ACCEGEN
BIOTECHNOLOGY

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AcceGen

Knockin Stable Cell Line
Product Manual

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1. Description

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 development, disease model construction, drug screening and assay development). It allows precise site-directed insertion of exogenous gene. For knockin stable cell lines, exogenous genes are inserted into specific genomic locations within target cells, which can help researchers study gene functions and establish cell models.

Using our customized service, you will receive high-quality technical support and professional customer service.

2. Knockin Stable Cell Line Generation Service

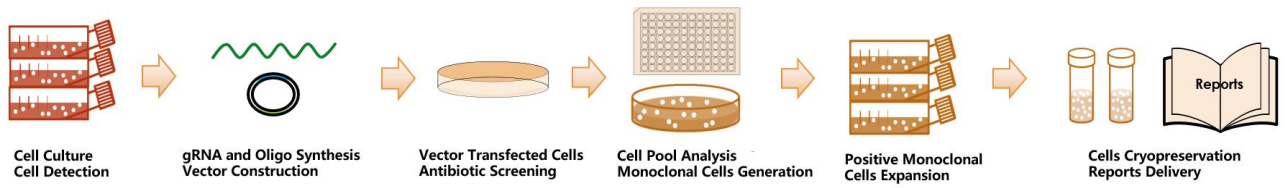
Service	Technical method	Gene expression evaluation	Method	Delivery
Knockin Stable Cell Line Generation	CRISPR/Cas9	Sequence	1. RNP 2. VRIUS-Free 3. VRIUS 4. Plasmid ……	Monoclonal cell line

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 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, a large number of successful cases have been completed, with more than 1,000 gene-editing projects successfully achieved in over 30 different cell types. In addition, we provide services such as cellular gene expression regulation and cellular function verification. Finally, professional laboratory and quality control reports will be delivered.

4. Experimental process

Cell Culture and Detection (2-3 weeks)	Vector Construction (2-3 weeks)	Vector Transfected Cells and Antibiotic Screening (3-4 weeks)	Cell Pool Analysis and Monoclonal Cell Lines Generation (4 weeks)	Positive Monoclonal Cells Generation (4-6 weeks)
•Bacteria and mycoplasma detection •The genotype of the target cells detection •Monoclonal cell formation experiment	•Design and synthesize gRNA and Oligo •Plasmid vector construction	•Plasmid vector transfection into cells •Screening positive cells with antibiotics	•Positive knock-in cells generation (cell pool or monoclonal cells) •Cells verification by sequence	•Positive monoclonal cells expansion and cryopreservation •Cell lines and reports delivery



Note: *The displayed timeline 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 knockin stable cell line

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

(3) Group cells according to experimental requirements

(4) Study the effect of gene knockout on cells through various experimental methods in terms of phenotype or gene function.

Default verification services

RT-PCR: Cellular RNA is extracted using a nucleic acid extraction kit, and sDNA is obtained by reverse transcription. SYBR Green or TaqMan Probe method can be used to detect the expression level of the target gene. Detect and observe the expression results of the target genes by agarose gel electrophoresis and gel imaging system.

Sanger sequencing: By providing precise sequencing of the specific target locus, it directly reads the DNA sequence to unequivocally confirm the presence of expected insertions, deletions, or point mutations at the

editing site. With its accurate results, it serves as the most fundamental and critical step for genotyping knockin cell lines, establishing a solid foundation for all subsequent research.

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, etc.

Whole genome sequencing: Whole Genome Sequencing (WGS) offers a "comprehensive map" of the gene-edited cells. It enables an unbiased scan of the entire genome to reconfirm on-target editing efficiency while comprehensively assessing potential off-target effects and genomic structural variations. This service represents the ultimate verification solution, ensuring the high quality, purity, and safety of your cell models for your in-depth research endeavors.

6. Delivery of Cell Lines and Report

1 vial of stable cell line (1×10^6 cells/vial) and KI 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

Case 1: Human Gene A (V617F+) HCT116 Cell Line

Project Description: Generation of HCT116 Stable Cell Lines for Introducing One Point Mutation in Gene A_ p.V617F Gene With CRISPR/Cas9 RNP Complexes.

