ACCEGEN
BIOTECHNOLOGY

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AcceGen

Knockdown Stable Cell Line
Product Manual

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1. Description

Knockdown stable cell lines are essential experimental tools for dissecting and characterizing the biological functions of target genes. They are broadly utilized in signaling pathway elucidation, target validation, antibody performance assessment, disease model development, drug screening, and assay optimization.

To achieve stable gene suppression, these cell lines commonly incorporate small interfering RNA (siRNA), short hairpin RNA (shRNA), or CRISPR interference (CRISPRi) systems, enabling sustained downregulation of one or more target genes. This controlled reduction in gene expression allows researchers to investigate gene function at the molecular, cellular, and phenotypic levels.

By attenuating endogenous gene expression, knockdown stable cell lines often recapitulate phenotypes similar to those observed in gene knockout models. Importantly, in cases where complete gene ablation results in lethality, knockdown systems provide a practical and indispensable alternative for functional studies across diverse biological contexts.

Through our customized services, you will receive reliable, high-quality support tailored to your specific research needs.

2. Knockdown Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Method	Delivery
Knockdown Stable Cell Line Generation	CRISPR/Cas9	qPCR	1. shRNA 2. siRNA 3. CRISPRi ……	Cell Pool/ Monoclonal cell line

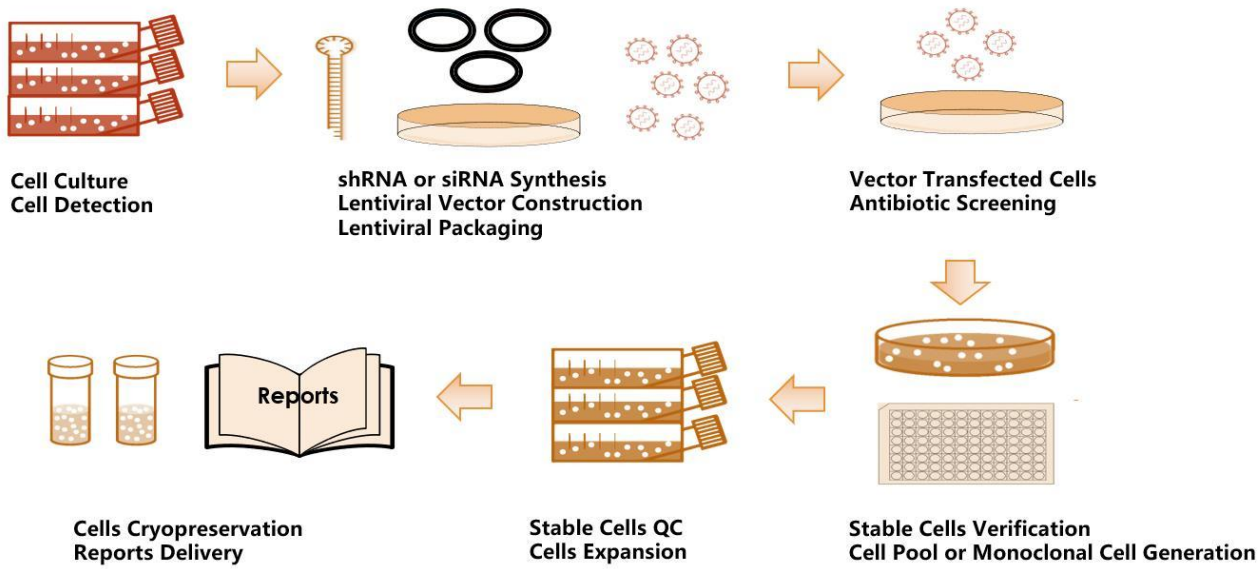
3. Technical advantages

Our lentiviral vector has high infection efficiency, facilitating quick and efficient integration of the target gene into the host cell genome, which enhancing RNAi efficiency and achieving long-term, stable and significant knockdown effects. In addition, we provide services such as cellular gene expression regulation

and cellular function verification. Furthermore, we offer comprehensive one-stop technical services. Finally, we deliver professional laboratory and quality control reports.

4. Experimental process

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	•Synthesize shRNA and optimize codon • 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



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

Important Note: *The efficiency of gene knockdown is influenced by the basal expression level of the target gene in the host cell line. Therefore, we strongly recommend performing an initial assessment of this basal expression prior to initiating the project.*

5. Application Operations and Detection Methods

Target gene function discovery and research

(1) Construct a gene knockdown stable cell line

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

(3) Group cells according to experimental requirements

(4) Study the effects of gene knockdown 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. SYBR Green or TaqMan Probe methods can be used to detect the expression level of the target gene.

