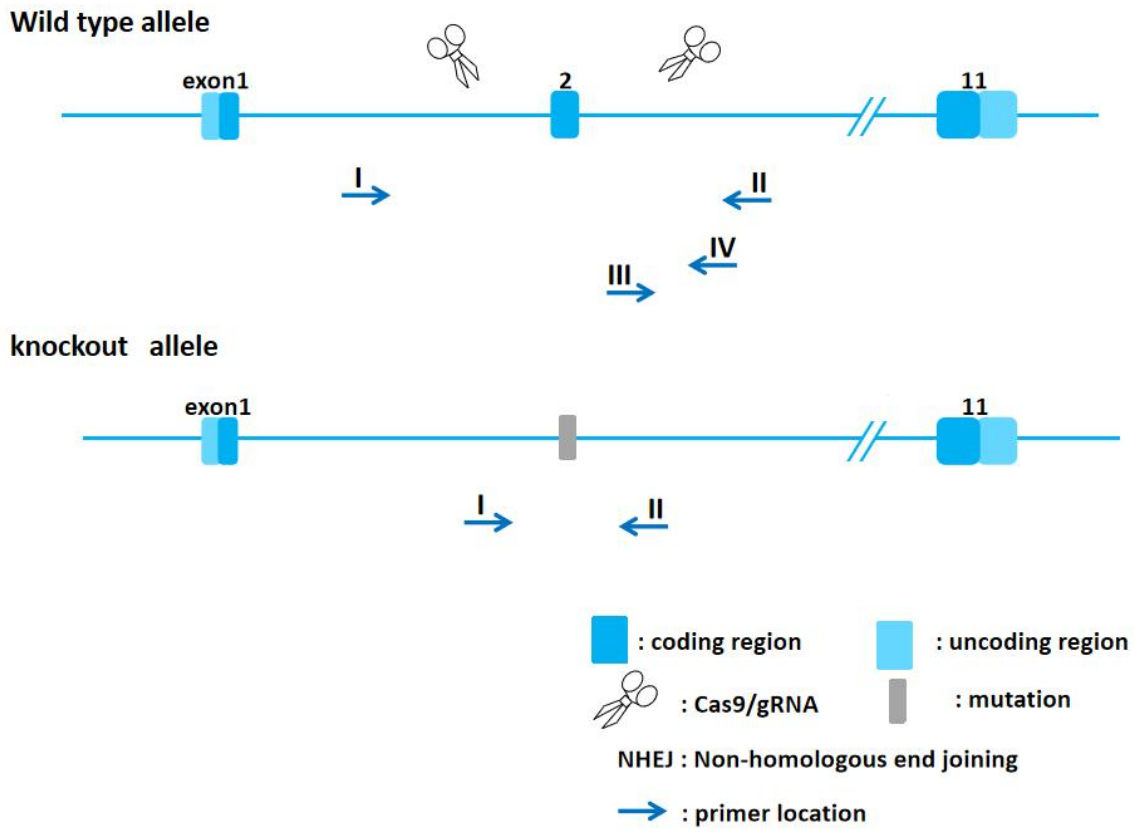
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Sucla2-KO Genotyping Protocol

Common Name	Sucla2-KO	**Cat. NO.**	NM-KO-231556
Strain of Origin	C57BL/6J	**Version**	V1

Genotyping strategy

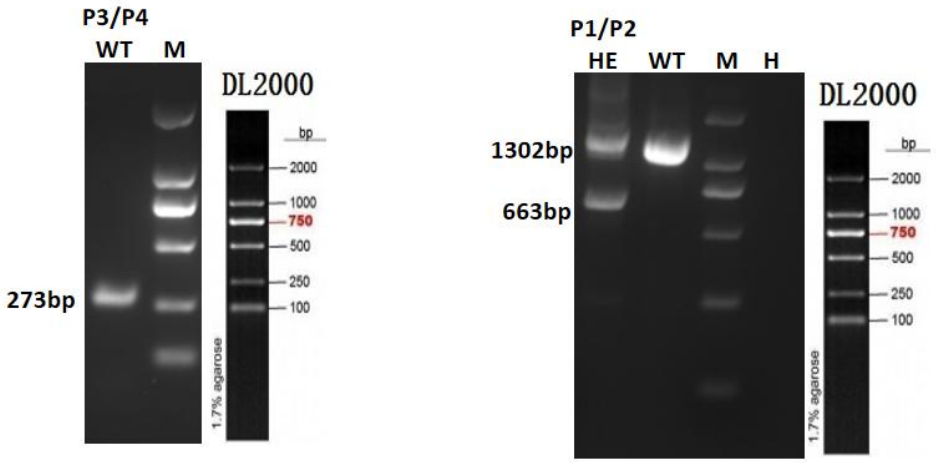


Primers

Primer	Sequence (5' →3')	Primer type
P1	GTGCGACCGGGTACCAATTA	Forward
P2	TCACCCTTTCCACACCACAC	Reverse
P3	TGATGACGTGGCCTTGCATA	Forward

P4	AGAGTAACTTTCTCCTAAAGTCTGT	Reverse

Expected results

Results	
Genotype	Knockout type: -639 bp Wild type: P1P2 =1302 bp; P3P4 =273 bp Heterozygote: P1P2 =1302 bp and 663 bp; P3P4 =273 bp Homozygote: P1P2 =663 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

PCR	Reaction Component	Volume (μl)
Reaction	ddH20	8.0

System	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	