ACCEGEN
BIOTECHNOLOGY

Overexpression Stable Cell Lines Product Manual

Overexpression Stable Cell Lines Product Information

1. Description

For the generation of overexpression stable cell lines, the target gene is cloned into the corresponding plasmid or viral vector using genetic engineering technology, which is then transfected into the cells, allowing for high and stable expression of the target gene. The technical methods include lentiviral transduction and plasmid transfection (piggyBac transposon system).

With our customized service, you can get our high-quality customer service experience. The overexpression stable cell line is an important research tool for target gene function discovery and research (e.g., signaling pathway research), target validation, antibody research, constructing models, drug screening and assay development.

2. Overexpression Stable Cell Line Generation Service

Service	Technical methods	Gene expression evaluation	Application	Delivery
Overexpression Stable Cell Line Generation	Lentivirus (Sequence length <9kb) piggyBac (Sequence length 9kb~ 15kb)	QPCR	Gene function research Protein engineering Recombinant antibodies development Drug discovery	Polyclonal cell line (Default) or Monoclonal cell line

3. Technical advantages

Our lentiviral vector has high infection efficiency and can quickly and efficiently integrate the target gene into the host cell genome. The overexpression efficiency of the target gene will also be significantly improved to achieve long-term, stable and significant expression effects. If you require larger fragments, our piggyBac transposition system offers the flexibility of gene editing to accomodate such needs. In addition, we can provide gene expression and cellular function verification services. Finally, professional laboratory reports and quality inspection reports will be delivered.

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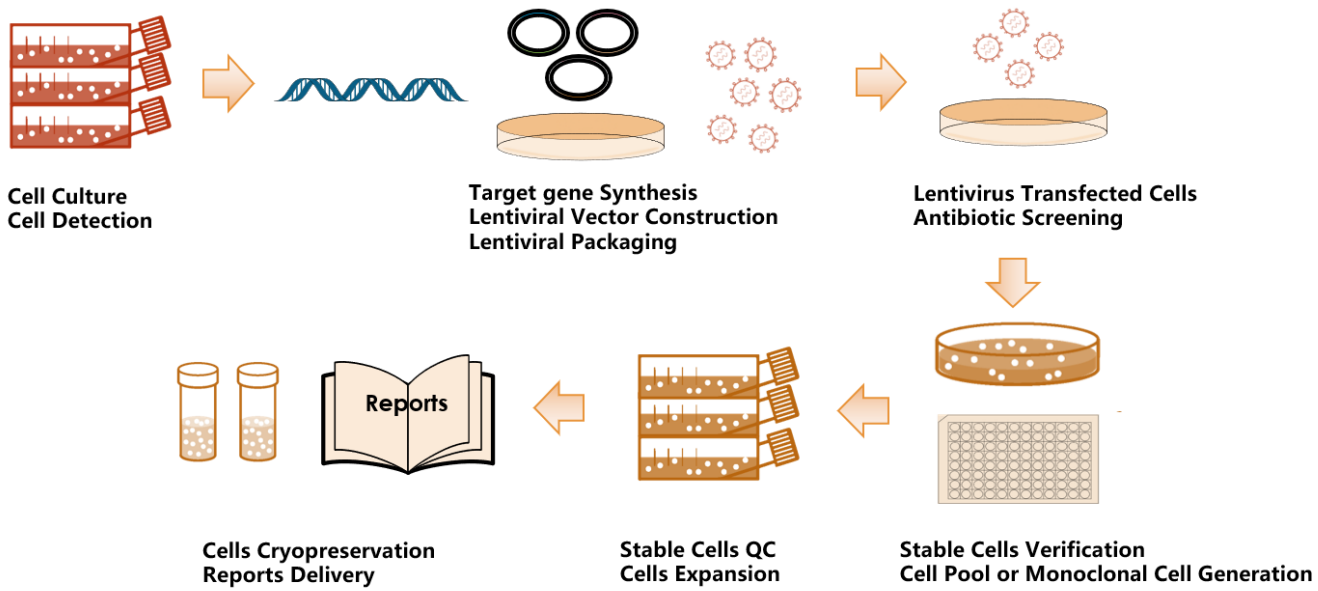
4. Experimental process

