# Post-print: Construction of a Chinese Hamster Ovary Cell Line Stably Expressing Human Albumin Using CRISPR/Cas9 Technology

**Authors:** Zhou Songtao, Chen Yun, Gong Xiaohai, Jin Jian, Li Huazhong

**Date:** 2019-01-17T00:00:00+00:00

## Abstract

CHO expression cell lines constructed via random integration of target genes frequently exhibit expression instability during long-term passaging, primarily due to position effects resulting from insertion of the target gene into unstable chromosomal regions. To resolve this problem, CRISPR/Cas9-mediated homologous recombination was employed to directly integrate the target gene into stable expression regions of CHO cell chromosomes, thereby overcoming long-term expression instability in CHO expression cell lines caused by position effects. Using this technology, we successfully obtained a total of 2 site-specific integration cell lines carrying the exogenous gene (human serum albumin gene); Western blot analysis revealed that the product in the cell supernatant exhibited human serum albumin antigenicity. Under adherent culture conditions, the two cell lines at passages 3, 12, 23, 35, and 50 exhibited comparable daily HSA expression levels, all maintained at approximately 0.5 pg/cell/day. One expression cell line was selected, and following suspension adaptation, under batch culture conditions, the expression concentration of suspension cells at passages 1, 25, and 50 remained stable at 13-14 mg/L in shake flasks. These results demonstrate the feasibility of site-specific integration of exogenous genes within the CHO cell genome; and following integration of exogenous genes into stable expression regions, the recombinant cell lines exhibit stable long-term expression of exogenous genes.

## Full Text

## Preamble

**Using CRISPR/Cas9 Technology to Construct a Human Serum Albumin Gene-Expressing Chinese Hamster Ovary Cell Line**

ZHOU Song-tao[1], CHEN Yun[2], GONG Xiao-hai[2], JIN Jian[2], *LI Hua-zhong[1]*
(1. School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China; 2. School of Pharmaceutical Sciences, Jiangnan University, Wuxi, Jiangsu 214122, China)

## Abstract

CHO expression cell lines constructed through random integration of target genes often exhibit unstable expression during long-term passaging due to insertion of the target gene into unstable chromosomal regions, a phenomenon primarily caused by position effects. To address this issue, we utilized CRISPR/Cas9-mediated homologous recombination to directly integrate the target gene into stable expression regions of the CHO cell chromosome, thereby overcoming long-term expression instability caused by position effects. Using this technology, we obtained two site-specific integration cell lines of the exogenous gene (human serum albumin gene). Western blot results demonstrated that the product in the cell supernatant possessed human serum albumin antigenicity. For both cell lines in adherent culture, the daily HSA expression mass per cell remained similar across passages 3, 12, 23, 35, and 50, consistently maintained at approximately 0.5 pg/cell/day. One selected expression cell line was adapted to suspension culture, and under batch culture conditions, the expression concentration in shake flasks remained stable at 13–14 mg/L for passages 1, 25, and 50. This study demonstrates the feasibility of site-specific integration of exogenous genes into the CHO cell genome and shows that recombinant cell lines can achieve stable long-term expression of exogenous genes when integrated into stable expression regions.

**Keywords:** CHO; site-specific integration; CRISPR/Cas9; protein expression
**Classification:** Q819
**Funding:** National High-Tech Development Project (2014AA021003), National High-Tech Development Project (2015AA020802)
**Corresponding Authors:** Jin Jian, Email: jianjin@jiangnan.edu.cn; Li Hua-zhong, Email: hzhli@jiangnan.edu.cn

---

Over the past three decades, Chinese hamster ovary (CHO) cells have been widely used for expressing various therapeutic proteins, including recombinant antibodies, monomeric proteins, and fusion proteins, establishing CHO as the predominant production cell line in biopharmaceutical manufacturing [1]. CHO cells are highly valued in biopharmaceutical applications for several reasons: first, they offer exceptional safety (the CHO genome carries no human viruses); second, CHO cells can be adapted to serum-free suspension culture and achieve rapid, high-density growth in such media; and finally, recombinant proteins expressed in CHO cells can obtain post-translational modifications similar to human proteins [2–4]. Traditional expression cell line construction relies on random integration of transgenes into the genome followed by multiple rounds

of monoclonal screening to obtain final expression cell lines. Although this method is straightforward, uncontrolled random integration creates substantial phenotypic variation among different monoclonal cells, necessitating extensive effort to screen for stably expressing cell lines [5, 6]. Developing a rational cell line construction method to circumvent the instability caused by random gene insertion represents the future direction of cell line development. It is generally accepted that site-specific integration (SSI) of transgenes into stable expression regions of the cell genome can effectively avoid instability from position effects, enabling rational construction of expression cell lines [1, 3, 6–8].

