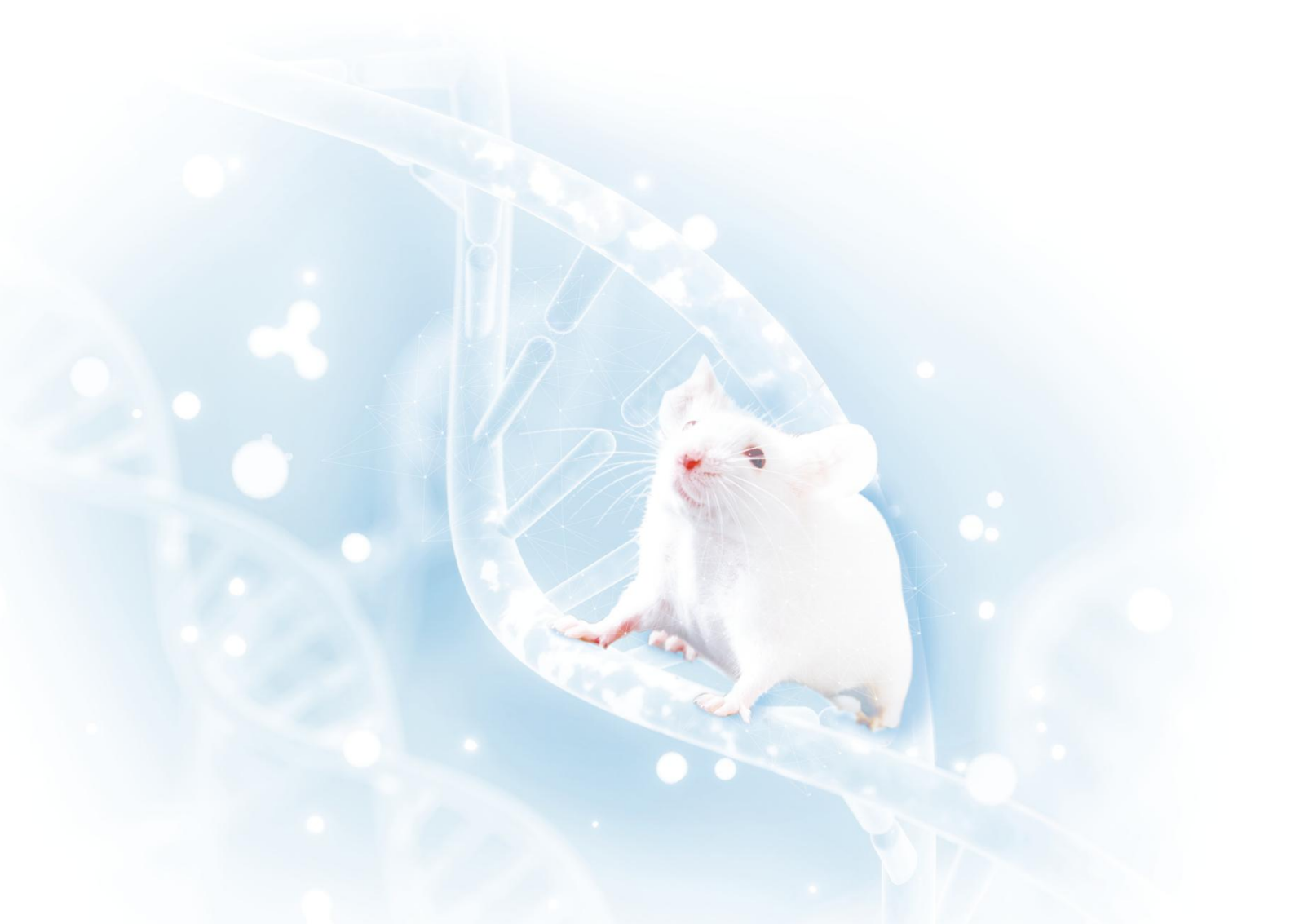
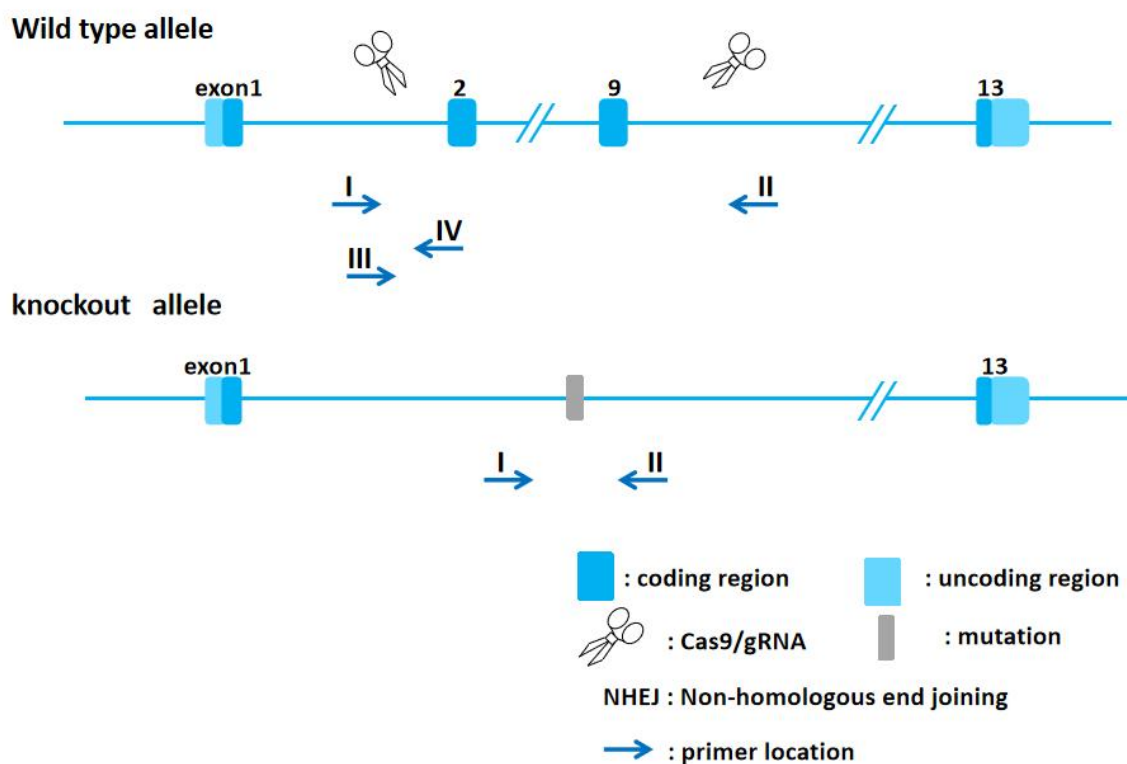


Cat-KO Genotyping Protocol



Common Name	Cat-K0	Cat. NO.	NM-K0-240164
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGACAAACATCAGCCCACG	Forward
P2	AAGACCTGAGGCTTTCGGTG	Reverse
P3	CGGCCGGCATCAATTCTTG	Forward
P4	GACCCGCGGTCATGATATT	Reverse

Expected results

Results	
Genotype	Knockout type: -13957 bp Wild type: P3P4=416 bp Heterozygote: P1P2 =731 bp; P3P4=416 bp Homozygote: P1P2 =731 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 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	20 sec	35 repeats to 2
	5	72° C	5 min	
	6	12° C	Hold	