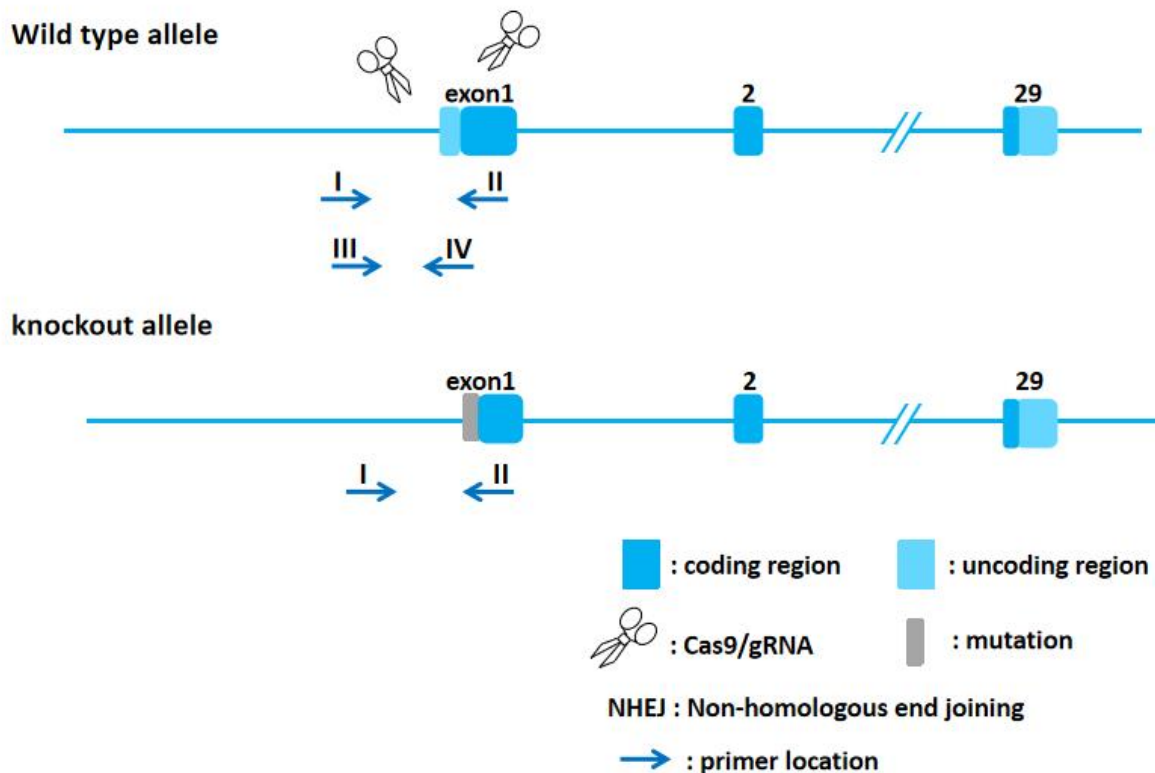


Nos1-KO Genotyping Protocol



Common Name	Nos1-KO	Cat. NO.	NM-KO-240406
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGCTCCAAGGTATTCGGTGTC	Forward
P2	ATCTCTGCTTTGGCTGGTCC	Reverse
P3	CTCCAAGGTATTCGGTGCCC	Forward
P4	TTGGGTTGGATCTGCTGGAC	Reverse

Expected results

Results	
Genotype	Knockout type: -719 bp Wild type: P1P2 =1136 bp; P3P4 =492 bp Heterozygote: P1P2 =1136 bp and 417 bp; P3P4 =492 bp Homozygote: P1P2 =417 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)
	ddH ₂ O			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	