Site-specific integration of target genes can be achieved using recombinase systems such as Cre/LoxP and Flp/FRT [9–11]; however, these methods require pre-integration of recombination sites into high-expression "hot spots" before subsequent site-specific integration of the target gene, making the process relatively complex [12–13]. Alternatively, CRISPR/Cas9-mediated site-specific integration offers a more direct approach. The CRISPR/Cas9 system is a widely utilized gene editing platform that enables rapid genome editing across nearly all species [14–15]. The active cleavage complex in CRISPR/Cas9 consists primarily of CRISPR-RNA (crRNA), trans-activating crRNA (tracrRNA), and Cas9 nuclease, which together induce DNA double-strand breaks (DSBs). Following DSB formation, two main DNA repair pathways are activated: non-homologous end-joining (NHEJ) and homology-directed repair (HDR) [6, 16]. When donor DNA containing sequences homologous to regions flanking the DSB is provided, the gene of interest (GOI) can be precisely inserted into the genomic DSB region via HDR [17]. Previous reports indicate that CRISPR/Cas9-mediated HDR targeting efficiency is approximately 10% [6]. In this study, we applied this method to achieve site-specific integration of our target gene.

Prior to this work, our research group established a method for screening stable expression sites in the CHO genome and identified several potential stable expression loci [24]. Here, we integrated the human serum albumin (HSA) gene into a previously reported stable expression site [24] to verify whether exogenous genes integrated at this locus could achieve stable expression.

## 1.1 Materials and Reagents

Codon-optimized Cas9 expression plasmid [6] was a gift from Dr. Kildegaard at the Technical University of Denmark; pSK-U6-gRNA expression plasmid was provided by Professor Lu Daru' s group at Fudan University; HSA donor plasmid was synthesized by Sangon Biotech; CHO-K1 cell line was purchased from ATCC; NEB Buffer 2 and BbsI-HF restriction enzyme were from NEB; T4 ligase was from Promega; Lipofectamine 3000, Ham' s F12K medium, and fetal bovine serum were from Thermo Fisher Scientific; nitrocellulose membranes were from Pall; anti-HSA antibody was from Gentex; bovine anti-rabbit secondary antibody was from Santa Cruz; DNA extraction kit, gel extraction kit, and proteinase K were from Tiangen Biotech (Beijing); puromycin, Triton, SDS-PAGE loading buffer, precast gels, skim milk, and TBST buffer were from Sangon

Biotech (Shanghai); PCR reagents were from Vazyme (Nanjing); ECL substrate was from Sheng' er (Shanghai); M2 and M4 suspension cell culture media were from Kangju (Suzhou); urinary microalbumin detection kit (latex turbidimetric method) was from Mingdian (Shanghai); and Human Serum Albumin ELISA kit was from Aibokang (Wuhan).

## 1.2 Instruments and Equipment

SimpliAmp A24811 PCR thermocycler (Life Technologies), Mini-PROTEAN Tetra electrophoresis cell, Trans-Blot transfer cell, Gel-Doc XR system imager, and 680 microplate reader (Bio-Rad), MoFlo XDP FACS cell sorter (Beckman Coulter).

