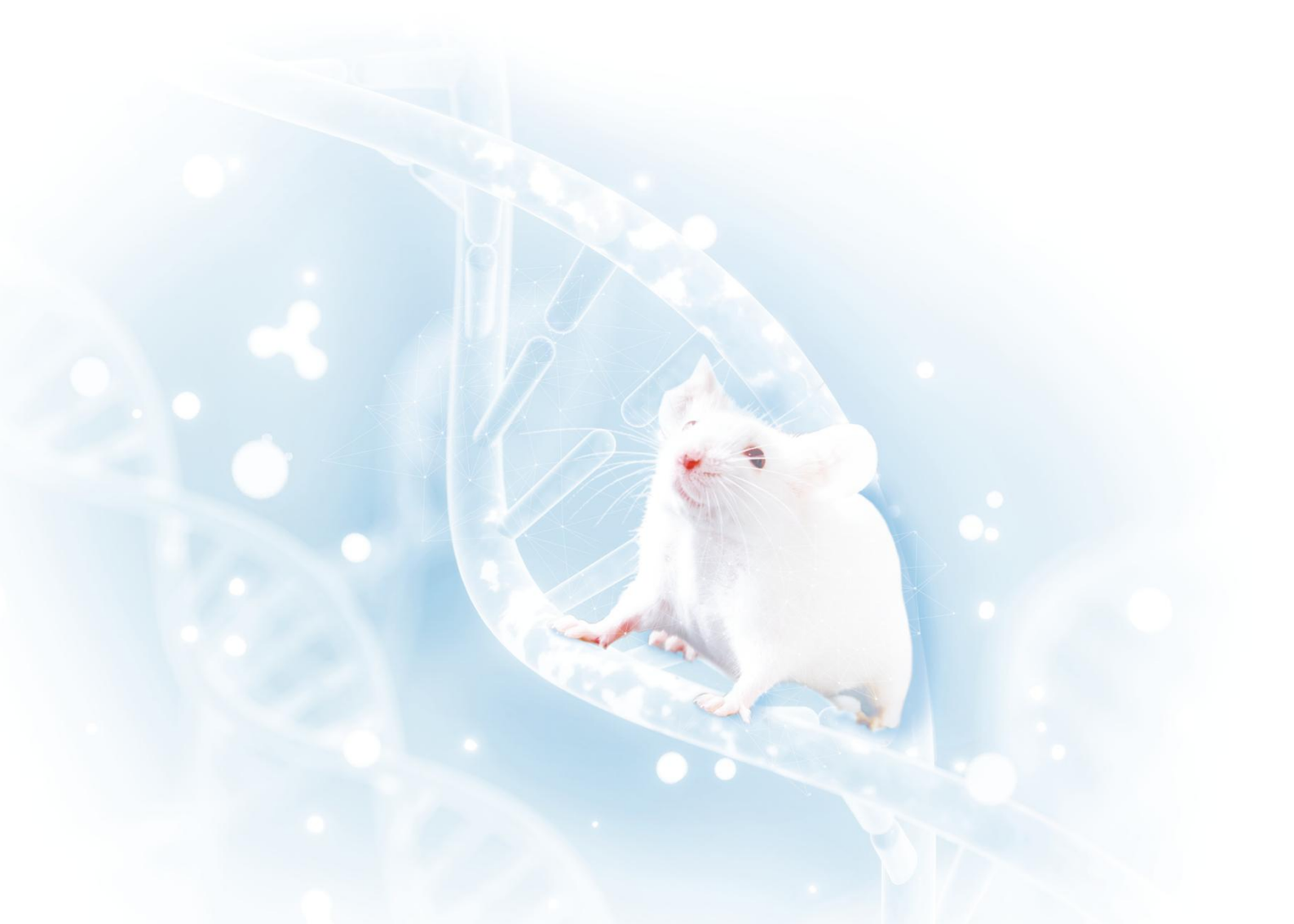
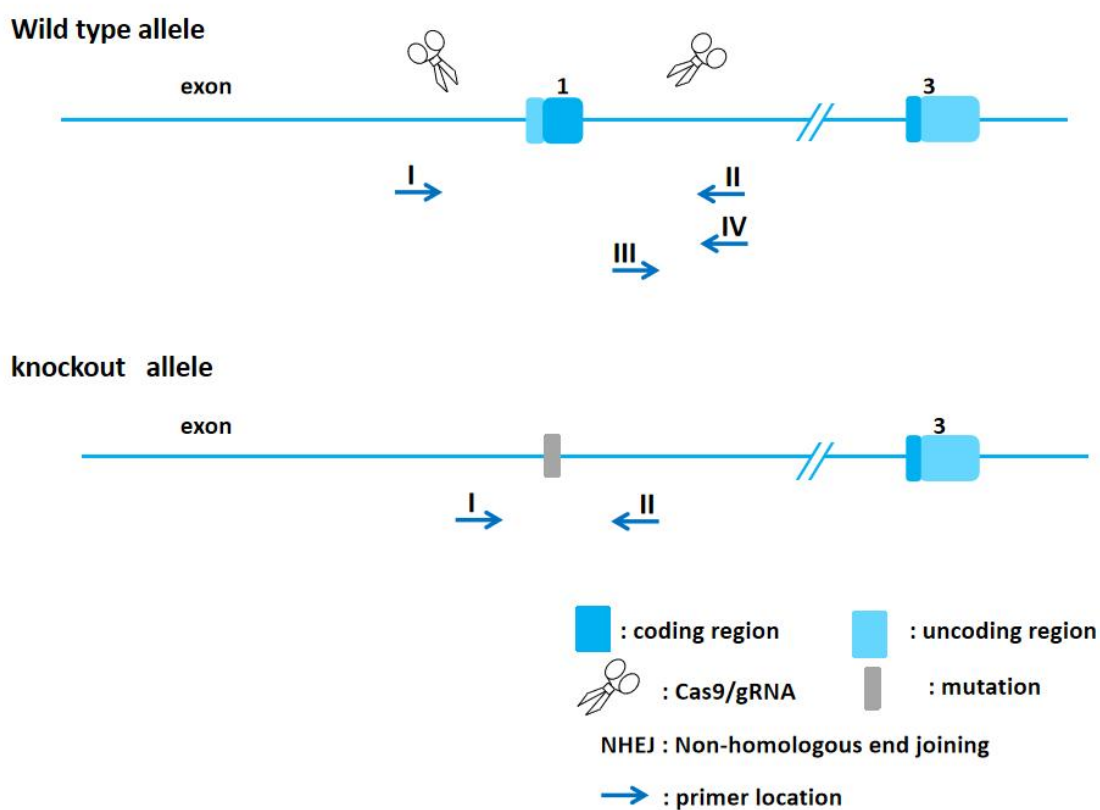


