

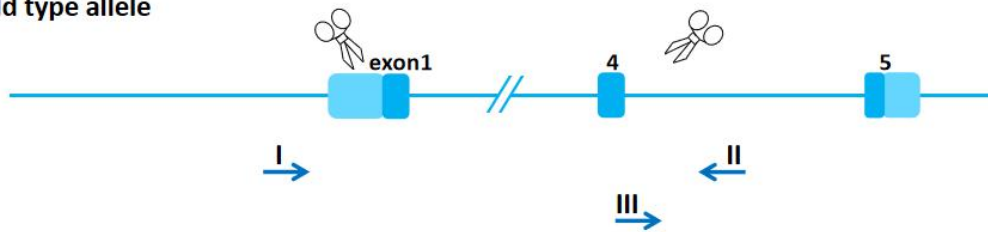
Akirin1-KO Genotyping Protocol



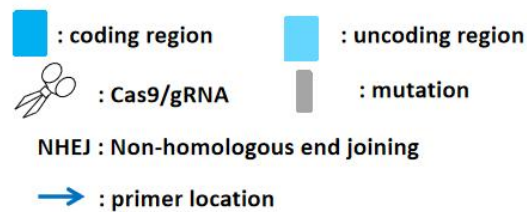
Common Name	Akirin1-KO	Cat. NO.	NM-KO-234313
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



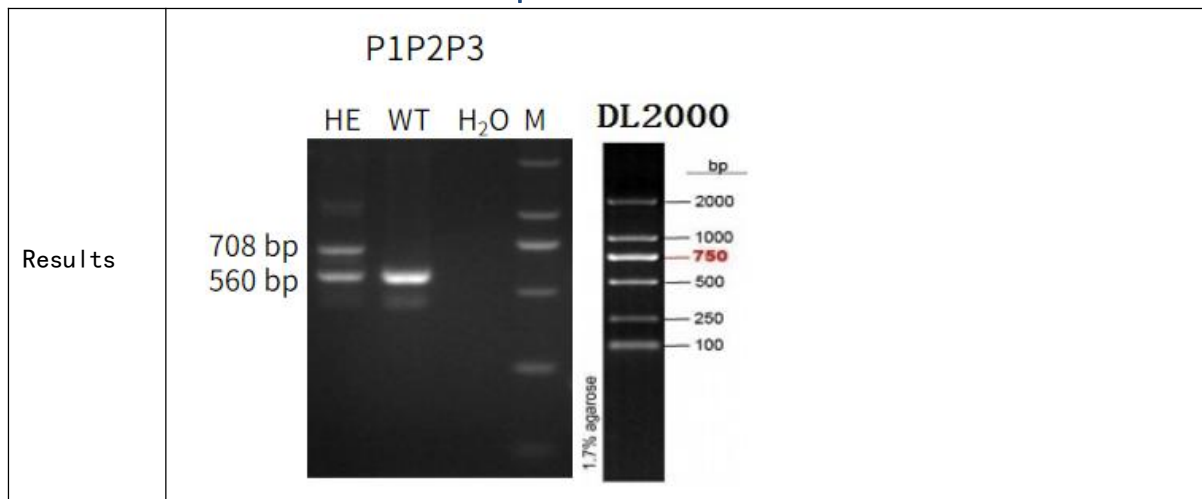
knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	ATCACCTCTTTTATCGGTCACGC	Forward
P2	GGAAACCAAAGCATTCTTAGCC	Reverse
P3	TACGGGACAAGGCCAACAAG	Forward

Expected results



Genotype	<p>Knockout type: -12437bp</p> <p>Wild type: P3P2=560 bp</p> <p>Heterozygote: P1P2 =708 bp; P3P2=560 bp</p> <p>Homozygote: P1P2 =708 bp</p>
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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 (μ l)		
	ddH ₂ O		7.5		
	2×Taq Plus Master Mix		10.0		
	P1 (10 pmol/ μ l)		0.5		
	P2 (10 pmol/ μ l)		0.5		
	P3 (10 pmol/ μ l)		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		