## 1.3.1 Construction of sgRNA Expression Plasmid and Evaluation of sgRNA-Guided Cas9 Targeting Efficiency

The target recognition sequence was located within the Kcmf1 gene in NW_006880285.1. To construct the sgRNA expression plasmid, two oligonucleotides were synthesized: sgRNA-1fwd: 5'-TTTGGAAAGAAGGTCTGATATCAAGT-3' and sgRNA-1rev: 5' -TAAAACTTGATATCAGACCTTCTTTC-3' . These oligonucleotides were annealed in a reaction mixture containing 4 L of 100 M sgRNA-1fwd, 4 L of 100 M sgRNA-1rev, 2 L of NEB Buffer 2, and 10 L of H2O, incubated at 95°C for 5 minutes, then cooled gradually to room temperature. The pSK-U6-gRNA plasmid vector was linearized with BbsI-HF at 37°C for 4 hours and purified using a gel extraction kit. The annealed oligonucleotides were ligated into the pSK-U6-gRNA vector using T4 ligase at 16°C overnight. The codon-optimized Cas9 plasmid and constructed sgRNA plasmid were co-transfected into CHO-K1 cells using Lipofectamine 3000. After three days, cellular DNA was isolated and used as template for PCR amplification and sequencing of sequences flanking the target site.

## 1.3.2 Cell Culture, Transfection, and Stable Cell Line Construction

CHO-K1 cells were cultured in complete medium (Ham' s F12K + 10% FBS) at 37°C with 5% CO . Cas9 plasmid (without selection marker), sgRNA plasmid (without selection marker), and HSA donor plasmid (containing a positive selection marker, puromycin resistance gene, and a negative selection marker, cop-GFP gene) were transfected at a 1:1:1 molar ratio into cells in 6-well plates using Lipofectamine 3000. Puromycin (4 g/mL) was added on day 3 as selection pressure to obtain stable cell pools. After 10 days of selection, cells were collected and single-cell clones were sorted by flow cytometry. To exclude random integration events, only non-fluorescent single cells were sorted and seeded into 96-well plates containing 100 L complete medium per well.

### 1.3.3 DNA Isolation and PCR Amplification

Collected cells were lysed in 100 L of 1% Triton solution with 20 L proteinase K, incubated at 56°C for 1 hour, then heated at 95°C for 5 minutes. After centrifugation at 13,000 × g for 10 minutes, the supernatant was used as template DNA for 5' and 3' junction PCR and out-out PCR. The 5' and 3' junction PCRs were used to verify site-specific integration, while out-out PCR determined whether the isolated clones were homozygous or heterozygous. PCR conditions were as follows: for 5' /3' junction PCR, initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 3 minutes, with a final extension at 72°C for 5 minutes. For out-out PCR: 95°C for 3 minutes, 30 cycles of 95°C for 15 seconds, 66°C for 15 seconds, and 72°C for 6 minutes, and final extension at 72°C for 5 minutes. For nested PCR, the out-out PCR product served as template with conditions: 95°C for 3 minutes, 30 cycles of 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 2 minutes, and final extension at 72°C for 5 minutes. All PCR products were sequenced. Primer sequences are listed in Table 1 .

**Table 1: PCR primers for site-specific integration verification**

| Primer Name | Sequence (5' -3' ) | Application |
|---|---|---|
| OoPCR_fwd | GCTACTCACCAAGTGCAGC | 5' junction PCR, out-out PCR primer and sequencing |
| HSA_rev | GGCAGCTTTATCAGCAGCTTG | 5' junction PCR primer |
| Puro_fwd | GAGGAAGTCTTCTAACATGCG | 3' junction PCR primer |
| OoPCR_rev | GGGTGGGCTAAGTACAAGG | 3' junction PCR, out-out PCR primer and sequencing |
| HSA_fwd | GGTTGATGTGATGTGCACTC | Out-out PCR primer |
| EF1_rev | CGGCGACTACTGCACTTATA | Out-out PCR primer |

### 1.3.4 Western Blot, ELISA, and Latex Turbidimetric Assay

For adherent cells, 250,000 cells were seeded in 6-well plates. After reaching confluence, the medium was replaced; after 24 hours, the supernatant was collected, mixed with SDS-PAGE loading buffer, and heated to 100°C for 10 minutes. Samples were electrophoresed on SDS-PAGE gels at 200 V for 40 minutes, then transferred to nitrocellulose membranes at 100 V for 70 minutes. Membranes were blocked with 5% skim milk in TBST for 2 hours at room temperature, then incubated sequentially with anti-HSA antibody and secondary antibody. Finally, membranes were developed with ECL substrate and imaged.

HSA concentration in adherent cell supernatants was measured by seeding

250,000 cells per well in 6-well plates. After confluence, the medium was replaced with fresh complete medium; after 24 hours, supernatants were collected for ELISA analysis according to the manufacturer' s protocol. Based on approximately 1.2 million cells per well at confluence, the average HSA mass expressed per cell per day was calculated.

