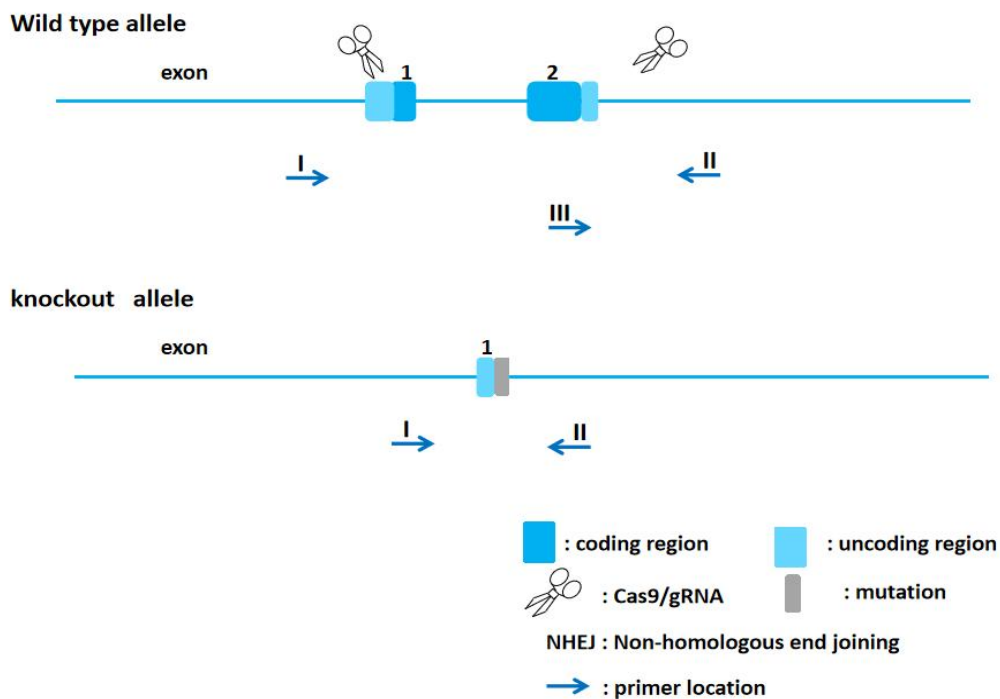


Ackr1-KO (2) Genotyping Protocol



Common Name	Ackr1-KO (2)	Cat. NO.	NM-KO-233760
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CTTTGTGGACCTCACCGTGT	Forward
P2	AAGTGACAAGGAGCACCCAG	Reverse
P3	CTTGATGCGATGCTGAATGTGA	Forward

Expected results

Results	P1P2P3 HE WT H₂O M DL2000 628 bp 473 bp bp 2000 1000 750 500 250 100 1% agarose
Genotype	Knockout type: -1742 bp Wild type: P2P3=628 bp Heterozygote: P1P2 =473 bp; P2P3=628 bp Homozygote: P1P2 =473 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
	P3 (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	