Lentivirus transduction:

Cell Culture and Detection (2-3 weeks)	Vector Construction and Lentivirus packaging (3-5weeks)	Lentivirus-mediated cell transduction (3-6 weeks)	Stable cell line QC (1 week)
• Bacteria and mycoplasma detection • The genotype of the target cells detection	• Target gene synthesis and codon optimization • Lentiviral vector construction • Lentiviral packaging and lentiviral titer determination	• Drug-resistance selection • Stable cell generation(cell pool or monoclonal cell)	• QPCR & stability test • Delivery of cell lines and reports



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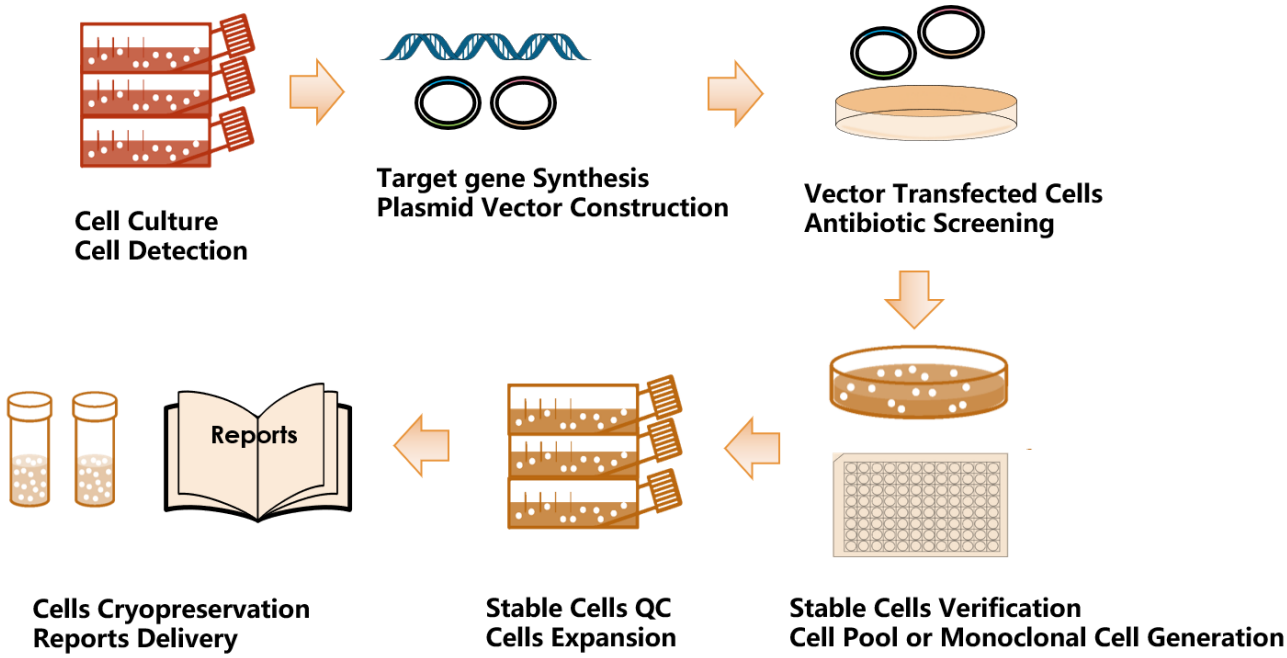
Plasmid transfection:

Cell Culture and Detection (2-3 weeks)	Vector Construction (3-5weeks)	Plasmid-mediated cell transduction (3-6 weeks)	Stable cell line QC (1 week)
• Bacteria and mycoplasma detection • The genotype of the target cells detection	• Target gene synthesis and codon optimization • The transposon donor plasmid and transposase helper plasmid construction	• Plasmids transfection into the target cells • Drug-resistance selection and stable cell generation(cell pool or monoclonal cell)	• QPCR & stability test • Delivery of cell lines and reports



5. Application Operations and Detection Methods

Target gene function discovery and research

a) Construct a gene overexpression stable cell line
b) Culture and expand the overexpression stable cell line with the suitable medium and serum
c) Group cells according to experimental requirements
d) Study the effect of gene overexpression on cells through various experimental methods in terms of phenotype or gene function.
e) Detection Methods:

RT-qPCR The process of extracting cellular RNA can be operated according to the nucleic acid extraction kit, and the target gene product can be obtained after reverse transcription. You can use SYBR Green or TaqMan Probe

method to detect the expression level of the target gene.