Value-added services

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 knockdown cells and control cells can then be obtained.

Flow Cytometry: Inoculate cells (5×10^5 cells/mL) into 6-well plates and culture them for 24 hours. Add the 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.

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

Human PCCA knockdown in HEK293 (shRNA)

Vector:

a) **hPCCA-shRNA** (Puro, EGFP)

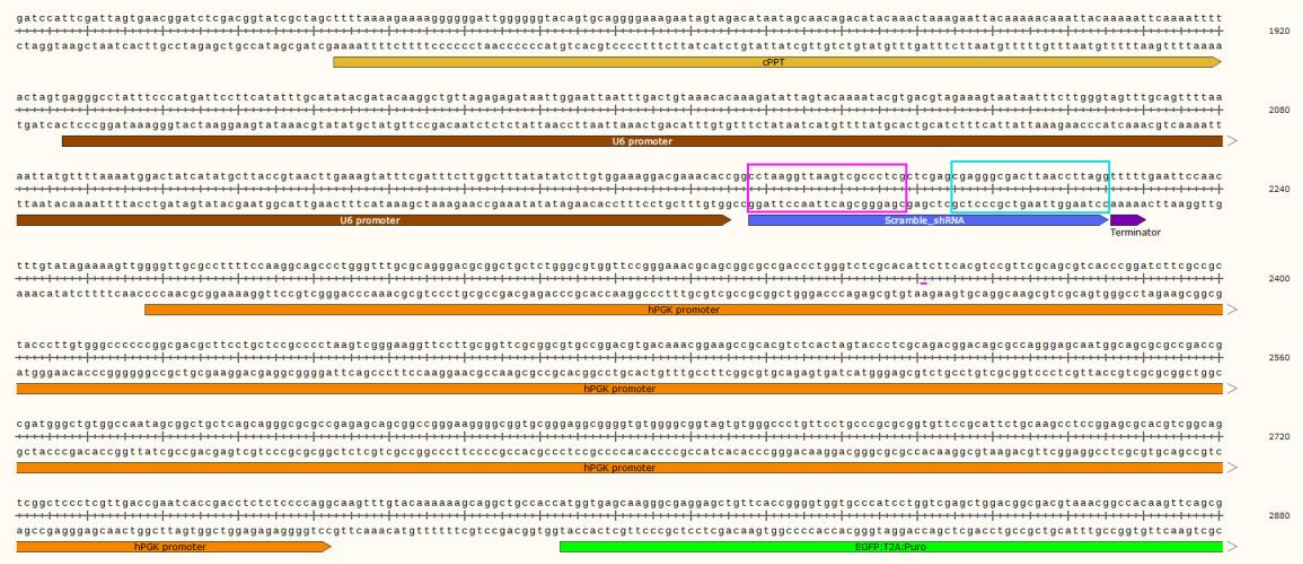
pLV[shRNA]-EGFP:T2A:Puro-U6> hPCCA-shRNA4(Puro, EGFP)

pLV[shRNA]-EGFP:T2A:Puro-U6> hPCCA-shRNA5(Puro, EGFP)

b) Control group

pLV[shRNA]-EGFP:T2A:Puro-U6>Scramble_shRNA (Puro, EGFP)

c) Plasmid map (Down/ scramble shRNA)

