ACCEGEN
BIOTECHNOLOGY

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AcceGen

Over-expression Stable Cell Line Product Manual

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1. Description

Overexpression stable cell lines serve as essential tools in functional genomics and mechanistic studies, including investigations of signaling pathways, target gene validation, antibody development, model construction, drug screening, and assay establishment. These engineered cell models provide a reliable platform for elucidating gene function and accelerating translational research.

For the generation of overexpression stable cell lines, the target gene is first cloned into an appropriate plasmid or viral vector through molecular cloning techniques and subsequently introduced into cells to achieve persistent and robust overexpression. Commonly used methodologies include lentiviral transduction and plasmid-based delivery systems, such as the piggyBac transposon system.

With our customized services, you will benefit from a high-quality and efficient technical service experience tailored to your research needs.

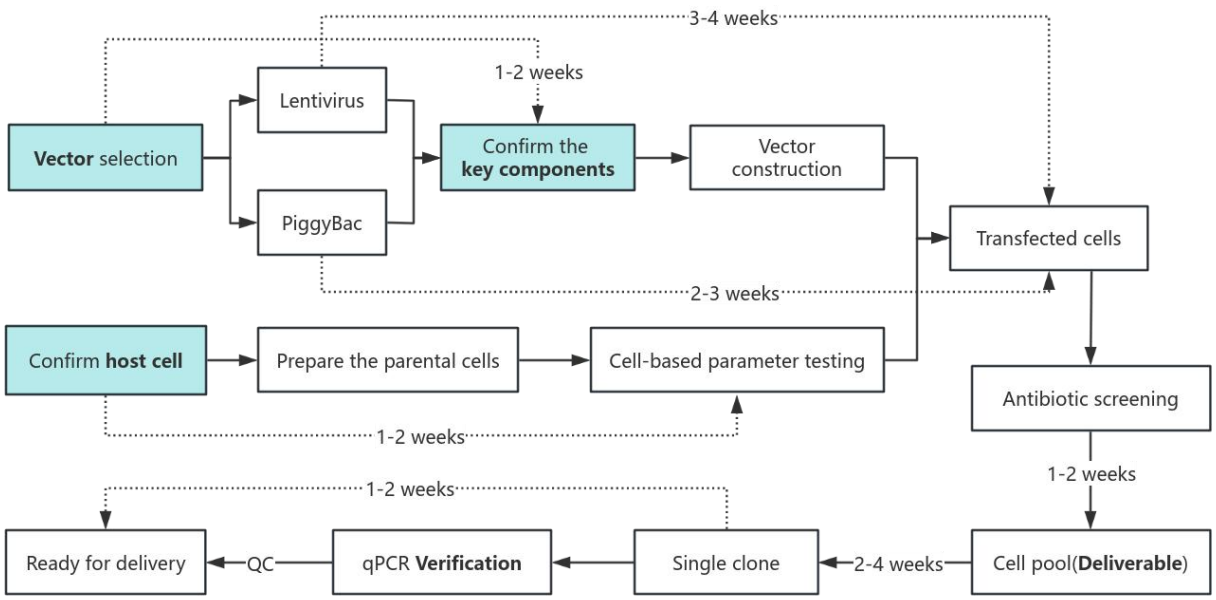
2. Over-expression Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Main method	Delivery
Over-expression Stable Cell Line Generation	CRISPR/Cas9	qPCR	1. VRIUS-Free 2. VRIUS ……	Cell pool / 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 is significantly enhanced, enabling long-term, stable and significant expression. If larger DNA fragments are required, our piggyBac transposition system offers the flexibility of gene editing capability to accommodate such needs. In addition, we provide gene expression and cellular function verification services. Finally, professional laboratory and quality control reports will be delivered.

4. Experimental process



* We will select the most suitable plan for you!

Note: *The displayed timeline is for reference only. The specific lead time depends on the project assessment.*

4.1 VIRUS-Free Technology

(1) Technical Introduction

VIRUS-Free is a technology that utilizes a transposon system for the construction and selection of stable cell lines, serving as an alternative to lentiviral infection-based methods. Its vector contains recognition regions for the transposon. Catalyzed by transposase, the gene of interest is excised from the vector or a

BAC molecule, forming a mini-circle structure, and is then inserted into a specific genomic sequence site, thereby achieving stable integration. This technology has now gained considerable clinical application in CAR-T cell therapy, fully demonstrating its characteristics of low cost, high efficiency, and stable gene expression.

