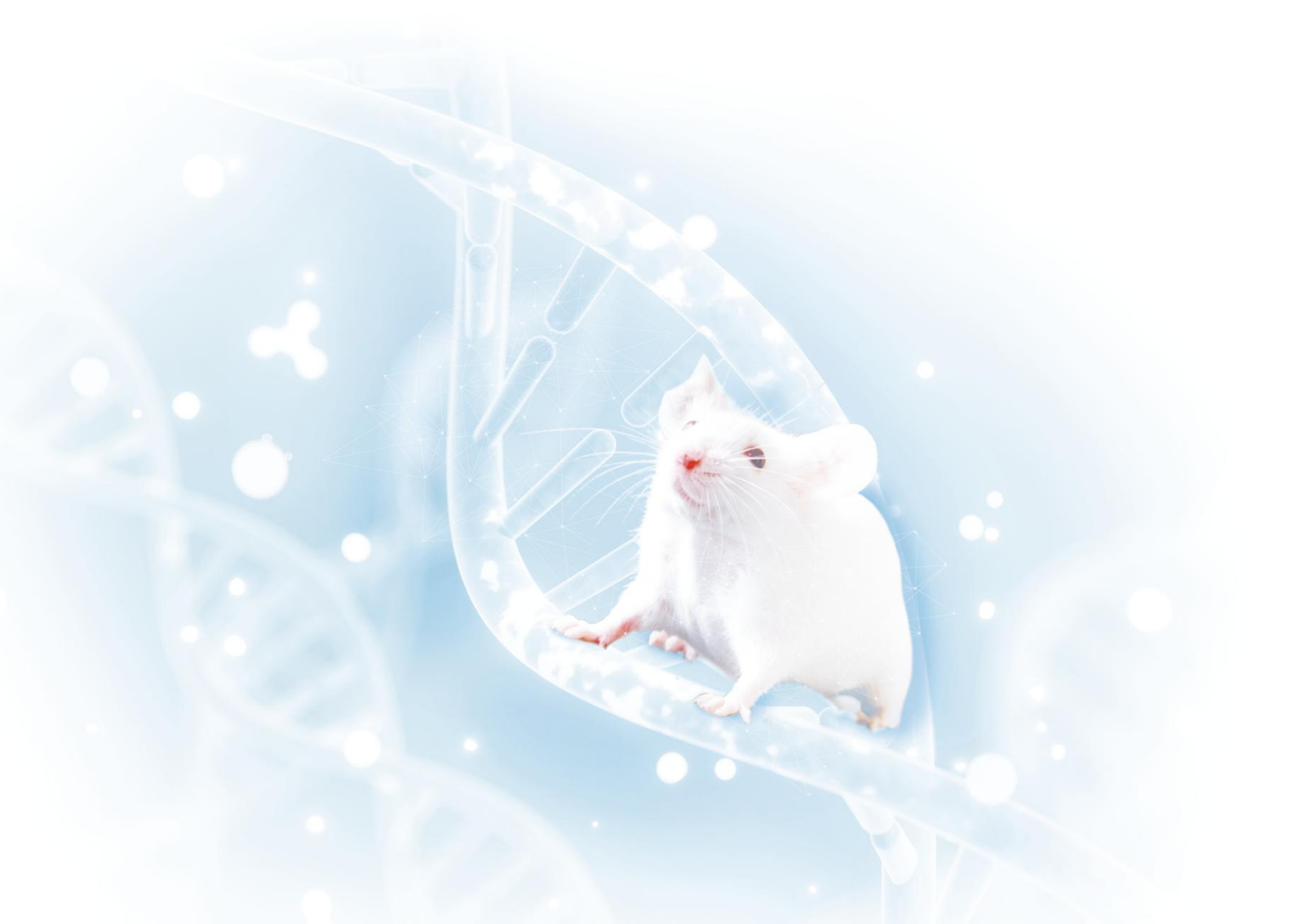


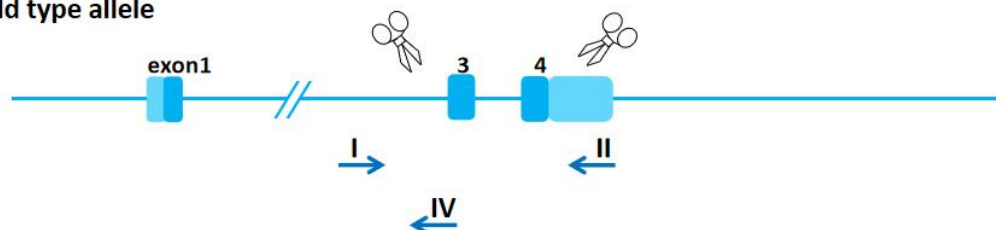
Tm2d2-K0 Genotyping Protocol



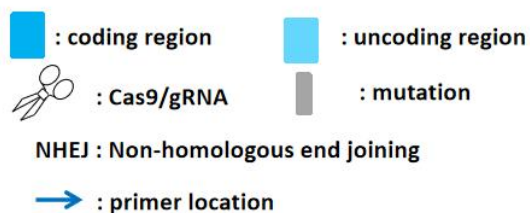
Common Name	Tm2d2-KO	Cat. NO.	NM-KO-233925
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



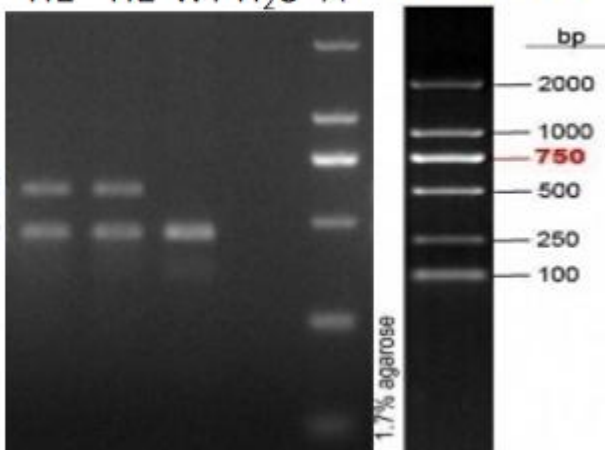
knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGGGGTAGACTACGCAAACC	Forward
P2	AAGAGCCAGCCACACAGAAA	Reverse
P4	TGCACATCACTGTACGCCTGAC	Reverse

Expected results

Results	P1P2P4 HE HE WT H₂O M DL2000 
Genotype	Knockout type: -2443bp Wild type: P1P4=447 bp Heterozygote: P1P2 =591 bp; P1P4=447 bp Homozygote: P1P2 =591 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
	P4 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
Cycling Reaction	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
	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	