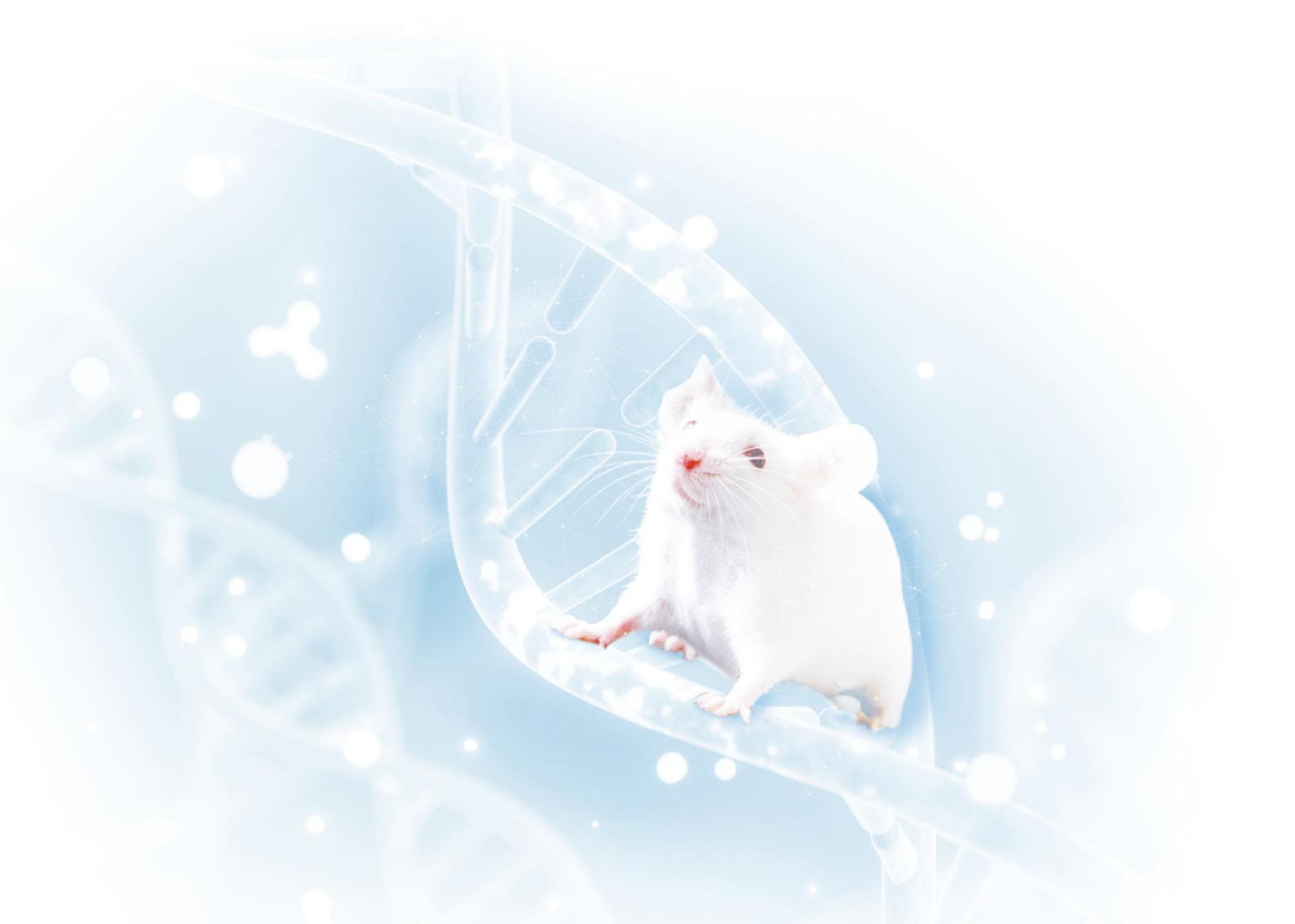


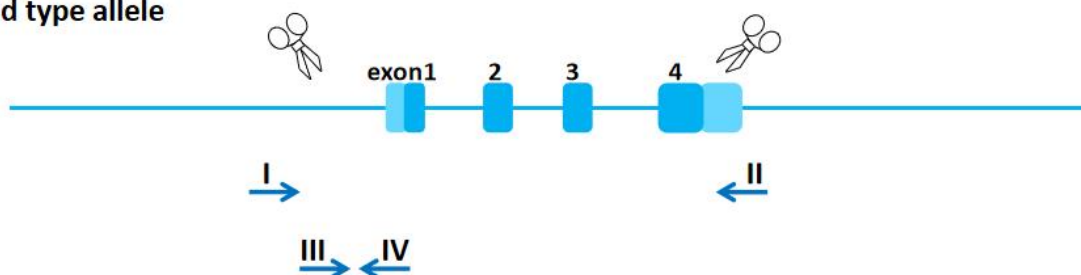
Cda-K0 Genotyping Protocol



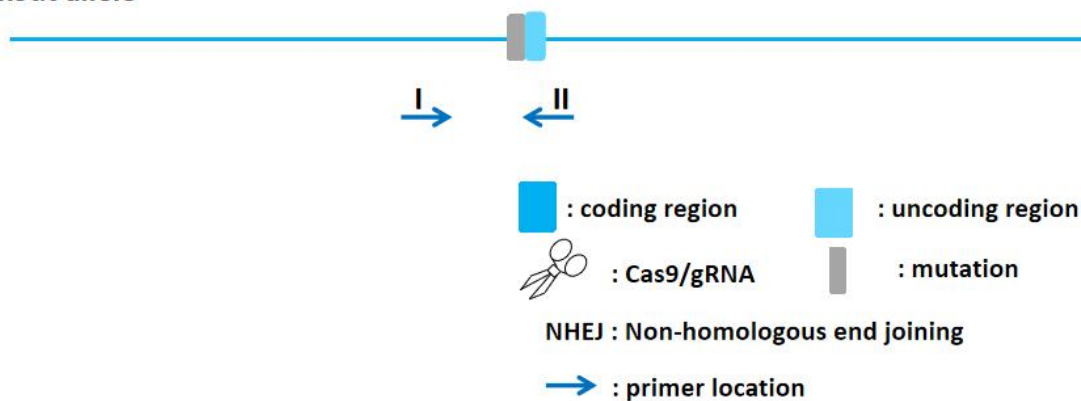
Common Name	Cda-K0	Cat. NO.	NM-K0-240281
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CAGCCTGAACCCTTCTCTCG	Forward
P2	GCACGTGCTGTGGCATTAT	Reverse
P3	AACTCCATATCCTCCGGGCT	Forward
P4	CTGACTTCTTGGCCTCACGA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% Tris/Borate LE GEL Agarose #B04031 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -29211 bp Wild type: P3P4 =299 bp Heterozygote: P1P2 =673 bp; P3P4 =299 bp Homozygote: P1P2 =673 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 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/ μ l) or P4 (10 pmol/ μ l)			0.5
	Genomic DNA			1.0
	Total			20
	2 $\times$ 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	