ACCEGEN
BIOTECHNOLOGY

Knockdown Stable Cell Lines
Product Manual

Knockdown Stable Cell Lines Product Information

1. Description

In knockdown stable cell lines, small interfering RNA (siRNA) or short hairpin RNA (shRNA) is used to downregulate or terminate the expression of one or more target genes, enabling the study of gene function.

Experience our customized service, and you will enjoy our high-quality customer service. The knockdown stable cell line is an important research tool for discovering and studying the function of target genes. They are particularly useful in various applications such as signaling pathway research, target validation, antibody research, model construction, drug screening, and assay development. By suppressing the expression of target gene in vivo, these knockdown cell lines appear the phenotype that mimics target gene deletion. In cases where gene knockout would be lethal, knockdown stable cell lines play an important role in numerous applications.

2. Knockdown Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Application	Delivery
Knockdown Stable Cell Line Generation	Lentivirus	QPCR	Gene function research Target discovery Target validation Compound screening	Polyclonal cell line 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 enhances the efficiency of the RNAi to achieve long-term, stable and significant 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 reports and quality inspection reports.

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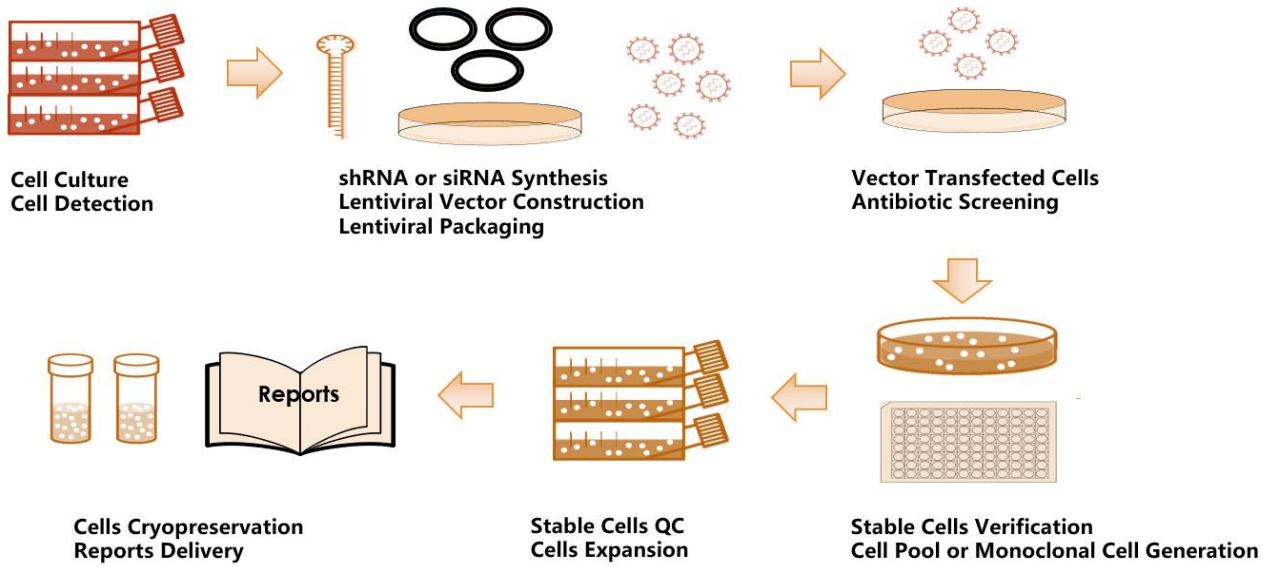
4. Experimental process

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



5. Application Operations and Detection Methods

Target gene function discovery and research

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

RT-qPCR The process of extracting cellular RNA can be operated using the nucleic acid extraction kit 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 can be lysed using RIPA or other reagents, and the supernatant can then be collected through centrifugation to determine the concentration of the target protein. The difference in protein expression between knockdown cells and control cells can be accessed.

RT-PCR The process of extracting cellular RNA can be performed using the nucleic acid extraction kit, and the target gene product can be obtained through reverse transcription and PCR amplification. The expression results of the target genes can be oberved and detected using agarose gel electrophoresis and gel imaging system.

Flow Cytometry Cells (5×10^5 cells/ml) can be inoculated into 6-well plates and cultured for 24 hours. The antibodies or stimulating factors required for the experiment can be added to the cells which are then incubated 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: HCC1806-hXRCC1-Knockdown Cell Line

