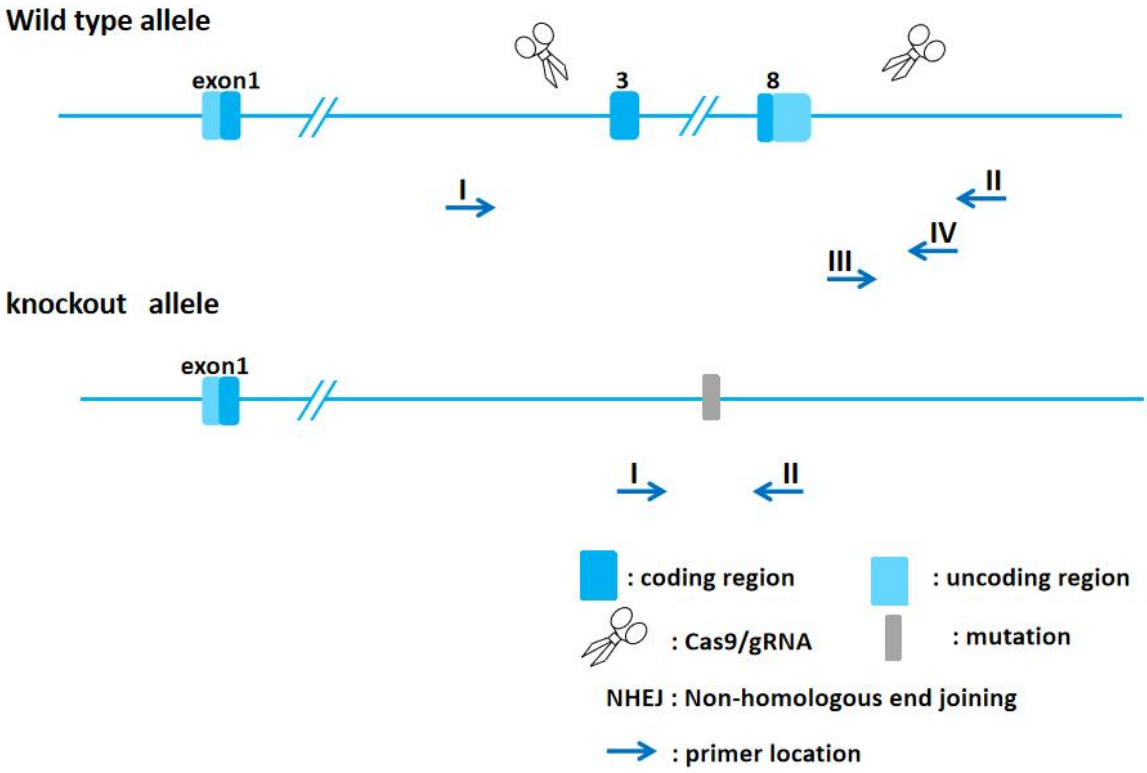
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Ccs-KO Genotyping Protocol

Common Name	Ccs-KO	**Cat. NO.**	NM-KO-240179
Strain of Origin	C57BL/6J	**Version**	V1

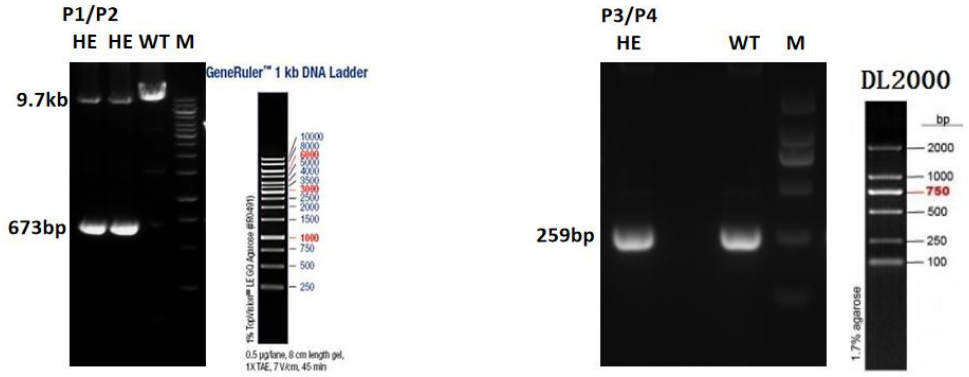
Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	TGTTCTGTGCCATGATAGCCTT	Forward
P2	CTGTGTCTTTTTGGCTCGGC	Reverse
P3	AGGCCTAAGTACAGGGCAGA	Forward
P4	AAGGACCCAGGGAGATCACA	Reverse

Expected results

Results	
Genotype	Knockout type: -9031 bp Wild type: P1P2 =9704 bp; P3P4 =259 bp Heterozygote: P1P2 =9704 bp and 673 bp; P3P4 =259 bp Homozygote: P1P2 =673 bp

Note: In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band.

Reaction &Cycling

	Reaction Component	Volume (μl)
PCR Reaction System	ddH20	8.0
	2 × Rapid Taq Master Mix	10.0
	P1(10 pmol/μl) or P3(10 pmol/μl)	0.5
	P2(10 pmol/μl) or P4(10 pmol/μl)	0.5

	Genomic DNA			1.0
	Total			20
	2 × Rapid Taq Master Mix from Vazyme(Code Number: P222-01)			
Cycling Reaction	Step	Temp	Time	Note
	1	95° C	5 min	
	2	95° C	20 sec	
	3	60° C	20 sec	
	4	72° C	20 sec	35 repeats to 2
	5	72° C	5 min	
	6	12° C	Hold	