ACCEGEN
BIOTECHNOLOGY

Knockin Stable Cell Lines
Product Manual

Knockin Cell Lines Product Information

1. Description

For knockin stable cell lines, exogenous genes are inserted into specific locations within target cells, which can help researchers study gene functions and establish cell models.

Using our customized service, you will obtain our high-quality customer service experience. The knockin 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. It allows for precise site-directed insertion of exogenous genes and serve as an important tool for studying gene functions and constructing disease models.

2. Knockin Stable Cell Line Generation Service

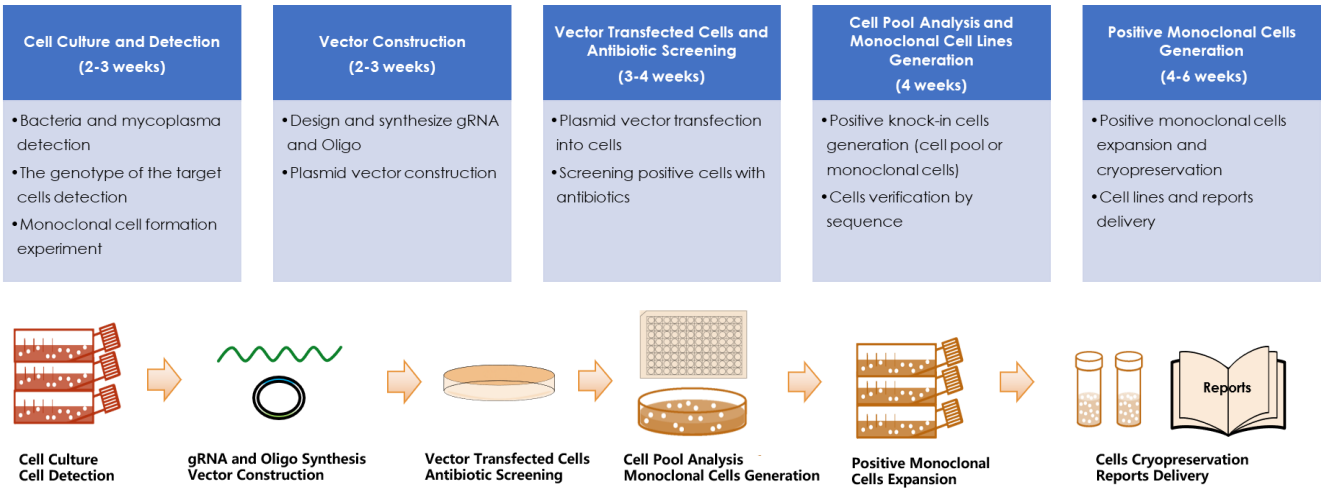
Service	Technical method	Gene expression evaluation	Application	Delivery
Knockin Stable Cell Line Generation	CRISPR/Cas9	Sequence	Gene function research Protein engineering Disease models creation	Monoclonal cell line

3. Technical advantages

We have optimized and upgraded the CRISPR/Cas9 technology, resulting in the development of a more efficient gene editing system. Our extensive experimental test optimization and cell line construction experiences allow us to enhance the efficiency of homologous recombination during gene knock-in and reduce the probability of random insertion. At present, there have been a large number of successful cases, and more than 1000 gene edits have been successfully achieved in more than 30 kinds of cells. In addition, we provide services such as cellular gene expression regulation and cellular function verification. Finally, professional laboratory reports and quality inspection reports will be delivered.

AcceGen Biotech
277 Fairfield Road,
Fairfield, NJ 07004, USA
T: +1-862-686-2696 | E: inquiry@accegen.com
F: +1-323-978-5598 | Homepage: www.accegen.com

4. Experimental process



5. Application Operations and Detection Methods

Target gene function discovery and research

a) Construct a gene knockin stable cell line

b) Culture and expand the knockin stable cell line with the suitable medium and serum

c) Group cells according to experimental requirements

d) Study the effect of gene knockin 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 knockin 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.

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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: THP 1-IDH2-Point Mutation Knockin Cell Line