Cldn11-KO Genotyping Protocol



Common Name	<i>Cldn11-KO</i>	Cat. NO.	NM-KO-2114558
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

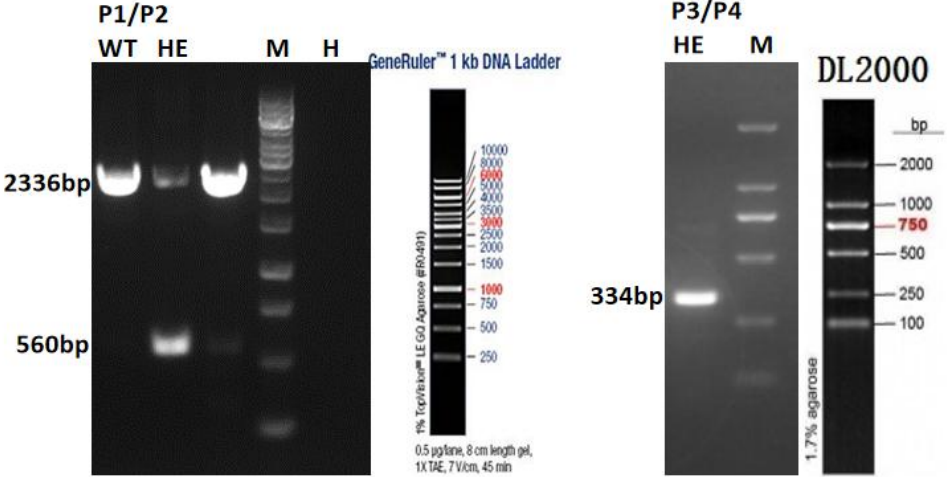


Primers

Primer	Sequence (5'→3')	Primer type
P1	AGAGCATTGTCTGGCTTGCT	Forward
P2	GGCCCTGAACTGAGAGAACC	Reverse
P3	ACACTGGTCAATCAATGGCT	Forward

P4	CTAATCCGGGCAGTCCAGAG	Reverse
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Expected results

Results	 The image displays two gel electrophoresis results. The left gel, labeled P1/P2, shows bands at 2336bp and 560bp for WT, HE, and M lanes, and a ladder (M) lane. The right gel, labeled P3/P4, shows a band at 334bp for HE and M lanes, and a ladder (DL2000) lane. A GeneRuler 1 kb DNA Ladder is also shown.
Genotype	Knockout type: -1776 bp Wild type: P1P2 =2336 bp; P3P4 =334 bp Heterozygote: P1P2 =2336 bp and 560 bp; P3P4 =334 bp Homozygote: P1P2 =560 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	ddH ₂ O	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	