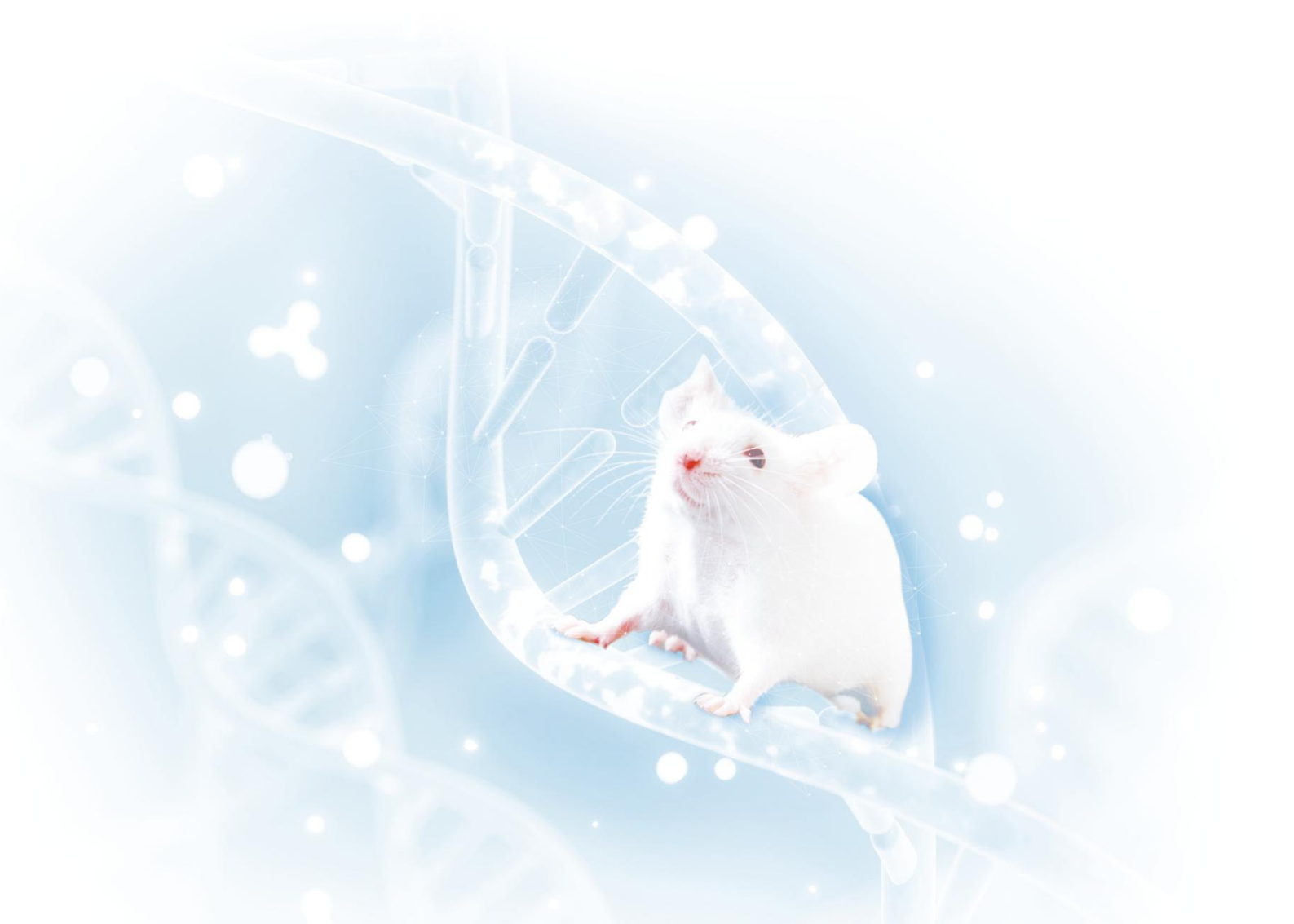
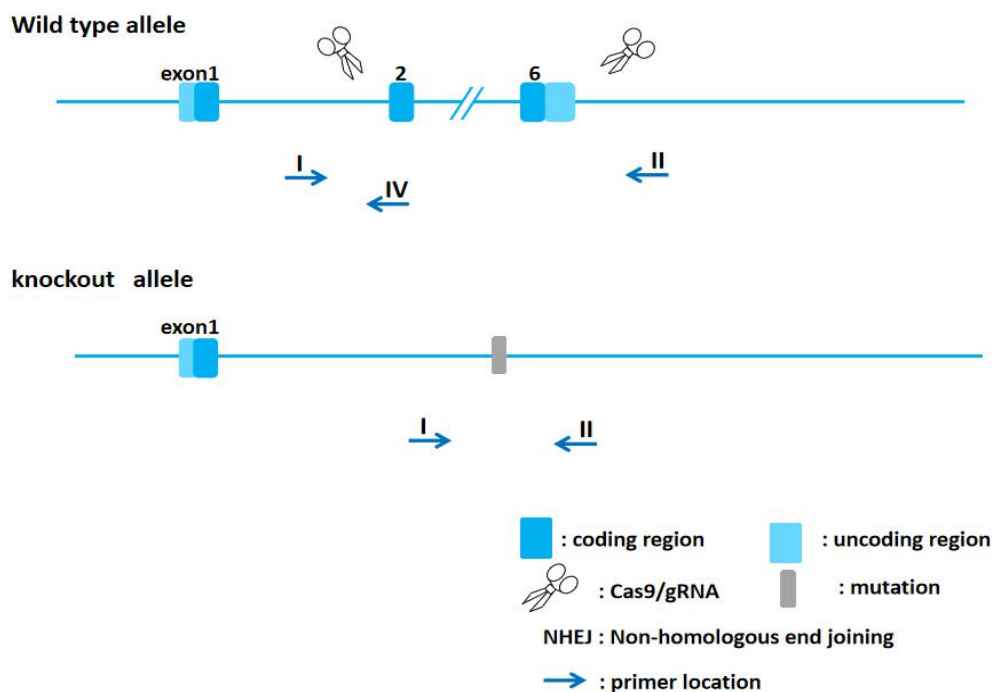


Nt5c1a-K0 Genotyping Protocol



Common Name	Nt5c1a-K0	Cat. NO.	NM-K0-231804
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGGTGAGGAAAGGGGAAGA	Forward
P2	CAGGTGCCATAGGTACCAGC	Reverse
P4	CTCCGTGTAGATCCTCTGTTCTC	Reverse

Expected results

Results	P1P2P4 HE HE WT H₂O M DL2000 606 bp 533 bp bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -8289 bp Wild type: P1P4 =606 bp Heterozygote: P1P2 =533 bp; P1P4=606 bp Homozygote: P1P2 =533 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 K0 band

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH ₂ O			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	