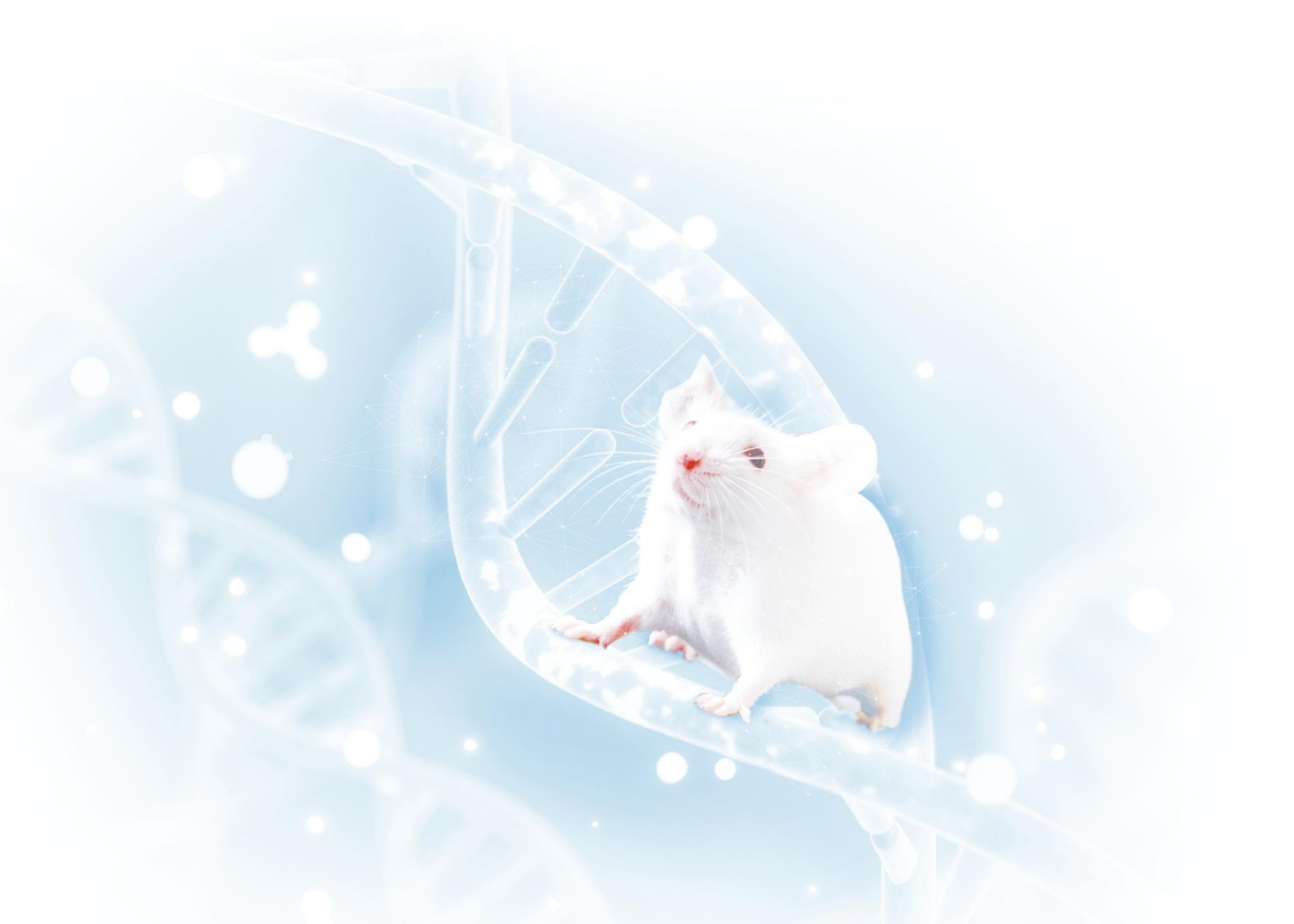
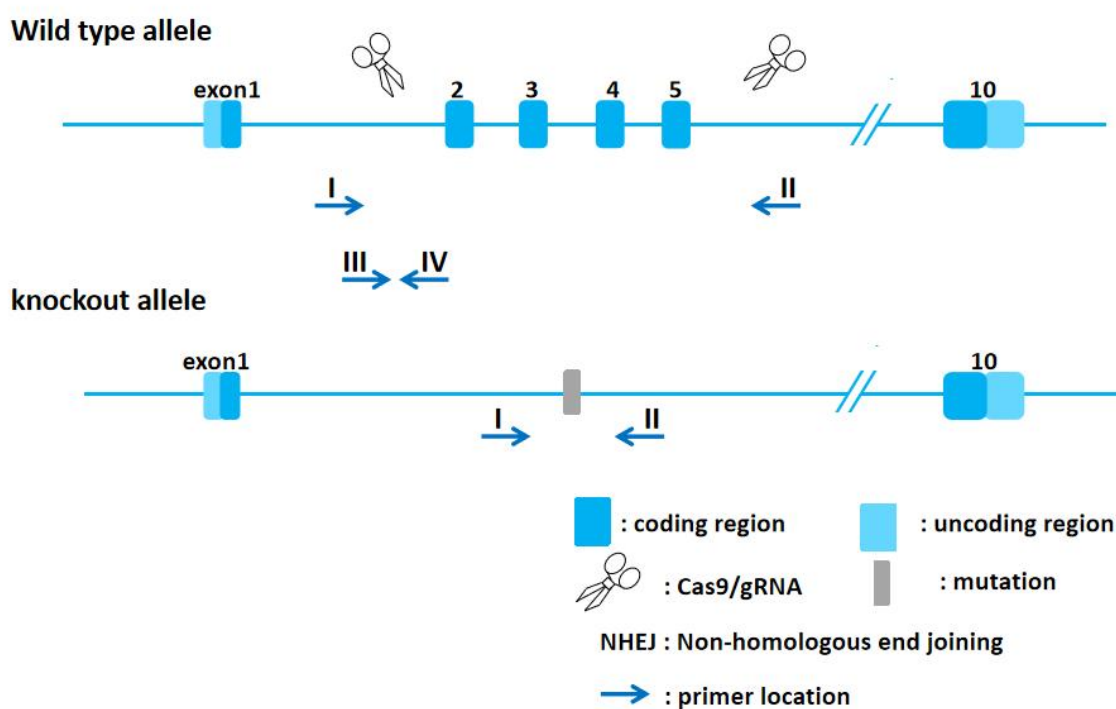


Tnfrsf1a-K0(2) Genotyping Protocol



Common Name	Tnfrsf1a-K0 (2)	Cat. NO.	NM-K0-241109
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGTGAAACCCGTACCCAGA	Forward
P2	GCCAGTGCTCTACCGTTGAT	Reverse
P3	ATTCTTGGTCGGGCAGAGG	Forward
P4	TGGTCTCTGGTGGCTCTGTA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 2525bp 512bp P1/P2 HE WT M 227bp P3/P4 HE WT M H DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -2013 bp Wild type: P1P2 =2525 bp; P3P4 =227 bp Heterozygote: P1P2 =2525 bp and 512 bp; P3P4 =227 bp Homozygote: P1P2 =512 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 Component	Volume (µl)
Reaction System	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	