For suspension cells, cells were seeded at 10  cells/mL in 20 mL M2+M4 (1:1) medium and cultured at 37°C, 5% CO , and 100 rpm for 6 days. HSA concentration in the supernatant was measured by latex turbidimetric assay according to the manufacturer'  s protocol.

### 1.3.5 Cell Suspension Adaptation

Adherent cells were initially cultured in complete medium. To adapt cells to suspension culture, M2+M4 (1:1) medium was gradually substituted for the original complete medium. Once cells were fully adapted to M2+M4 (1:1) medium, they were transferred to shake flasks for culture at 100 rpm. If cell density fell below 10  cells/mL due to cell death, adherent cells were added to maintain a minimum density of 10  cells/mL.

## 2 Results and Analysis

### 2.1 Site Editability Verification

Based on a previously identified stable expression site [24], we planned to integrate the HSA gene near this locus. A potential CRISPR/Cas9 target sequence adjacent to the stable expression region was identified: 5' -GAAAGAAGGTCTGATATCAAAGG-3' . To confirm whether this site could be used for subsequent site-specific integration studies, we verified the targeting efficiency of this sgRNA. Sequencing results are shown in Figure 1 [Figure 1: see original paper].

**Figure 1** Sequencing chromatogram showing that sgRNA could guide Cas9 to specifically cleave the target genomic sequence.

The sequencing chromatogram exhibited persistent overlapping peaks beginning at the sgRNA target sequence and continuing to the end of the read, indicating insertions/deletions at the target site. This confirms that the target sequence can mediate site-specific integration via CRISPR/Cas9-induced HDR.

### 2.2 Construction of Exogenous Gene Knock-In Cell Lines via Site-Specific Integration

The knock-in system comprised three components: Cas9 expression plasmid, sgRNA expression plasmid, and HSA donor plasmid. The donor plasmid design is illustrated in Figure 2 [Figure 2: see original paper]: it contains 600 bp homology arms flanking the Cas9 cleavage site (CRISPR + PAM), with the puromycin resistance gene and HSA gene positioned between the arms, and the

cop-GFP gene placed outside the arms to exclude random integration events. Upon precise integration of the HSA gene at the target site, cells would express HSA and puromycin resistance but not green fluorescence.

**Figure 2** HSA donor plasmid map and schematic of HSA gene integration into a hot spot in the CHO genome.

After transfecting the three plasmids, stable cell pools were obtained through puromycin selection. Non-fluorescent cells were then sorted by FACS into single cells in 96-well plates. Following one week of culture, DNA from different clones was extracted for 5' and 3' junction PCR analysis. We successfully obtained two site-specific integration monoclonal cell lines, both showing prominent PCR bands for 5' and 3' junctions (Figure 3a [Figure 3: see original paper]–3b). The amplification products were approximately 1.5 kb, consistent with our design (Figure 3a). Sequencing of the 5' /3' genome-donor boundaries confirmed precise integration of the targeting cassette at the target site (Figure 3c). Out-out PCR revealed both cell lines were heterozygous, with the targeting cassette fully integrated at the locus: the expected amplification product was obtained (wild-type amplicon: 1.2 kb + targeting cassette: 4.3 kb  5.5 kb, Figure 3d, lanes 1–2). Additionally, out-out PCR products were sequenced (Table 2 ), verifying the accuracy of the knocked-in HSA sequence.

**Figure 3** Verification of HSA site-specific integration monoclonal cell lines. (a) 5' junction PCR results of two knock-in cell lines; (b) 3' junction PCR results of two knock-in cell lines; (c) Sanger sequencing results of 5' /3' junction PCR products; (d) out-out PCR results of two knock-in cell lines.

**Table 2: HSA site-specific integration sequencing results (including CMV promoter and SV40 poly(A) sequence)**

[The full HSA integration sequence is preserved here exactly as in the original]

## 2.3 Expression Level Detection of Site-Specific Integration Cell Lines

After successful site-specific integration of the HSA gene into the stable expression site, Western blot was performed to verify accurate expression. Results showed a protein band of approximately 67 kDa in the supernatants of both cell lines (Figure 4a [Figure 4: see original paper]), consistent with the molecular weight of HSA, confirming that the integrated HSA gene was expressed and secreted correctly. ELISA analysis revealed that both recombinant cell lines stably secreted HSA protein at approximately 0.5 pg/cell/day across different passages (p3, p12, p26, p35, p50) (Figure 4b).

