ACCEGEN
BIOTECHNOLOGY

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AcceGen

Knockout Stable Cell Line
Product Manual

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1. Description

Knockout stable cell lines are generated by introducing loss-of-function mutations into specific target genes within the mammalian genome using genetic engineering techniques, thereby achieving complete or partial gene inactivation. These engineered models provide a controlled and reproducible system for studying gene ablation and serve as an essential experimental platform for elucidating gene function, biological pathways, and pathological mechanisms.

As critical tools in functional genomics and molecular mechanism research, knockout stable cell lines are widely employed in the investigation of signaling networks, biological target validation, antibody specificity and affinity assessment, disease model development, drug screening, and assay establishment. By systematically analyzing the molecular and phenotypic alterations that arise following gene disruption, researchers can precisely define the role of the target gene in cellular homeostasis, disease processes, and regulatory pathways, while also validating the specificity and functional efficacy of target-directed molecules.

With our customized services, you will receive high-quality, reliable, and research-oriented technical support designed to advance and accelerate your scientific discovery.

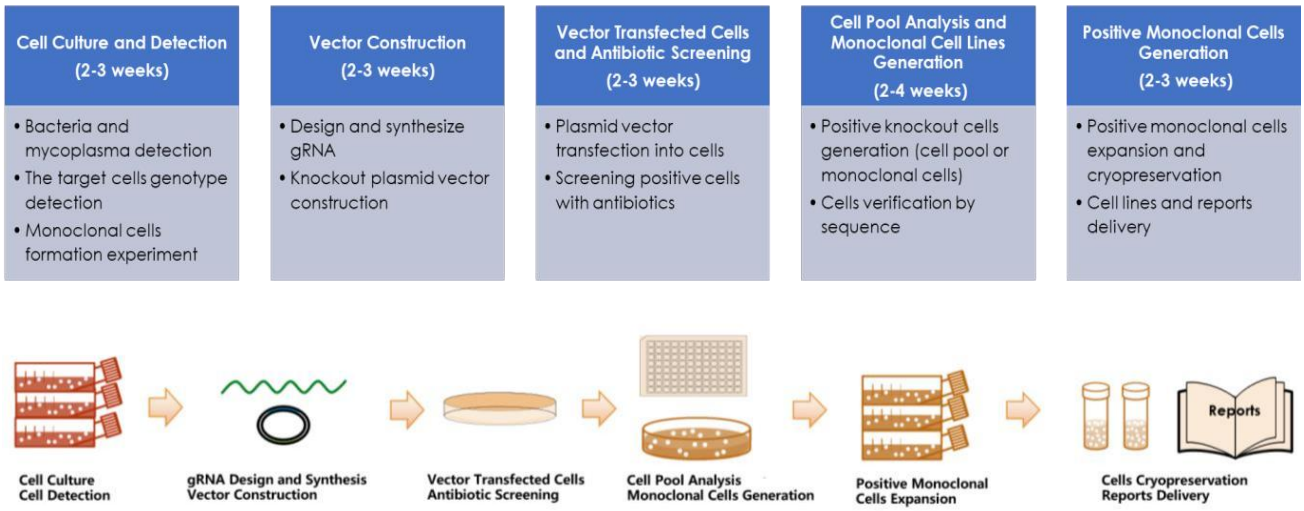
2. Knockout Stable Cell Line Generation Service

Service	Technical method	Gene expression evaluation	Method	Delivery
Knockout 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 and developed a more efficient gene editing system, which can realize multiple knockout strategies such as frameshift knockout, fragment knockout, and multiple gene knockout in host cells. We have extensive experience in constructing knockout stable cell lines, including but not limited to A549, CHO, HepG2. In addition, we provide services such as cellular gene expression regulation and cellfunction verification. Finally, professional laboratory and quality control reports will be delivered.

4. Experimental process



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

Construct a gene knockout stable cell line

Culture and expand the knockout stable cell line using suitable medium and serum

Group cells according to experimental requirements

Study the effects 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, followed by reverse transcription and PCR amplification. The expression of the target gene is detected and analyzed by agarose gel electrophoresis and a gel imaging system.

Sanger sequencing: This method directly reads the DNA sequence of the specific target locus to unequivocally confirm the presence of expected insertions, deletions, or point mutations at the editing site. With its accurate and straightforward results, it serves as the most fundamental and critical step for genotyping knockout 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 knockout 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.

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 cell models for in-depth research application.

