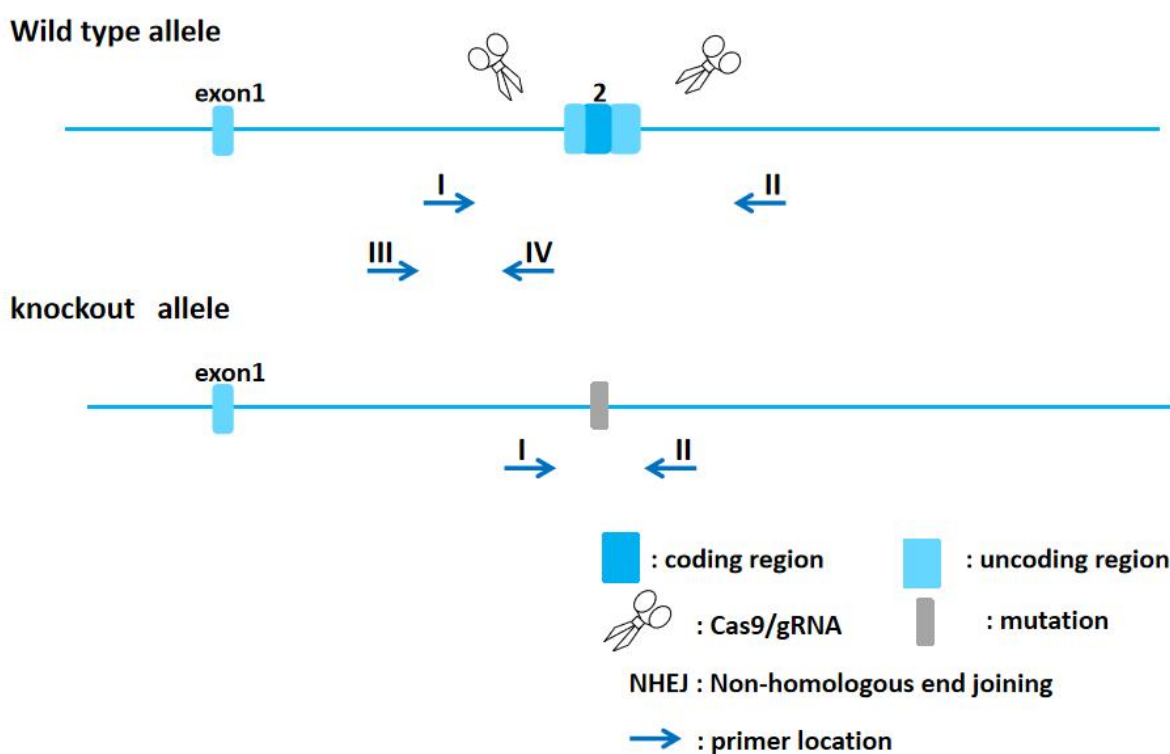


Cited2-KO Genotyping Protocol



Common Name	Cited2-KO	Cat. NO.	NM-KO-232371
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	AAGGGAACGGCTCGGAATTT	Forward
P2	CTGGCACCTAATGCATGGGA	Reverse
P3	GCGGAGCTGGGGAATAACAA	Forward
P4	GTCTCAAGCTGCAGACCACC	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100
Genotype	Wild type: P1P2 =3044 bp; P3P4=301 bp Heterozygote: P1P2 =3044 bp and 743 bp; P3P4=301 bp Homozygote: P1P2 =743 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (µl)
	ddH ₂ O			8.0
	2×Taq Plus 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×Taq Plus Master Mix from Vazyme(Code Number: P222-1)			
Cycling	Step	Temp	Time	Note

Reaction	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	