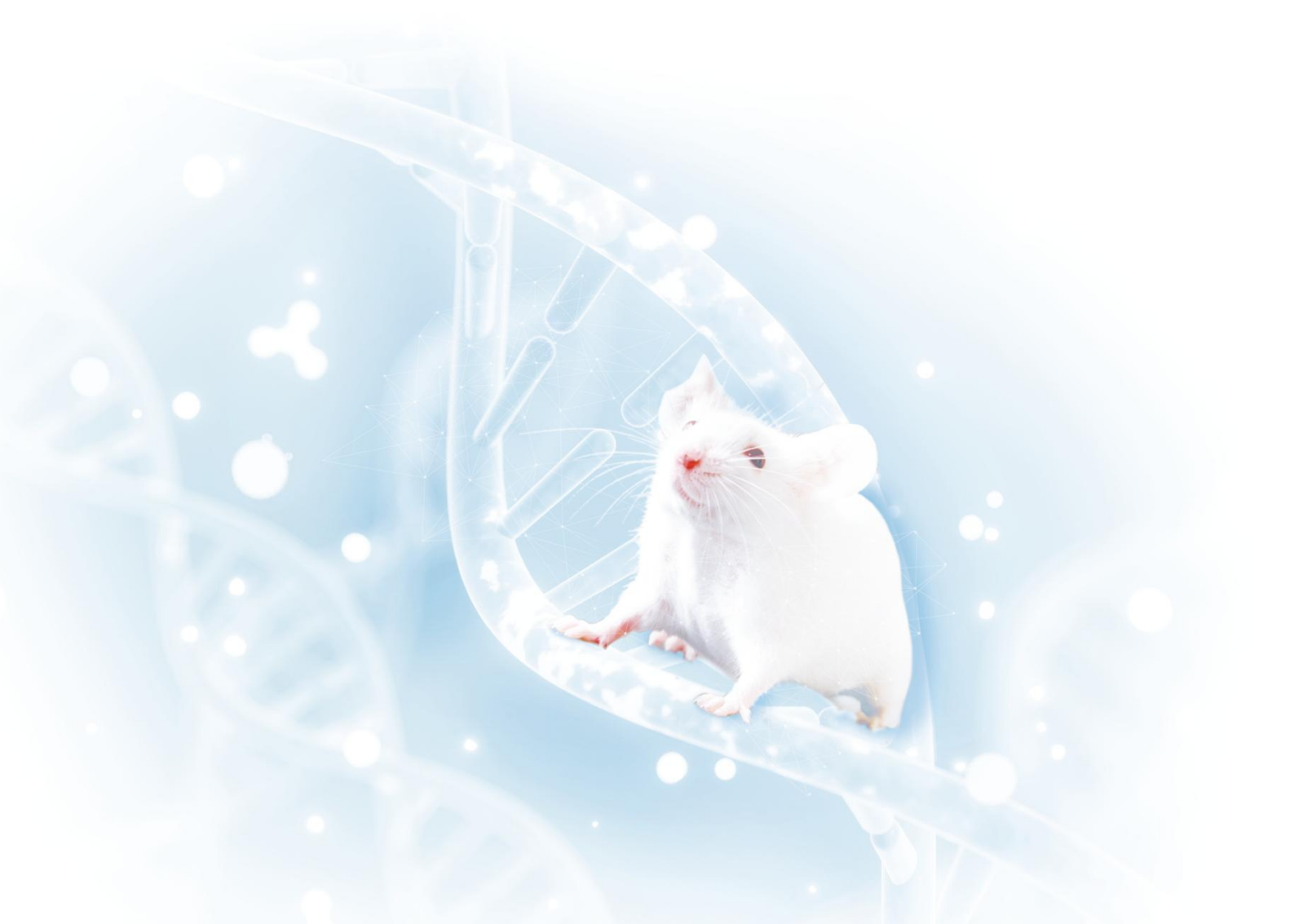


Adora3-2A-CreERT2 Genotyping Protocol



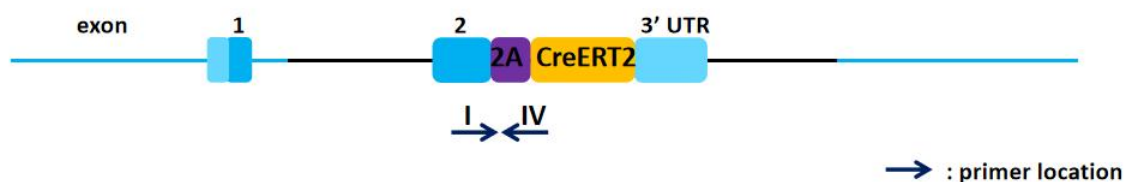
Common Name	Adora3-2A-CreERT2	Cat. NO.	NM-KI-240958
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



Targeted allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCTTCTTGTTTGCCTGTGC	Forward
P2	GGATGCATTGCCTTCACGTC	Reverse
P4	TGCGAACCTCATCACTCGTT	Reverse

Expected results

Results	P1+P2+P4 491bp 390bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Wild type: P1P2 =491 bp Heterozygote: P1P2 =491 bp; P1P4 =390 bp Homozygote: P1P4 =390 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH ₂ O			7.5
	2×Rapid Taq 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×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	