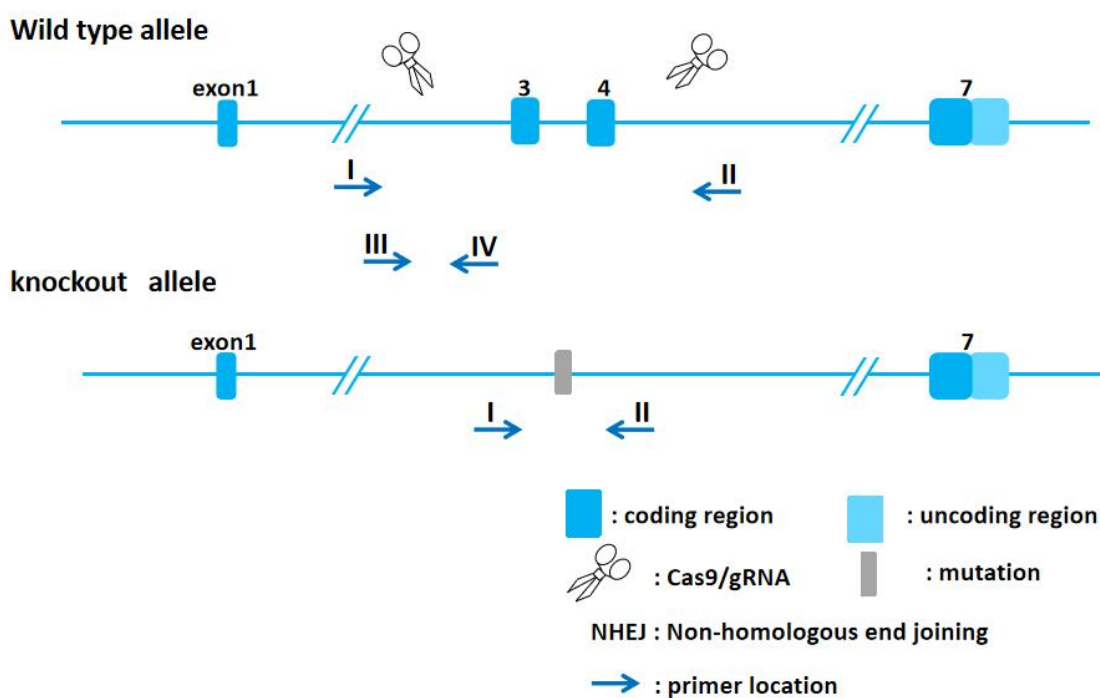


Tmco1-K0 (2) Genotyping Protocol



Common Name	Tmco1-KO (2)	Cat. NO.	NM-KO-233864
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AACTTCTGACTGGGCTTGG	Forward
P2	GGACAAGCAAGGCTAAGCAA	Reverse
P3	CACTGCCACCATCTGGCTAC	Forward
P4	TCAGAAACATGCCGTGCTACA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% Tris/Borate LE Gels Agarose #80491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -3223 bp Wild type: P3P4 =202 bp Heterozygote: P1P2 =645 bp; P3P4 =202 bp Homozygote: P1P2 =645 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	