ACCEGEN
BIOTECHNOLOGY

Knockout Stable Cell Lines

Product Manual

Knockout Stable Cell Lines Product Information

1. Description

The knockout stable cell line uses genetic engineering technology to induce loss-of-function mutations at the target gene in mammalian cells. It is a key tool for exploring protein function, analyzing the effects of gene loss, and studying disease models.

With our customized service, you can get our high-quality customer service experience. The knockout 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. Researchers can study the effect of gene knockout on cells and verify the specificity of the target antibody.

2. Knockout Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Application	Delivery
Knockout Stable Cell Line Generation	CRISPR/Cas9	Sequence	Gene function research Protein engineering Recombinant antibodies development Drug discovery	Monoclonal cell line

3. Technical advantages

We have optimized and upgraded the CRISPR/Cas9 technology and developed a more efficient gene editing system, which can realize multiple knockout strategies such as frameshift knockout, fragment knockout, and multiple gene knockout of host cell genes. We have a lot of experience in constructing knockout stable cell lines, including but not limited to A549, CHO, HepG2 and other cell lines. In addition, we provide services such as cellular gene expression regulation and cell function verification. Finally, professional laboratory reports and quality inspection reports will be delivered.

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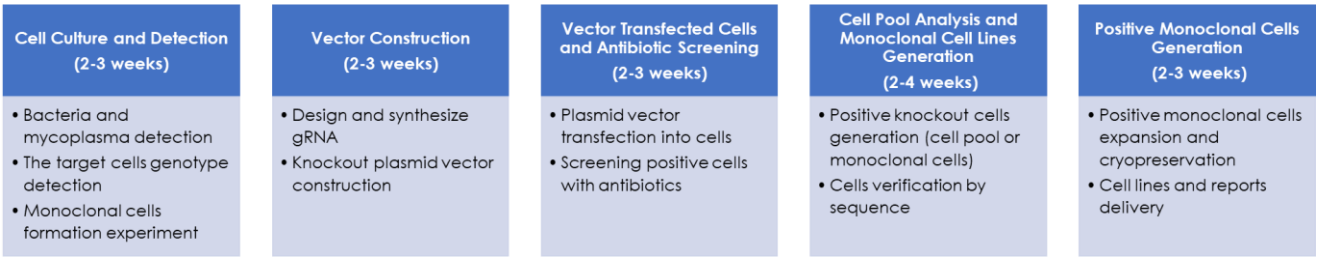
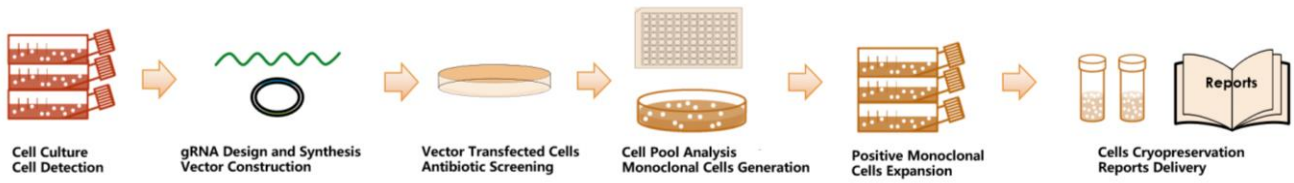
4. Experimental process





5. Application Operations and Detection Methods

Target gene function discovery and research

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

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 knockout 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.

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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 sequencing 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.