Western blotting Cells were lysed using RIPA or other reagents, and the cell supernatant can be taken after centrifugation to determine the concentration of the target protein. Then the difference in protein expression between overexpression cells and control cells can be obtained.

RT-PCR The process of extracting cellular RNA can be operated according to the nucleic acid extraction kit, and the target gene product can be obtained after reverse transcription and PCR amplification. Detect and observe the expression results of the target genes by agarose gel electrophoresis and gel imaging system.

Flow Cytometry Inoculate cells (5×10^5 cells/ml) into 6-well plates and culture them for 24 hours. Add antibodies or stimulating factors required for the experiment to the cells and incubate for several hours. Finally, a flow cytometer can be used to detect cell cycle and apoptosis, etc.

Statistical analysis Experimental data can be analyzed using software such as SPSS, GraphPad Prism, Flow Jo, and Excel.

6. Delivery of Cell Lines and Report

1 vial of stable cell line (1×10^6 cells/vial) and qPCR test report will be delivered to the customer. If the project requires the customer to provide a host cell, we will request the customer to fill in AcceGen Custom Stable Cell Line Service Requisition Form.

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7. Experimental case

Case1: THP 1-hMR1-Overexpression Cell Line

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

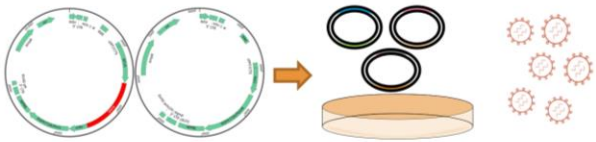

Fig.1a Lentiviral preparation

3. Lentivirus-mediated cell transduction and drug-resistance selection
4. Stable cell line QC (qPCR results)

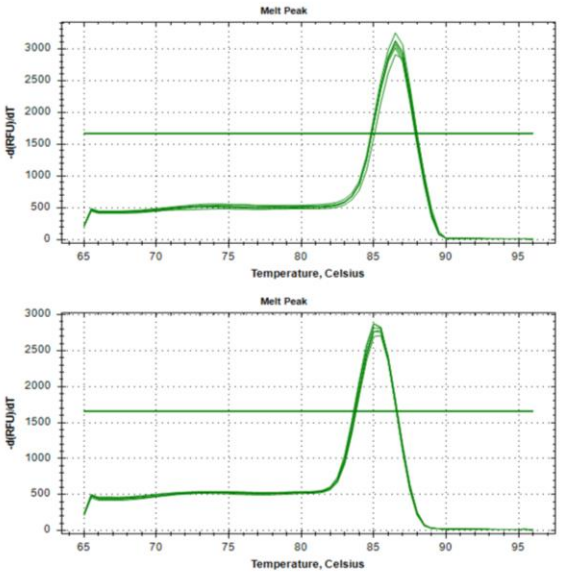

Fig.1b Melting curve

Gene	primer	Sequence	Length
hMR1	hMR1-F1	GGTTGGGTACGTGGACTCG	249 bp
	hMR1-R1	GTGGTGCTTCCATCCTCCAG	

group	① hMR1	② hGAPDH	ΔCT (①-②)	ΔΔCT (hMR1-Ctrl)	$2^{-\Delta\Delta CT}$	expression
hMR1	24.01	19.53	4.48	-1.78	3.4342	343.43%
Ctrl	21.77	19.07	2.7	0	1	100.0%

