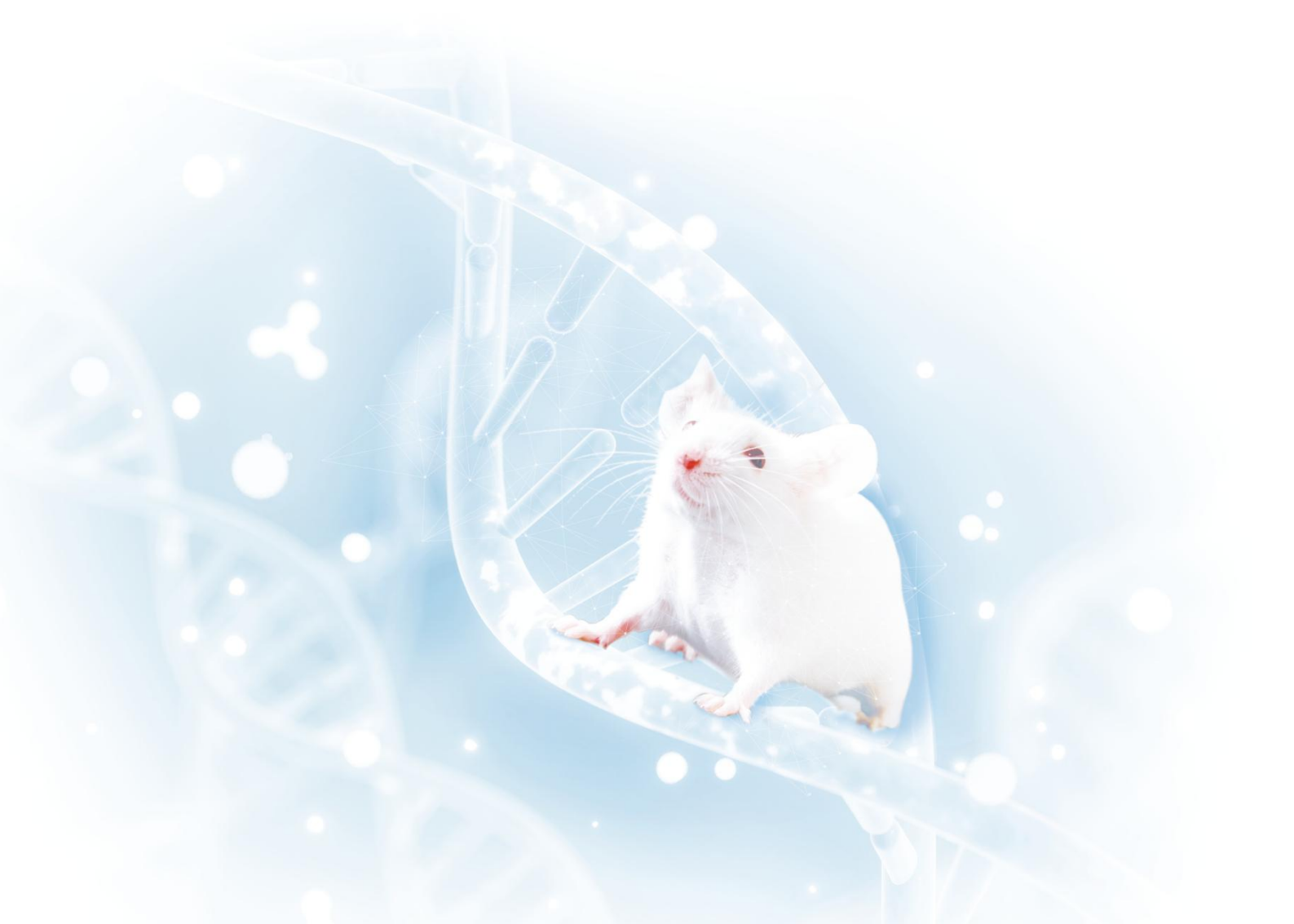
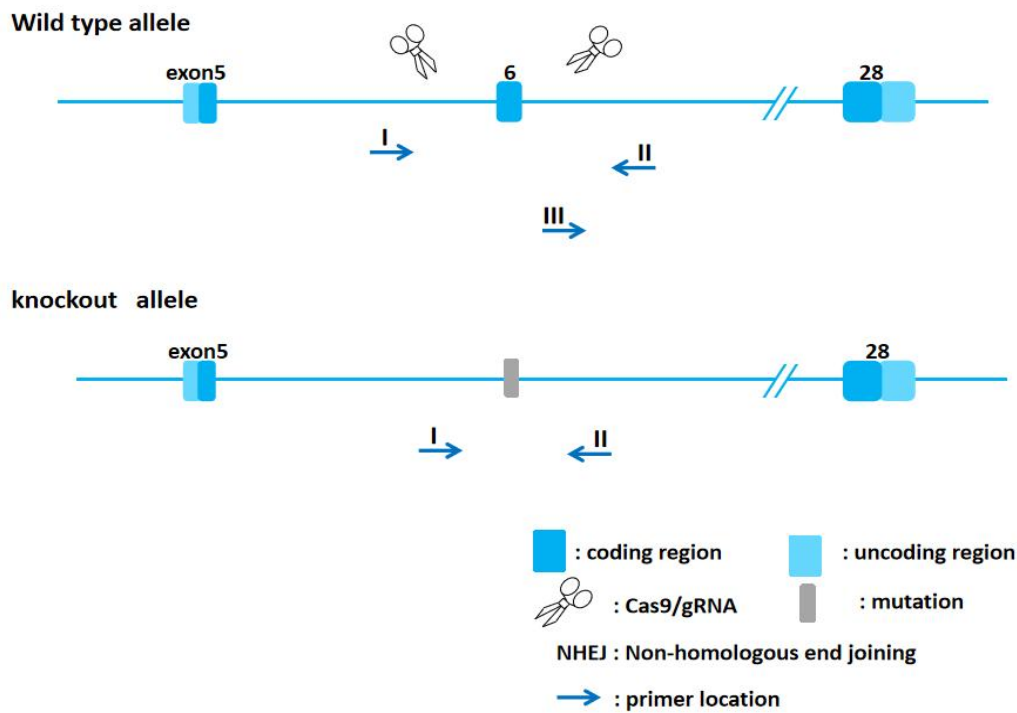


