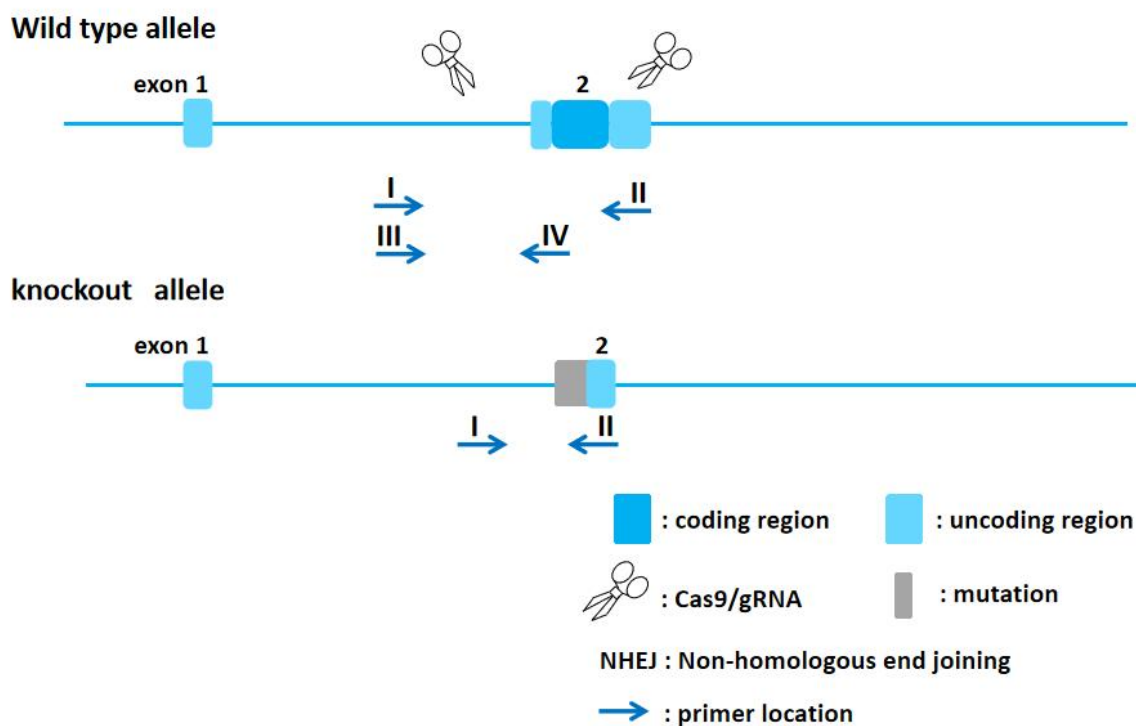


Tnfaip8l1-KO Genotyping Protocol



Common Name	Tnfaip8l1-KO	Cat. NO.	NM-KO-240945
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GCACAAGAACTCTCCGTGGT	Forward
P2	ATTTGGCAGGGGTAAGGGTC	Reverse
P3	AAGGGGCACAAGAACTCTCC	Forward
P4	GGCCTGGTACAGTTCATCCA	Reverse

Expected results

Results	P1/P2 HE HE WT M H 1213bp 575bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT H M 418bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -638bp Wild type: P1P2 =1213 bp; P3P4 =418 bp Heterozygote: P1P2 =1213 bp and 575 bp; P3P4 =418 bp Homozygote: P1P2 =575 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	30 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	