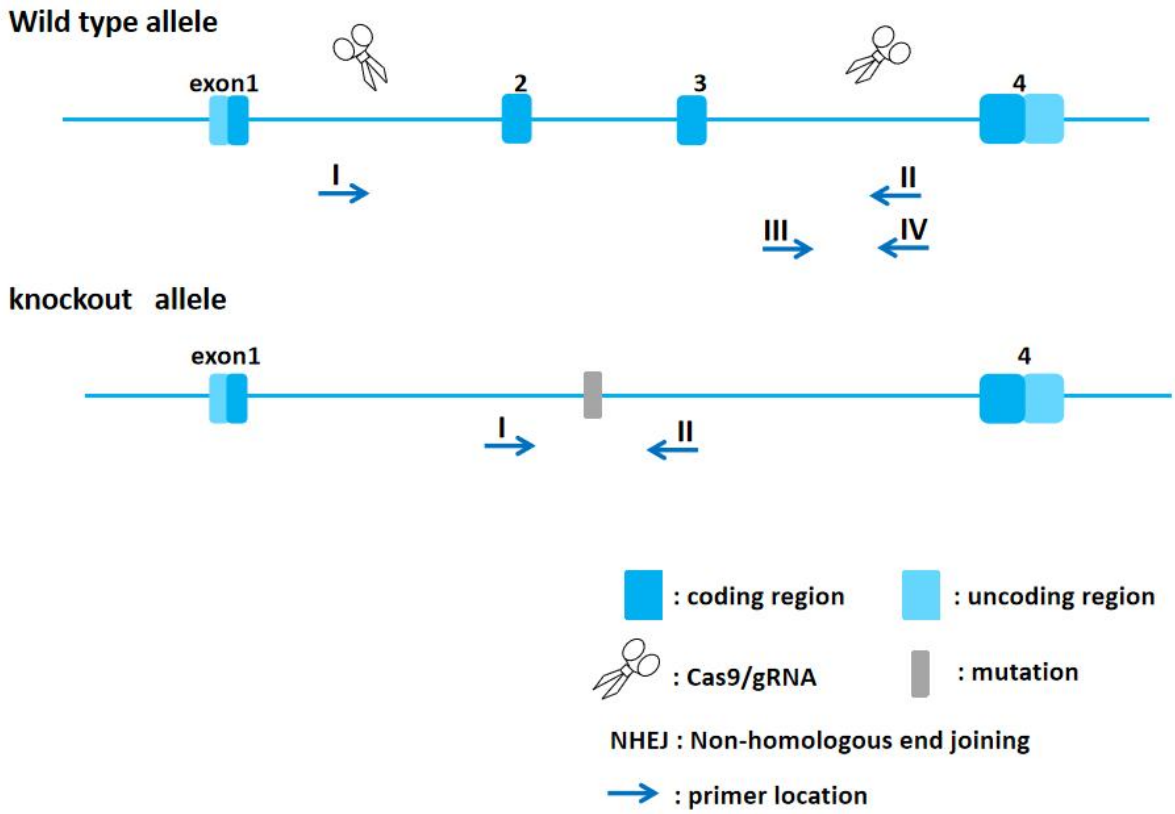
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Cd300lb-KO Genotyping Protocol

Common Name	Cd300lb-KO	Cat. NO.	NM-KO-240802
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

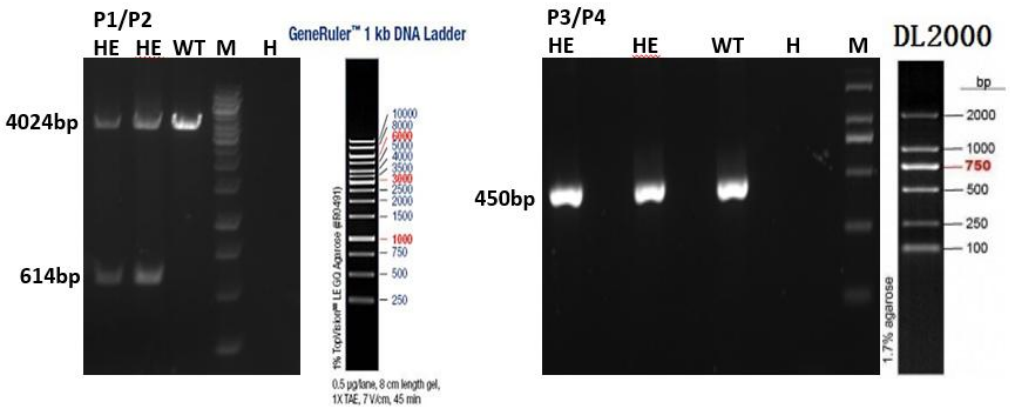


Primers

Primer	Sequence (5'→3')	Primer type
P1	GTGAGGAGGAGAGATGACCCT	Forward
P2	TCTCTCCCTTGGCAGTCACA	Reverse
P3	CCATGCTCCTGCCTTGATGAT	Forward

P4	TCTGCCCTCAACAATGGCTC	Reverse

Expected results

Results	
Genotype	Knockout type: -3410bp Wild type: P1P2 =4024 bp; P3P4 =450 bp Heterozygote: P1P2 =4024 bp and 614 bp; P3P4 =450 bp Homozygote: P1P2 =614 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	ddH2O	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	40 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	