

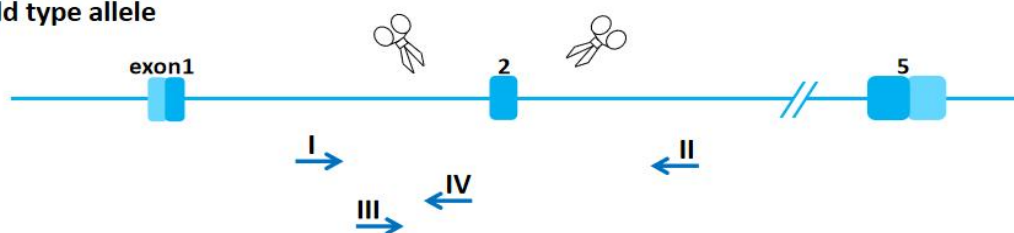
Aicda-K0 Genotyping Protocol



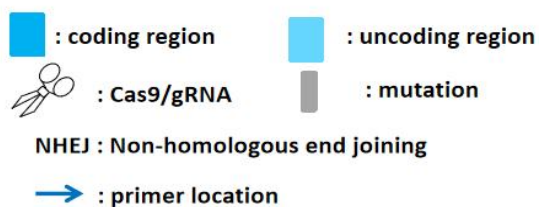
Common Name	Aicda-KO	Cat. NO.	NM-KO-234890
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCAGGCATGCATGTACACAG	Forward
P2	GGTCATTGGCCTCTCTGGTC	Reverse
P3	GGCATGCATGTACACAGAGC	Forward
P4	AAGTCAGGGCAAGGCTTCTC	Reverse

Expected results

Results	
Genotype	Knockout type: -332 bp Wild type: P1P2 =1050 bp; P3P4=387 bp Heterozygote: P1P2 =1050 bp and 718 bp; P3P4=387 bp Homozygote: P1P2 =718 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 System	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	