(2)Advantages

Streamlined Workflow, No Viral Packaging Required

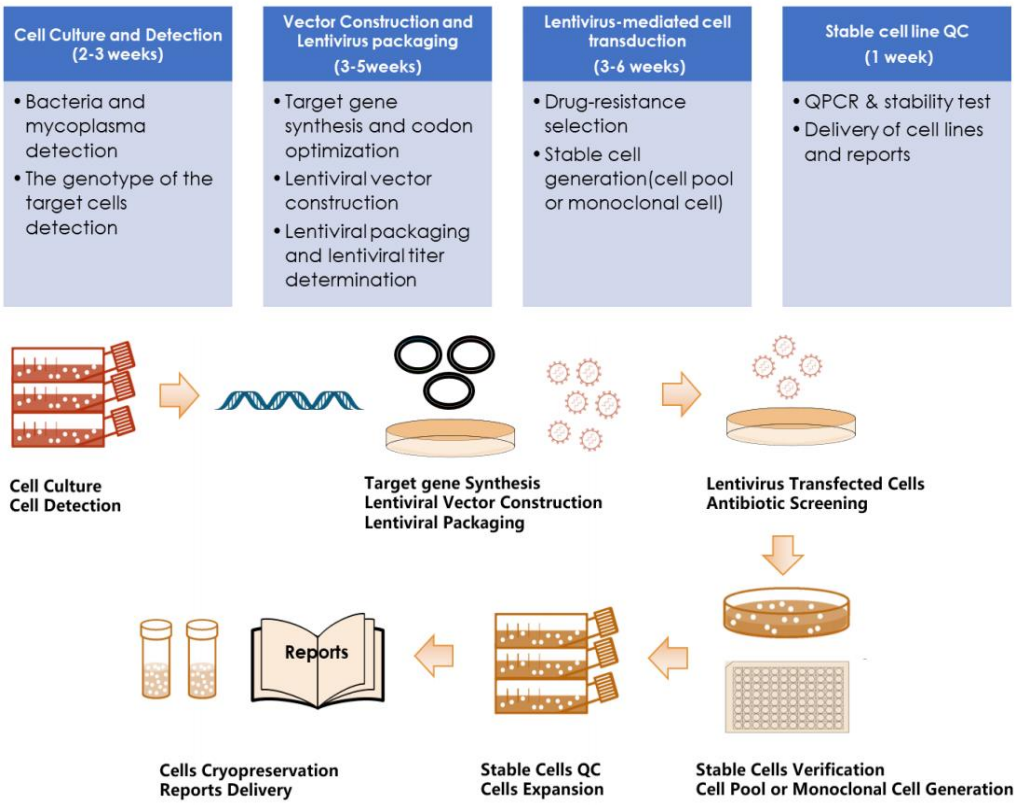
Stable Phenotype with High-Efficiency Expression (Beyond 20 Passages)

No Restriction on Gene Insert Size (Up to 100 Kb)

High Transfection Efficiency in Suspension Cells

Complete and Scarless Removal of the Transgene

4.2 Lentivirus transduction



Note: *The displayed period is for reference only. The specific lead time depends on the project assessment.*

5. Application Operations and Detection Methods

Target gene function discovery and research

(1) Construct a gene overexpression stable cell line

(2) Culture and expand the overexpression stable cell line using suitable medium and serum

(3) Group cells according to experimental requirements

(4) Study the effects of gene overexpression on cells through various experimental methods in terms of phenotype or gene function

Default verification services

RT-qPCR: Cellular RNA is extracted using a nucleic acid extraction kit, and sDNA is obtained by reverse transcription. You can use SYBR Green or TaqMan Probe method to detect the expression level of the target gene.

Value-added services

(1) **Western blotting:** Cells are lysed using RIPA or other reagents, and the cell supernatant is collected after centrifugation to determine the concentration of the target protein. The difference in protein expression between overexpressed cells and control cells can then be obtained.

(2) **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.

Should you require any further characterization, please do not hesitate to contact us. Our team is ready to support your research needs.

6. Delivery of Cell Lines and Report

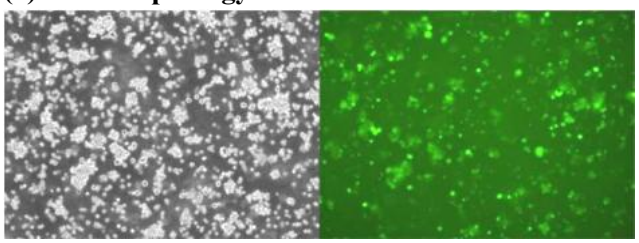
1 vial of stable cell line (≥ 0.5×10^6 cells/vial) and customized project report will be delivered to the customer. If the project requires the customer to provide a host cell, the customer will be request to fill in AcceGen Custom Stable Cell Line Service Requisition Form.

7. Experimental case report

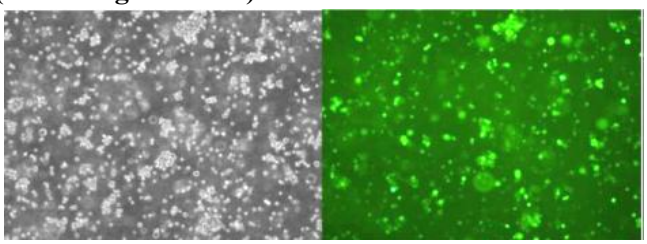
7.1 Suspension Cell

Case 1: hMR1Over-expression THP-1 Cell Line

(1) The morphology of the cell lines is shown below (100X magnification):



THP-1- hMR1



NC

Validation of Over-expression

RT-qPCR:

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
hMR1	F	GGTTGGGTACGTGGACTCG	249
	R	GTGGTGCTTCCATCCTCCAG	

Result

Group	hMR1	hGAPDH	Δ CT	$2^{-\Delta\Delta CT}$
hMR1	21.77	19.07	2.70	3.434
NC	24.01	19.53	4.48	1

