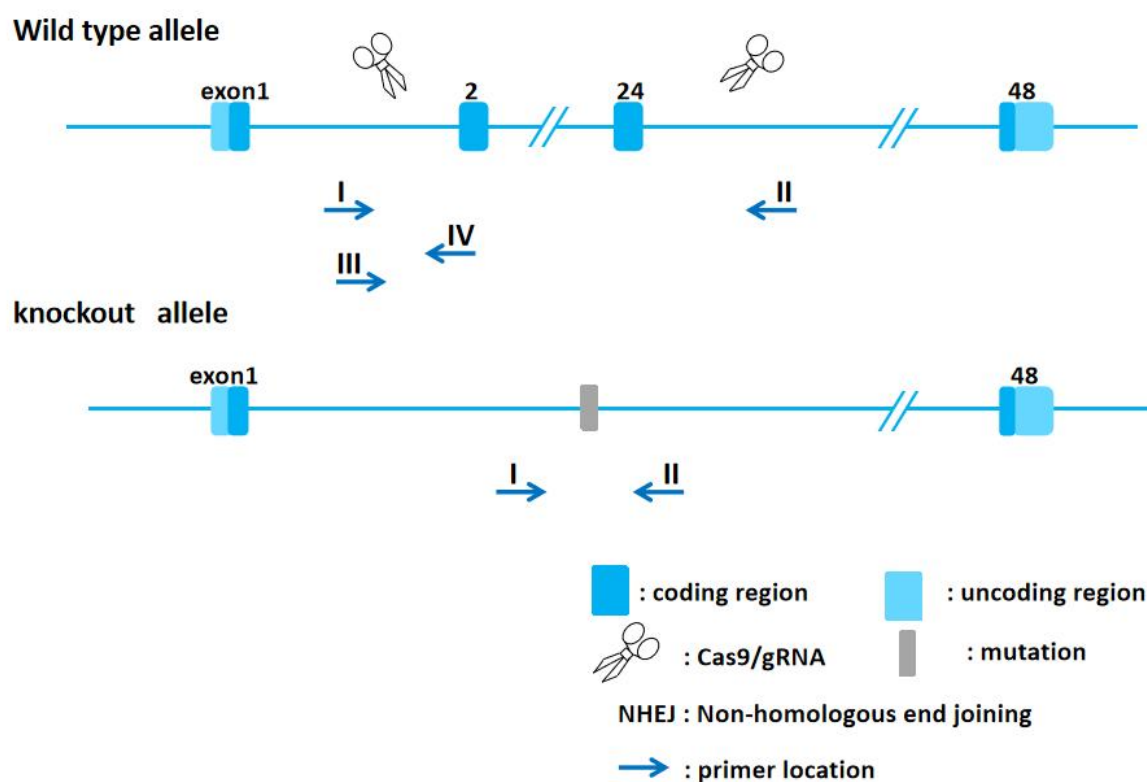


Tg-KO Genotyping Protocol



Common Name	Tg-KO	Cat. NO.	NM-KO-240168
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGATGAGCTGAATCGCCTGAG	Forward
P2	TGGGGGTTCTGGTTTGTGA	Reverse
P3	CCTCCCAGGAAAGCACAAATG	Forward
P4	CTTCAGAGCACTGGGGAACATA	Reverse

Expected results

Results	P3/P4 HE WT M 293bp DL2000 bp 2000 1000 750 500 250 100 GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 P1/P2 M HE HE 520bp 1.7% agarose 1% TopView™ LE GQ Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -46044 bp Wild type: P3P4 =293 bp Heterozygote: P1P2 =520 bp; P3P4 =293 bp Homozygote: P1P2 =520 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 Component	Volume (µl)
Reaction	ddH2O	8.0
System	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	