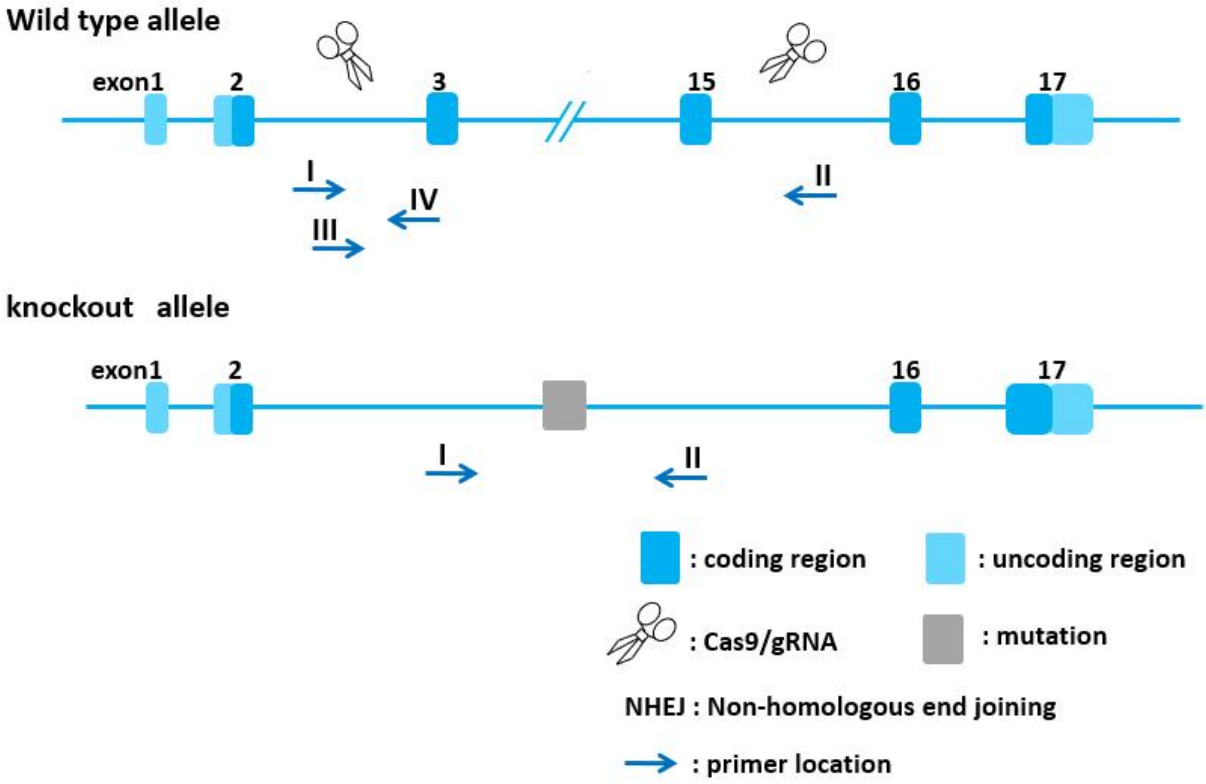
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Abcb8-KO Genotyping Protocol

Common Name	Abcb8-KO	Cat. NO.	NM-KO-240865
Strain of Origin	C57BL/6J	Version	V1

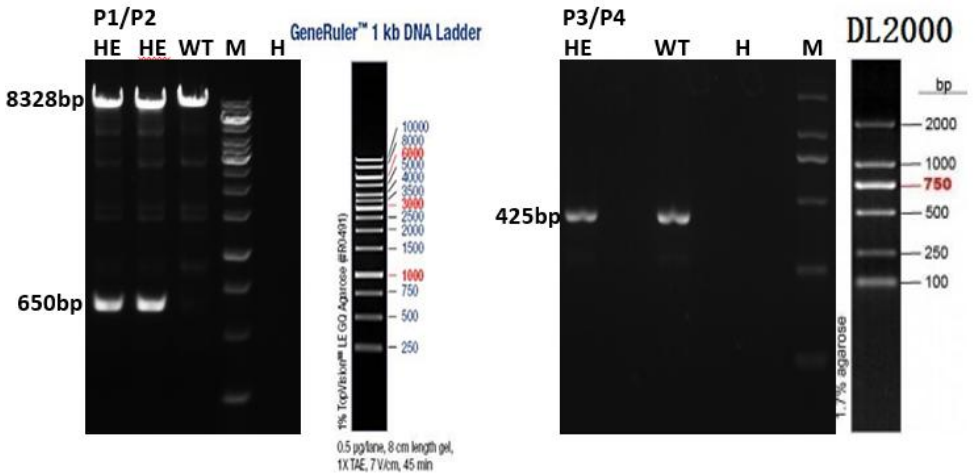
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCTTCCCGAGGTCCTGAGTT	Forward
P2	TTTCTTGTCCTGGGCTGTGG	Reverse
P3	TGATGTGTCTGAAGTCAGCTAAGG	Forward
P4	TACAGTCCGCAGGAGGTGTG	Reverse

Expected results

Results	
Genotype	Knockout type: -7678bp Wild type: P1P2 =8328 bp; P3P4 =425 bp Heterozygote: P1P2 =8328 bp and 650 bp; P3P4 =425 bp Homozygote: P1P2 =650 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	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°C	5 min	
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	1min 30 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	