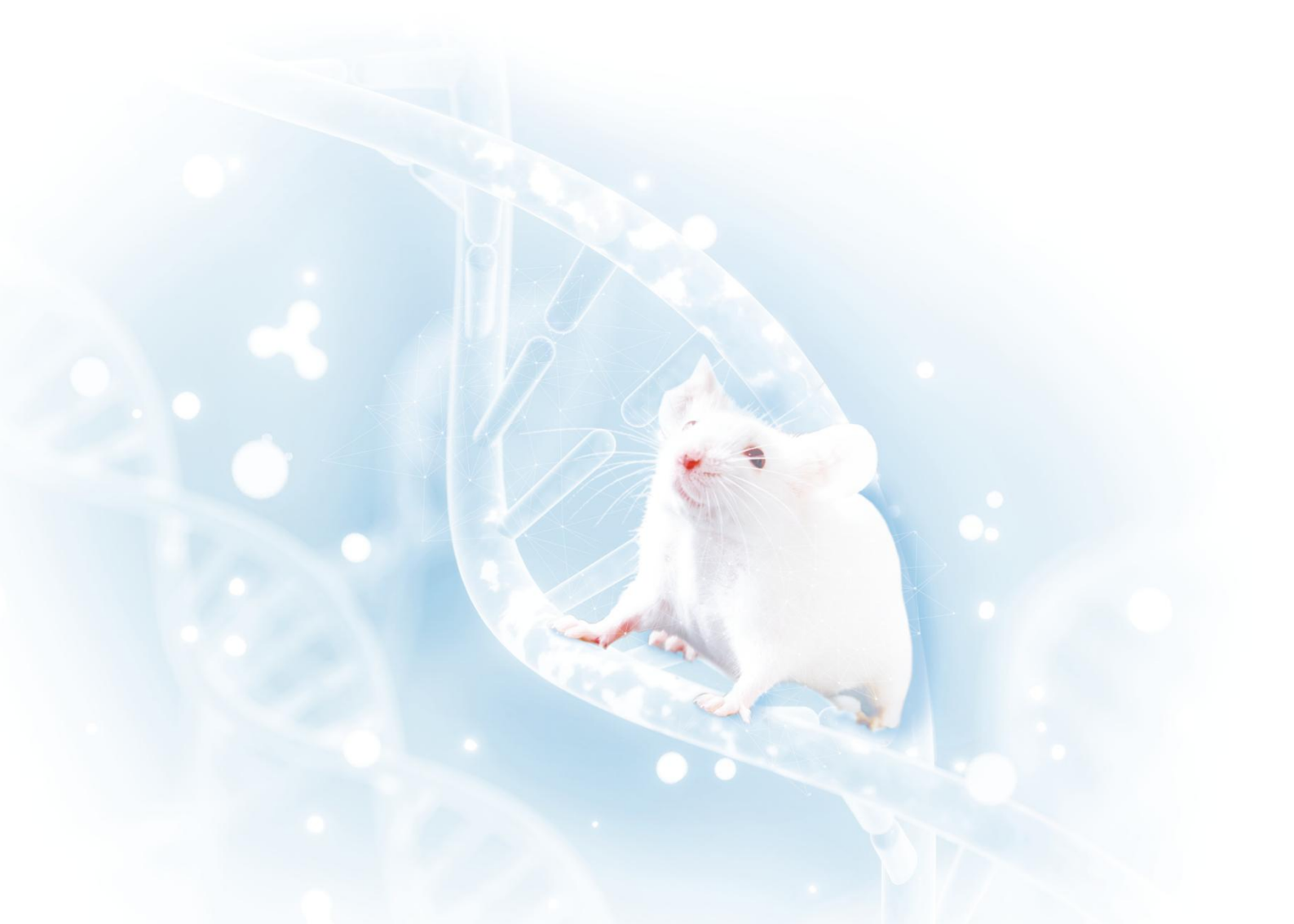
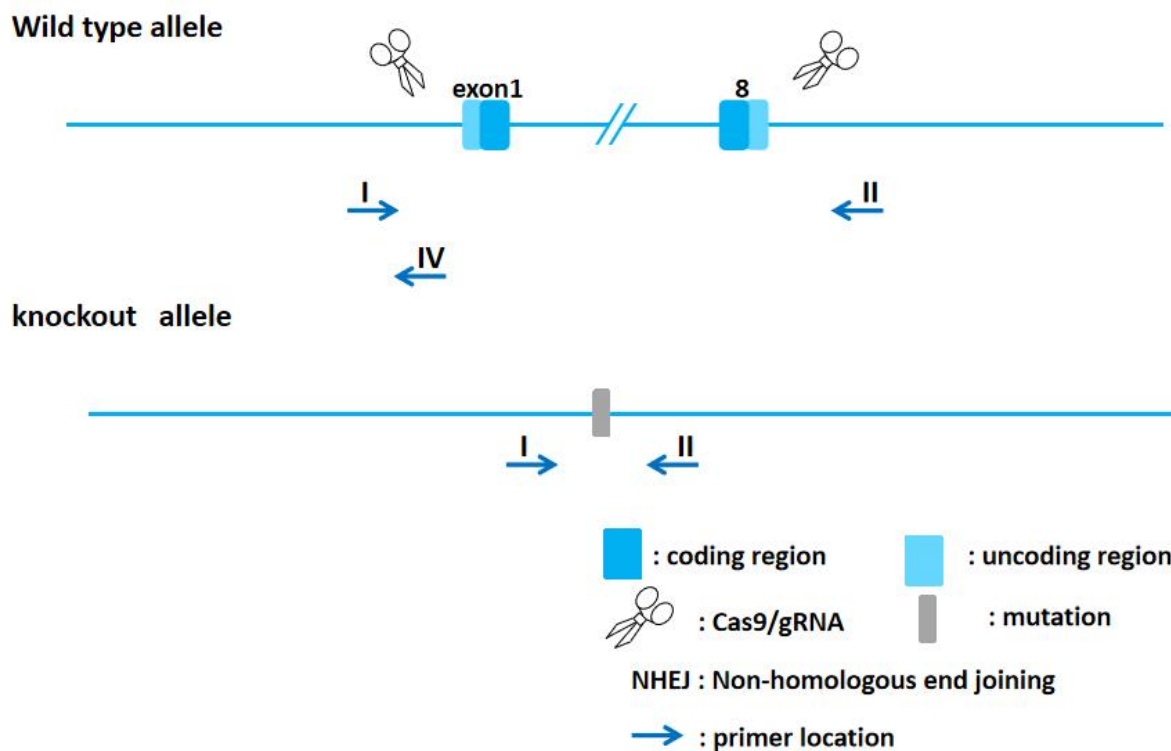


Ccdc74a-K0 Genotyping Protocol



Common Name	Ccdc74a-KO	Cat. NO.	NM-KO-231498
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GAAGATCCTGGGGTTCACGG	Forward
P2	TCCAGAAGCTATGGGGGACA	Reverse
P4	CTCAGTACTGGCTGCTGGAG	Reverse

Expected results

Results	P1P2P4 HE WT M DL2000 486 bp 288 bp 1.7% agarose bp 2000 1000 750 500 250 100
Genotype	Knockout type: -4327bp Wild type: P1P4=288 bp Heterozygote: P1P2 =486 bp; P1P4=288 bp Homozygote: P1P2 =486 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
Cycling Reaction	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
	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	