGG-3'.

12. The use according to claim 1, wherein the target sequence is
20 5'-GGGGTAAATGGATGGTTGGTTGG-3'.

13. The use according to claim 1, wherein the target sequence is
5'-TGGGGCACAAGGGTGAGGTGGGG-3'.

14. The use according to claim 1, wherein the target sequence is 5'-ACAGAGAAACCCTGTCTTTGGGG-3'.

5 15. The use according to claim 1, wherein the target sequence is 5'-CACAGAGAAACCCTGTCTTTGGG-3'.

16. The use according to claim 1, wherein the target sequence is 5'-AGTGAGTTCTAAGACAGCCAGGG-3'.

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17. The use according to claim 1, wherein the target sequence is 5'-ACACAGAGAAACCCTGTCTTTGG-3'.

18. The use according to claim 1, wherein the target sequence is 15 5'-GTTTCTCTGTGTAACAGCCCTGG-3'.

19. The use according to claim 1, wherein the target sequence is 5'-ACTCACTCTGTAGACCAGGCTGG-3'.

20 20. A targeting vector, wherein the targeting vector comprises the target sequence of 5' NNNNNNNNNNNNNNNNNNNNNNGG3' according to claim 1, which can be used for site editing and can be identified by CRISPR/Cas9 technology.

21. A recombinant donor vector for expressing foreign genes in CHO cells,

wherein the recombinant donor vector comprises a homologous arm within a range of 500 bp-800 bp upstream and downstream of the 1020651th base of the site NW_006882456.1 according to claim 1.

5 22. The recombinant donor vector according to claim 21, wherein the vector is prepared by inserting protein genes into a region between 5' arm and 3' arm of a plasmid, so that a nucleotide sequence is located at the downstream of a promoter and is regulated by the promoter to obtain a recombinant CHO cell-expressed plasmid.

10 23. A CHO recombinant cell line for stably expressing foreign proteins, wherein the vector or plasmid according to claim 20, 21 or 22 is used in the construction process of the recombinant cell line.

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