6. Delivery of Cell Lines and Report

1 vial of stable cell line (1×10^6 cells/vial) and a knockout project report will be delivered to the customer. If the project requires the customer to provide a host cell, the customer will be requested to fill in AcceGen Custom Stable Cell Line Service Requisition Form.

7. Experimental case report

7.1 Homozygous knockout

Case 1: Human Gene A Knockout Cell Line (Expi293)

Project Description: Generation of an Expi293F Stable Cell Line for Human Gene A Knockout (CRISPR-Cas9) with Ribonucleoprotein (RNP).

Experimental procedure:

RNP complex formation: Form the RNP complexes for cell electroporation, and the gRNA sequences are designed for the target gene.

gRNA-A1: ACACAGCATAATCATTAAGG TGG

gRNA-A2: CTGCTACCTAAGCACGTGAG AGG

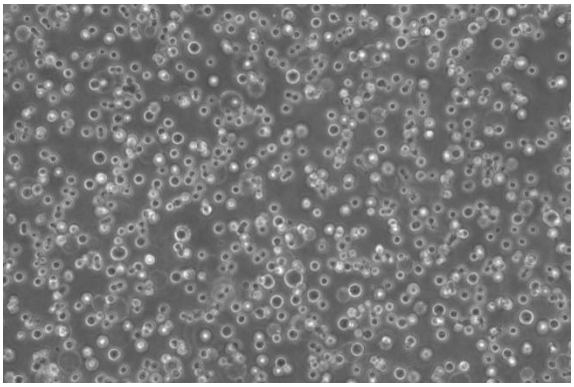
Cell electroporation: Target cells are electroporated with one or more RNP complexes.

Generation of stable cell line from single clone:

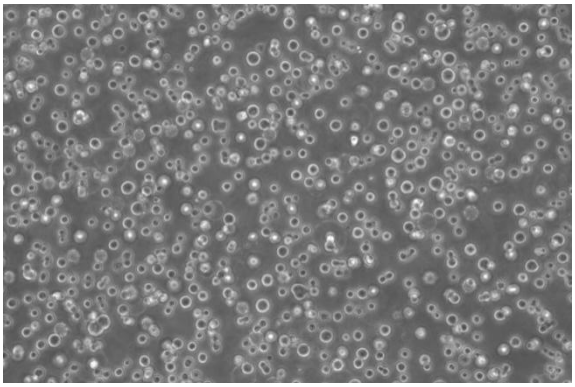
- Plate the electroporated cells and culture them in appropriate medium.
- Genome DNA is extracted, followed by a T7E1 mismatch cleavage assay or Sanger sequencing to determine the remaining transfected cells containing the edits.
- Isolate ~96 cell clones from the plates and expand to 24 wells.
- Retrieve genomic DNA from the single clones, perform genotyping PCR and Sanger sequencing for the target region.
- Identify positive single clones with the genomic deletions on one or more copies of target gene.
- After QC completion. expand and cryopreserve the monoclonal cell lines, including sterility testing for bacteria and fungi, and mycoplasma detection.

Generation of the Knockout cell lines: Expi293F cells are transfected with RNP complexes. After transfection, selected cells are monocloned and identified by genotyping PCR and Sanger sequencing.

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



Clone 1



Clone 2

Validation of Knockout

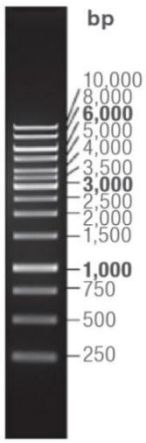
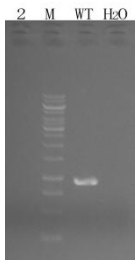
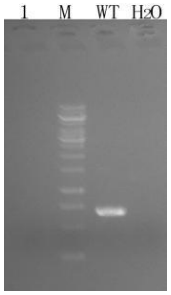
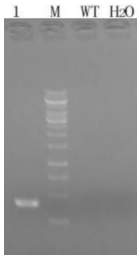
Genotyping PCR:

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

Primer information

Target Gene	Primer Name	Primer Sequence (5'->3')
[gRNA#1]	F:	GACTTTGATCTCTTTTCCAAGGTGT
	R:	AGGCTAGTGTTGTTGATTCCAA
[gRNA#2]	F:	TGGATCCTGTTCATGGGTGC
	R:	ACACCAAGTGTGCCTTCTCC
[KO]	F:	GACTTTGATCTCTTTTCCAAGGTGT
	R:	ACACCAAGTGTGCCTTCTCC

