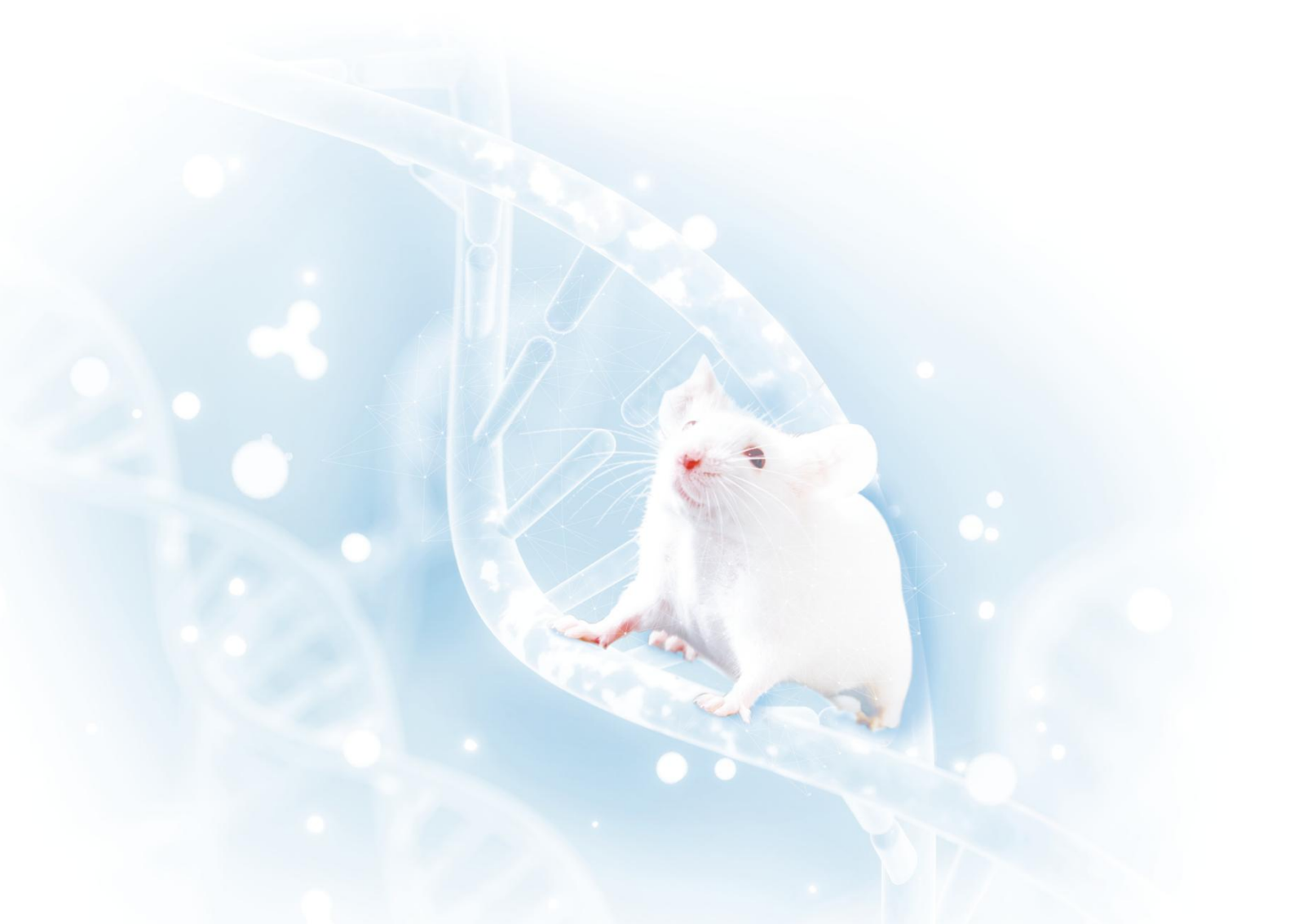


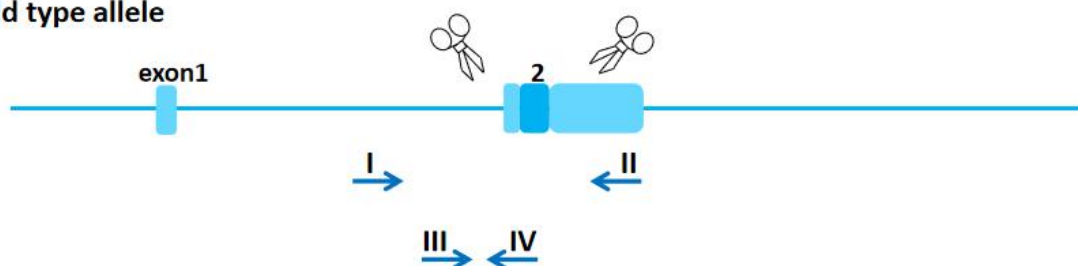
Trmt10c-K0 Genotyping Protocol



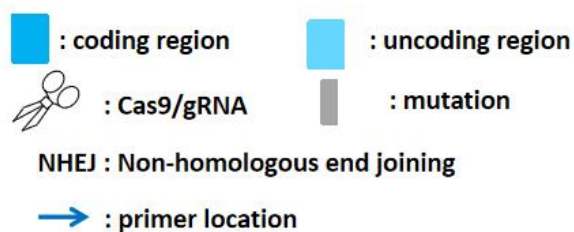
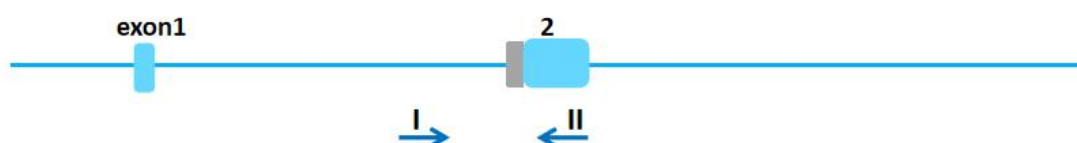
Common Name	Trmt10c-KO	Cat. NO.	NM-KO-234572
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCCAAAGGGACCTTGGAGAC	Forward
P2	GGTTGAACCTGGCATAGGCT	Reverse
P3	GGTAGCTGAGAAGTGGCCTG	Forward
P4	GGCACCAGACATCTGGCAA	Reverse

Expected results

Results	
Genotype	Knockout type: -1533bp Wild type: P1P2 =2181 bp; P3P4=299 bp Heterozygote: P1P2 =2181 bp and 648 bp; P3P4=299 bp Homozygote: P1P2 =648 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)
	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	