**Figure 4** Western blot and expression level analysis of HSA site-specific integration cell lines. (a) Identification of secreted protein in supernatants from two HSA monoclonal cell lines; (b) HSA expression and secretion levels of two monoclonal cell lines at different passages.

## 2.4 Cell Suspension Adaptation and Batch Expression

Since industrial production primarily uses CHO suspension cells, we adapted the adherent cells to suspension culture to evaluate expression levels and stability for industrial application potential. Successful suspension adaptation was defined as the ability of cell density to double within two days after inoculation into shake flasks. For one selected HSA cell line, cell density doubled on the second day, and after dilution, doubled again the following day. Extended observation confirmed successful suspension adaptation (Figure 5a [Figure 5: see original paper]).

Based on previous results [24], exogenous genes integrated at this stable expression site continue to express stably after suspension adaptation. Therefore, we measured HSA expression levels directly after suspension adaptation. Figure 5b shows that HSA expression levels remained stable at 13–14 mg/L across different passages, demonstrating excellent expression stability after HSA gene integration at the target site.

**Figure 5** Suspension adaptation of HSA site-specific integration cell line and expression level analysis. (a) Cell density of HSA knock-in cell line recovered to original levels by day 2 after 2-fold dilution; (b) HSA expression levels of suspension cells at different passages.

Our research group previously identified stable expression sites in the CHO genome using lentivirus-based random integration [24]. This study employed CRISPR/Cas9 technology to site-specifically integrate the HSA gene near one such site and evaluated its expression level to assess the potential of this technology for future industrial applications.

Compared with traditional random integration methods, site-specific integration of target genes into stable expression loci offers several advantages. First, cell line construction no longer requires extensive monoclonal screening. Figure 4b shows that two different cell lines obtained through site-specific integration both expressed HSA at approximately 0.5 pg/cell/day, consistent with previous reports [15] that protein expression levels are generally consistent when exogenous genes are integrated at a specific site. Therefore, expression cell lines generated via site-specific integration eliminate the need for additional rounds of monoclonal selection to identify high producers. Furthermore, previous studies reported that model cells maintained stable fluorescent protein expression after 50 passages post-suspension adaptation, indicating excellent stability of this locus. In contrast, random integration lacks such reference model cells, making it impossible to confirm whether selected monoclonal cells can maintain stable exogenous gene expression after suspension adaptation. Consequently, cell lines constructed by random integration may exhibit unstable expression following suspension adaptation.

Additionally, we found that both positive monoclonal cell lines obtained after HSA integration at the stable expression site were heterozygous, which differs

significantly from previous reports showing homozygous integration rates exceeding 80% [6]. Although heterozygous cell lines contain one fewer gene copy than homozygous lines, this characteristic can be leveraged to integrate a fixed gene cassette such as LoxP or FRT at the hot spot to further enhance site-specific integration efficiency [19]. If the cassette were integrated homozygously, unpredictable recombination events could occur during subsequent integration steps, which would be detrimental for constructing stable expression cell lines in industrial applications.

To further improve expression levels, optimization of secretion signals could be considered to increase target protein yield [23]. Additionally, optimization of fed-batch processes through pathway engineering could achieve higher protein expression [3]. Other gene screening methods such as CRISPR/Cas9-based loss-of-function screening could identify targets for further enhancing exogenous gene expression. These approaches are more meaningful for site-specific integration-based cell lines than for random integration, as genetic diversity in random integration reduces target universality. Numerous studies have reported strategies for identifying such targets, which will be valuable for further improving expression levels in CHO site-specific integration cell lines [20–22].

In summary, this study successfully detected stable HSA expression after site-specific integration into a stable expression region. We anticipate that with continued technological refinement, this novel construction method will be increasingly adopted by industrial manufacturers.

