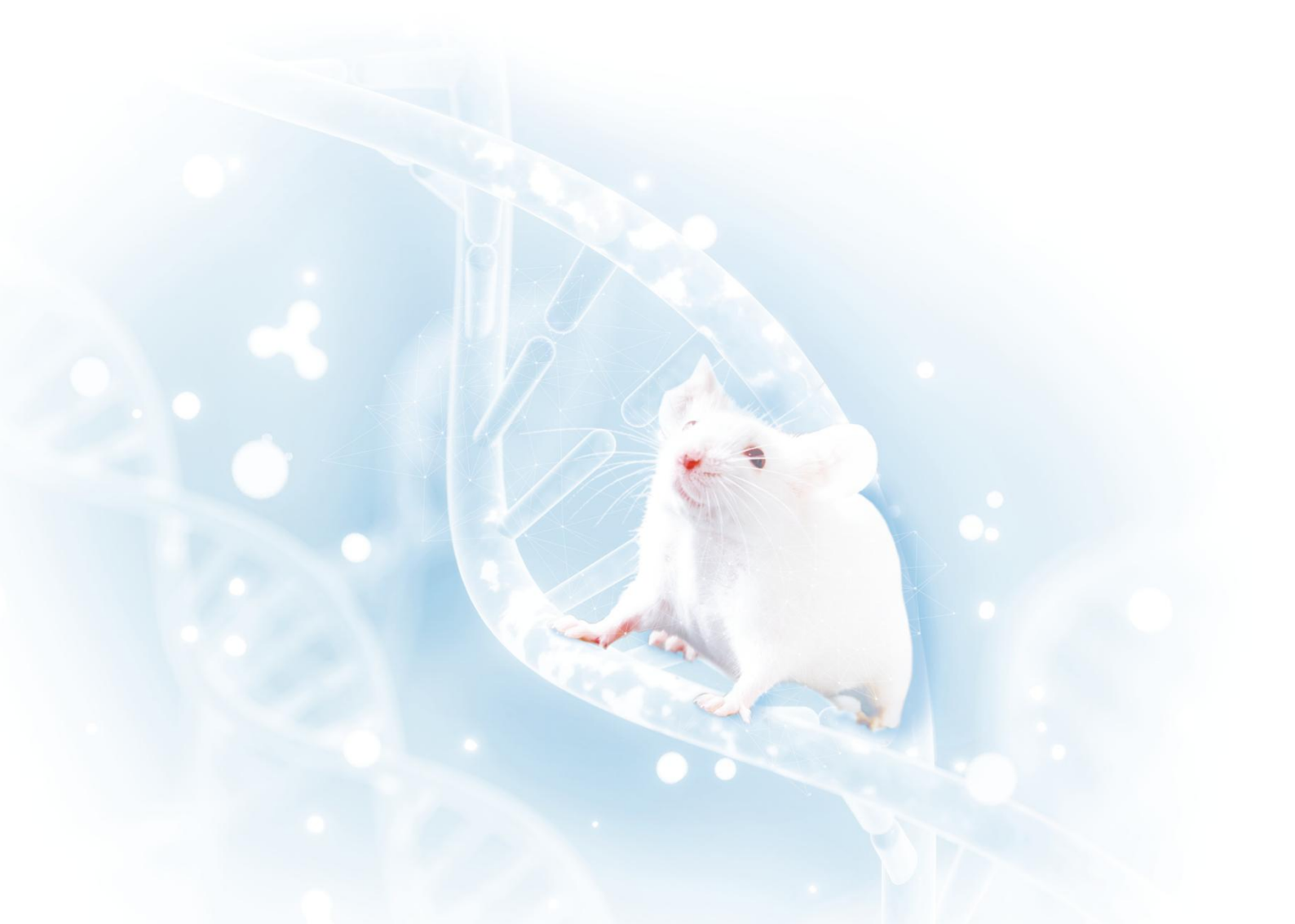


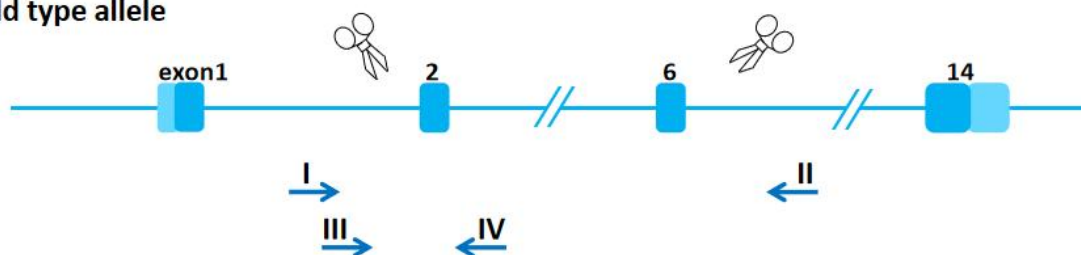
Clnn-KO Genotyping Protocol



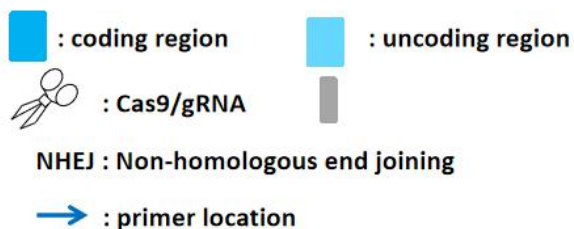
Common Name	Clmn-KO	Cat. NO.	NM-KO-240340
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CTGTGAGATCCACGCCATGT	Forward
P2	GCTCTCCCTCCTCAAGGTA	Reverse
P3	GTTCTGCTTTCTCACGTTGGG	Forward
P4	CCTCCCTGCCACCATACAGA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 0.5 µg/lane, 8 cm length gel, 1XTAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -14796bp Wild type: P3P4=288 bp Heterozygote: P1P2 =496 bp; P3P4=288 bp Homozygote: P1P2 =496 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 K0 band

Reaction &Cycling

	Reaction Component	Volume (µl)
PCR	ddH2O	8.0
Reaction	2×Rapid Taq Master Mix	10.0
System	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	