

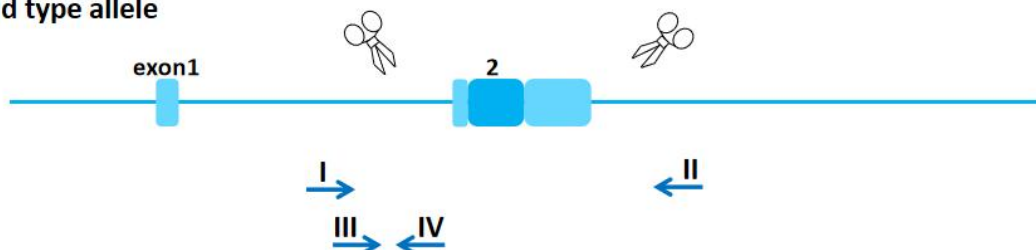
Tmem119-KO Genotyping Protocol



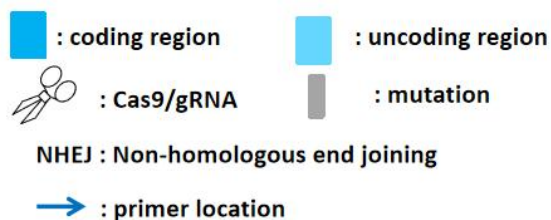
Common Name	Tmem119-KO	Cat. NO.	NM-KO-242141
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



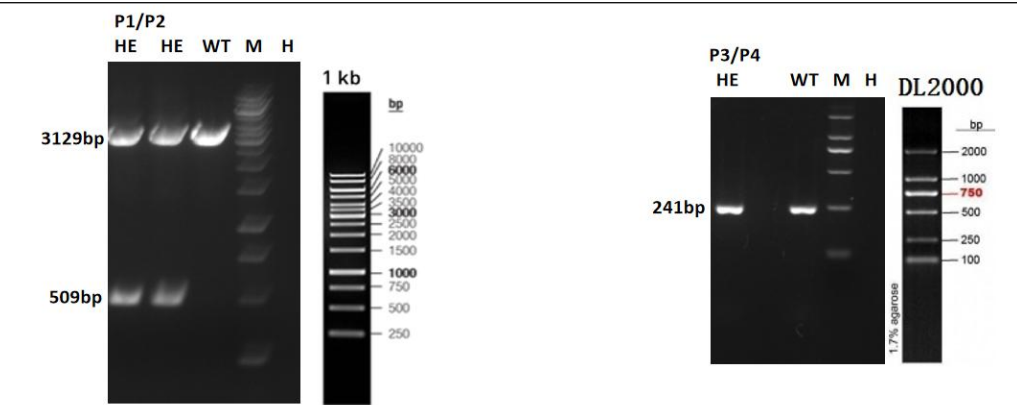
knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	AATGACAGCTCTTCACCGGG	Forward
P2	CATGTTCCCAACCCCTACC	Reverse
P3	AGGTCCCACCCAGTAAGAGG	Forward
P4	CGGTGTTTCGAACCCAGAGT	Reverse

Expected results

Results	
Genotype	Knockout type: -2621+1 bp Wild type: P1P2 =3129 bp; P3P4 =241 bp Heterozygote: P1P2 =3129 bp and 509 bp; P3P4 =241 bp Homozygote: P1P2 =509 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			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	