7. Experimental case

Case1: HEK293-hERRA-Knockout Cell Line

Fig.1a gRNA

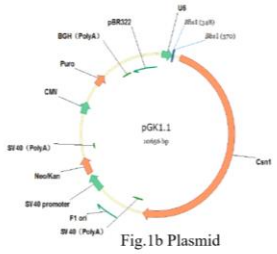

Fig.1b Plasmid

3. Plasmid vector transfection into cells
4. Positive stable cells generation

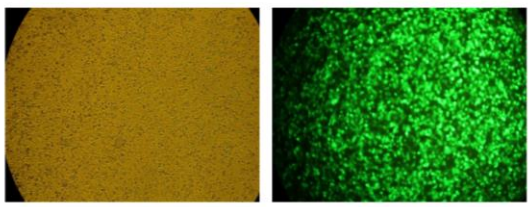

Fig.1c Electroporation results

5. Validation results (PCR and Sequencing) Identification for positive clone

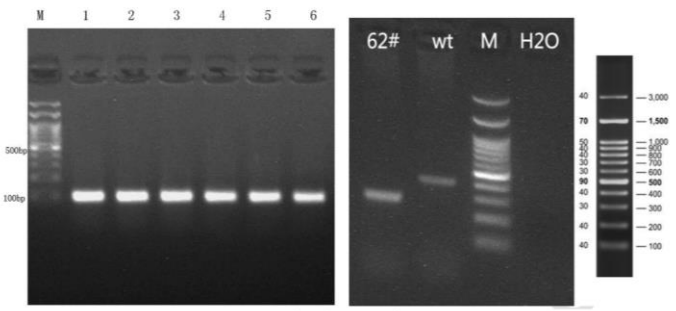

Fig.1d PCR results

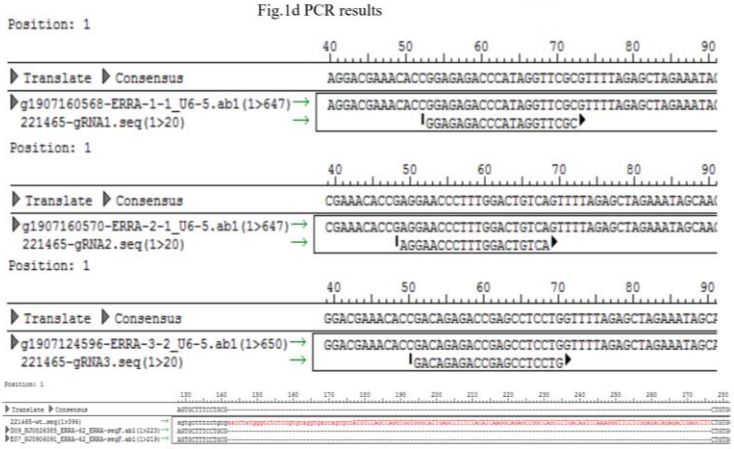

Fig.1e Sequencing results

6. Positive monoclonal cells expansion
7. Cell cryopreservation and reports delivery

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Case2: HEK293-hERRG-Knockout Cell Line

1. Design and synthesize gRNA
2. Plasmid vector construction

aatatatacctattgagccataattggaatatttagcgtttattgtaggatatttggattgtttttcaaaacacaaaatgctctagggtttataaaagaggg
atgatactactagtgatcttgtagtgctctggtctgatcctgcagataacaagattgtctcacatttgttggtggctgaaccggagaagatctatgccAT
GCCTGACCCTACTGTCCCCGACAGTGACATCAAAGCCCTCACTACACTGTGTGACTTGGCCGACCG
AGAGTTGGTGGTTATCATTGGATGGGCGAAGCATATTCCAGgtacattttctgaaaagaaaaagaaaagaaaaggtt
gcaattaattccctaggttatgtatatagatttgggtgaattcaaatttttctcatttagacttaaaaaaaccctttacatttatttttaatcataatttagagtttt
gcatatcctaatagaatccttagactttat

ERRG-gRNA1: ACATTTGTTGGCTGAACCGG (gRNA1 46 Guide 76)
ERRG-gRNA2: GGGCTTTGATGTCACTGTCGGGG (gRNA2 80 Guide 88)
ERRG-gRNA3: TGACTTGGCCGACCGAGAGTTGG (gRNA3 136 Guide 98)

