

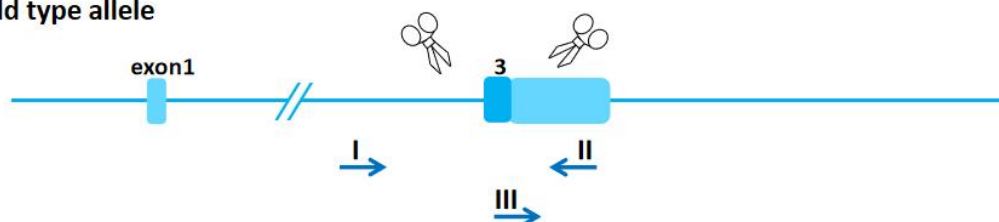
Adora1-KO Genotyping Protocol



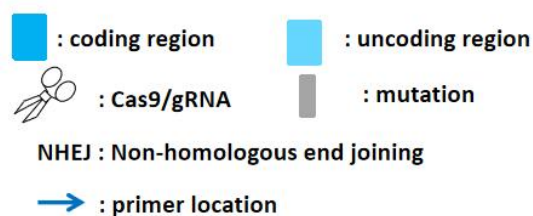
Common Name	Adora1-KO	Cat. NO.	NM-KO-2110009
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGTTATAGTCCAGCGGTGAG	Forward
P2	CAGAGAACAGCCAGGAATGAT	Reverse
P3	CCCTCATCCTCTTCTCTTGC	Forward

Expected results

Results	P1P2P3
Genotype	Knockout type: -991bp Wild type: P3P2=567 bp Heterozygote: P1P2 =450 b; P3P2=567 bp Homozygote: P1P2 =450 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
	P3 (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	