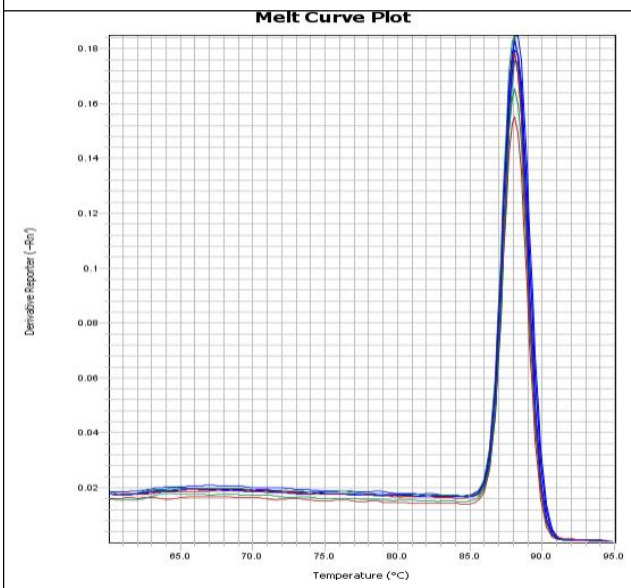
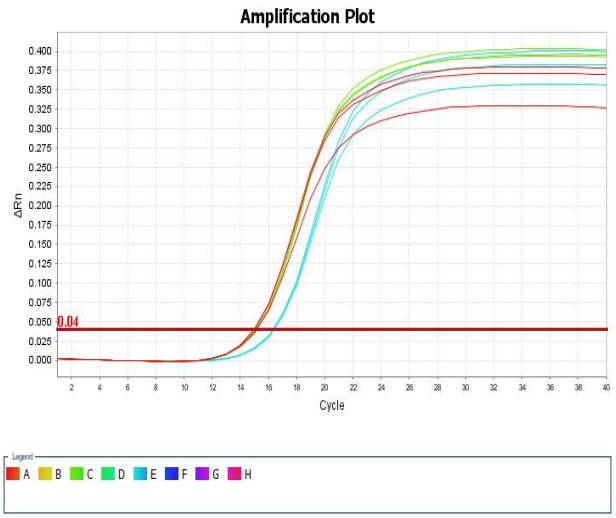
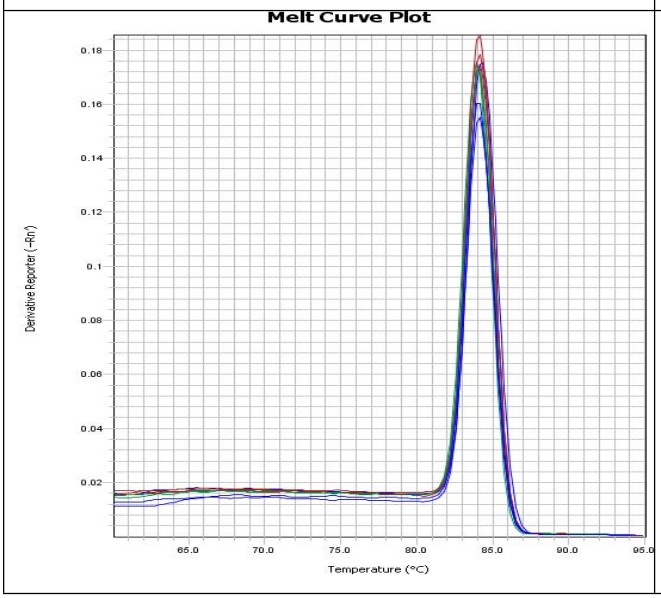
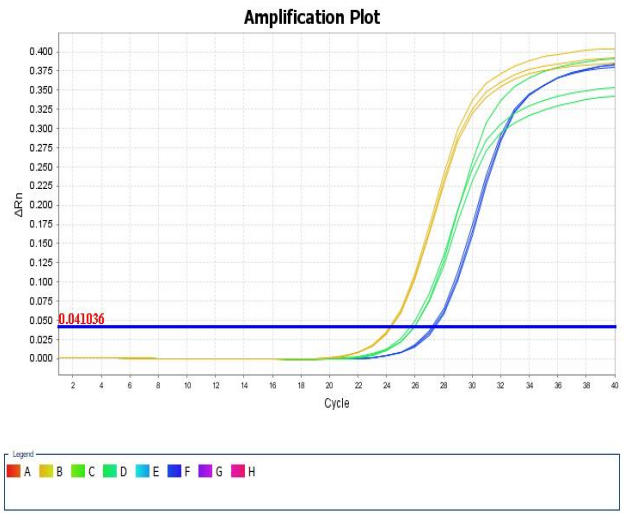


qPCR detection

a)Primer information

Target Gene	Primer Name	Primer Sequence (5'->3')
hsa-PCCA	F:	AAGCTACCTCAACATGGATGC
	R:	GTGTCAGGTCCAATGAAAACGA
hsa-GAPDH	F:	ATCATCAGCAATGCCTCCT
	R:	CATCACGCCACAGTTTCC

b)Melt curve and Amplification curve

GAPDH melt curve	GAPDH Amplification curve
	
PCCA melt curve	**PCCA Amplification curve**
	

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