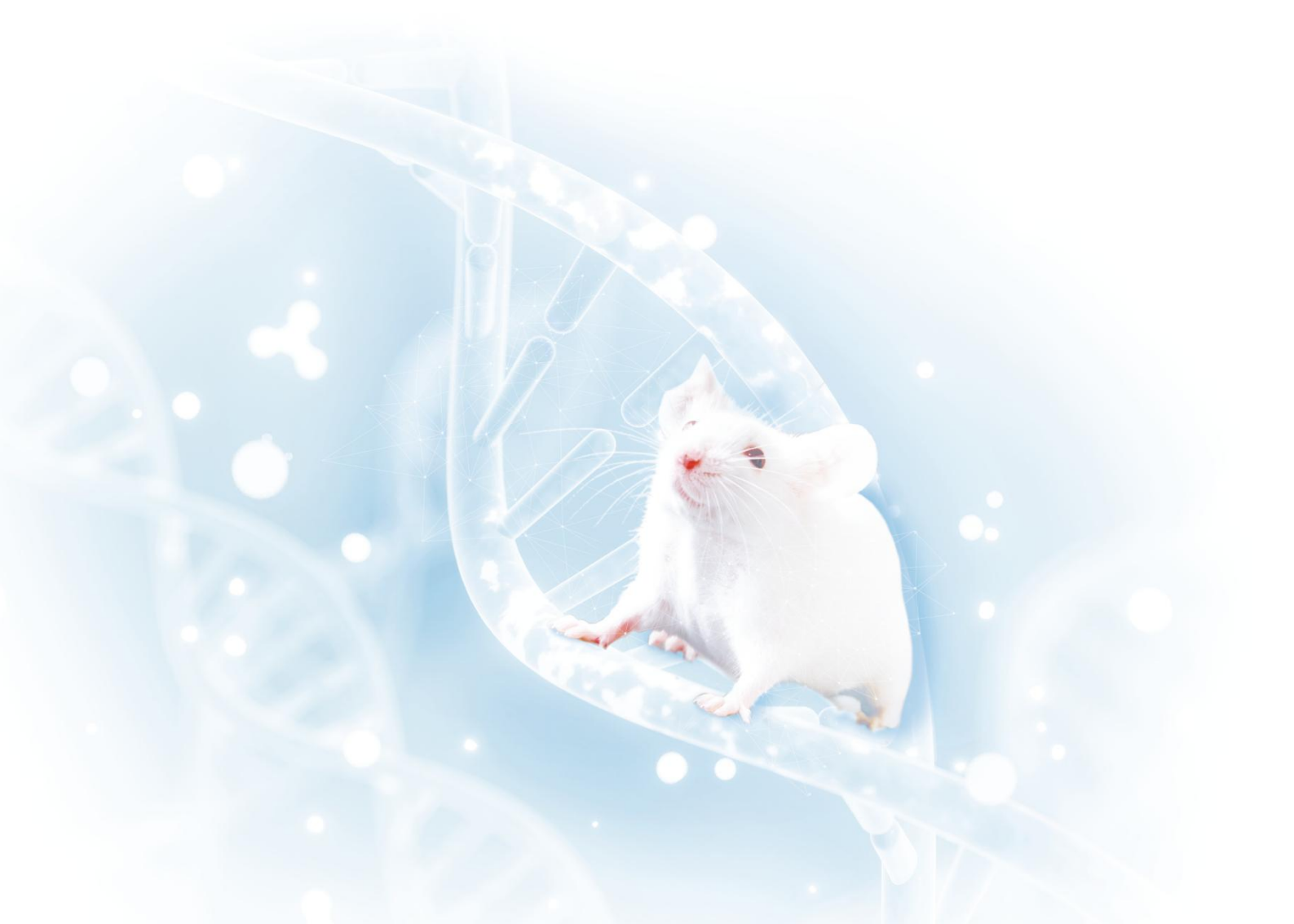


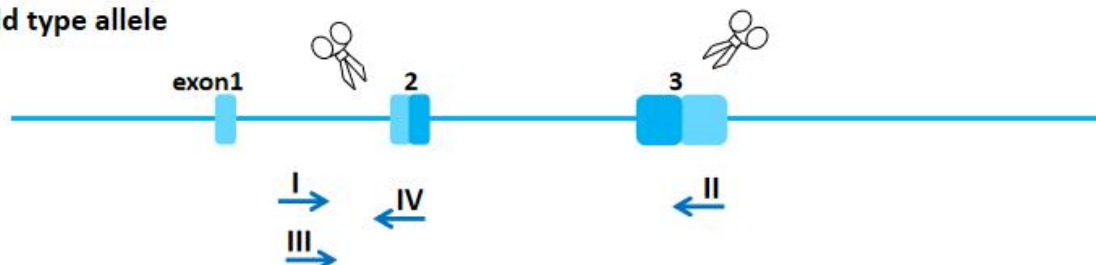
Tlr13-KO Genotyping Protocol



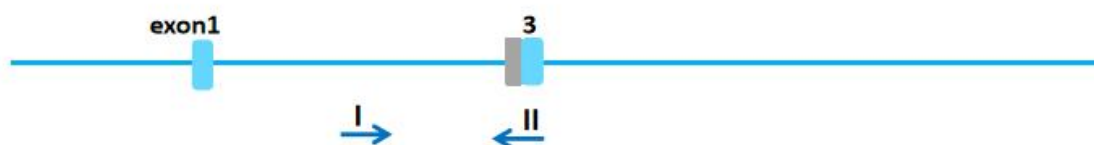
Common Name	Tlr13-KO	Cat. NO.	NM-KO-241687
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



 : coding region  : uncoding region

 : Cas9/gRNA  : mutation

NHEJ : Non-homologous end joining

 : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	AGCTGCCATGTGGATACTGG	Forward
P2	AGTCTCCCATGACCACAACTG	Reverse
P3	CCATGAAGTAGGGCAGGCAA	Forward
P4	AGGATCCTGTAGAGCCCACT	Reverse

Expected results

Results	P1/P2 HE HE HE WT M H 12916bp 624bp GeneRuler™ 1 kb DNA Ladder 10000 9000 8000 7000 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 435bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -12292bp Wild type: P1P2 =12916 bp; P3P4 =435 bp Heterozygote: P1P2 =12916 bp and 624 bp; P3P4 =435 bp Homozygote: P1P2 =624 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	