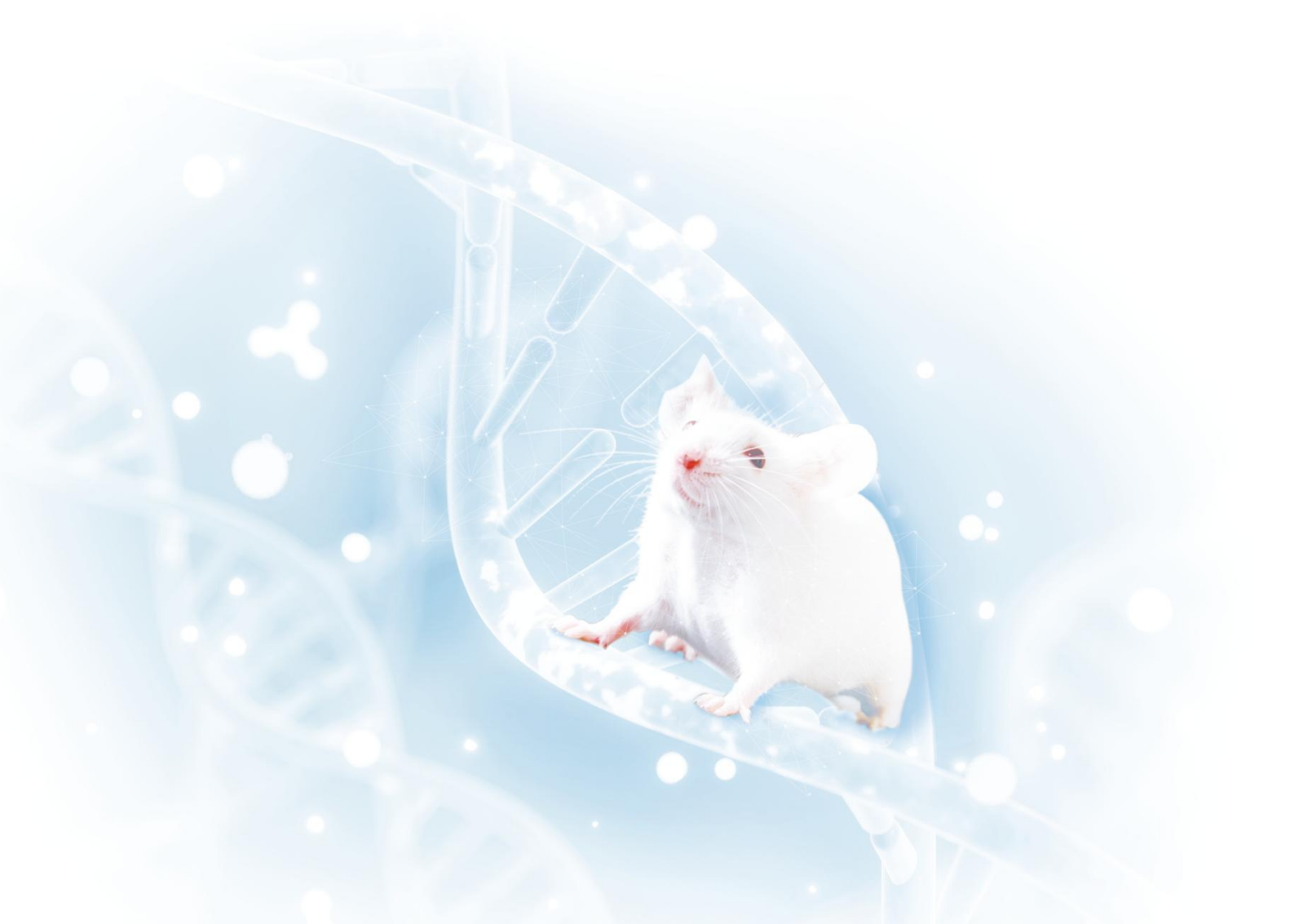
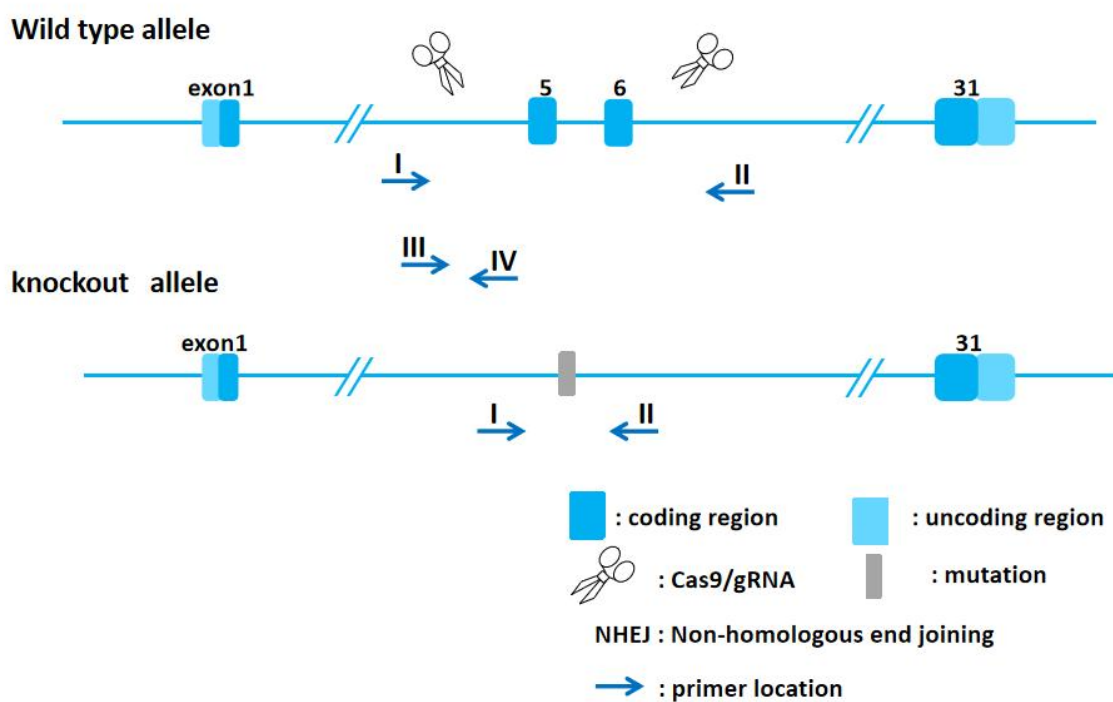


