

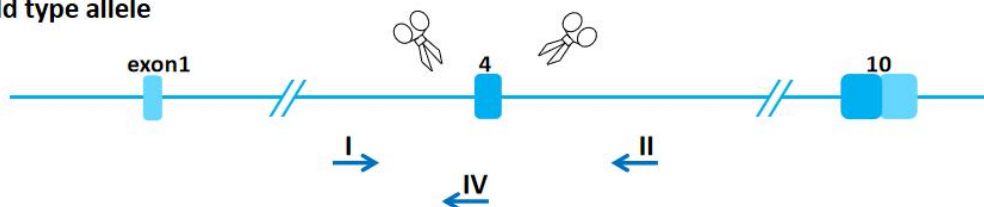
Tmem268-KO Genotyping Protocol



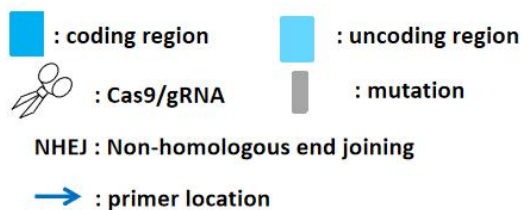
Common Name	Tmem268-KO	Cat. NO.	NM-KO-231847
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



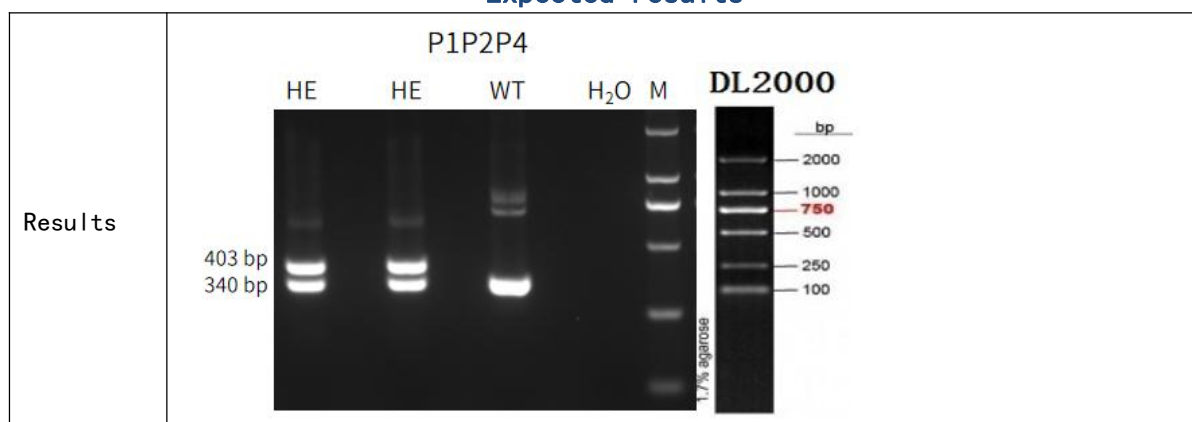
knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGCCTTGTGACATGGGACTC	Forward
P2	GTGTTGCGTGTGGTCCTCTA	Reverse
P4	TCCCAGGTCAAAGGAGATAGGTG	Reverse

Expected results



Genotype	Knockout type: -307bp
	Wild type: P1P4=340 bp
	Heterozygote: P1P2 =403 bp; P1P4=340 bp
	Homozygote: P1P2 =403 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			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
	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	