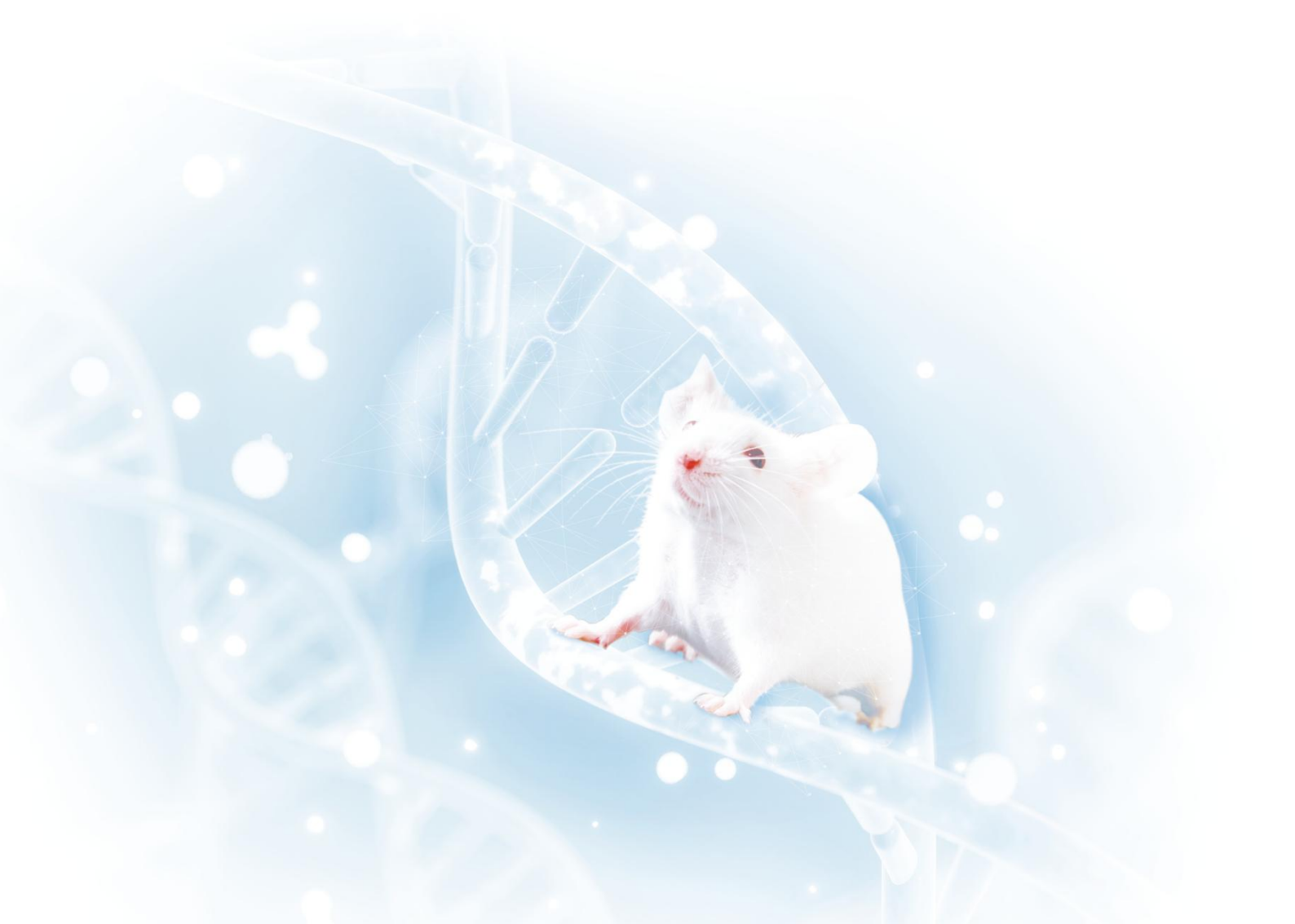
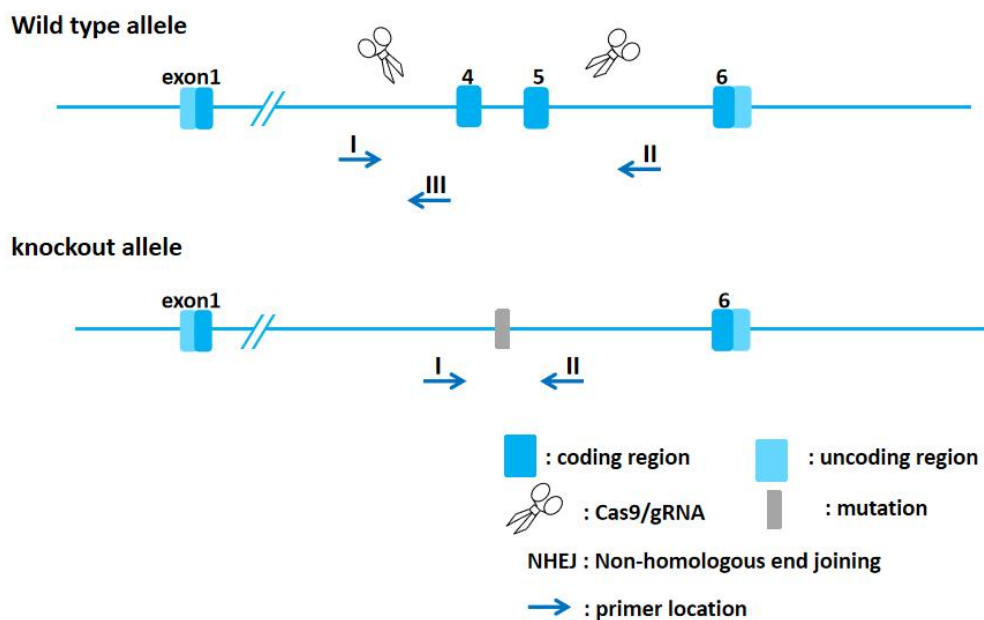


Tm2d1-K0 Genotyping Protocol



Common Name	Tm2d1-KO	Cat. NO.	NM-KO-233926
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCTACACCGACCTTAGTCCG	Forward
P2	TTAAGTGCTTAAGGGCGGCA	Reverse
P3	GGTGAACGTTGTAGGGGACA	Reverse

Expected results

Results	P1P2P3
Genotype	Knockout type: -5525 bp Wild type: P1P3 =448 bp Heterozygote: P1P2 =736 bp; P1P3=448 bp Homozygote: P1P2 =736 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 K0 band

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			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	