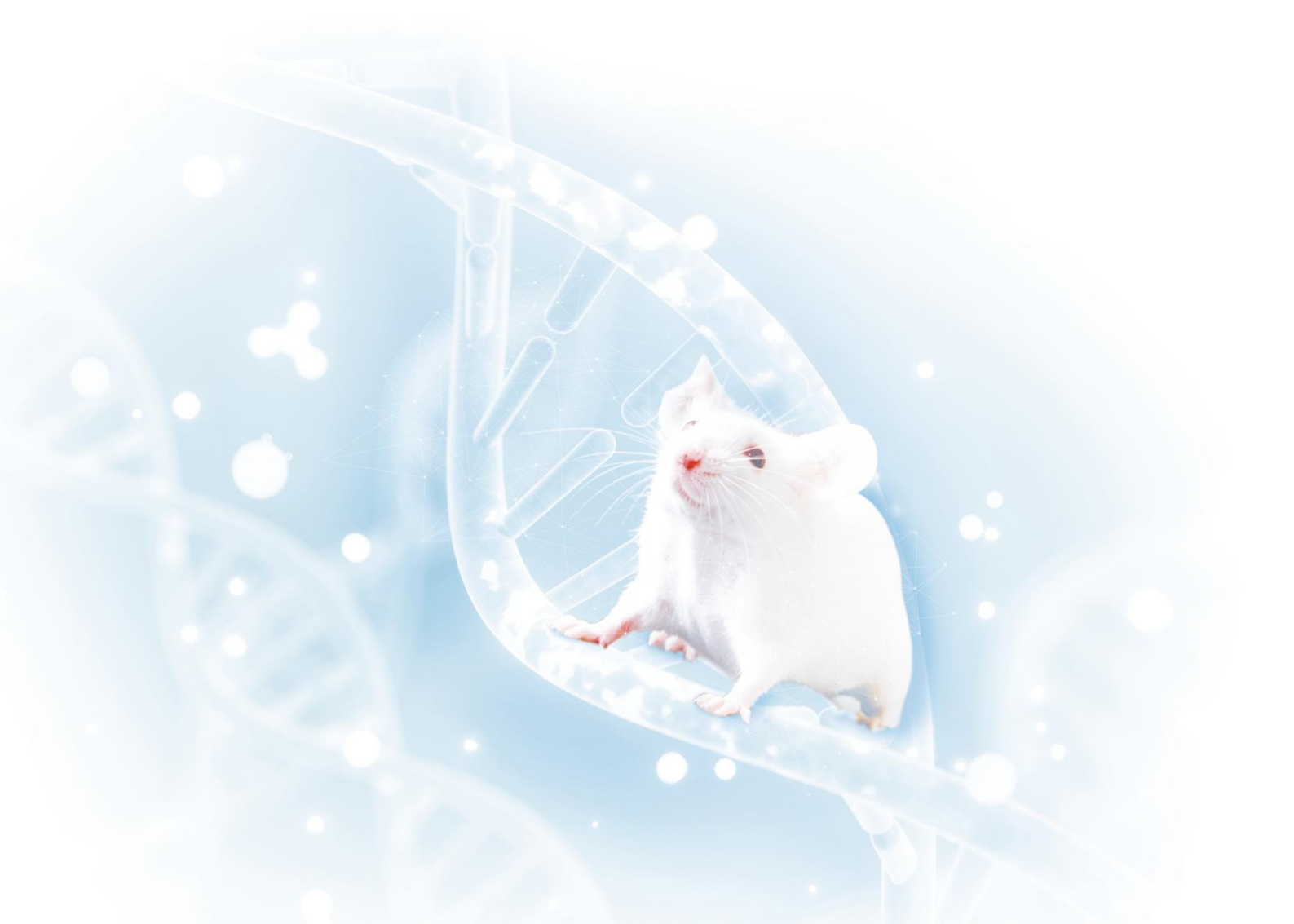
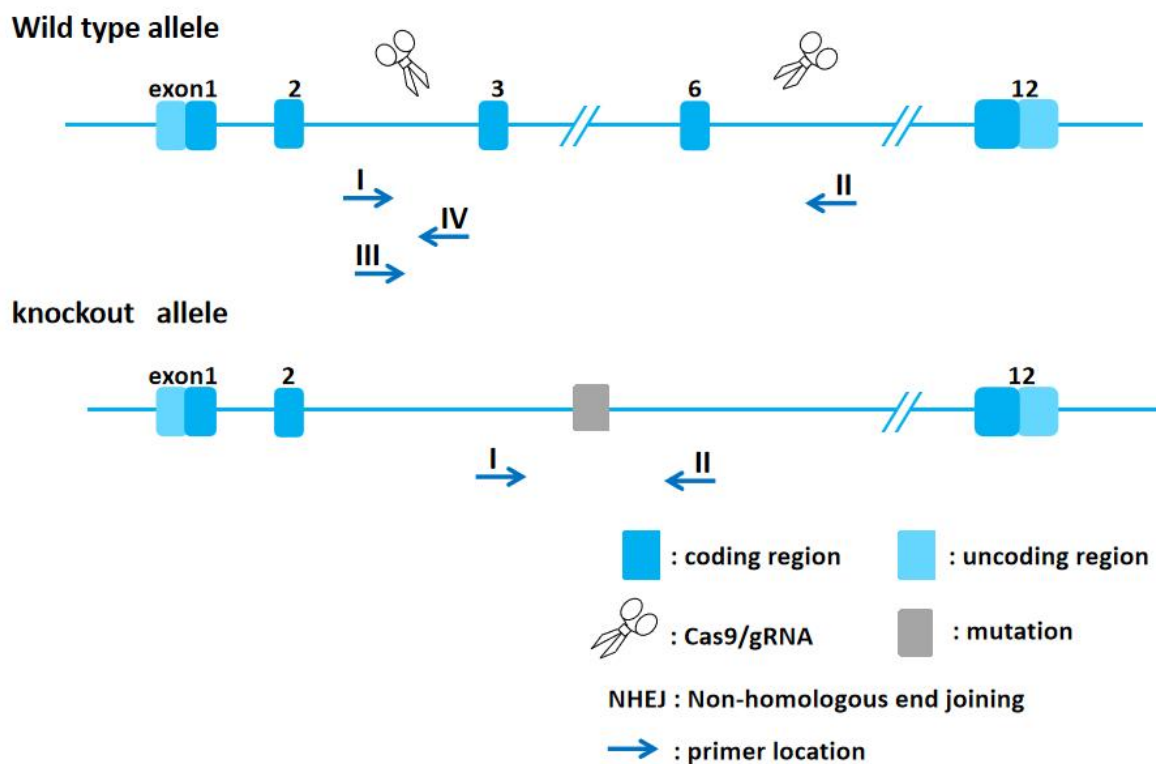


Card9-KO(2) Genotyping Protocol



Common Name	Card9-KO(2)	Cat. NO.	NM-KO-241193
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TGGTCTCTAGAAGGGGAGCC	Forward
P2	ACCTCTAAATCCGGGGACCA	Reverse
P3	CGATGGCACACACCTTTAGC	Forward
P4	GCTGGTCCTTGCCGTAAGT	Reverse

Expected results

Results	
Genotype	Knockout type: -1842bp Wild type: P1P2 =2462 bp; P3P4 =337 bp Heterozygote: P1P2 =2462 bp and 620 bp; P3P4 =337 bp Homozygote: P1P2 =620 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)
	ddH ₂ O			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	