

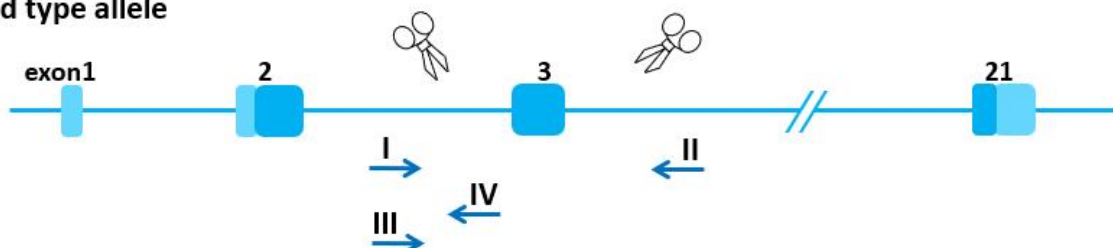
Acs1-KO Genotyping Protocol



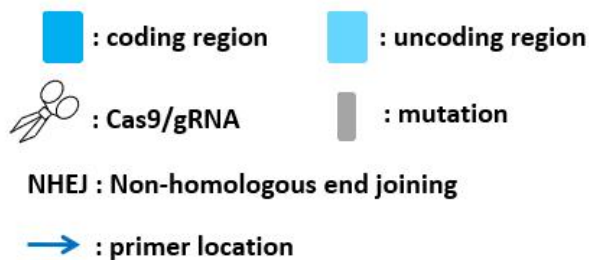
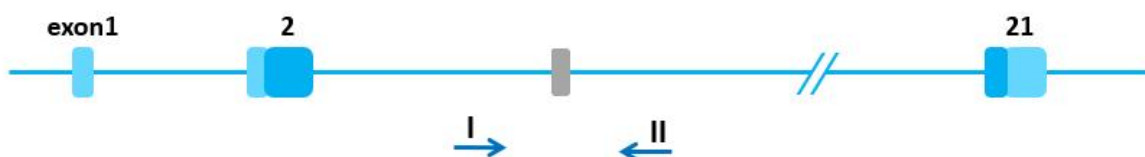
Common Name	Acsl1-KO	Cat. NO.	NM-KO-232917
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	GTGGGTGACTCGTGGAAGTAAAA	Forward
P2	TGAGGGCTGATTCAAGGTGTATGT	Reverse
P3	ACCTAACTGACAAATAGAGATGGCA	Forward
P4	CAGGGGCGAGCAGGATGTTA	Reverse

Expected results

Results	P1/P2 HE HE WT M H 1224bp 559bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE WT M H 281bp DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -665bp Wild type: P1P2 =1224 bp; P3P4 =281 bp Heterozygote: P1P2 =1224 bp and 559 bp; P3P4 =281 bp Homozygote: P1P2 =559 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)
	ddH ₂ O			8.0
	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	

