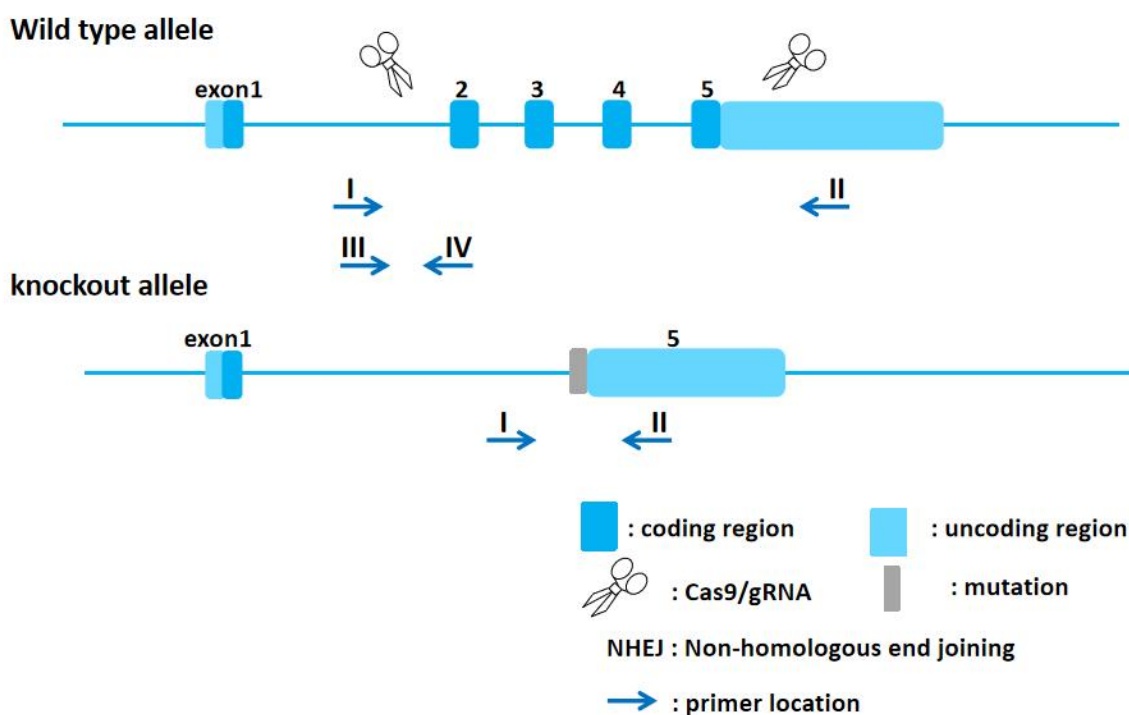


Timp2-KO Genotyping Protocol



Common Name	Timp2-KO	Cat. NO.	NM-KO-240286
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCACCATGTGTCACCACTCC	Forward
P2	TGCATGTGACAGTGCCAGAG	Reverse
P3	CCAAGGGCTGGTAAGGTGAG	Forward
P4	ACTGAATCCTCTTGATGGGGT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% Topo/BioLor™ LE G0 Agarose (#R0491) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -16690 bp Wild type: P3P4 =417 bp Heterozygote: P1P2 =553 bp; P3P4 =417 bp Homozygote: P1P2 =553 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	ddH ₂ O	8.0
System	2 × Rapid Taq Master Mix	10.0
	P1(10 pmol/µl) or P3(10 pmol/µl)	0.5

	P2(10 pmol/ μ l) or P4(10 pmol/ μ l)			0.5
	Genomic DNA			1.0
	Total			20
	2 $\times$ 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	