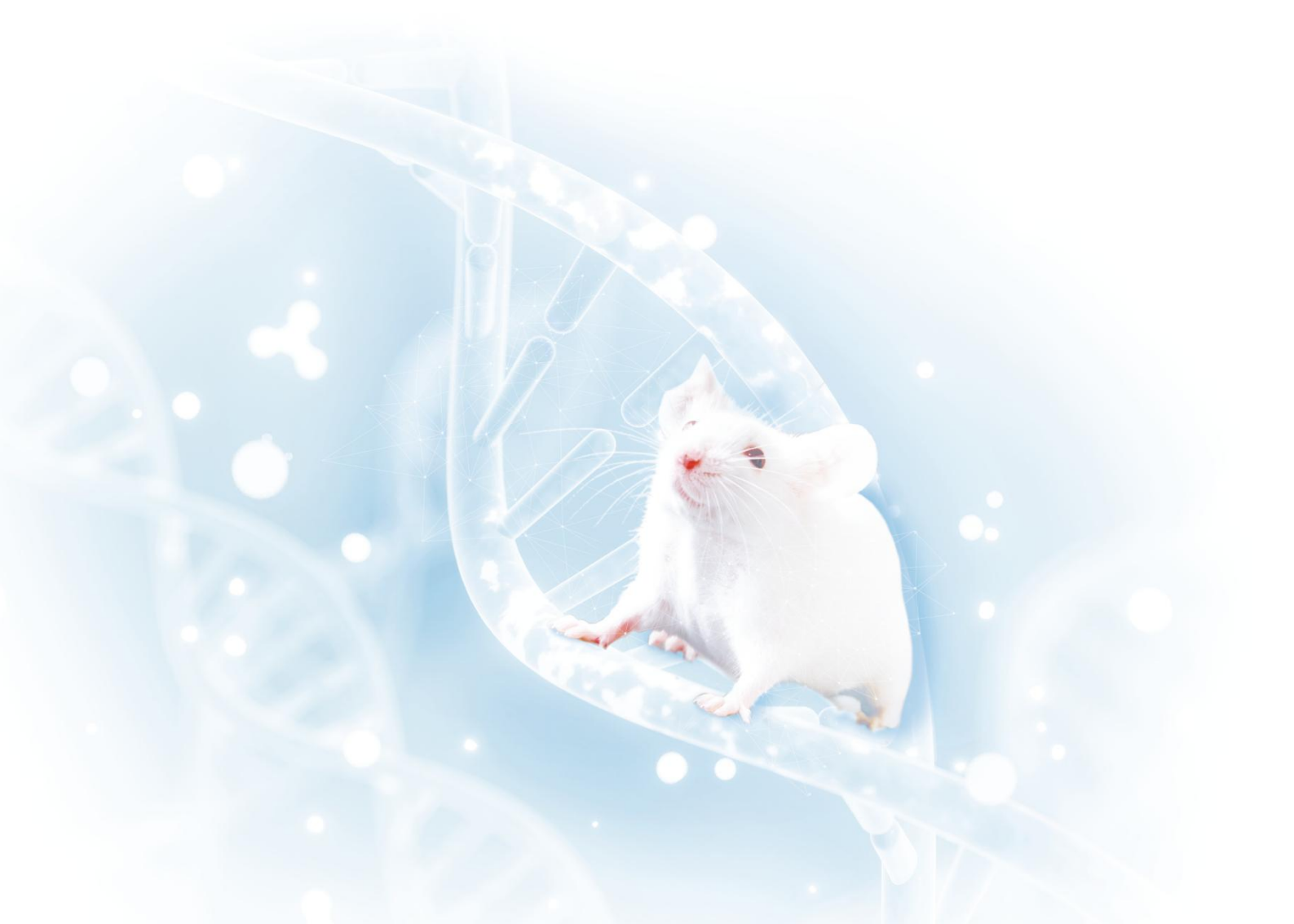


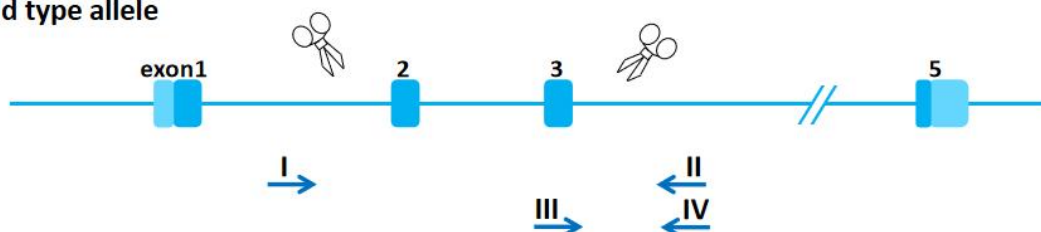
Cidea-KO Genotyping Protocol



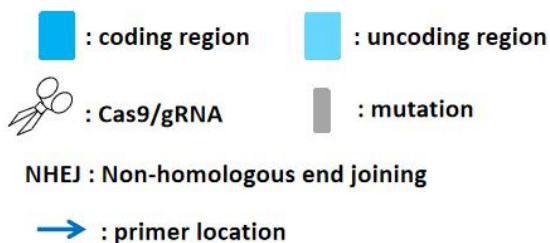
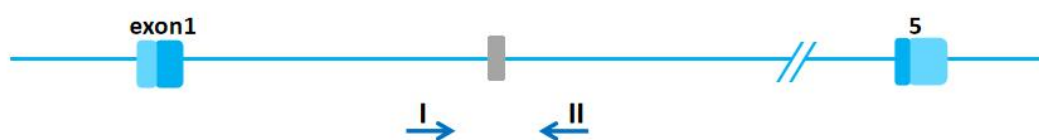
Common Name	Cidea-KO	Cat. NO.	NM-KO-240655
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	CCACAAACTGGTCCTCTGCT	Forward
P2	CTCAGCAAGGAGGCAGAACT	Reverse
P3	TCTTGCTGCTCCCTTGCAAAC	Forward
P4	AGCAAGGAGGCAGAACTGATT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 HE HE WT M 3116bp 691bp 1% TspI/SbfI LE GA Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 500bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2425bp Wild type: P1P2 =3116 bp; P3P4 =500 bp Heterozygote: P1P2 =3116 bp and 691 bp; P3P4 =500 bp Homozygote: P1P2 =691 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/ μ l) or P4(10 pmol/ μ l)			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	