## References

[1] Wurm F M. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnology*, 2004, 22(11): 1393–1398.
[2] Wurm F M, Hacker D. First CHO genome. *Nat Biotechnology*, 2011, 29(8): 718–720.
[3] Kim J, Kim Y G, Lee G. CHO cells in biotechnology for production of recombinant proteins: current state and further potential. *Applied Microbiology & Biotechnology*, 2012, 93(3): 917–930.
[4] Fischer S, Handrick R, Otte K. The art of CHO cell engineering: A comprehensive retrospect and future perspectives. *Biotechnology Advances*, 2015, 33(8): 1878–1896.
[5] Wilson C, Bellen H J, Gehring W J. Position Effects on Eukaryotic Gene Expression. *Annual Review of Cell Biology*, 1990, 6(1): 679–714.
[6] Lee J S, Kallehauge T B, Pedersen L E, et al. Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway. *Scientific Reports*, 2015, 5: 8572.
[7] Jakociunas T, Jensen M K, Keasling J D. CRISPR/Cas9 advances engineering of microbial cell factories. *Metabolic Engineering*, 2016, 34: 44–59.
[8] Lee J S, Grav L M, Pedersen L E, et al. Accelerated homology-directed targeted integration of transgenes in Chinese hamster ovary cells via CRISPR/Cas9 and fluorescent enrichment. *Biotechnology and Bioengineering*, 2016, 113(11):

2518–2523.
[9] Kito M, Itami S, Fukano Y, et al. Construction of engineered CHO strains for high-level production of recombinant proteins. *Applied Microbiology & Biotechnology*, 2002, 60(4): 442–448.
[10] Gao J, Cha S, Jonsson R, et al. Detection of anti-type 3 muscarinic acetylcholine receptor autoantibodies in the sera of Sjogren' s syndrome patients by use of a transfected cell line assay. *Arthritis Rheum*, 2004, 50(8): 2615–2621.
[11] Huang Y, Li Y, Wang Y G, et al. An efficient and targeted gene integration system for high-level antibody expression. *Journal of Immunological Methods*, 2007, 322(1): 28–39.
[12] Schwenk F, Baron U, Rajewsky K, et al. A cre-transgenic mouse strain for the ubiquitous deletion of loxp-flanked gene segments including deletion in germ cells. *Nucleic Acids Research*, 1995, 23(24): 5080–5081.
[13] Yang-Nim P, Daniel M, Eisenberg E, et al. Application of the FLP/FRT system for conditional gene deletion in yeast *Saccharomyces cerevisiae*. *Yeast*, 2011, 28(9): 673–681.
[14] Cong L, Ran F A, Cox D, et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science*, 2013, 339(6121): 819–823.
[15] Lee J S, Grav L M, Lewis N E, et al. CRISPR/Cas9-mediated genome engineering of CHO cell factories: Application and perspectives. *Biotechnology Journal*, 2015, 10(7): 979–994.
[16] Mali P, Yang L, Esvelt K M, et al. RNA-guided human genome engineering via Cas9. *Science*, 2013, 339(6121): 823–826.
[17] Hockemeyer D, Soldner F, Beard C, et al. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat Biotechnology*, 2009, 27(9): 851–857.
[18] Stemmer M, Thumberger T, Del S, et al. CCTop: An Intuitive, Flexible and Reliable CRISPR/Cas9 Target Prediction Tool. *PLoS One*, 2015, 10(4): e0124633.
[19] Takata Y, Kondo S, Goda N, et al. Comparison of efficiency between FLPe and Cre for recombinase-mediated cassette exchange in vitro and in adenovirus vector production. *Genes to Cells*, 2011, 16(7): 765–777.
[20] Gilbert L A, Horlbeck M A, Adamson B, et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell*, 2014, 159(3): 647–661.
[21] Shalem O, Sanjana N E, Hartenian E, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*, 2014, 343(6166): 84–87.
[22] Wang T, Wei J J, Sabatini D M, et al. Genetic screens in human cells using the CRISPR-Cas9 system. *Science*, 2014, 343(6166): 80–84.
[23] Xu D, Chen Y, Jin J. Effect of signal peptide on the expression and secretion of hepatocyte growth factor in CHO. *Chinese Journal of Cell Biology*, 2016, 38(12): 1–7.
[24] Zhou S T, Ding X F, Yang L, et al. Discovery of stable expression hot spots in the genome of Chinese hamster ovary cells using lentivirus-based random integration method. [2018-11-14]. https://doi.org/10.1101/467936