Generation of the mutation cell lines: HCT116 cells were transfected with Cas9-gRNA and ssDNA(hFOXG1*[c.256dupC]) below.

gRNA:

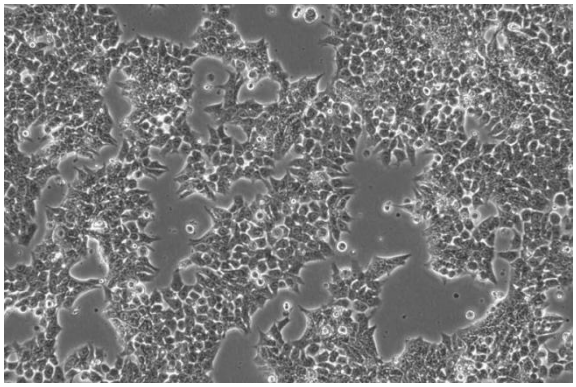
AATTATGGAGTATGTGTCTG TGG

ssDNA:

AAGCAGCAAGTATGATGAGCAAGCTTTCTCACAAGCATTTGGTTTTAAATTATGGAGTATGTTTC TGTGGAGACGAGAGTAAGTAAAACTACAGGCTTTCTAATGCCTTTCTCAGAGCAT

The selected cells were mono-cloned and identified by genotyping PCR and Sanger sequencing.

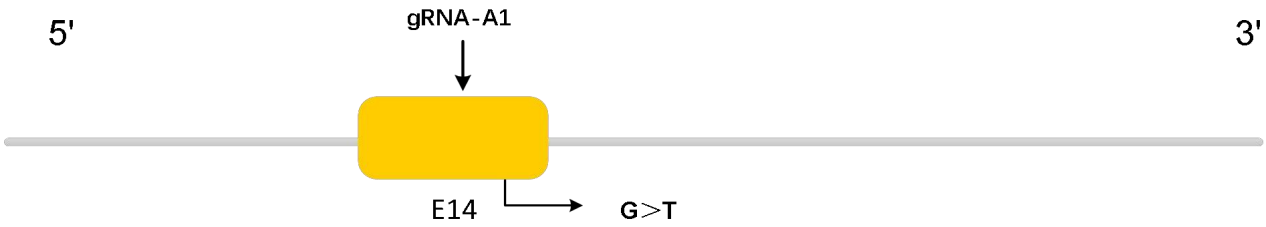
The morphology of positive clones is shown below (100X magnification):



1) Validation of Gene B Mutation

Genotyping PCR:

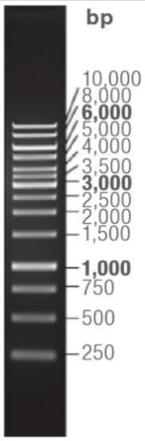
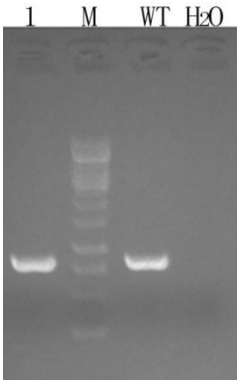
PCR was performed on the genomic DNA extracted from the stably transfected cells.



a) Primer information

Target Gene	Primer Name	Primer Sequence (5'->3')	Product Size (bp)
Gene A (gene of interest)	F:	ATGGCAGTTGCAGGTCCATA	845
	R:	CTAGACACTGGGTTGCCGTAA	

b) Result

Marker	Region 1 (845 bp)
	

Genotyping PCR and Sanger sequencing showed that the Gene A Mutation was detected, and single clone, Clone 1 was validated to be heterozygote.

