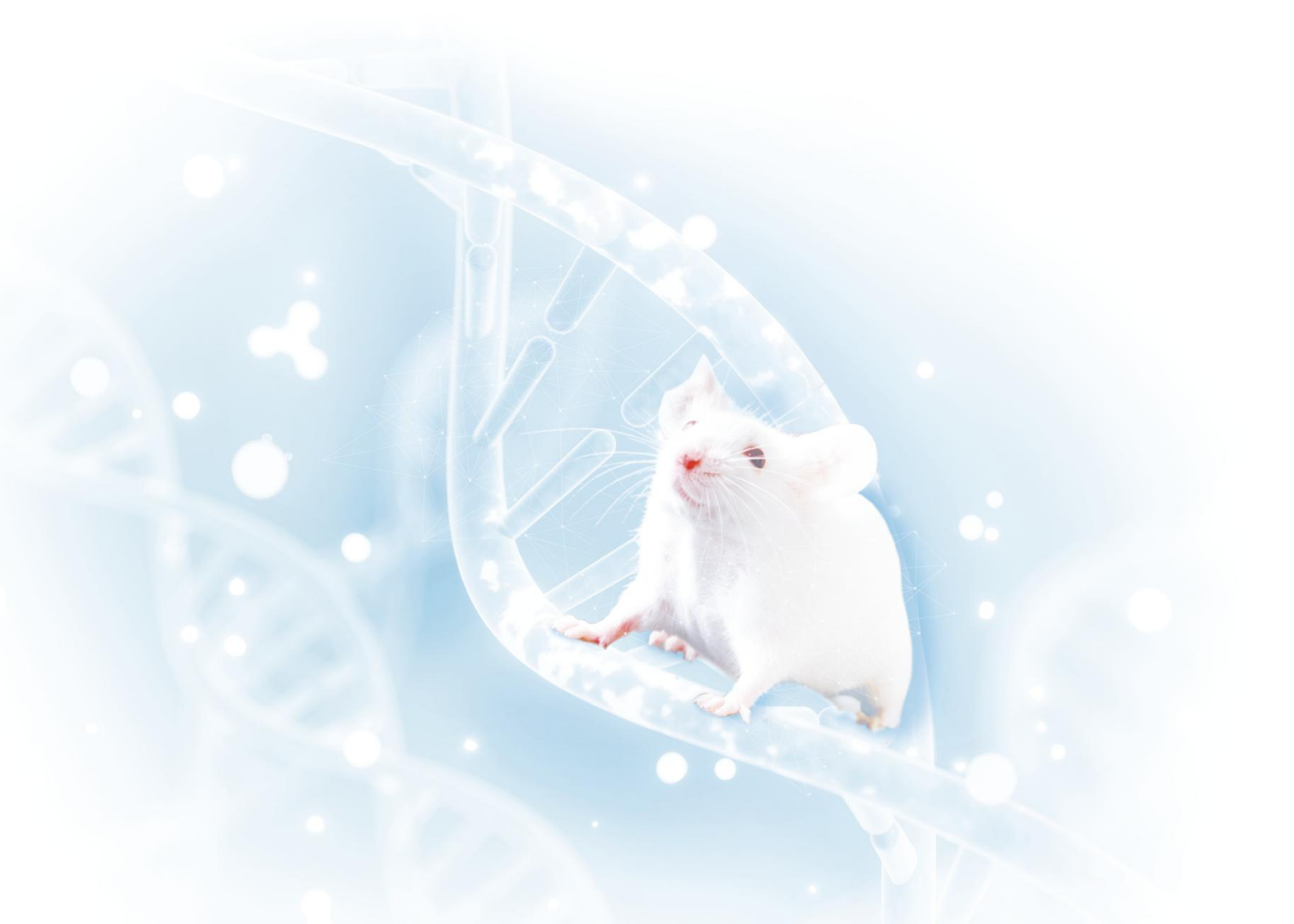


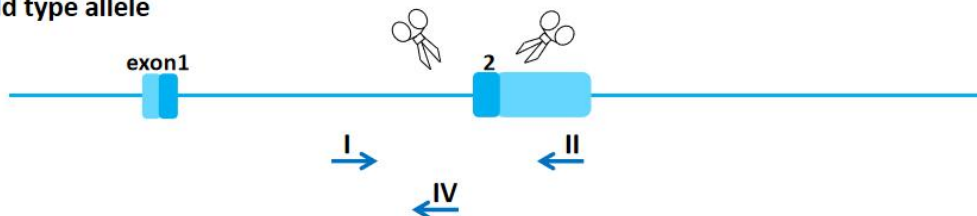
Adora3-K0 Genotyping Protocol



Common Name	Adora3-KO	Cat. NO.	NM-KO-225075
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

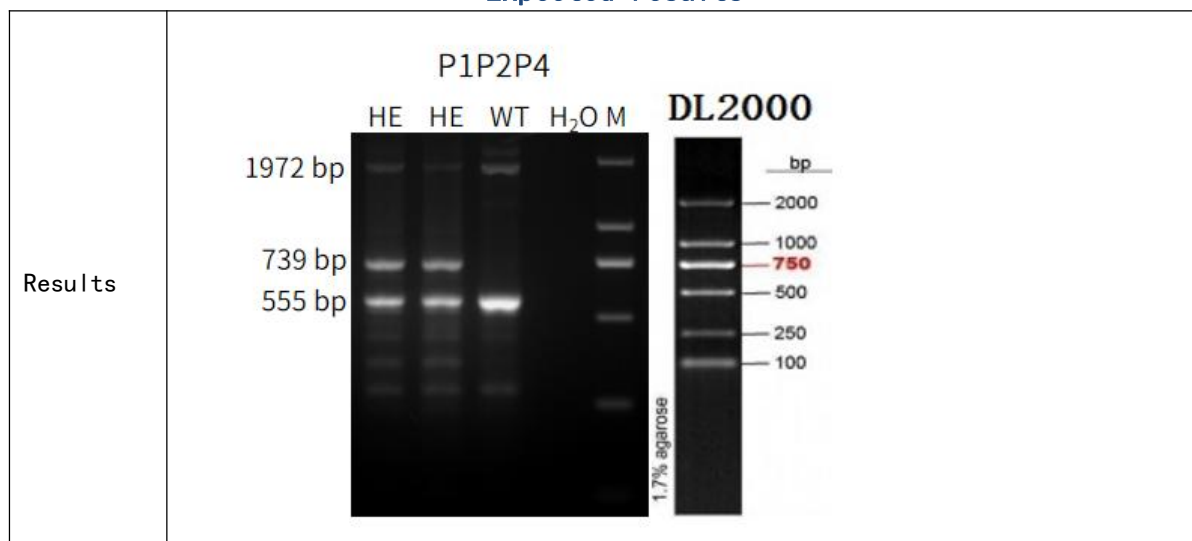


: coding region : uncoding region
 : Cas9/gRNA : mutation
 NHEJ : Non-homologous end joining
→ : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	CAGCAGGCTTCGAGGAGATT	Forward
P2	GCCTGGTCTACACAGCAAGT	Reverse
P4	CCTGGGAGAAAACAAGAAAGACAA	Reverse

Expected results



Genotype	Knockout type: -1236+3bp
	Wild type: P1P2 =1972 bp; P1P4=555 bp
	Heterozygote: P1P2 =1972 bp and 739 bp; P1P4=555 bp
	Homozygote: P1P2 =739 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
	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	