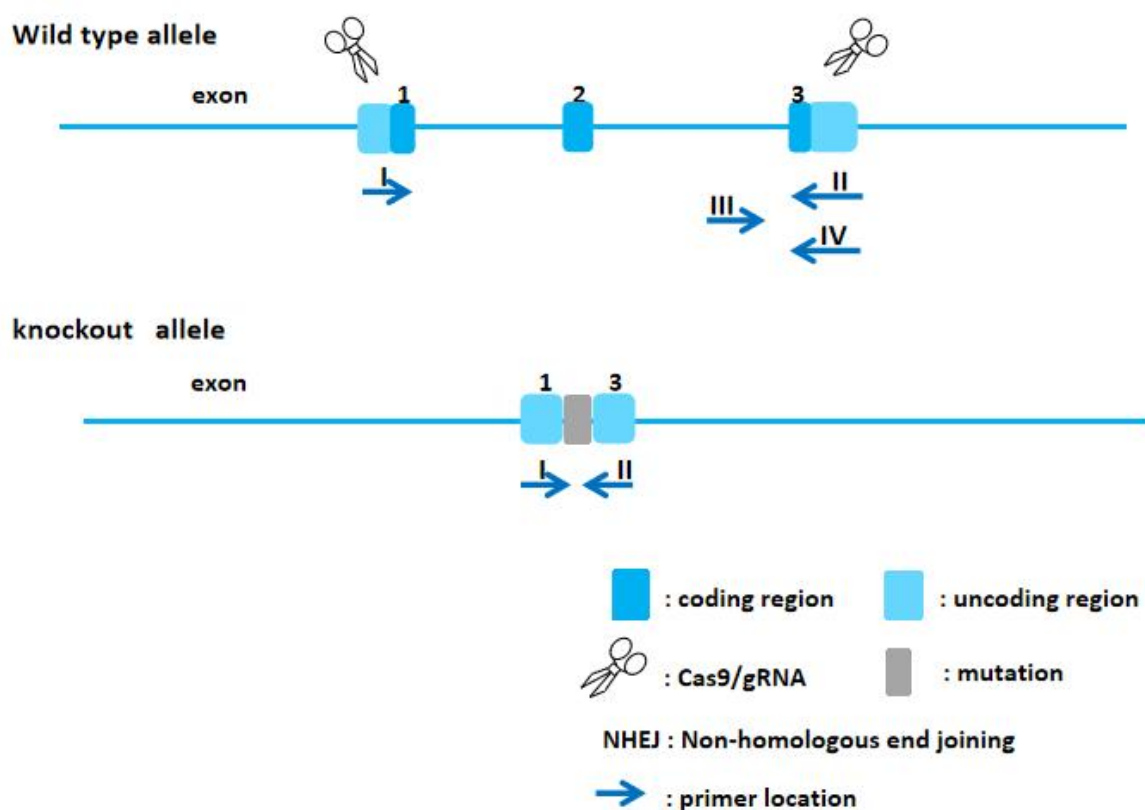


Cisd2-KO Genotyping Protocol



Common Name	<i>Cisd2</i> -KO	Cat. NO.	NM-KO-234735
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	CCCGTGTCACAGAAACTCCA	Forward
P2	AGCAGAGTTCCACAAAAGGC	Reverse
P3	ACACTGGCTCCAAGAGCATT	Forward
P4	TTTGGCCACAGTCAAACCTT	Reverse

Expected results

Results	P1/P2 HE WT M GeneRuler™ 1 kb DNA Ladder 751bp P3/P4 HE HE WT H M DL2000 460bp 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -14748+11bp Wild type: P3P4 =460 bp Heterozygote: P1P2 =751 bp; P3P4 =460 bp Homozygote: P1P2 =751 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	ddH ₂ O	8.0
System	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
	2xRapid 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	