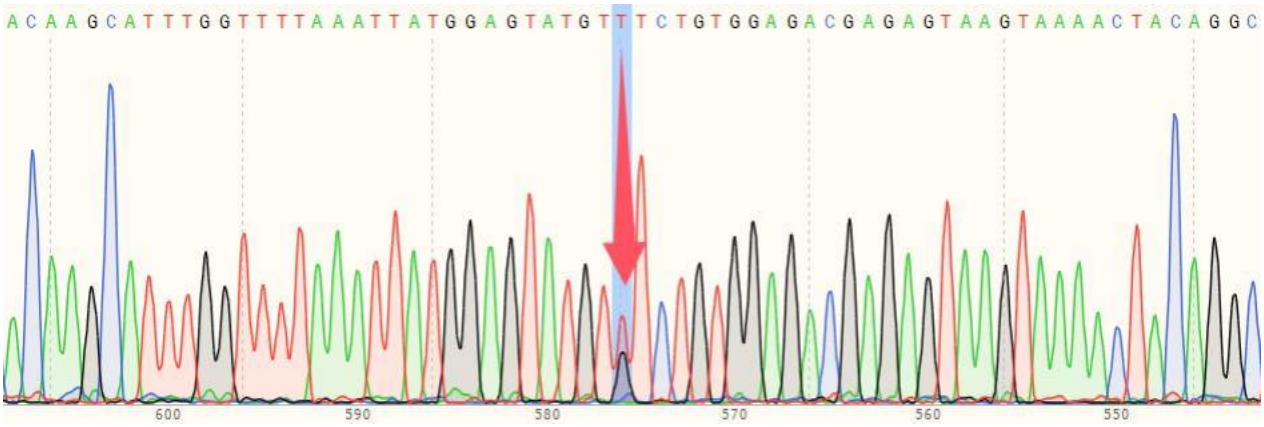
Sanger Sequencing

a) WT:

AAGCAGCAAGTATGATGAGCAAGCTTTCTCACAAGCATTTGGTTTTAAATTATGGAGTATGTGTCT
GTGGAGACGAGAGTAAGTAAAACTACAGGCTTTCTAATGCCTTTCTCAGAGCAT

b) Clone 1:

AAGCAGCAAGTATGATGAGCAAGCTTTCTCACAAGCATTTGGTTTTAAATTATGGAGTATGTT(G)T
CTGTGGAGACGAGAGTAAGTAAAACTACAGGCTTTCTAATGCCTTTCTCAGAGCAT

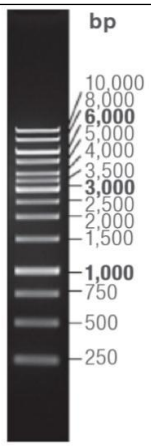
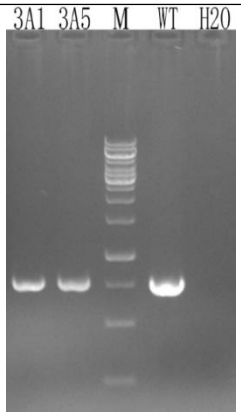


c) Conclusion

One single clone, Clone 1, with Gene A Mutation was obtained successfully.

Case 2: C2C12 cell Gene B (P351L) site-directed mutation (homozygous delivery)

Genotyping PCR Result:

Marker	3A1 3A5
	

Sanger Sequencing

a) WT:

GGCTTGGAACCTCTCTCTGCCCCGGCTGCAGATTTTATTAGAGCTGCTGCCGCGTCTCCTGGGTCG
AGCCCACCCCCAGAGGATCCCCGAACTGCCTCACCAGCGAGGCG

b) **MT:**

GGCTTGGAACCTCTCTCTGCCCCGGCTGCAGATTTTATTAGAGCTGCTGCTGCGTCTGGCTCGGTCG
AGCCCACCCCCAGAGGATCCCCGAACTGCCTCACCAGCGAGGCG

Case 3: MCF-7 Human ESR1-Point Mutation Knockin Cell Line

1) **Project Description:** Generation of MCF-7 Stable Cell Lines for Introducing One Point Mutation in Human ESR1 Gene With CRISPR/Cas9 RNP Complexes.

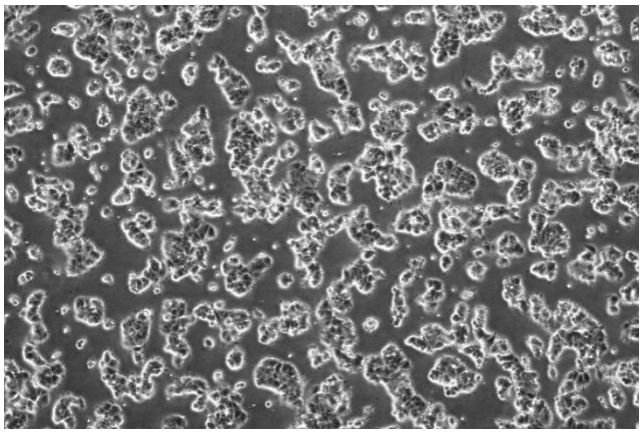
2) **Generation of the mutation cell line:** MCF-7 cells were transfected with Cas9-gRNA and ssDNA (hESR1*(p.Y537S)) below.

