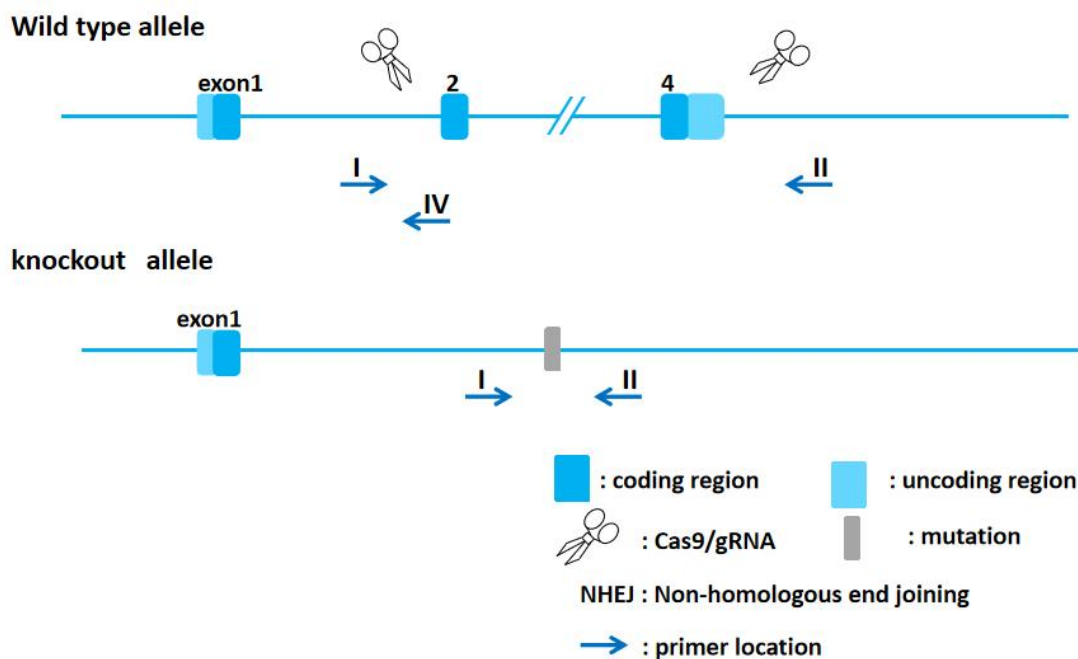


Cldn1-KO Genotyping Protocol



Common Name	Cldn1-KO	Cat. NO.	NM-KO-231380
Strain of Origin	C57BL/6J	Version	V1

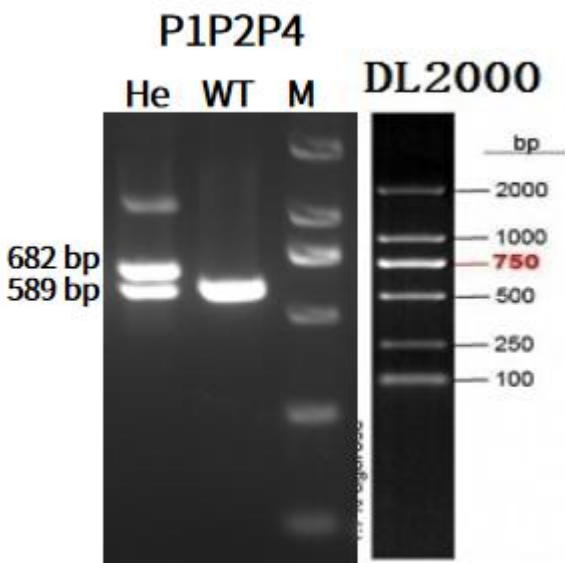
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCCCTGAAAACCTCATTTGCTTG	Forward
P2	GCCTGGACCTCACCTTTTGAAC	Reverse
P4	CACATCTTCTGCACCTCATCATCTT	Reverse

Expected results

Results	
Genotype	Knockout type: -7933+1bp Wild type: P1P4=589 bp Heterozygote: P1P2 =682 bp; P1P4=589 bp Homozygote: P1P2 =682 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			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P4 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
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	