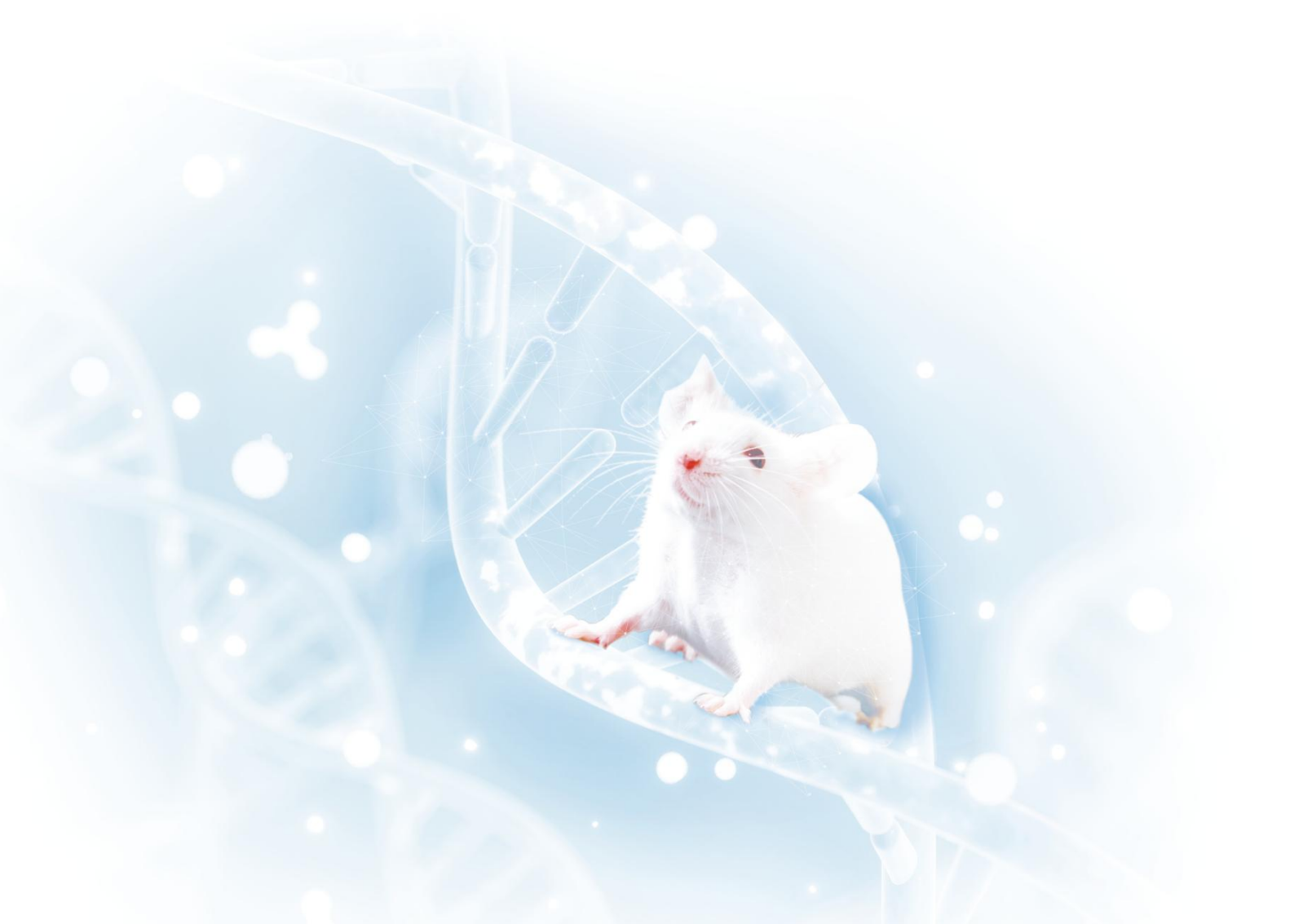
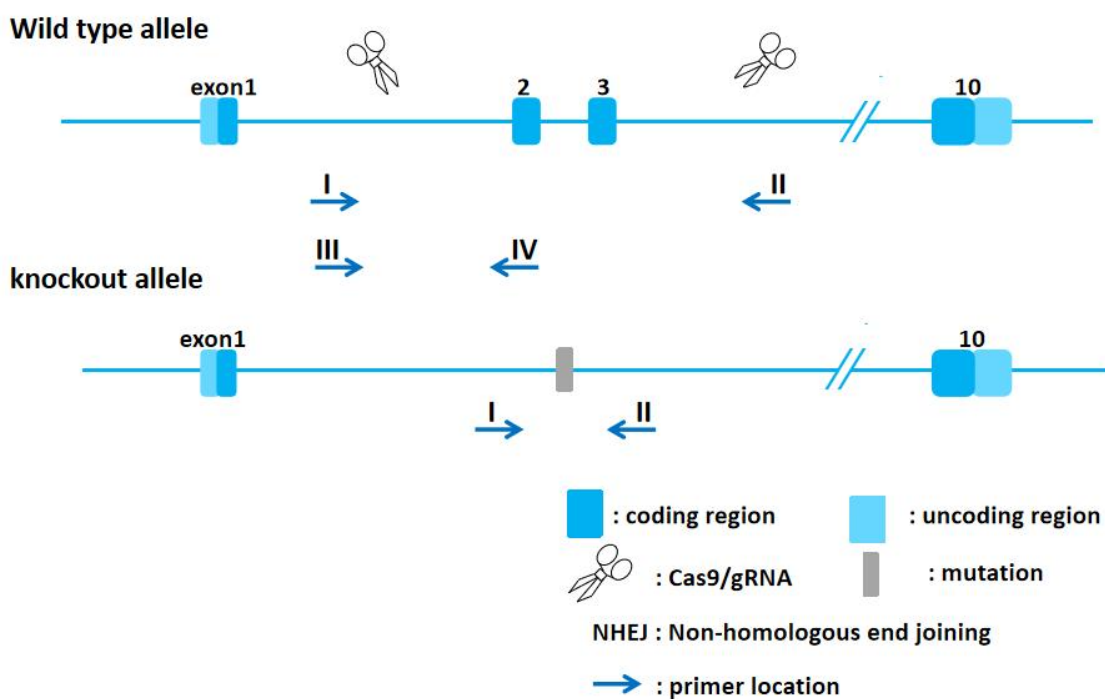