Conclusion

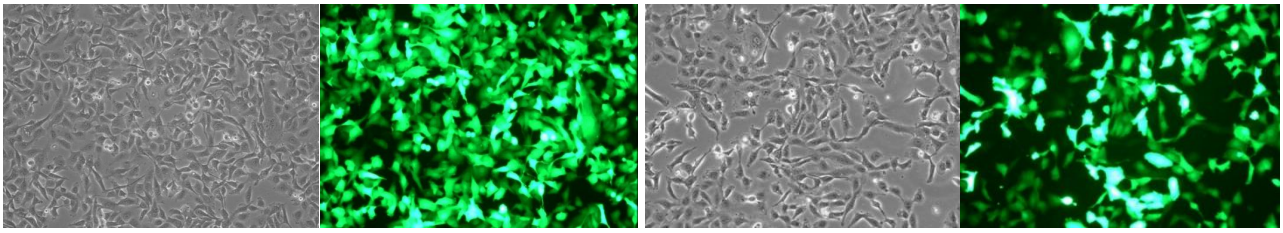
The RT-qPCR results show that hMR1 mRNA is efficiently expressed in THP-1#hMR1#Poolcells.

7.2 Adherent Cell

Case 1: hFAP Over-expression HT-1080 Cell Line (Vector carries GFP)

Proposal Name: Generation of HT-1080 Stable Cell Line for hFAP[NM 004460.5, 2283 bp] fusion protein Overexpression with Plasmid DNA.

The morphology of the cell lines is shown below (100X magnification):



HT-1080- hFAP NC

Validation of Over-expression

HT-1080#Control#Pool RT-qPCR was performed on total RNA extracted from the three samples: HT-1080#hFAP#Poolcells, HT-1080#Control#Poolcells cells and ddH_2O.

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
hFAP	F	TGAGAGCACTCACACTGAAGG	212
	R	CCGATCAGGTGATAAGCCGT	

Result

Group	hMR1	hGAPDH	Δ CT	$2^{-\Delta\Delta CT}$
hFAP	14.38	15.08	-0.69	49702.12
NC	30.29	15.39	14.91	1
H_2O	Undetectable	Undetectable	N/A	N/A

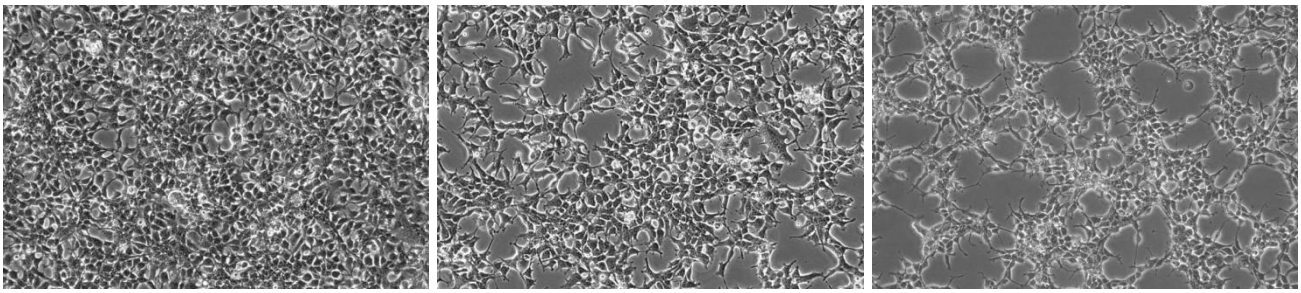
Conclusion

The RT-qPCR results show that hFAP mRNA is efficiently expressed in HT-1080#hFAP#Poolcells.

Case 2: hTROP2 Over-expression HEK-293Cell Line (Vector does not carry GFP)

Proposal Name: Generation of HEK-293 Stable Cell Line for hTACSTD2 [NM_002353.3，891 bp] fusion protein Overexpression with Lentivirus.

The morphology of the cell lines is shown below (100X magnification):



Poolcells NC Single clone

Validation of the lentivirus insertion and GOI expression

RT-qPCR:

RT-qPCR was performed on total RNA extracted from the four samples: HEK-293#hTACSTD2#Poolcells, NC, Single clone and ddH_2O.

Primer information

Gene	Primer	Primer Sequence (5'->3')	Length (bp)
hTACSTD2 (gene of interest)	F	ACCCACCACATCCTCATTGAC	233
	R	CTCTCGAAGTAGTAGGCGGC	

Result

Group	hMR1	hGAPDH	Δ CT	$2^{-\Delta\Delta CT}$
Pool cells	13.57	16.53	-2.96	245163.58
NC	31.45	16.51	14.94	1
Single clone	13.13	15.62	-2.49	170906.62
NC	31.70	16.81	14.90	1
H_2O	Undetectable	Undetectable	N/A	N/A

Conclusion

The RT-qPCR results show that hTACSTD2 mRNA is efficiently expressed in HEK-293#hTACSTD2#Poolcells and Single clone.

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