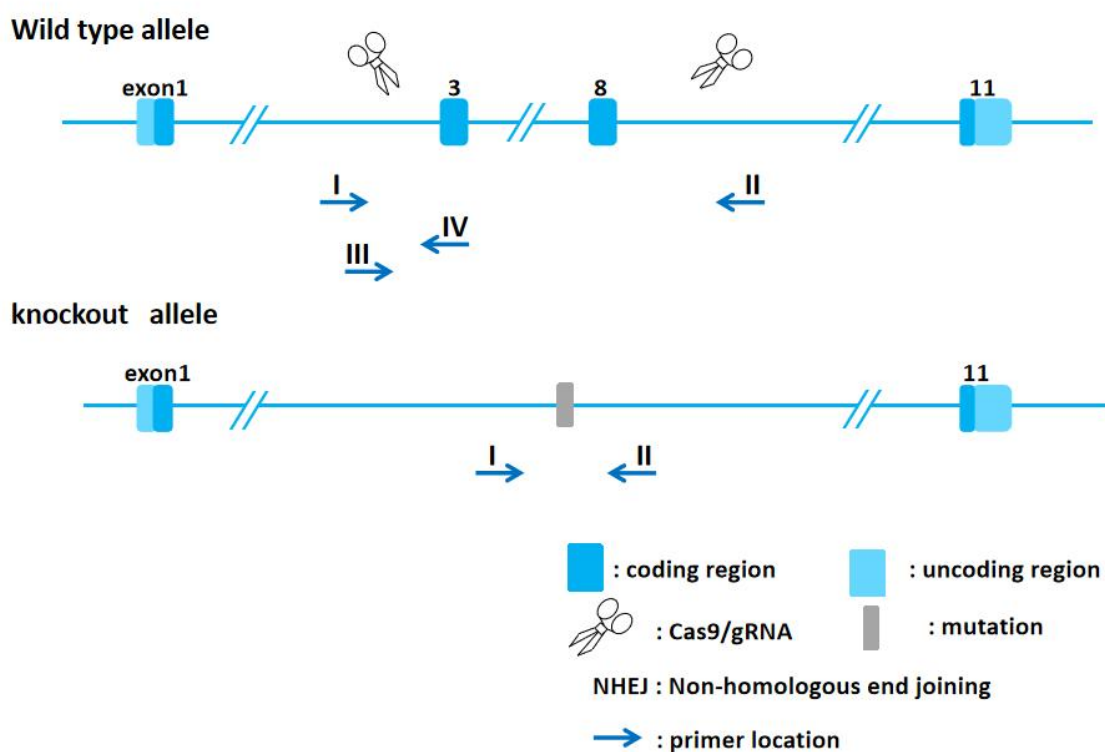


Acd-KO Genotyping Protocol



Common Name	Acid-K0	Cat. NO.	NM-K0-240274
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCAGAGACCCGGGTAGAGAA	Forward
P2	GGTGTGAGCACAGACCTCTC	Reverse
P3	TGCTACTTGTACGCGGAAGG	Forward
P4	CTTACCACTCACCGCATGGT	Reverse

Expected results

Results	P1/P2 HE HE WT M 1918bp 697bp GeneRuler™ 1 kb DNA Ladder 1% TspRI/NotI LE GO Agarose (BioLabs) 0.5 µg/lane, 8 cm length gel, 1X TBE, 7 V/cm, 45 min P3/P4 HE WT M 258bp DL2000 1.7% agarose bp 2000 1000 750 500 250 100
Genotype	Knockout type: -1221 bp Wild type: P1P2 =1918 bp; P3P4 =258 bp Heterozygote: P1P2 =1918 bp and 697 bp; P3P4 =258 bp Homozygote: P1P2 =697 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 K0 band.

Reaction &Cycling

PCR	Reaction Component	Volume (µl)
Reaction	ddH2O	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
	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	