gRNA: TCTCCAGCAGCAGGTCATAG AGG

ssDNA:

TAAAGTAGTCCTTTCTGTGTCTTCCCACCTACAGTAACAAAGGCATGGAGCATCTGTACAGCATG
AAGTGCAAGAACGTGGTGCCCCTCTCTGACCTGCTGCTGGAGATGCTGGACGCCCACCGCCT

3) The morphology of positive clones is shown below (40X magnification):



4) Validation of hESR1 Mutation

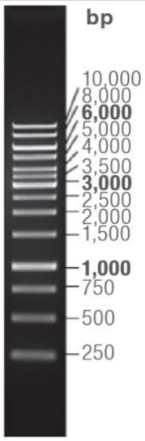
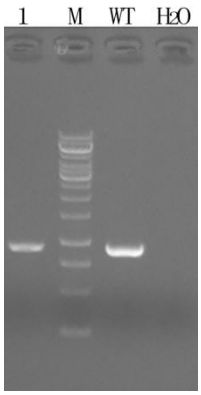
PCR

PCR was performed on the genomic DNA extracted from the stably transfected cells.

a) Primer information

Target Gene	Primer Name	Primer Sequence (5'->3')	Product Size (bp)
ESR1	F:	AATACCCACTCCTGCTTGGC	928
	R:	GAGCTACGGGAATCCTCACG	

b) Result

Marker	Region 1 (928 bp)
	

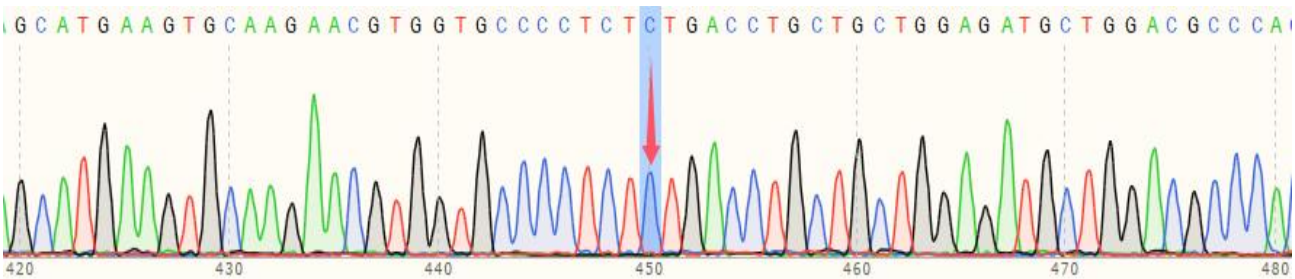
Sanger

WT:

TAAAGTAGTCCTTTCTGTGTCTTCCCACCTACAGTAACAAAGGCATGGAGCATCTGTACAGCATG
AAGTGCAAGAACGTGGTGCCCCTCTATGACCTGCTGCTGGAGATGCTGGACGCCCACCGCCT

Clone 1:

TAAAGTAGTCCTTTCTGTGTCTTCCCACCTACAGTAACAAAGGCATGGAGCATCTGTACAGCATG
AAGTGCAAGAACGTGGTGCCCCTCTCTGACCTGCTGCTGGAGATGCTGGACGCCCACCGCCT



Genotyping PCR and Sanger sequencing showed that the ESR1 mutation was detected, and single clone, Clone 1 was validated to be homozygous.

8. Successful Cases (Examples)

Host Cell	Gene	Genetic modification	Methods
RAW264.7	Leptin	3.5 Kb-KI	RNP
SW480	NFE2L2	4.6 Kb-KI	RNP
HEK293	FOXG1	0.7 Kb-KI	RNP
HEK293	FOXG1	0.6 Kb-KI	RNP
U2OS	ATG13	5.8 Kb-KI	VIRUS-Free
U2OS	ATG16L1	5.8 Kb-KI	VIRUS-Free
Continuing to update...			

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