1. Synthesize shRNA and optimize codon
2. Lentiviral vector construction and lentiviral packaging

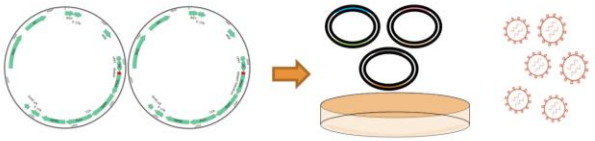
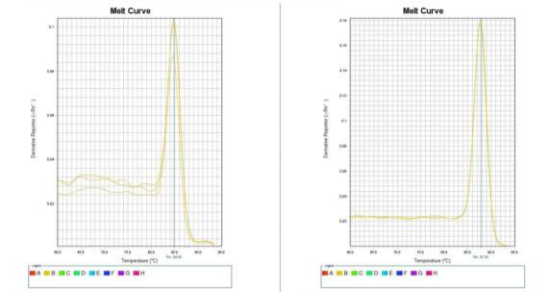


Fig.1a Lentiviral preparation

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



HCC1806-XRCC1-RNA1+XRCC1 melt curve | HCC1806-XRCC1-RNA1+h_actin melt curve

HCC1806-XRCC1-RNA2+XRCC1 melt curve | HCC1806-XRCC1-RNA2+h_actin melt curve

Fig.1b Melting curve

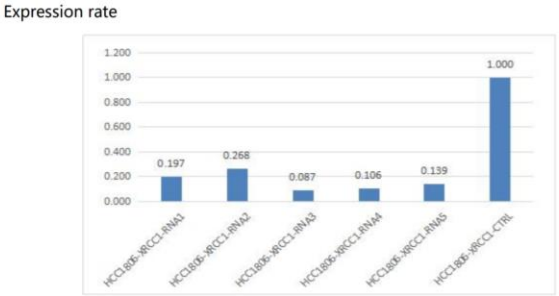
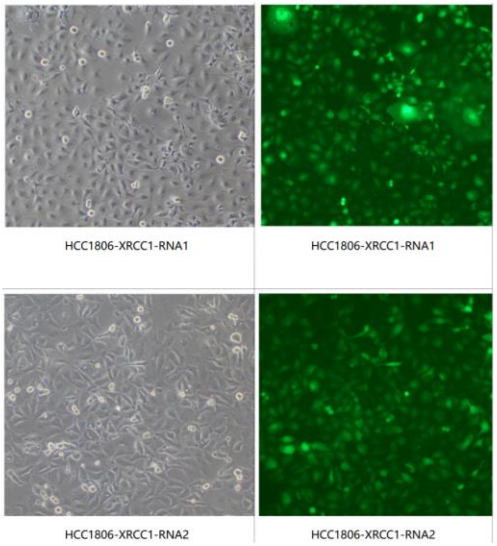


Fig.1c qPCR results

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



HCC1806-XRCC1-RNA1 | HCC1806-XRCC1-RNA1

HCC1806-XRCC1-RNA2 | HCC1806-XRCC1-RNA2

Fig.1d Knockdown cells generation

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Case2: HCC1806-hXRCC1-Knockdown Cell Line