Result

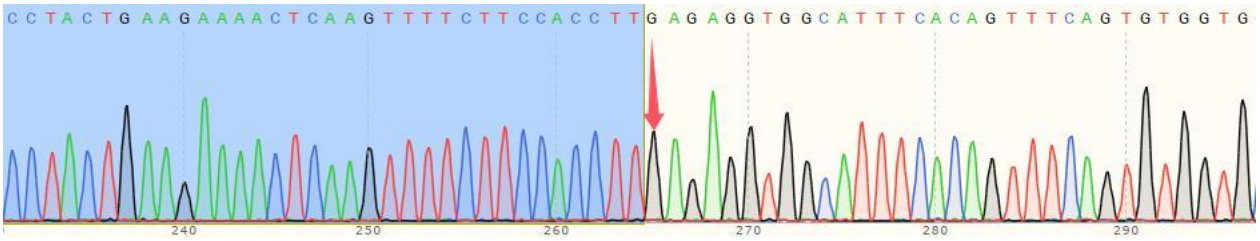
Marker	Region 1 (WT: 962 bp, heterozygote: 962 bp, homozygous: 0 bp)	Region 2 (WT: 681 bp, heterozygote: 681 bp, homozygous: 0 bp)	Region 3 (WT: 15393 bp, heterozygote: 15393/~416bp, homozygous: ~416 bp)
	 	 	 

Genotyping PCR results showed that the Expi293F knockout was detected, and single clones, Clone 1 and Clone 2 were validated to be homozygous.

Sanger Sequencing

Clone 1:

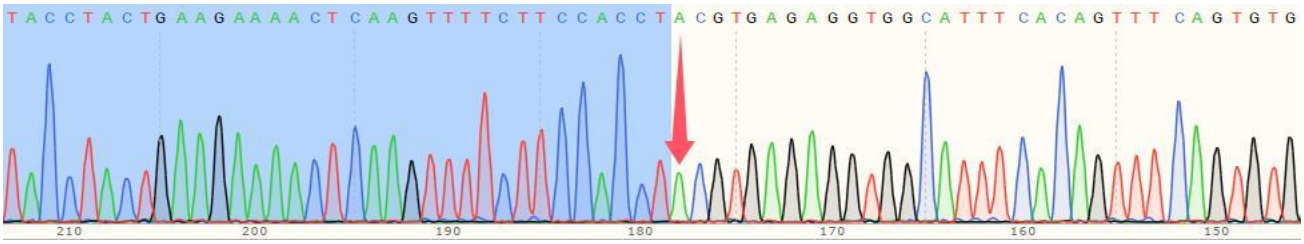
CCTACTGAAGAAAACTCAAGTTTTCTTCCACCTT-del 14976 bp-GAGAGGTGGCATTTCACAGTTTCAGTGTGGTGCTG



Clone 2:

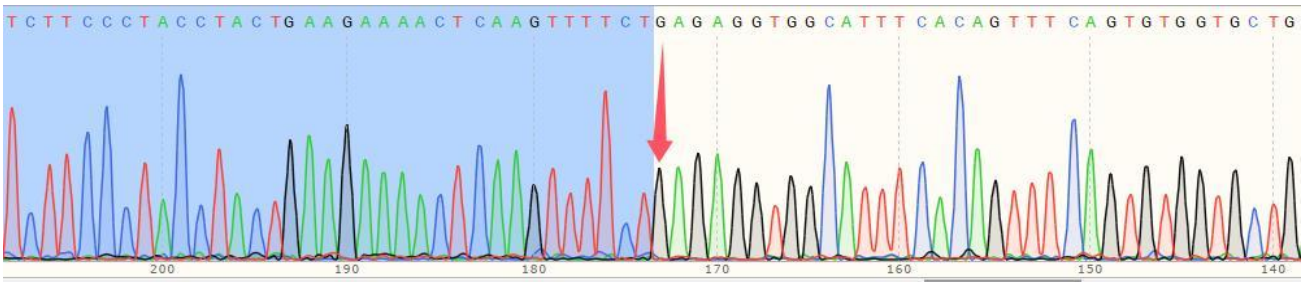
TACCTACTGAAGAAAACTCAAGTTTTCTTCCACCT-del 14973 bp-

