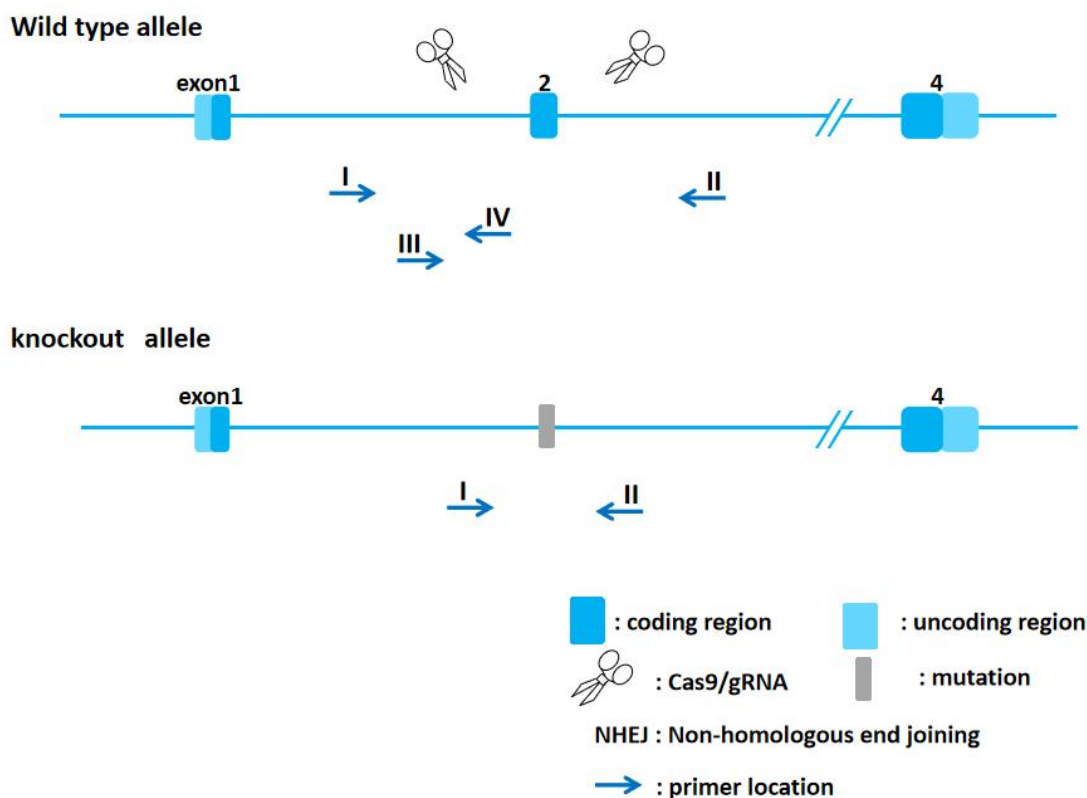


Vgll3-KO Genotyping Protocol



Common Name	Vgll3-KO	Cat. NO.	NM-KO-242029
Strain of Origin	C57BL/6J	Version	V1

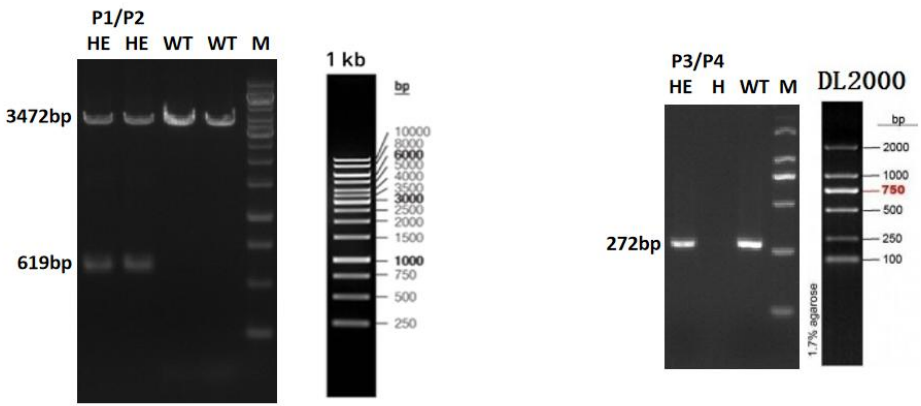
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGTCAGCCATTCCAGGGTTG	Forward
P2	GGAACCAGGTGAGCTTGGAA	Reverse
P3	GGTGCTTATCACTTATCACAGAGC	Forward
P4	ACCCATAGAGTCGTTTCTGCG	Reverse

Expected results

Results	
Genotype	Knockout type: -2853 bp Wild type: P1P2 =3472 bp; P3P4 =272 bp Heterozygote: P1P2 =3472 bp and 619 bp; P3P4 =272 bp Homozygote: P1P2 =619 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	