Abcc4-K0 Genotyping Protocol



Common Name	Abcc4-KO	Cat. NO.	NM-KO-232766
Strain of Origin	C57BL/6J	Version	V1

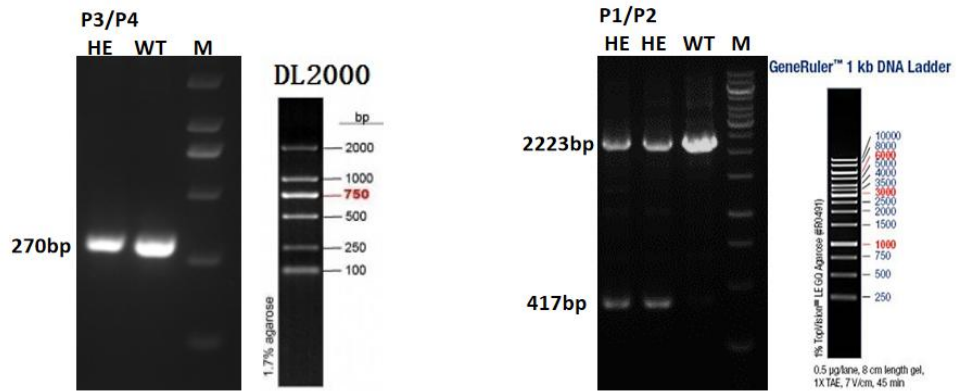
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCGCCGCTTCTCTTTCATT	Forward
P2	TCTGTAAAGTTGGCCCTGC	Reverse
P3	AGAAGCCATGACCACGTGTA	Forward
P4	GCCATGACGACAGCCCTAAT	Reverse

Expected results

Results	
Genotype	Knockout type: -1806 bp Wild type: P1P2 =2223 bp; P3P4 =270 bp Heterozygote: P1P2 =2223 bp and 417 bp; P3P4 =270 bp Homozygote: P1P2 =417 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° 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	