Tnfrsf11a-K0 Genotyping Protocol



Common Name	Tnfrsf11a-K0	Cat. NO.	NM-K0-240373
Strain of Origin	C57BL/6J	Version	V1

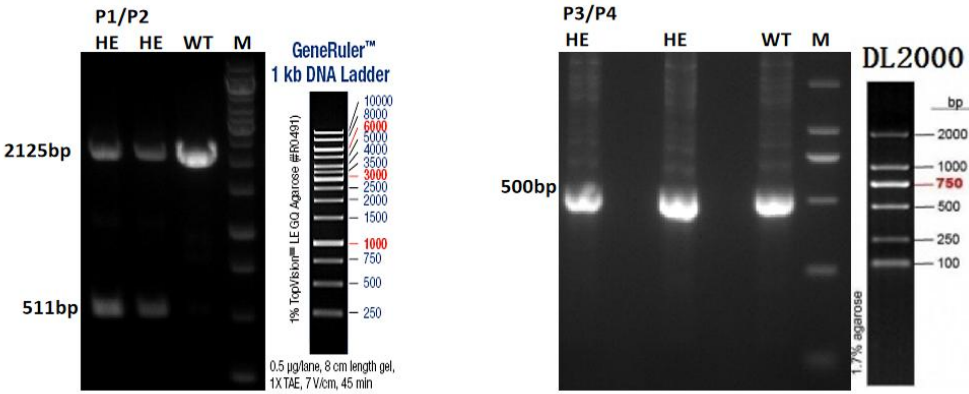
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGTCCCACTGACACAGGAG	Forward
P2	GGTTGCCCAACCAGAAGATCA	Reverse
P3	ACAGGAGAAGTGTGGTCCCT	Forward
P4	CACCGTCCGAGATGCTCATA	Reverse

Expected results

Results	 P1/P2 HE HE WT M 2125bp 511bp 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 M 500bp DL2000 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -1614 bp Wild type: P1P2 =2125 bp; P3P4 =500 bp Heterozygote: P1P2 =2125 bp and 511 bp; P3P4 =500 bp Homozygote: P1P2 =511 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 K0 band.

Reaction &Cycling

PCR Reaction System	Reaction Component	Volume (µl)
	ddH2O	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	