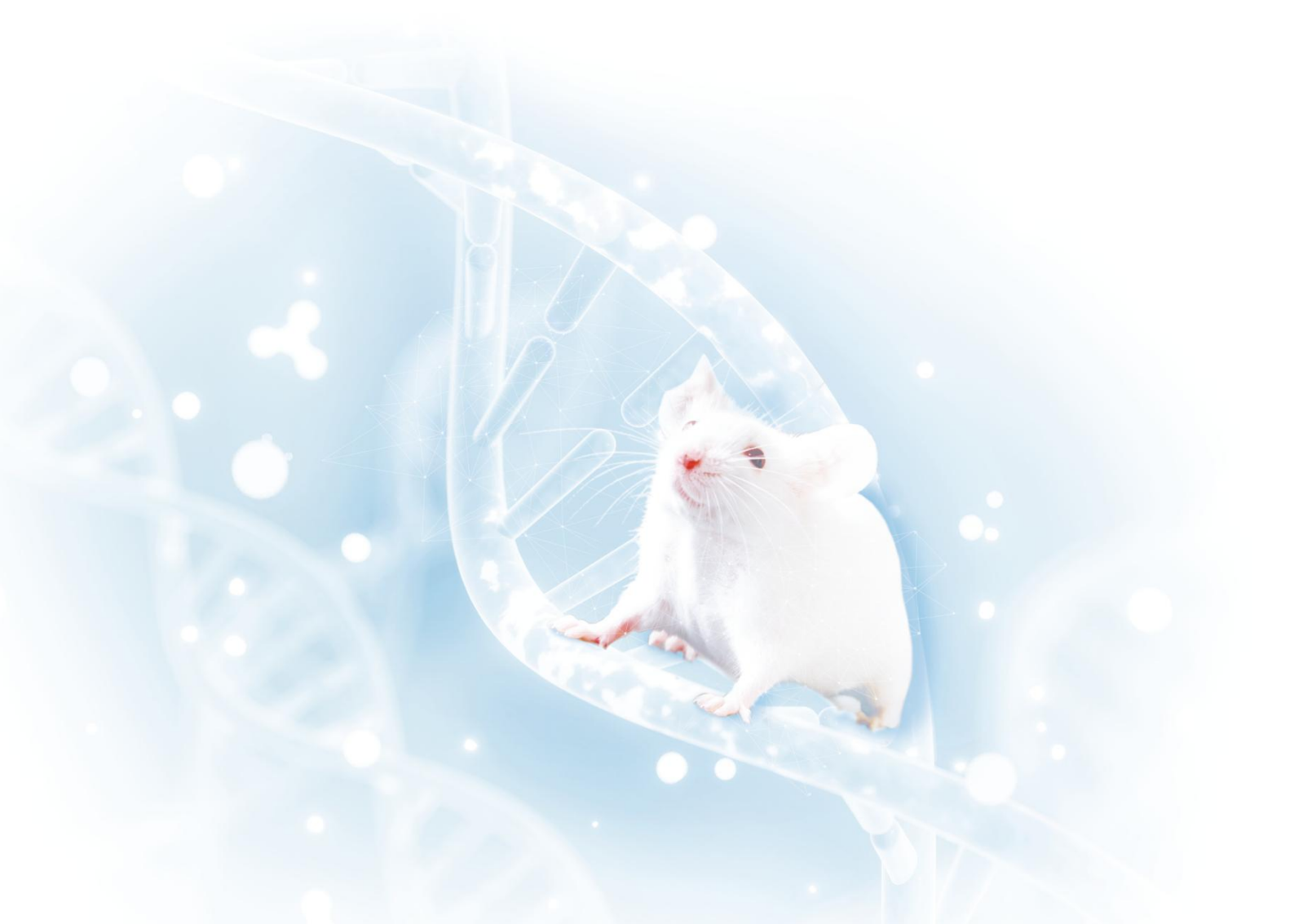
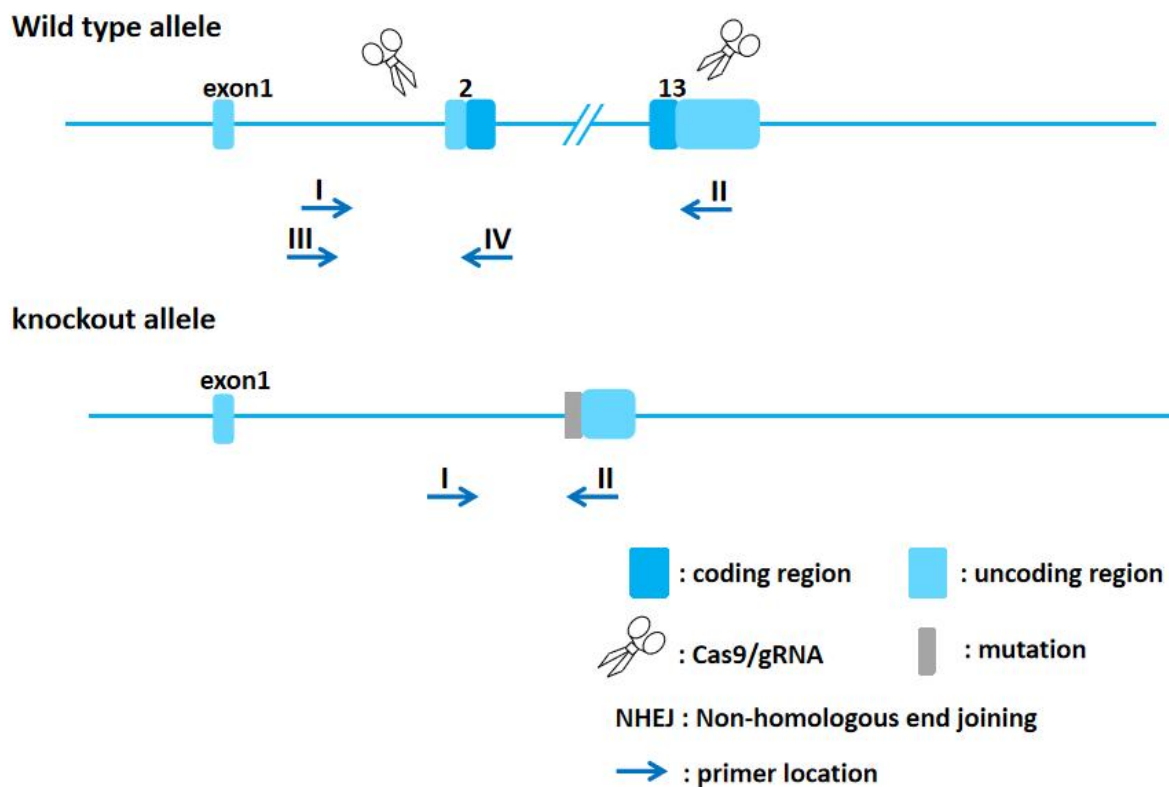


Blk-KO Genotyping Protocol



Common Name	Blk-KO	Cat. NO.	NM-KO-240558
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TATGCCAGGTGATGTCTGCG	Forward
P2	TTTCGGGACTATCGGGGTCT	Reverse
P3	GCTCCTGTCCCTGAAAACTGA	Forward
P4	GGTCCAGGCATGACTCCAAT	Reverse

Expected results

Results	0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -14532 bp Wild type: P3P4 =499 bp Heterozygote: P1P2 =451 bp; P3P4 =499 bp Homozygote: P1P2 =451 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	