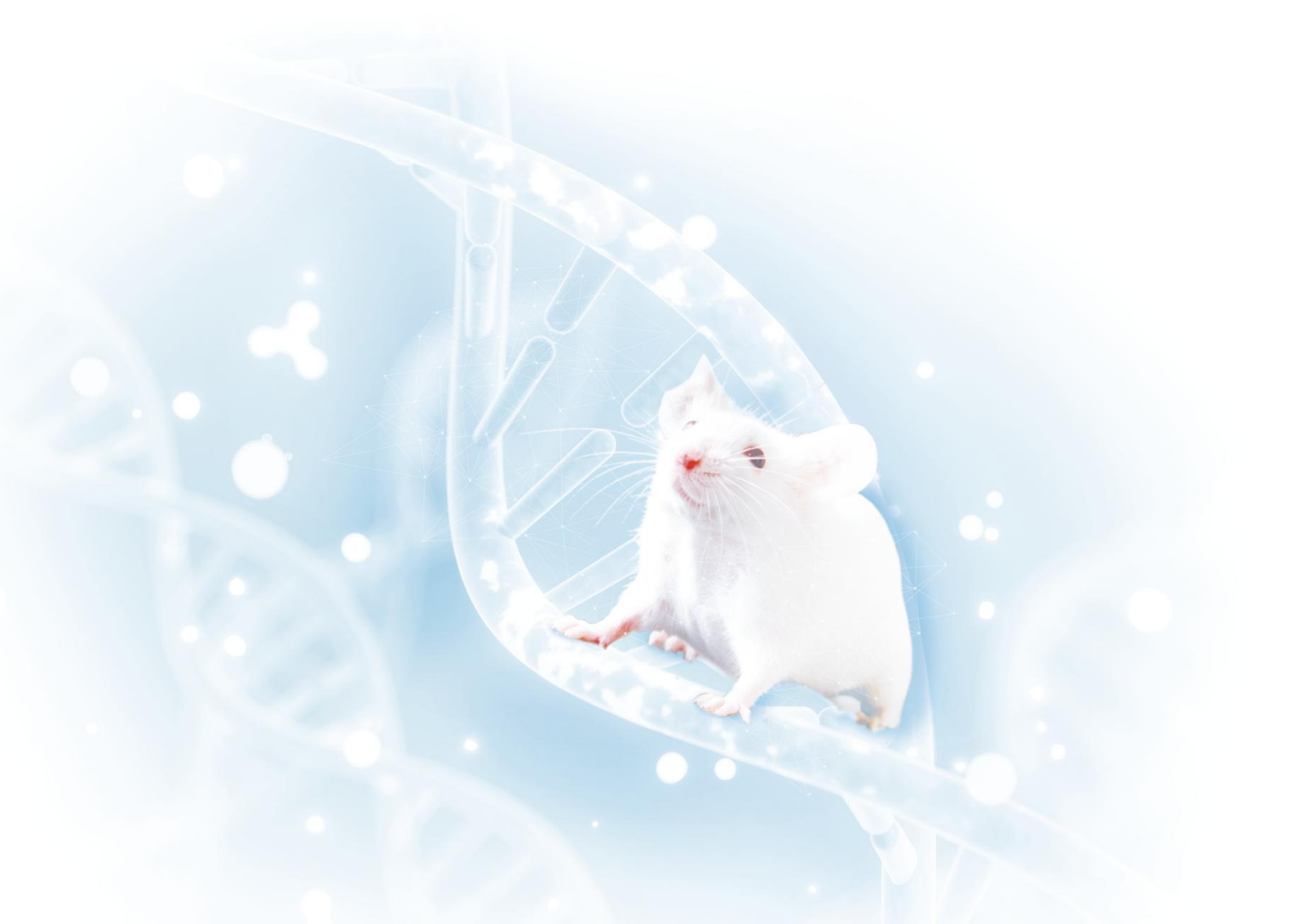
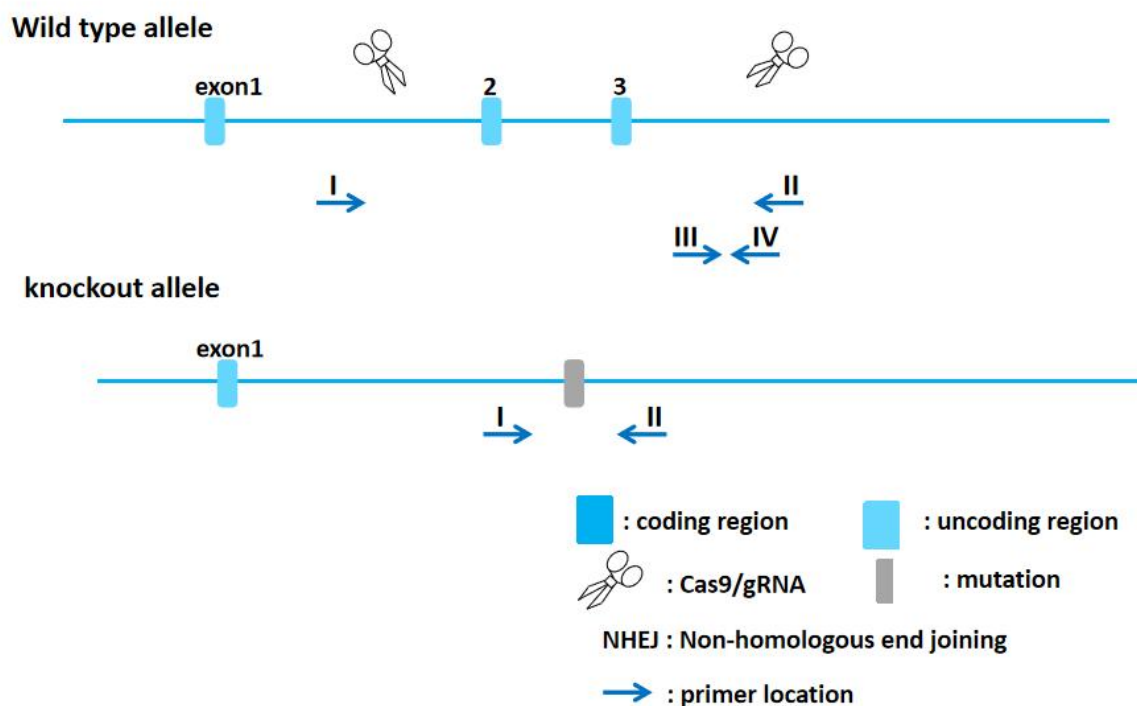


Gad1os-KO Genotyping Protocol



Common Name	Gad1os-KO	Cat. NO.	NM-KO-242018
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TTGCTGATGGCTTGGGTAGT	Forward
P2	CCAGGAAGCCACCCACATTT	Reverse
P3	TAGCCACCATGTTGGTGTG	Forward
P4	TGCTCACAAATGCACTGGAA	Reverse

Expected results

Results	
Genotype	Knockout type: -3656 bp Wild type: P1P2 =4305 bp; P3P4 =261 bp Heterozygote: P1P2 =4305 bp and 649 bp; P3P4 =261 bp Homozygote: P1P2 =649 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	