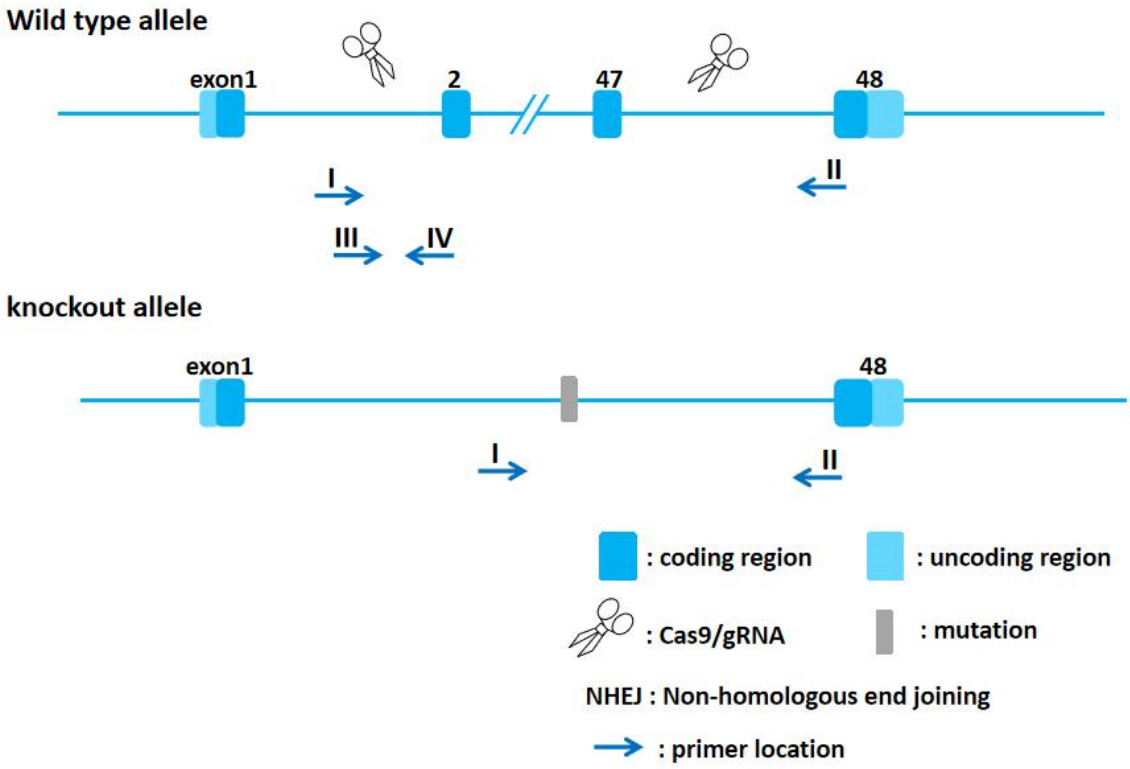
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Cacna1f-KO Genotyping Protocol

Common Name	Cacna1f-KO	Cat. NO.	NM-KO-241309
Strain of Origin	C57BL/6J	Version	V1

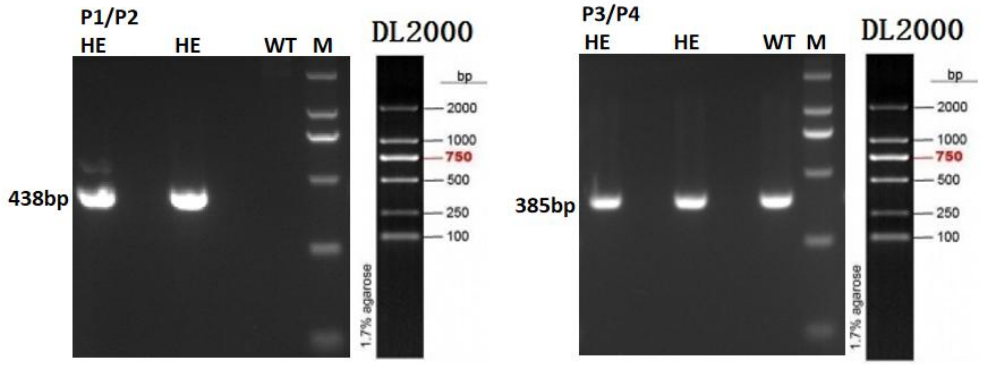
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGACCCACGCAGACACAAAA	Forward
P2	GAGACCTAGGCCTTCGGAGA	Reverse
P3	TGTCCTGGAGTTGAAATGCACA	Forward
P4	TGGGTCCTTCTTCTTGGGGT	Reverse

Expected results

Results	
Genotype	Knockout type：-25979 bp Wild type：P3P4 =385 bp Heterozygote：P1P2 =438 bp；P3P4 =385 bp Homozygote：P1P2 =438 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 System	Reaction Component			Volume (μl)
	ddH2O			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℃	5 min	
	2	95℃	20 sec	
	3	60℃	20 sec	
	4	72℃	20 sec	35 repeats to 2
	5	72℃	5 min	
	6	12℃	Hold	