1. Synthesize shRNA and optimize codon
2. Lentiviral vector construction and lentiviral packaging

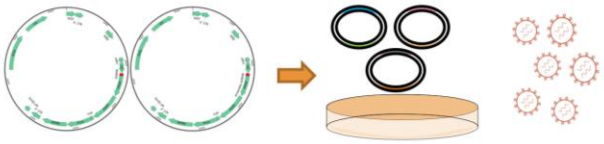

Fig.2a Lentiviral preparation

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

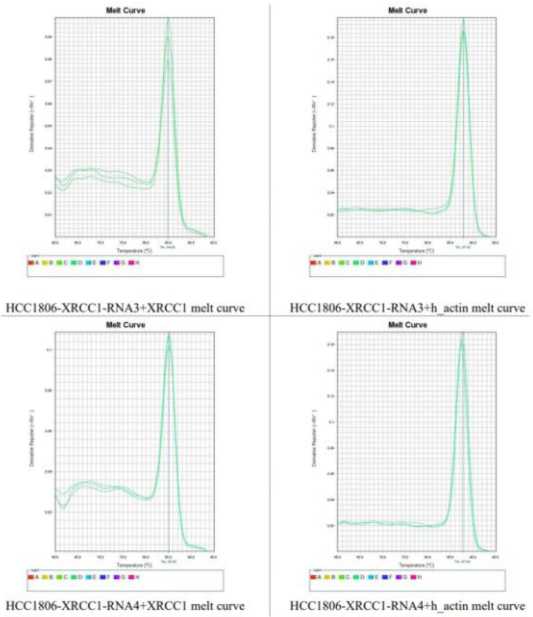

Fig.2b Melting curve

Expression rate

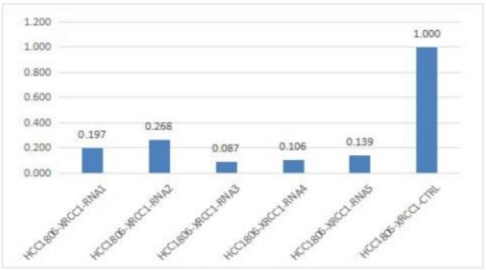

Fig.2c qPCR results

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

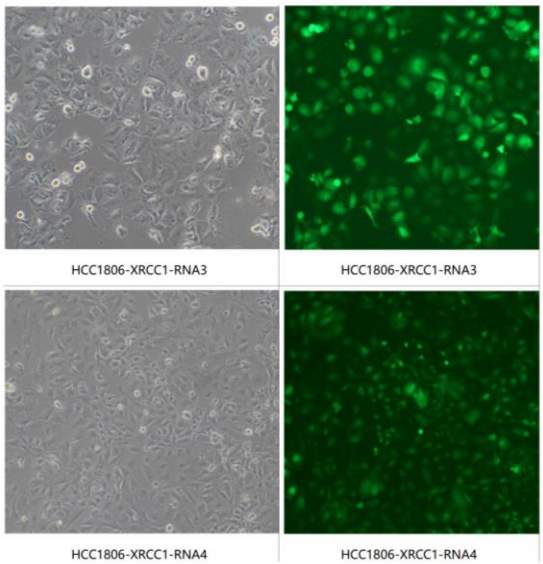

Fig.2d Knockdown cells generation

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Case3: HCC1806-hXRCC1-Knockdown Cell Line

1. Synthesize shRNA and optimize codon
2. Lentiviral vector construction and lentiviral packaging

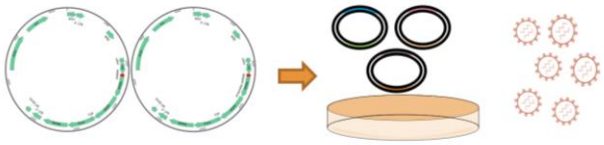
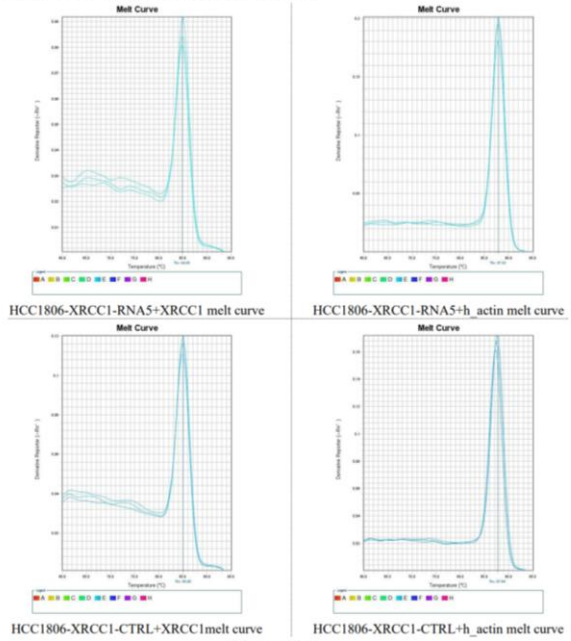


Fig.3a Lentiviral preparation

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



HCC1806-XRCC1-RNA5+XRCC1 melt curve | HCC1806-XRCC1-RNA5+h_actin melt curve

HCC1806-XRCC1-CTRL+XRCC1melt curve | HCC1806-XRCC1-CTRL+h_actin melt curve

Fig.3b Melting curve

Expression rate

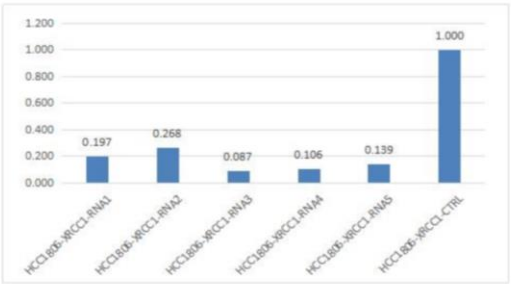
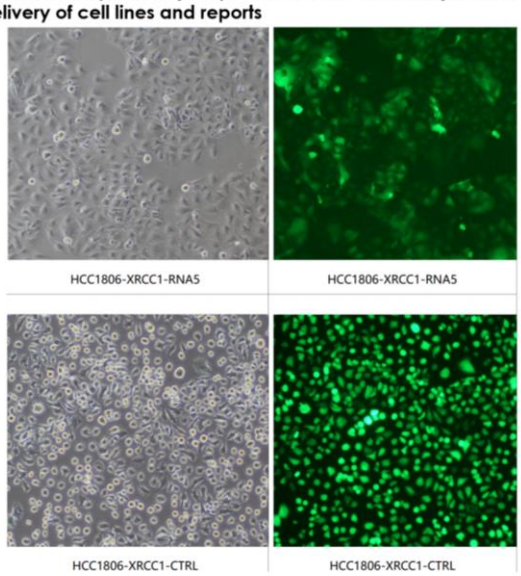


Fig.3c qPCR results

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



HCC1806-XRCC1-RNA5 | HCC1806-XRCC1-RNA5

HCC1806-XRCC1-CTRL | HCC1806-XRCC1-CTRL

Fig.3d Knockdown cells generation

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