Fig.1c qPCR results

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

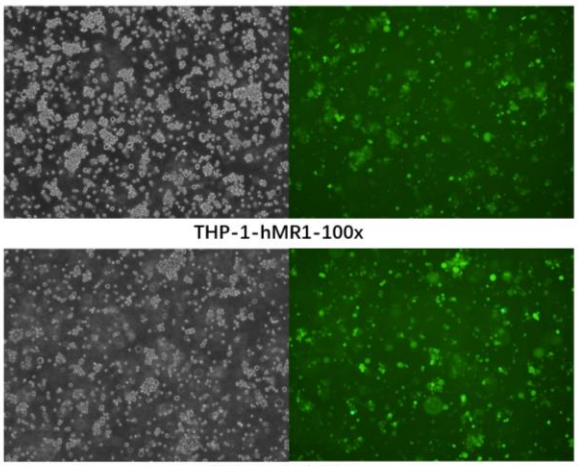

Fig.1d THP 1 overexpressing hMR1 cells

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Case2: HEK293-hGAPDH-Overexpression Cell Line

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

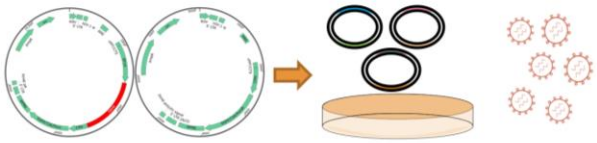

Fig.2a Lentiviral preparation

3. Lentivirus-mediated cell transduction and drug-resistance selection
4. Stable cell line QC (qPCR results)

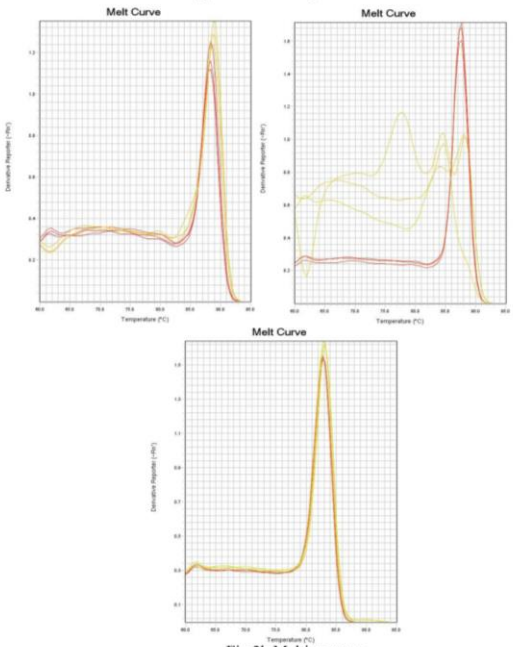

Fig.2b Melting curve

Gene	primer	Sequence	Length
G1	G1-F	GCAAGCTGACCCTGAAGCTG	115 bp
	G1-R	ATGTGGTCGGGGTACCTGGC	
G2	G2-F	TGGTGACCACCCTGACCTGG	278 bp
	G2-R	GATGTACACGTTGTGGCTGA	

group	G1	hGAPDH	ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$	expression
G1+G2	19.92	16.65	3.27	0	1	100.0%
Ctrl	33.364	16.142	17.222	13.951	0	0

Fig.2c qPCR results

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

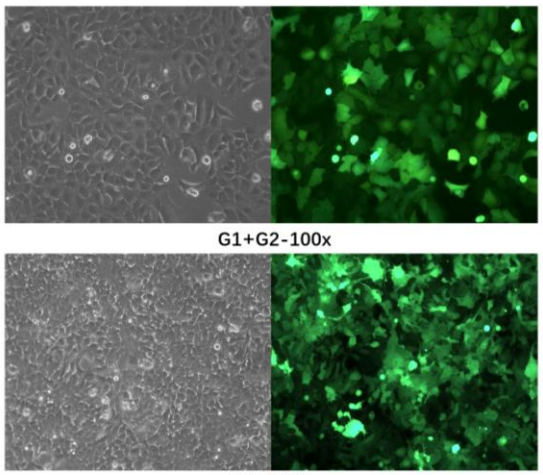

Fig.2d HEK293 overexpressing hGAPDH cells

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