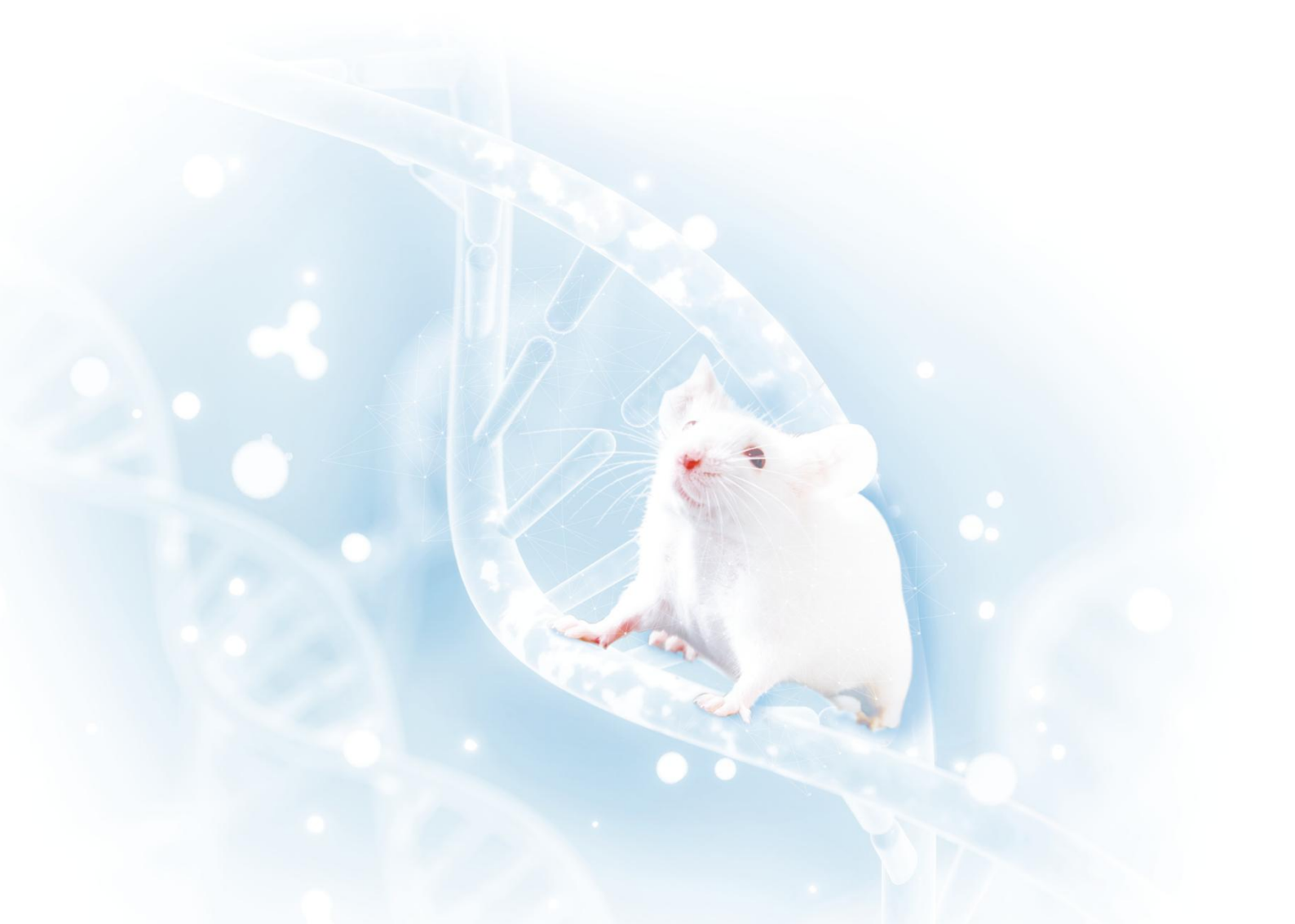


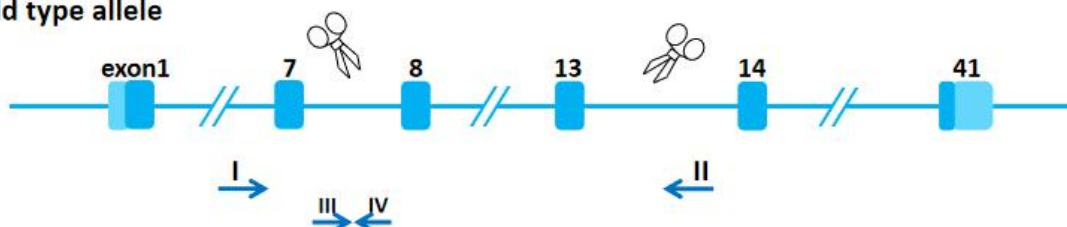
C4b-KO(2) Genotyping Protocol



Common Name	C4b-KO(2)	Cat. NO.	NM-KO-232731
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



: coding region : uncoding region

: Cas9/gRNA : mutation

NHEJ : Non-homologous end joining

→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	TCTCTAGTACCTGGCCACCC	Forward
P2	ACTGCCCCAATGACTAAGGC	Reverse
P3	TCACCGTGTAGTCCTGTCTGGC	Forward
P4	CCTAGCATTAGGAGCATGGAGG	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 HE HE WT M 2686bp 554bp 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 237bp DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -2132bp Wild type: P1P2 =2686 bp; P3P4 =237 bp Heterozygote: P1P2 =2686 bp and 554 bp; P3P4 =237 bp Homozygote: P1P2 =554 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	