

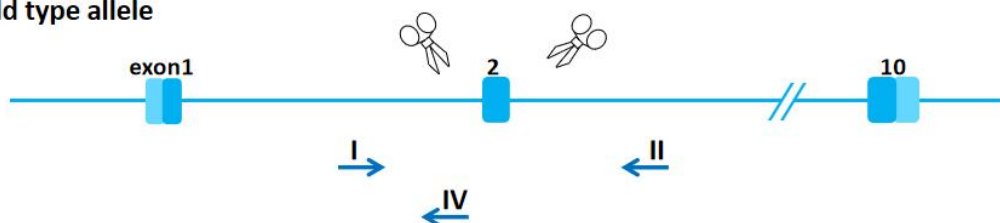
Acads-KO Genotyping Protocol



Common Name	Acads-KO	Cat. NO.	NM-KO-234833
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

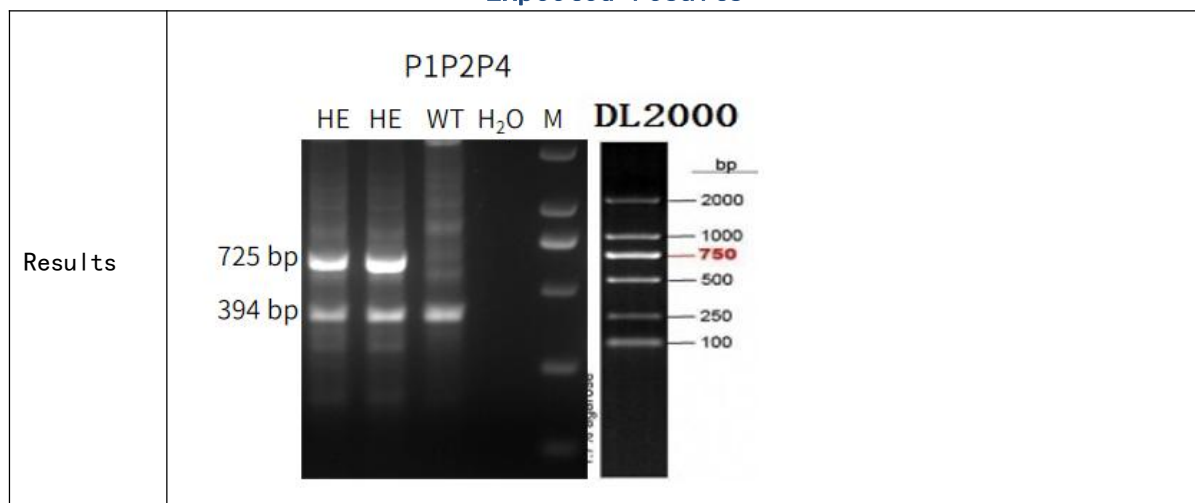


: coding region : uncoding region
 : Cas9/gRNA : mutation
 NHEJ : Non-homologous end joining
→ : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCCTGGGCTTTTCTGTAACAC	Forward
P2	ACAAGAGGTGGGCTGGAGAG	Reverse
P4	TGTAAGAAGAACAACGCTGAGGT	Reverse

Expected results



Genotype	Knockout type: -671bp
	Wild type: P1P4=394 bp
	Heterozygote: P1P2 =725 bp; P1P4=394 bp
	Homozygote: P1P2 =725 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			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	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	