

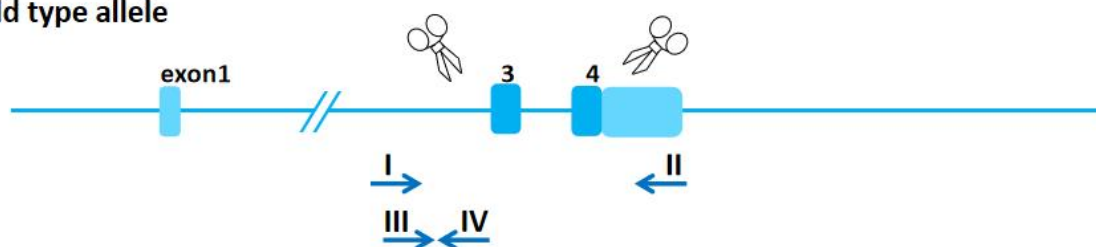
Trmt61a-K0 (2) Genotyping Protocol



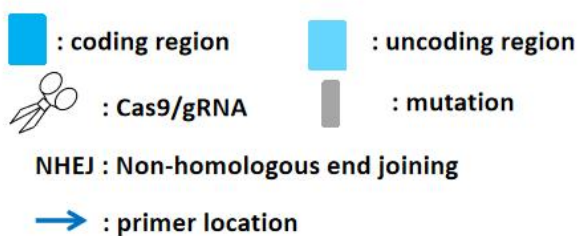
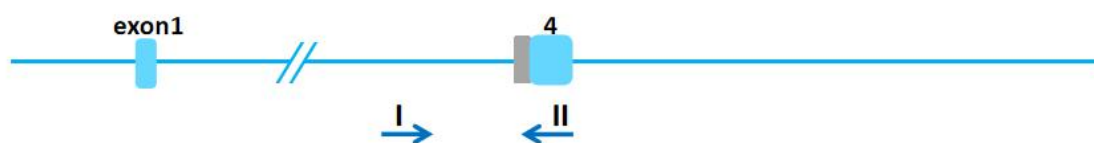
Common Name	Trmt61a-K0 (2)	Cat. NO.	NM-K0-240949
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGAGAAGGGTGGTGCAGTTG	Forward
P2	GGTCCCTCCATCAGCCTTAG	Reverse
P3	GCTGGTGTGTGGAGAGGAT	Forward
P4	TCAGTCTTGGGTTTGGGGTG	Reverse

Expected results

Results	
Genotype	Knockout type: -2675+11bp Wild type: P1P2 =3041 bp; P3P4=286 bp Heterozygote: P1P2 =3041 bp and 377 bp; P3P4=286 bp Homozygote: P1P2 =377 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×Taq Plus 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×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	