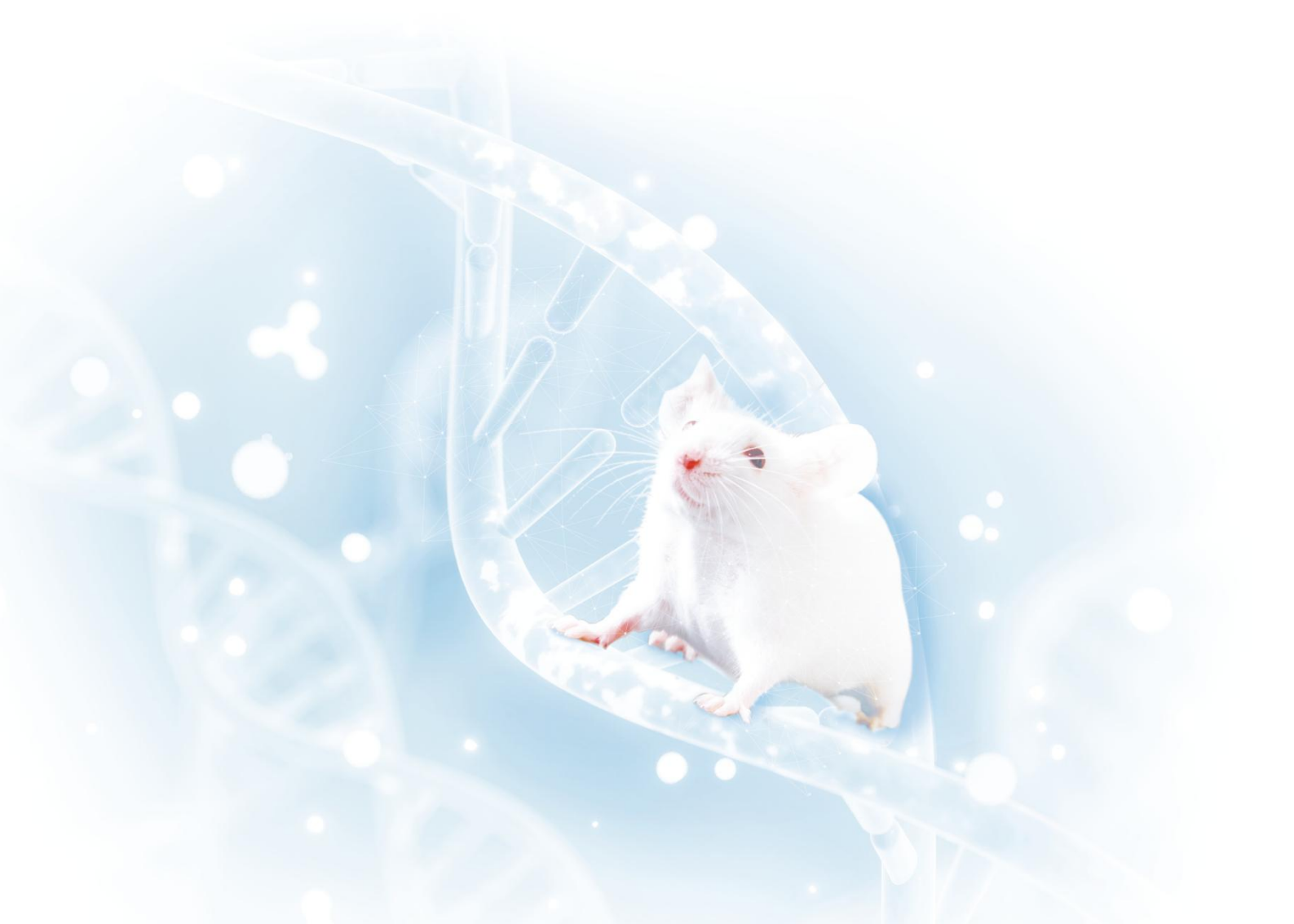
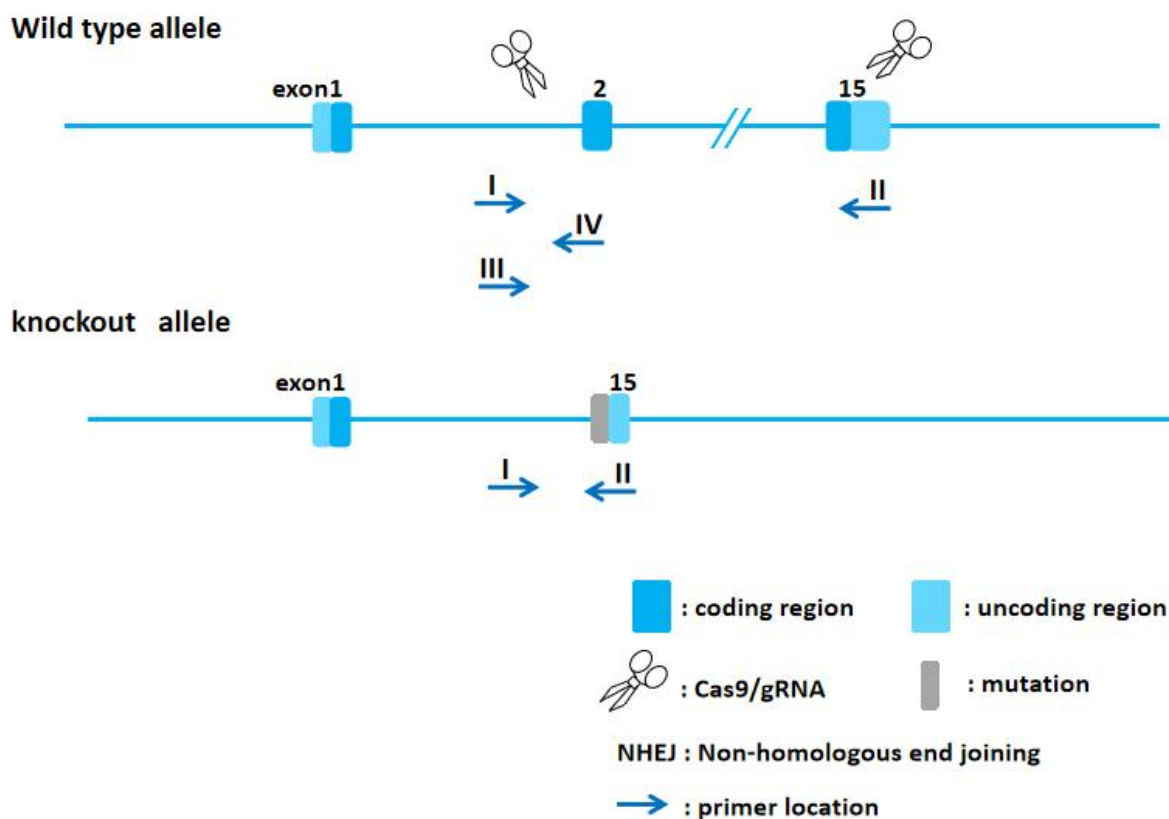


Accsl-KO Genotyping Protocol



Common Name	Accs1-KO	Cat. NO.	NM-KO-241493
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GGGATTGAACAACACAAGCCC	Forward
P2	GAATCCGGGGAACAGCCTTT	Reverse
P3	TCCTAGAGGGCCTTACTGCT	Forward
P4	TCCTGCACAAATTTGAAGCCC	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder DL2000
Genotype	Knockout type: -10636bp Wild type: P1P2 =11158 bp; P3P4 =388 bp Heterozygote: P1P2 =11158 bp and 522 bp; P3P4 =388 bp Homozygote: P1P2 =522 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	