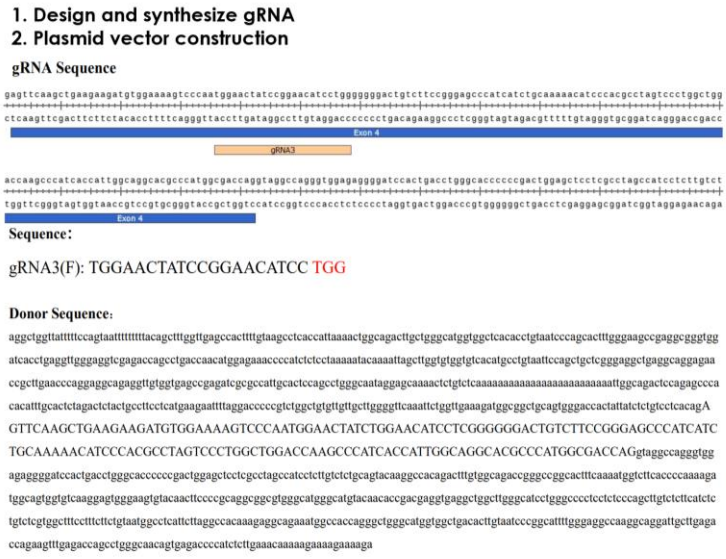

Fig.1a gRNA

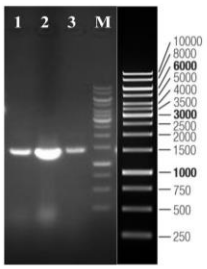

Fig.1b PCR results

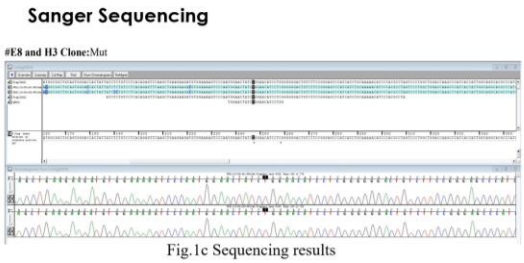

Fig.1c Sequencing results

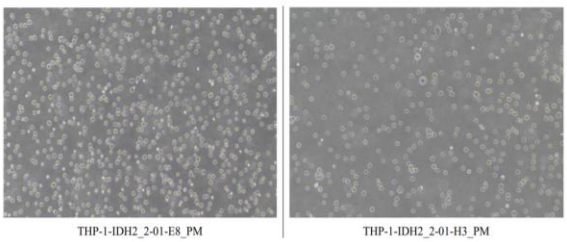

Fig.1d Stable cells generation

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Case2: THP 1-IDH2-Point Mutation Knockin Cell Line

1. Design and synthesize gRNA
2. Plasmid vector construction

gRNA Sequence

Sequence:

gRNA3(F): TGGACCAAGCCCATCACCAT TGG

Donor Sequence:

ttgctgggcatggtggctcacacctgtaatcccagcactttgggaagccgaggcgggtggatcacctgaggttgggaggtcgagaccagcctgaccaacatggagaaaccccatctctctaaaaatacaaaatt
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Fig.2a gRNA

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

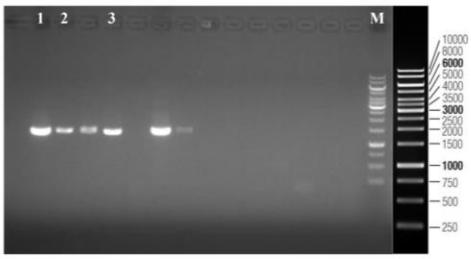


Fig.2b PCR results

Sanger Sequencing

#D03 and D05 Clone:Mut

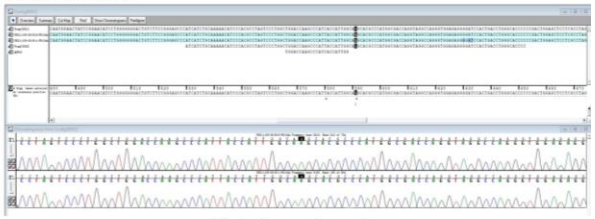
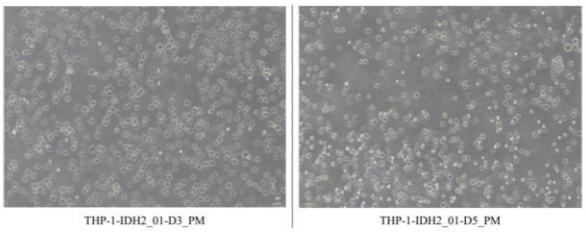


Fig.2c Sequencing results

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



THP-1-IDH2_01-D3_PM THP-1-IDH2_01-D5_PM

Fig.2d Stable cells generation

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Case3: MCF7-ESR1-Point Mutation Knockin Cell Line

1. Design and synthesize gRNA
2. Plasmid vector construction

gRNA Sequence

Sequence:

gRNA1(R):TCCAGCAGCAGGTCATAGAG GGG

Oligo Sequence:

tacagTAACAAAGGCATGGAGCATCTGTACAGCATGAAGTGCAAGAACGTCGTGCCCCTCAGCGACCTGCTGCTGGAGATGC
TGGACGCCCACCGCCTACATGCGCCCACTAGCCGTGGAGGG

Fig.3a gRNA

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

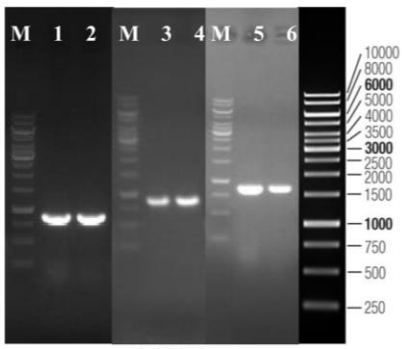


Fig.3b PCR results

Sanger Sequencing

#E10,A02 and A08 Clone: Mut +WT

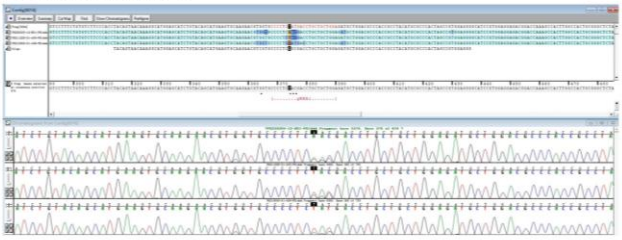
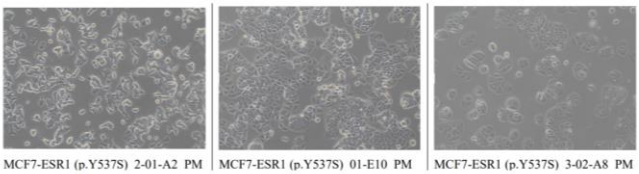


Fig.3c Sequencing results

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



MCF7-ESR1 (p.Y537S) 2-01-A2 PM | MCF7-ESR1 (p.Y537S) 01-E10 PM | MCF7-ESR1 (p.Y537S) 3-02-A8 PM

Fig.3d Stable cells generation

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