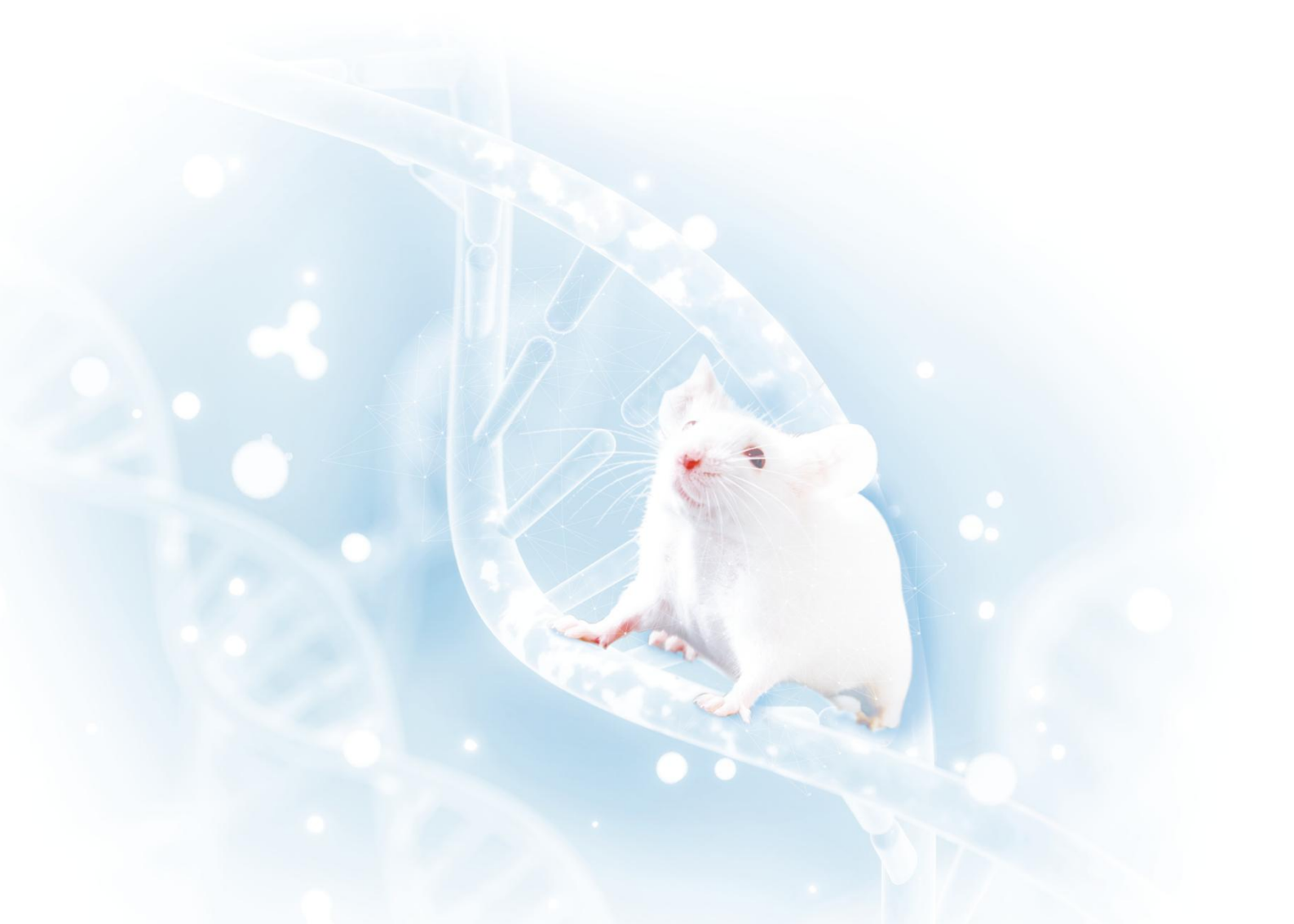
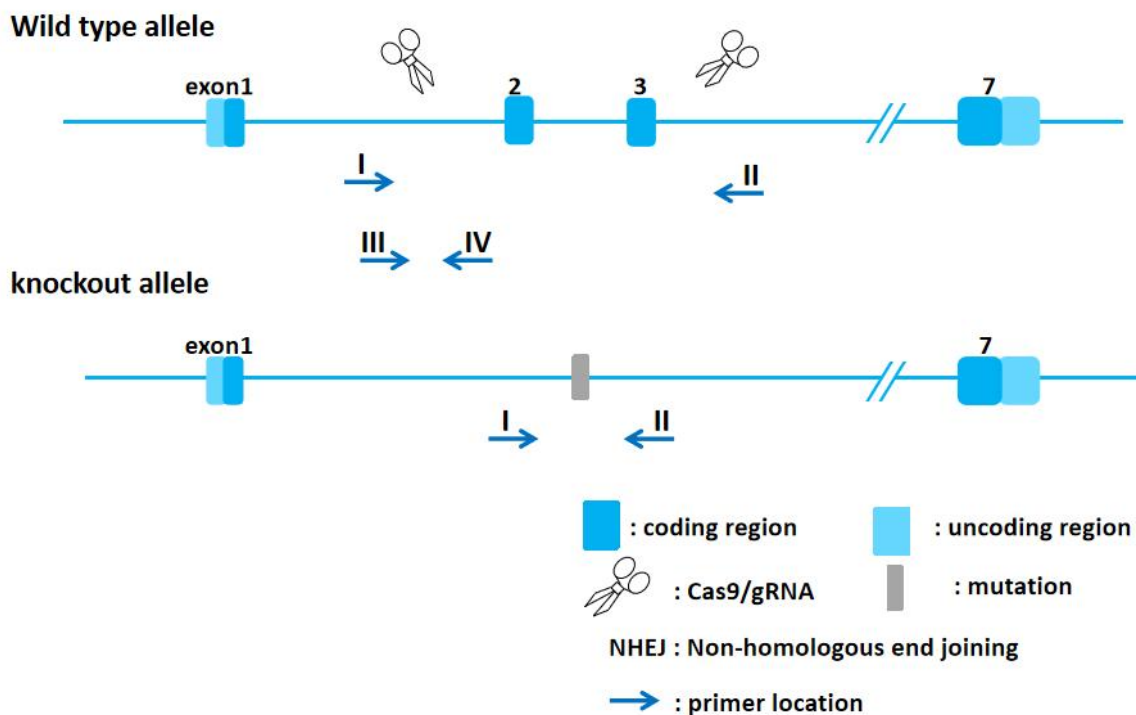


Cd300lf-KO Genotyping Protocol



Common Name	Cd300lf-KO	Cat. NO.	NM-KO-200096
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GCAATGGGATCCGATGTCCT	Forward
P2	TGGTTGTGACTGCCATGTGT	Reverse
P3	TGTGCAACACCACACCTGAA	Forward
P4	CGACATCCTGCTGTGTCAGA	Reverse

Expected results

Results	P1/P2 HE WT HE WT M H 3524bp 793bp 1 kb bp 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 P3/P4 HE WT M 265bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2731 bp Wild type: P1P2 =3524 bp; P3P4 =265 bp Heterozygote: P1P2 =3524 bp and 793 bp; P3P4 =265 bp Homozygote: P1P2 =793 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	