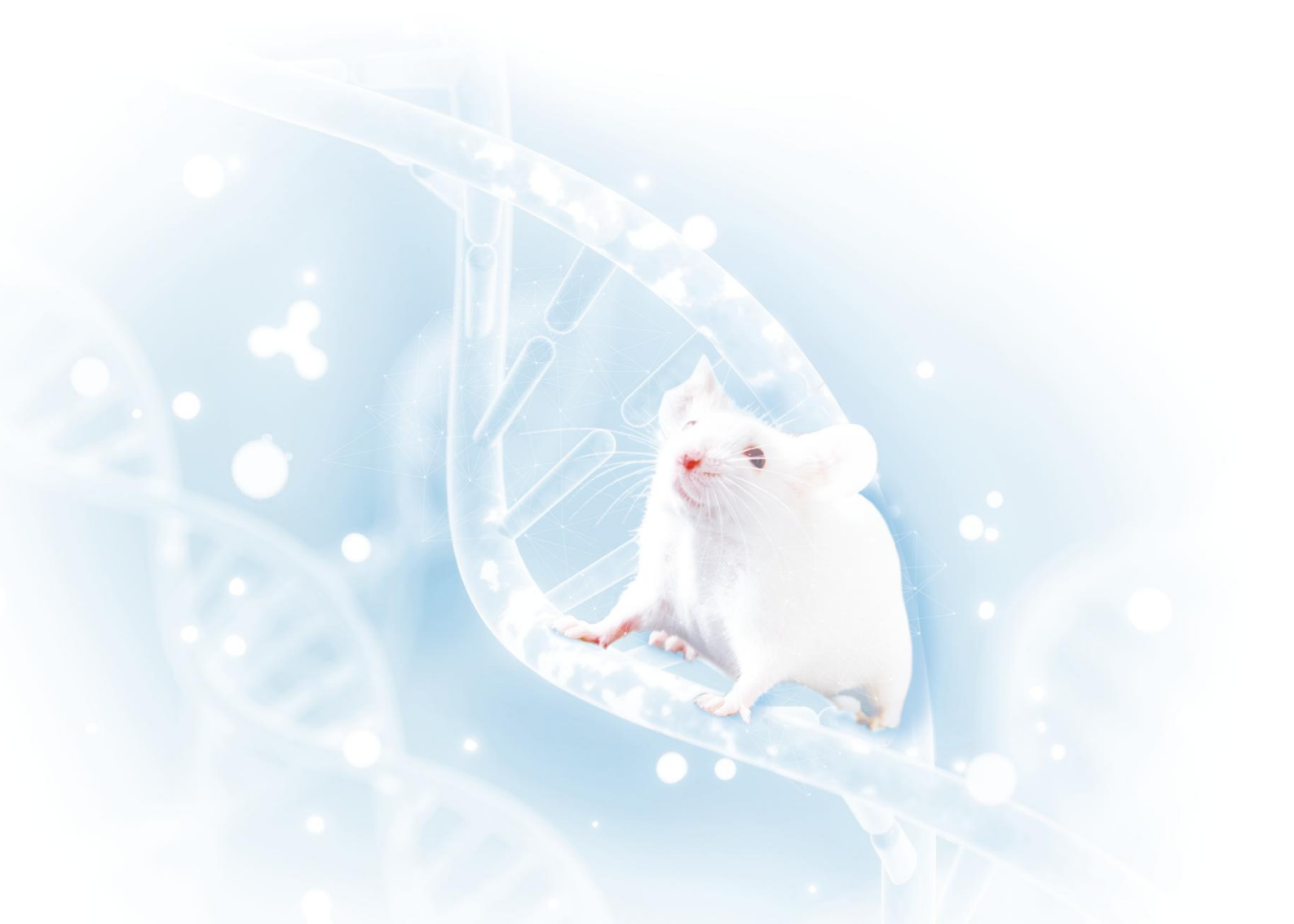
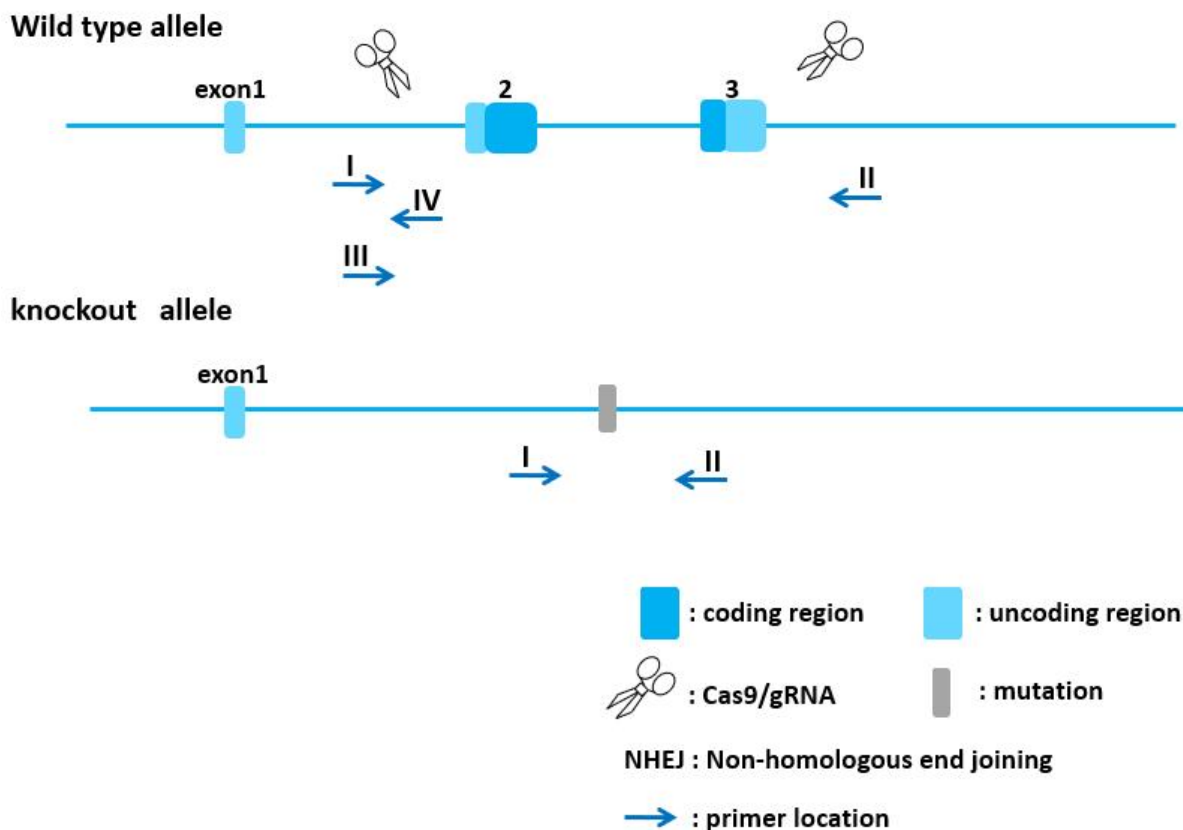


Cdkn1a-KO(2) Genotyping Protocol



Common Name	Cdkn1a-KO(2)	Cat. NO.	NM-KO-226095
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	ACGCTCATGGCTTCTGTTTCT	Forward
P2	CCCACCCTAGACCCACAATG	Reverse
P3	TATGCTGCCACAACCACACT	Forward
P4	CCTACCCTGCTGTCTTGGTG	Reverse

Expected results

Results	P1/P2 HE WT M GeneRuler™ 1 kb DNA Ladder 2693bp 769bp 1% Tgpt/taq™ LE G2 Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT H M DL2000 356bp 1.7% agarose bp 2000 1000 750 500 250 100
Genotype	Knockout type: -1924bp Wild type: P1P2 =2693 bp; P3P4 =356 bp Heterozygote: P1P2 =2693 bp and 769 bp; P3P4 =356 bp Homozygote: P1P2 =769 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)
	ddH2O			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	