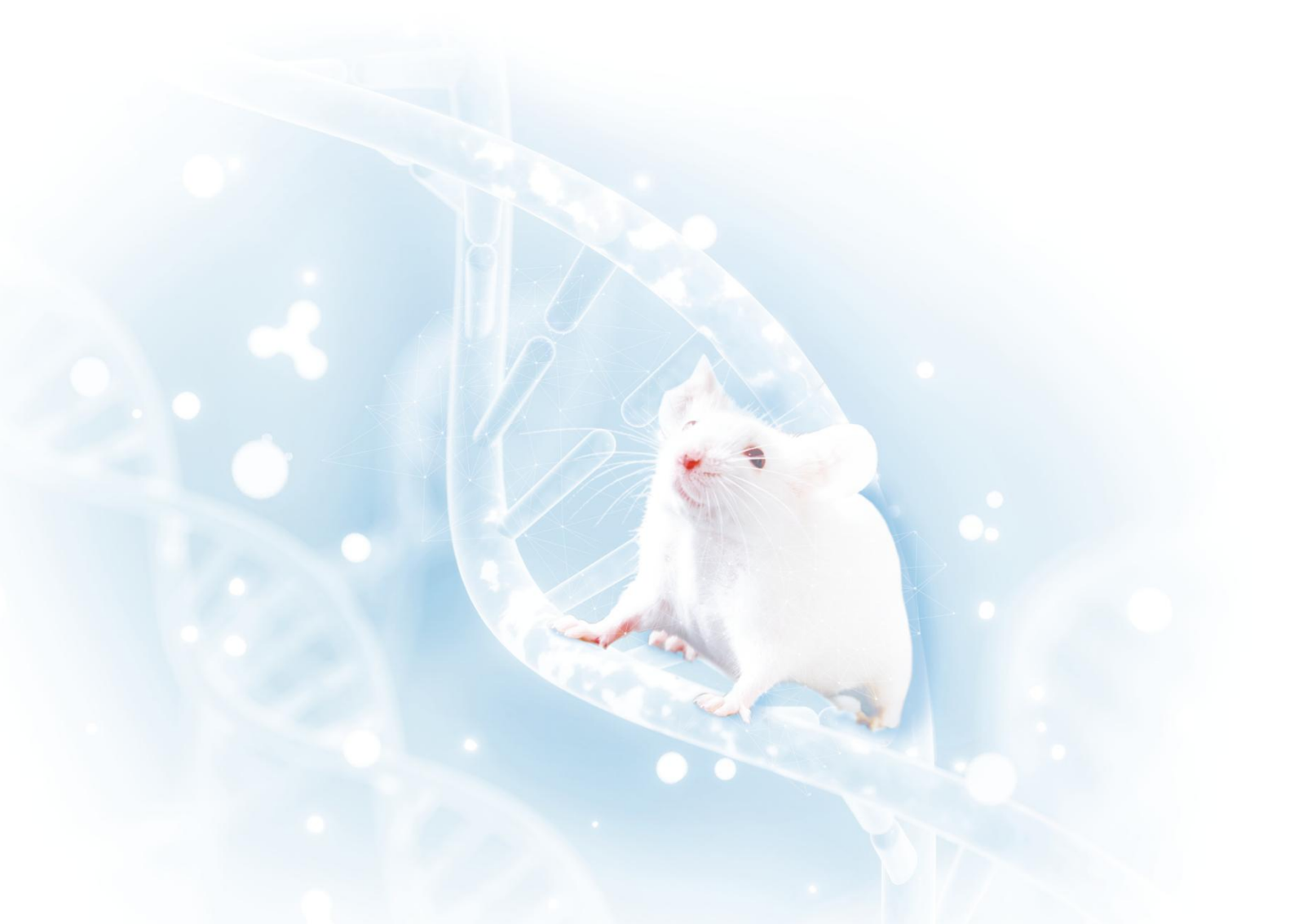
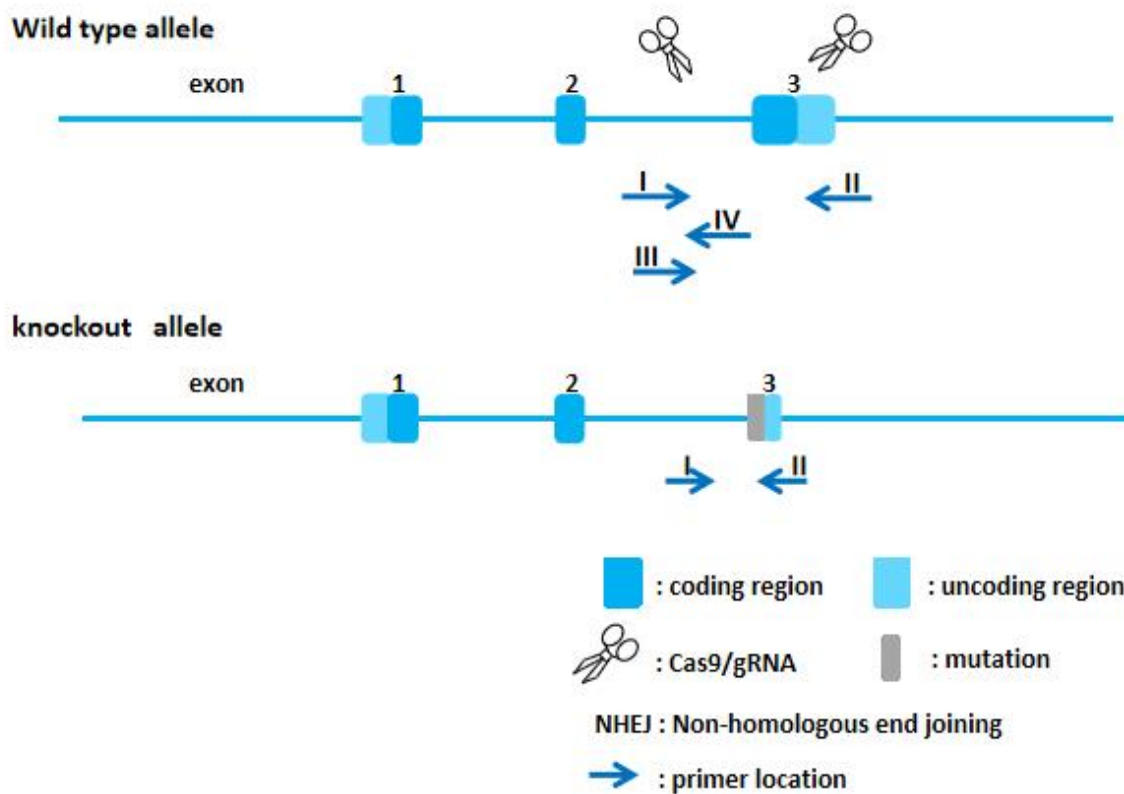


Xk-KO Genotyping Protocol



Common Name	Xk-KO	Cat. NO.	NM-KO-240543
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	CGTGAGAGGATTTGAGGCCA	Forward
P2	GGCTACTGAGACAACAGGCT	Reverse
P3	AGTGAGCACTGGGAAATAGC	Forward
P4	CAAGAGTCCTCATGCCAGTG	Reverse

Expected results

Results	P1/P2 HE WT M H GeneRuler™ 1 kb DNA Ladder 1603bp 556bp 1% TBE, 100V, 80mA, 45 min P3/P4 HE HE WT H M DL2000 281bp 1.7% agarose bp 2000 1000 750 500 250 100
Genotype	Knockout type: -1047bp Wild type: P1P2 =1603 bp; P3P4 =281 bp Heterozygote: P1P2 =1603 bp and 556 bp; P3P4 =281 bp Homozygote: P1P2 =556 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	