ACGTGAGAGGTGGCATTTCACAGTTTCAGTGTGGT



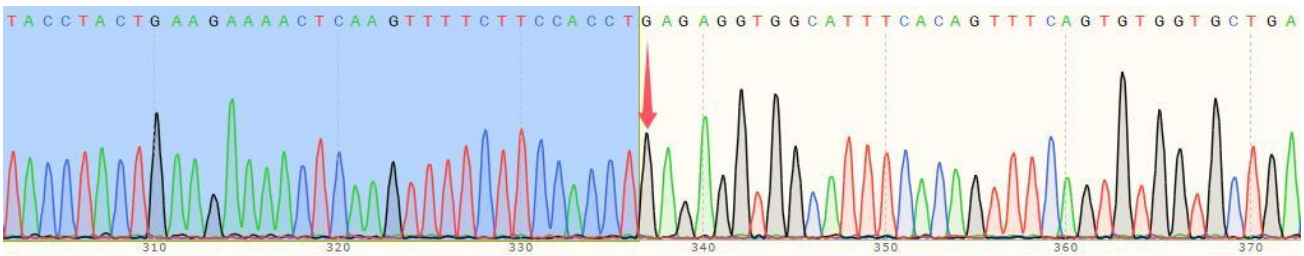
TCTTCCCTACCTACTGAAGAAAACTCAAGTTTTCT-del 14984 bp-

GAGAGGTGGCATTTCACAGTTTCAGTGTGGTGCTG



TACCTACTGAAGAAAACTCAAGTTTTCTTCCACCT-del 14977 bp-

GAGAGGTGGCATTTCACAGTTTCAGTGTGGTGCTG



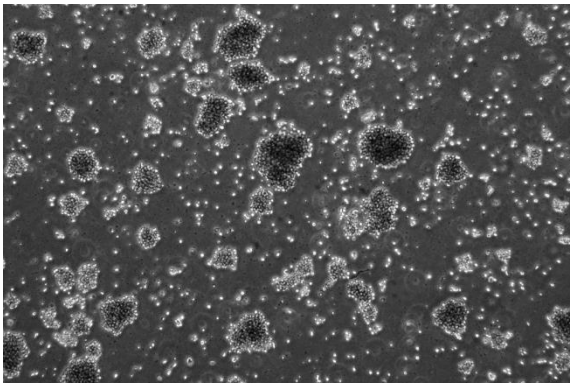
Conclusion

Two single clones, Clone 1 and Clone 2, with Human Gene A knockout were obtained successfully.

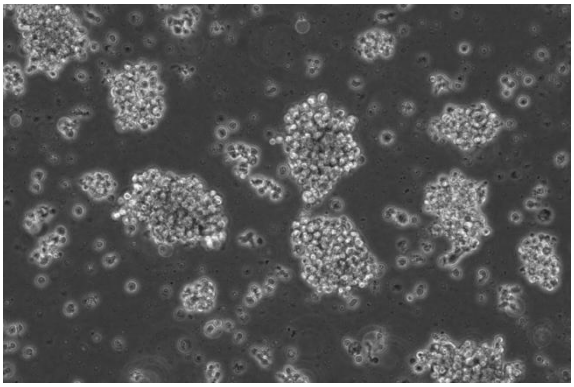
Case 2: Human Gene B Knockout Cell Line (NK-92)

KO Region: Exon 3~4 (~8.2 Kb)

The morphology of positive clones is shown below (40X&100X magnification):



40X

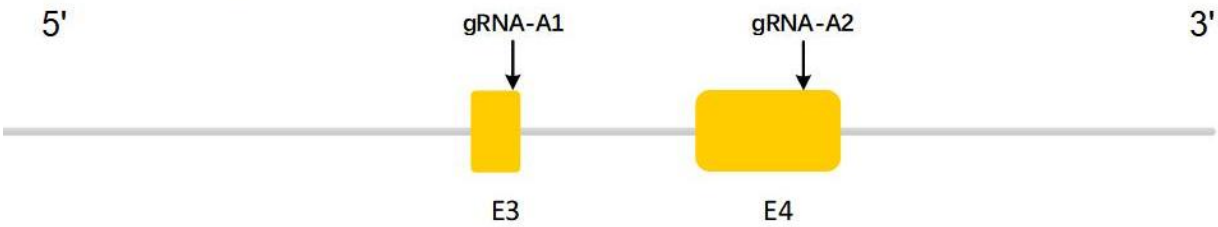


100X

Validation of KO

Genotyping PCR:

