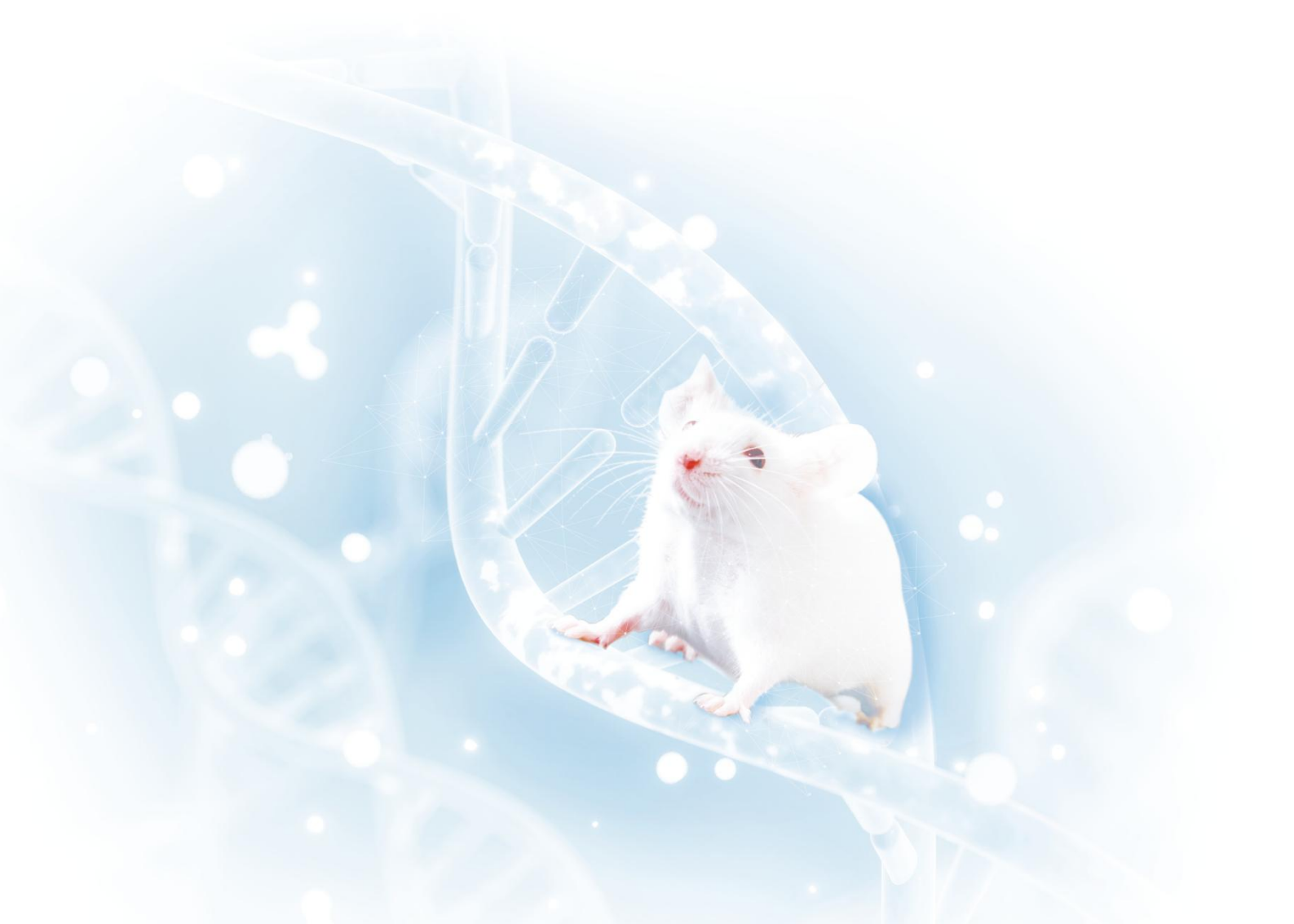


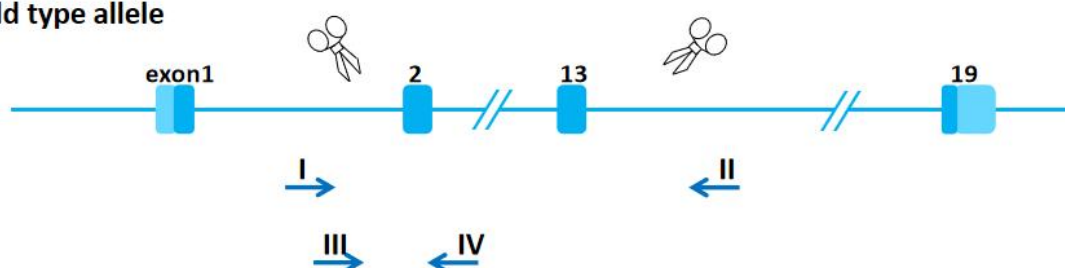
Abcd4-K0 Genotyping Protocol



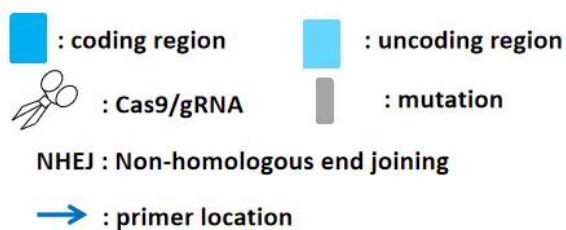
Common Name	Abcd4-KO	Cat. NO.	NM-KO-2110692
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GGCGACAAACACTTGGACCTT	Forward
P2	CGGCCCATAAATAATGCACAG	Reverse
P3	CAAGCCCACCGAGTTTACCC	Forward

P4	CCGGTTCTTCCTATTGCCC	Reverse
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Expected results

Results	
Genotype	Knockout type: -9815 bp Wild type: P3P4 =457 bp Heterozygote: P1P2 =669 bp; P3P4 =457 bp Homozygote: P1P2 =669 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 Component	Volume (μl)
Reaction	ddH2O	8.0
System	2 × Rapid Taq Master Mix	10.0

	P1 (10 pmol/μl) or P3 (10 pmol/μl)			0.5
	P2 (10 pmol/μl) or 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	