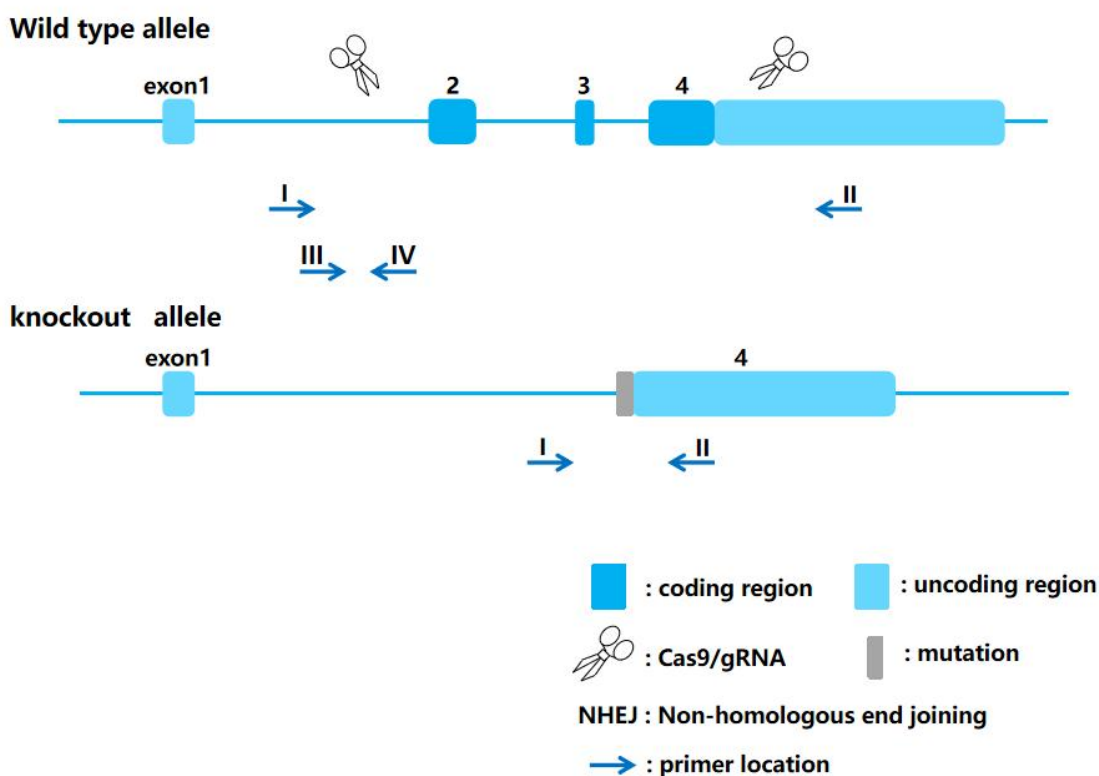


Has3-KO Genotyping Protocol



Common Name	Has3-KO	Cat. NO.	NM-KO-252579
Strain of Origin	C57BL/6J	Version	V1

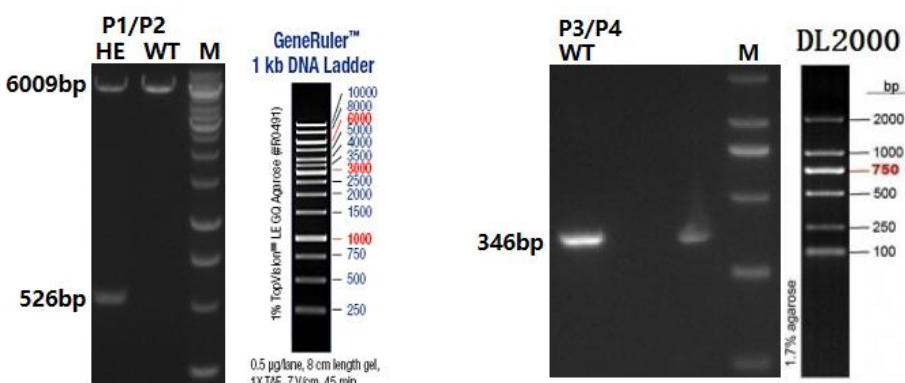
Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	CCCCTCCAGGGTACACTTA	Forward
P2	TTCAGCTGGGCTAGGAGGAT	Reverse
P3	AACCCCAGCAACTTAGGCTC	Forward
P4	TAGTGGATCAGGCAGGGACA	Reverse

Expected results

Results	 GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE gel 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
Genotype	Knockout type: -5483 bp Wild type: P1P2 =6009 bp; P3P4 =346 bp Heterozygote: P1P2 =6009 bp and 526 bp; P3P4 =346 bp Homozygote: P1P2 =526 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 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	