Fig.2a gRNA

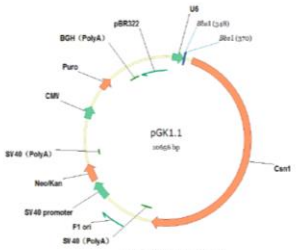

Fig.2b Plasmid

3. Plasmid vector transfection into cells
4. Positive stable cells generation

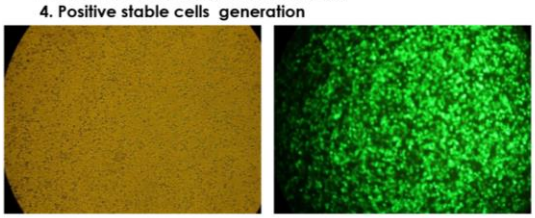

Fig.2c Electroporation results

5. Validation results (PCR and Sequencing) Identification for positive clone

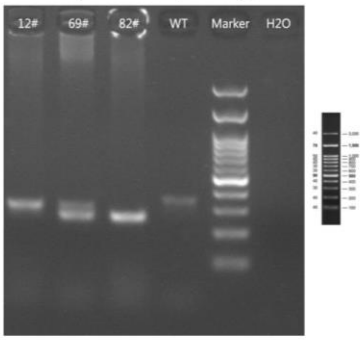

Fig.2d PCR results

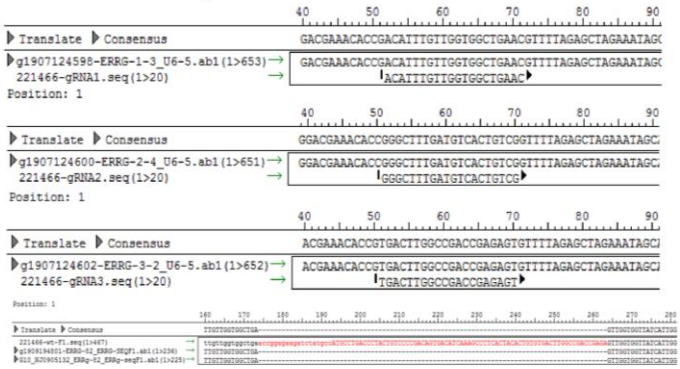

Fig.2e Sequencing results

6. Positive monoclonal cells expansion
7. Cell cryopreservation and reports delivery

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Case3: 293T-hCRBN-Knockout Cell Line

1. Design and synthesize gRNA
2. Plasmid vector construction

gRNA Sequence

Fig.3a gRNA

3. Plasmid vector transfection into cells
4. Positive stable cells generation
5. Validation results (PCR and Sequencing) Identification for positive clone

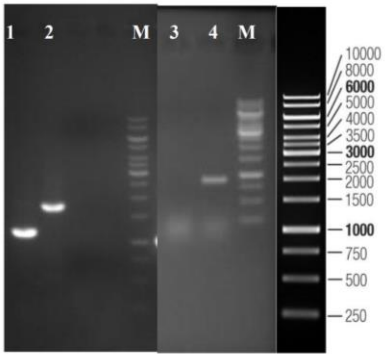

Fig.3b PCR results

Sanger Sequencing

Fig.3c Sequencing results

6. Positive monoclonal cells expansion
7. Cell cryopreservation and reports delivery

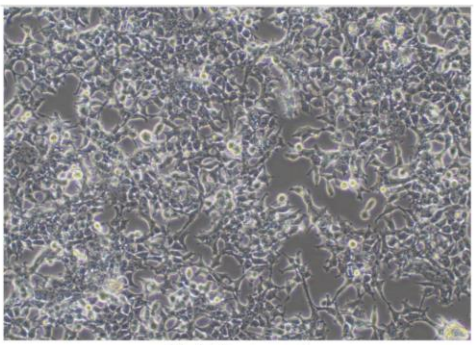

Fig.3d Knockout cells

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