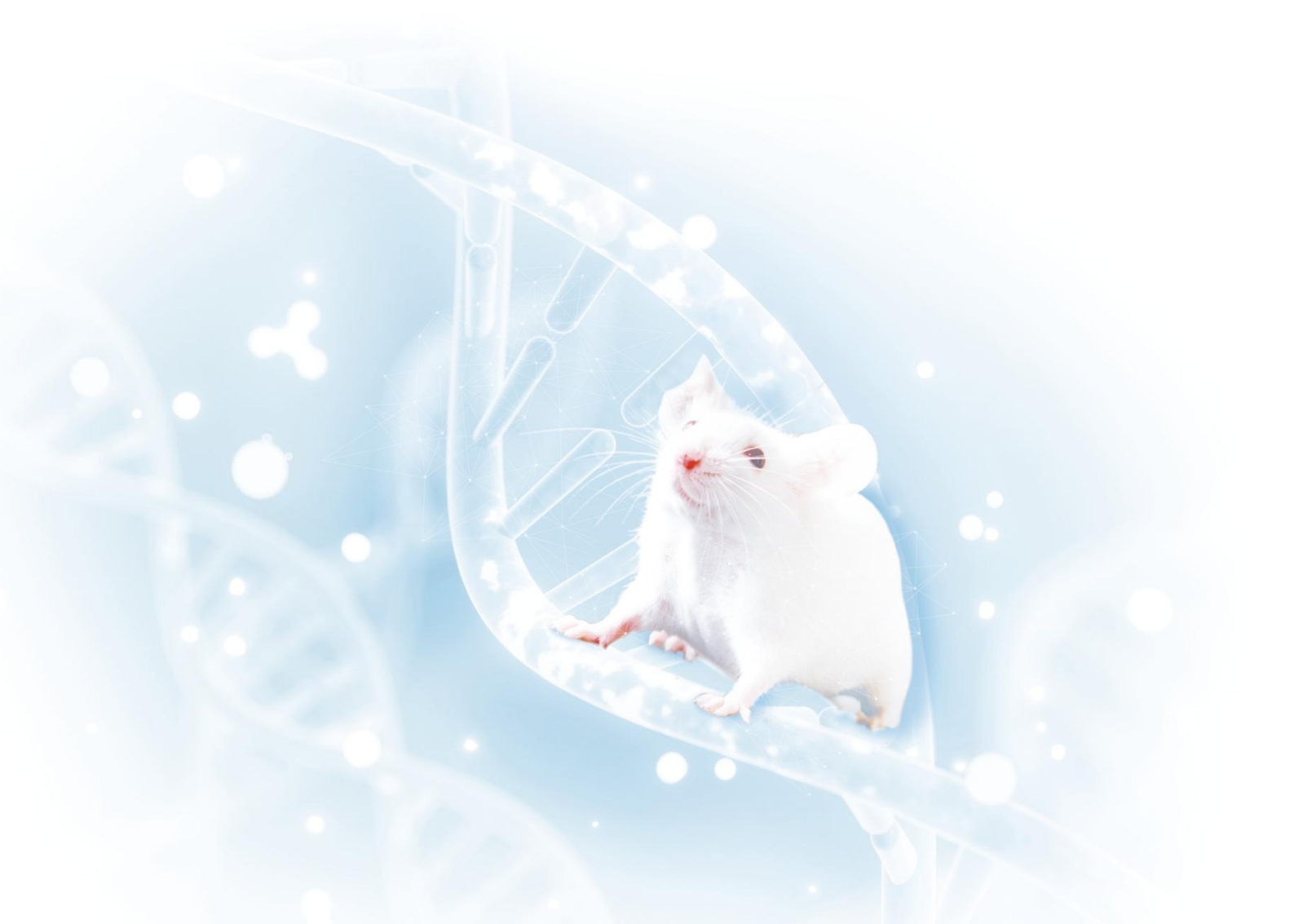
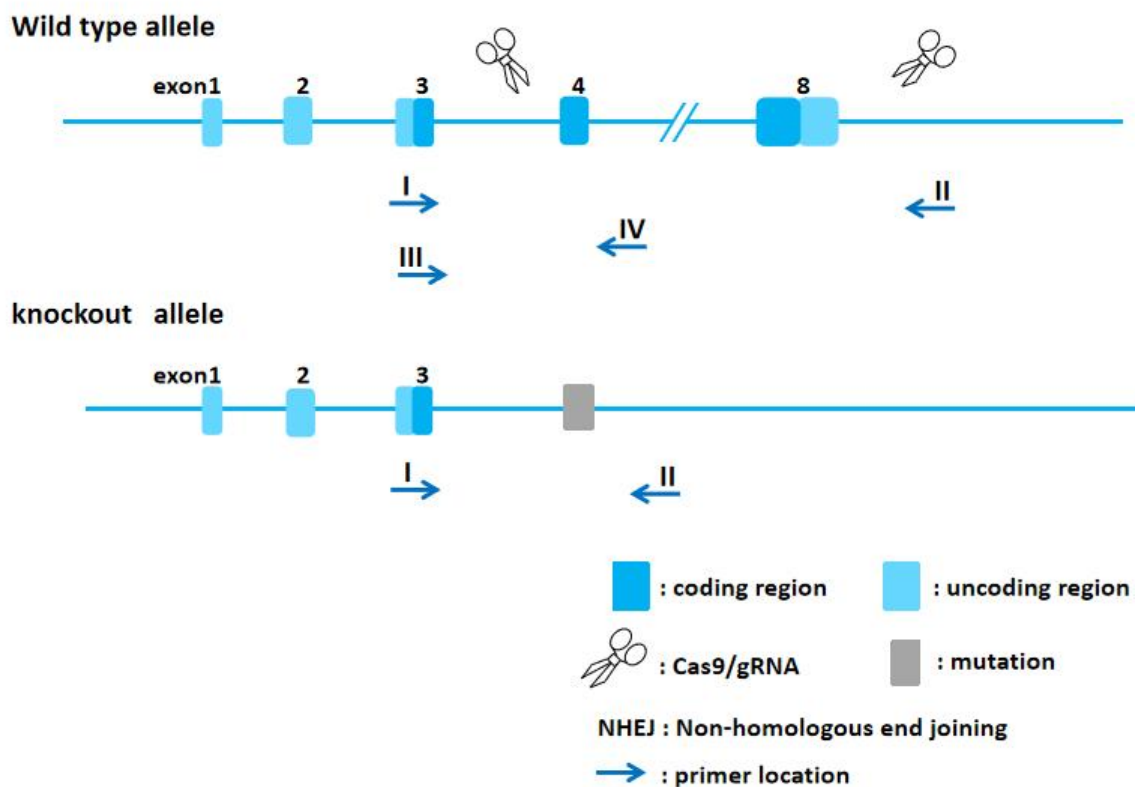


Aif1-KO Genotyping Protocol



Common Name	Aif1-KO	Cat. NO.	NM-KO-240427
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	ATGTGGAAGTGATGCCTGGG	Forward
P2	GCTGGCCATGAACTCAGACT	Reverse
P3	TGCCAGCCTAAGACAACCAG	Forward
P4	GTTTGGACGGCAGATCCTCA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 M WT HE HE 1962bp 518bp P3/P4 HE HE WT H M 430bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7V/cm, 45 min
Genotype	Knockout type: -1448+4bp Wild type: P1P2 =1962 bp; P3P4 =430 bp Heterozygote: P1P2 =1962 bp and 518 bp; P3P4 =430 bp Homozygote: P1P2 =518 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)
	ddH ₂ O			8.0
	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	