

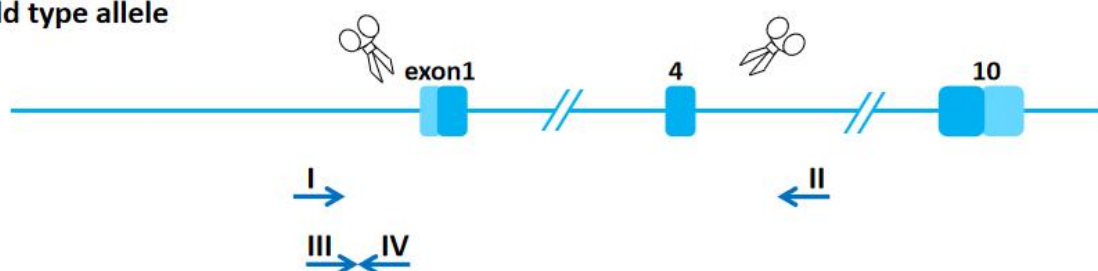
Akr1b7-K0 Genotyping Protocol



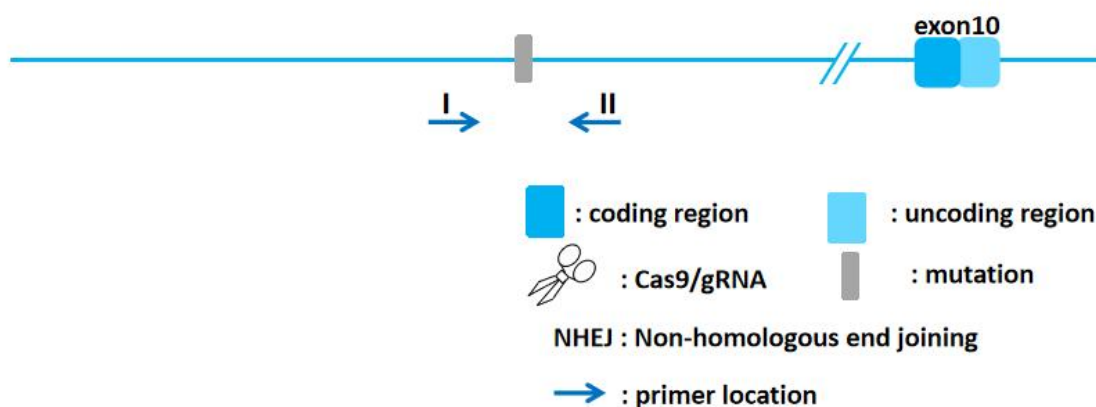
Common Name	Akr1b7-K0	Cat. NO.	NM-K0-234845
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AACCCTCTGCACATACTGGG	Forward
P2	AGAGCCTCTGACTTGCAGTA	Reverse
P3	CTGCTCTAACCGAGGACCCA	Forward
P4	CTGTGAAACGCAAGGATGGT	Reverse

Expected results

Results	
Genotype	Knockout type: -6146bp Wild type: P1P2 =6826 bp; P3P4=378 bp Heterozygote: P1P2 =6826 bp and 680 bp; P3P4=378 bp Homozygote: P1P2 =680 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	ddH2O	8.0
System	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	