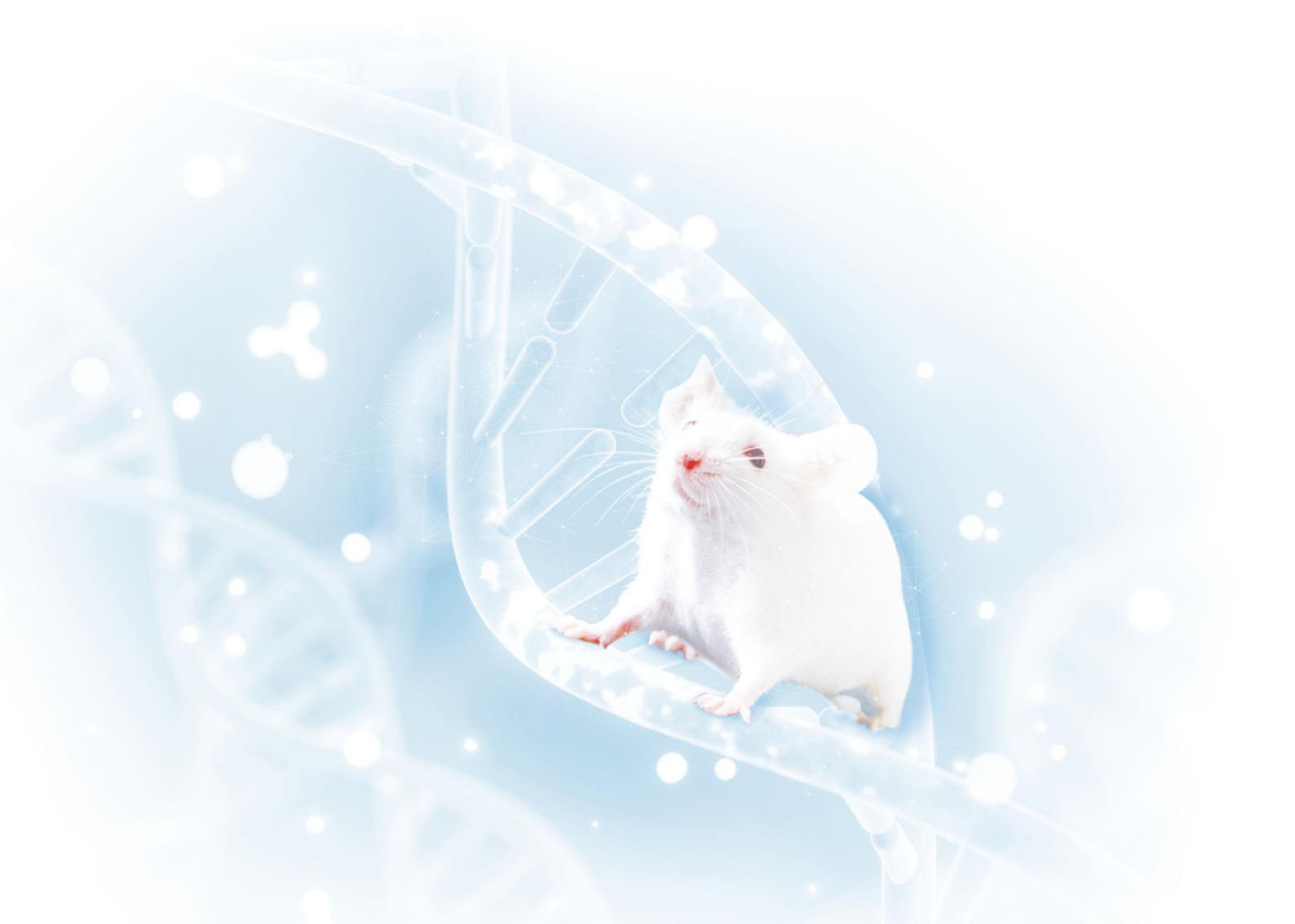
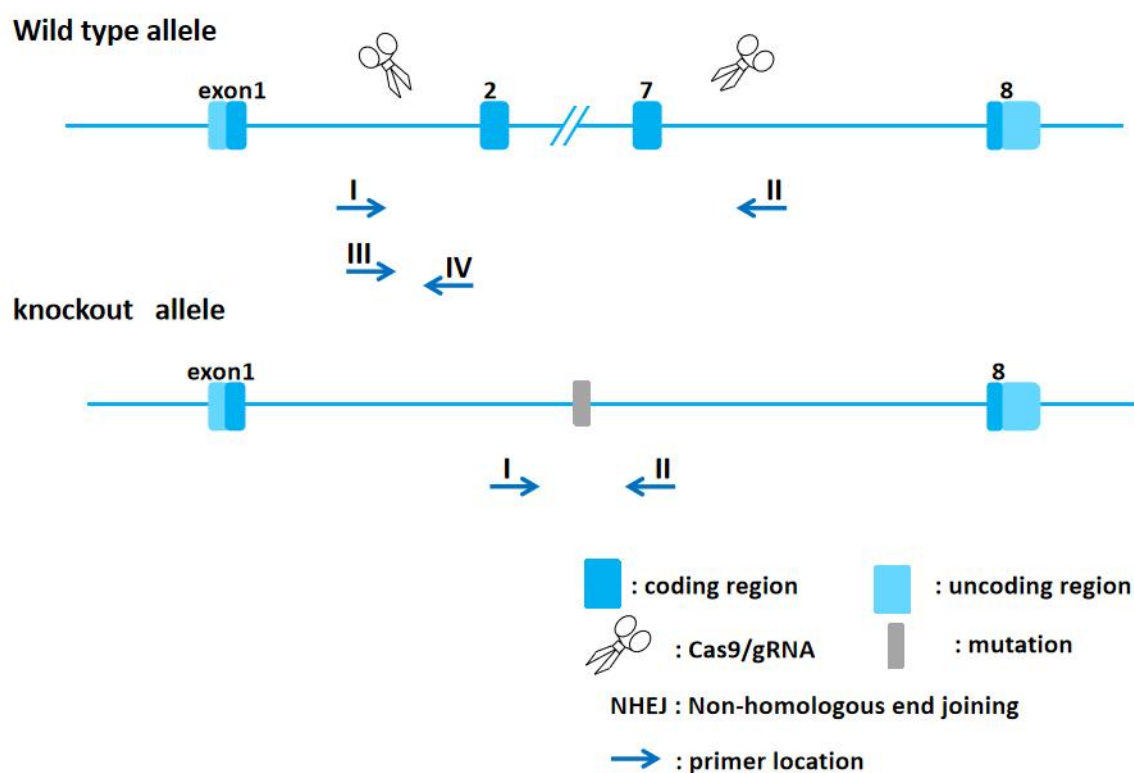


Car4-KO Genotyping Protocol



Common Name	Car4-K0	Cat. NO.	NM-K0-234803
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	ACAGAGCTGTGTCAGAGCAG	Forward
P2	CTCAGGTCCCCACATGAACC	Reverse
P3	CCAGAAGATTGGCATGCTGG	Forward

P4	GAGCCTTAGGTGAGCCCGTA	Reverse
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Expected results

Results	P3/P4 HE HE WT M 334bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose P1/P2 HE M WT HE 3141bp 540bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3000 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -2601 bp Wild type: P1P2 =3141 bp; P3P4=334 bp Heterozygote: P1P2 =3141 bp and 540 bp; P3P4=334 bp Homozygote: P1P2 =540 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	20 sec	35 repeats to 2
	5	72° C	5 min	
	6	12° C	Hold	