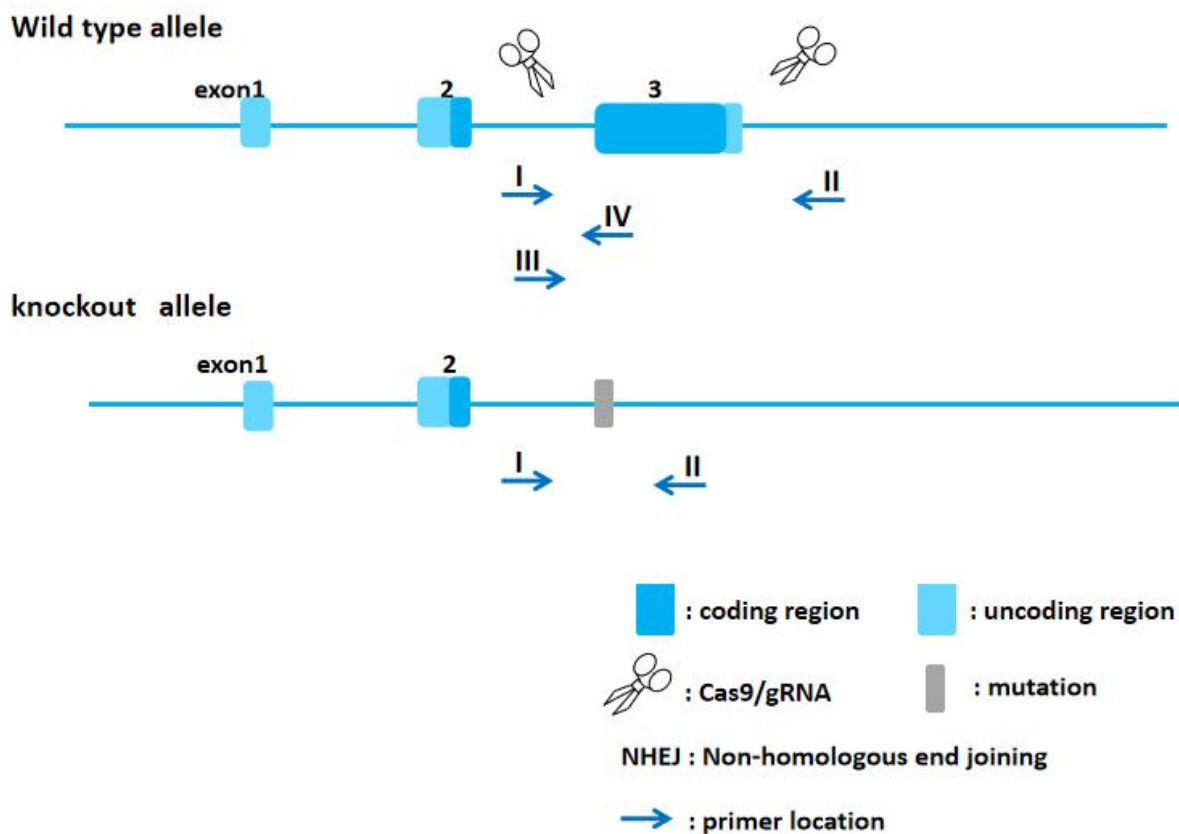


Tlr8-KO Genotyping Protocol



Common Name	Tlr8-KO	Cat. NO.	NM-KO-240544
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGCTTCTGTGGTGCAGACTC	Forward
P2	ATCTCCTCTCTCACTGCGGA	Reverse
P3	CTCTGTGCTTCCGCCCTTC	Forward
P4	GGTCCAGAGGACAGCAGAC	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2: HE, HE, WT, M P3/P4: HE, HE, WT, H, M DL2000 4180bp, 598bp, 345bp 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -3582bp Wild type: P1P2 =4180 bp; P3P4 =345 bp Heterozygote: P1P2 =4180 bp and 598 bp; P3P4 =345 bp Homozygote: P1P2 =598 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	