

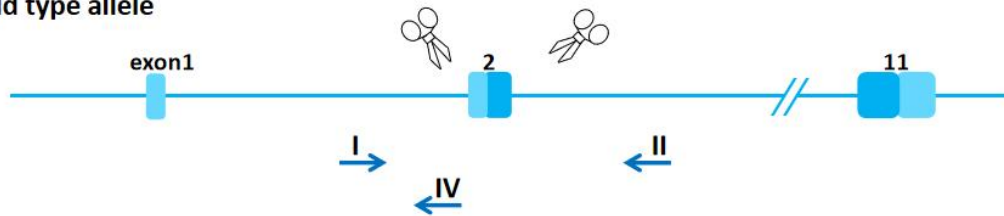
Akap1-KO Genotyping Protocol



Common Name	Akap1-KO	Cat. NO.	NM-KO-2103716
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



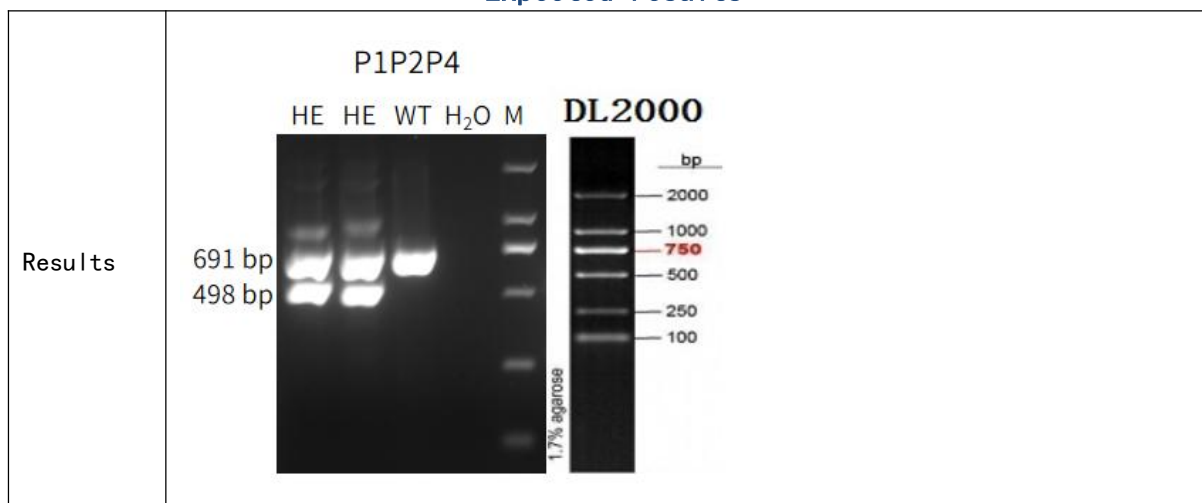
knockout allele



Primers

Primer	Sequence (5' →3')	Primer type
P1	AGACGCTGGAGATCCTGCTTGAAT	Forward
P2	CTGCGGCTGTCCTGGGTGAA	Reverse
P4	AGTACGCTGGGCATGGTGAT	Reverse

Expected results



Genotype	Knockout type: -2324bp
	Wild type: P1P4=691 bp
	Heterozygote: P1P2 =498 bp; P1P4=691 bp
	Homozygote: P1P2 =498 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		
	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		