

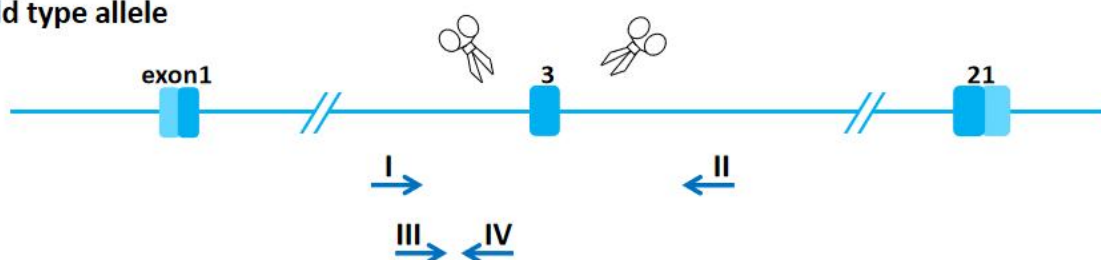
Acs16-K0 (2) Genotyping Protocol



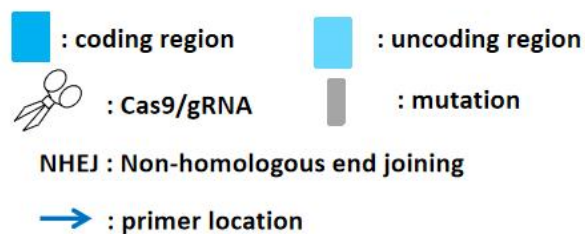
Common Name	Acs16-KO (2)	Cat. NO.	NM-KO-235041
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GAGTCCCTCTTGGGCAGAAC	Forward
P2	CCACCAAGAACAGGGTGGAA	Reverse
P3	TCTCGGGGCTTAGCAGTAGA	Forward
P4	CATGGCCTGTTACACCAGA	Reverse

Expected results

Results	
Genotype	Knockout type: -708bp Wild type: P1P2 =1567 bp; P3P4=282 bp Heterozygote: P1P2 =1567 bp and 859 bp; P3P4=282 bp Homozygote: P1P2 =859 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 System	ddH2O	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 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	