

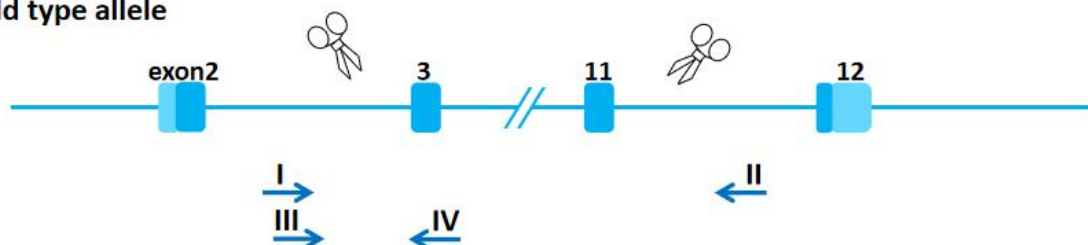
Alad-KO Genotyping Protocol



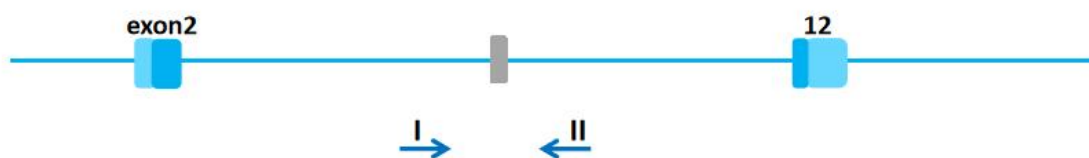
Common Name	Alad-KO	Cat. NO.	NM-KO-241480
Strain of Origin	C57BL/6J	Version	V1

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	GGAACCGTACAAGCCCTGAA	Forward
P2	GTGGACCTTTCTGGTGAGGG	Reverse
P3	AGAGTCCAAGTCCTCCCAGT	Forward
P4	CAGCCTCTGCCACAAATGTC	Reverse

Expected results

Results	
Genotype	Knockout type: -3577 bp Wild type: P1P2 =4197 bp; P3P4 =493 bp Heterozygote: P1P2 =4197 bp and 620 bp; P3P4 =493 bp Homozygote: P1P2 =620 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			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	