Trpm8 Genotyping Protocol



Common Name	Trpm8-KO	Cat. NO.	NM-KO-233013
Strain of Origin	C57BL/6J	Version	V1

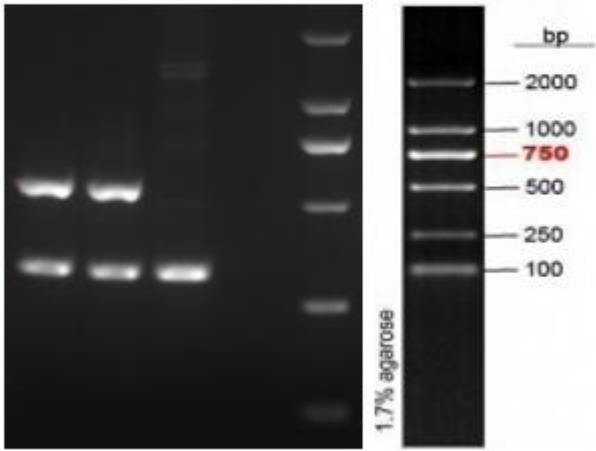
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCTGGTGC GTG TAAAATGGC	Forward
P2	ACCACCACCACTGTGTGTTA	Reverse
P3	TGTAGTTGTTCCCTGTTCTTGTC	Forward

Expected results

Results	P1P2P3 HE HE WT H₂O M  537 bp 310 bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -734+2 bp Wild type: P2P3 =310 bp Heterozygote: P1P2 =537 bp; P2P3=310 bp Homozygote: P1P2 =537 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			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P3 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
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	