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



Sanger Sequencing

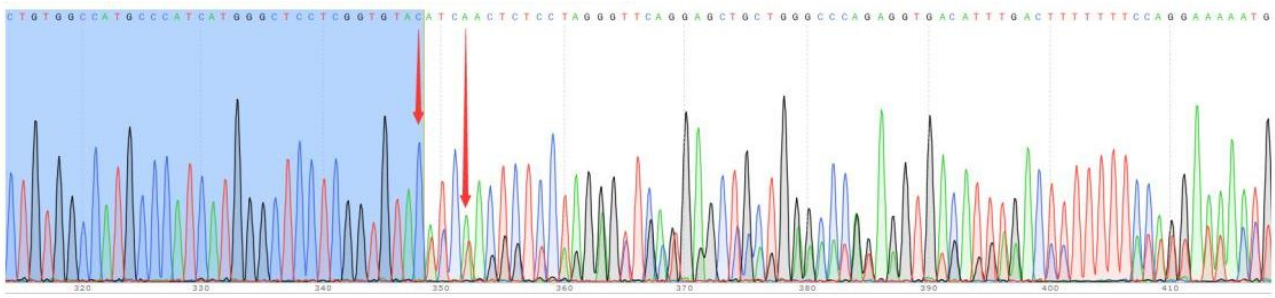
Clone 1:

GGCCATGCCCATCATGGGCTCCTCGGTGTACATCA - del 8459 bp -

ACTCTCCTAGCGTTCAGCAGCTCCTGGGCCCAGAG

CTGTGGCCATGCCCATCATGGGCTCCTCGGTGTAC - del 8522 bp - in 30 bp-

AAAATGTAAGTGTGAGGAAACCCTTTTTATTTTAT



8. Successful Cases (Examples)

Host Cell	Gene	Exons	Methods
HEK293	B2M	Exon 1	RNP
KGN	ESR1	Exon 4	RNP
TCMK-1	DDAH2	Exon 1~6	RNP
MDA-MB-231	ADORA2A	Exon 3~4	VIRUS-Free
K562	c-Myc	Exon 2~3	VIRUS-Free
U937	HMGB1	Exon 2~4	VIRUS-Free
786-O	ADAMTS5	Point mutation	VIRUS-Free
THP-1	SIRT4	Point mutation, T < G	VIRUS-Free
OVCAR-3	CAV1	2.4 Kb	VIRUS-Free
A2780	CARS1	Exon 3~7	VIRUS
Continuing to update...			

9. Spot Inventory List

Field	Host Cell	Gene	Cat.No
Cancer-related	786-O	hBAP1	ABC-KH1333
	MDA-MB-231	hSTK11	ABC-KH14803
	NCI-H1975	hTP53	ABC-KH15931
	U87-MG	hTP53	ABC-KH15931
	A172	hTP53	ABC-KH15931
	SW480	hTP53	ABC-KH15931
	MOLM-13	hPTEN	ABC-KH12362
	22RV1	hPTEN	ABC-KH12362
	MeT-5A	hBAP1	ABC-KH1333
	NCIN87	hSOX9	ABC-KH14453
	PANC-1	hFOSL1	ABC-KH5652
	THP-1	hTET2	ABC-KH15294
	U251	hRERE	ABC-KH12844
	HT-29	hGDF15	ABC-KH5982

Neurodegenerative Diseases & Neurological Diseases	SH-SY5Y	hPLA2G6	ABC-KH11684
	SH-SY5Y	hPLD3	ABC-KH11722
	SH-SY5Y	hPIEZO2	ABC-KH11545
Immune and Inflammatory Diseases	THP-1	hTBK1	ABC-KH15152
	THP-1	hIL6R	ABC-KH7354
	THP-1	hTGFBR1	ABC-KH15350
	THP-1	hCEBPB	ABC-KH2898
Metabolic Diseases	BEAS-2B	hFTO	ABC-KH5762
	THP-1	hPPARG	ABC-KH11947
	293T	hLDLR	ABC-KH8365
	Huh-7	hHSD17B13	ABC-KH7028
	Caco2	hAPOA1	ABC-KH0799
	HepG2	hAPOA4	ABC-KH0802
	HepG2	hDEGS1	ABC-KH4103
Development and Signal Transduction	HEK293	hATP7A	ABC-KH1214
	HeLa	hHGS	ABC-KH6729
	Hep3B2.1-7	hRAB18	ABC-KH12533
	Hep3B2.1-7	hMFSD2A	ABC-KH9238
	LX-2	hTPP1	ABC-KH15987
	A375	hSYVN1	ABC-KH15008
Others	THP-1	hBCAT2	ABC-KH1376
	HCT116	hARRDC4	ABC-KH1023
	MIA PaCa-2	hSNAP23	ABC-KH14344
	293T	hFKBP1A	ABC-KH5573
	HeLa	hLTA	ABC-KH8773
Our services are more than that...			

Note: Subject to actual inventory availability provided by the Sales Department.

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