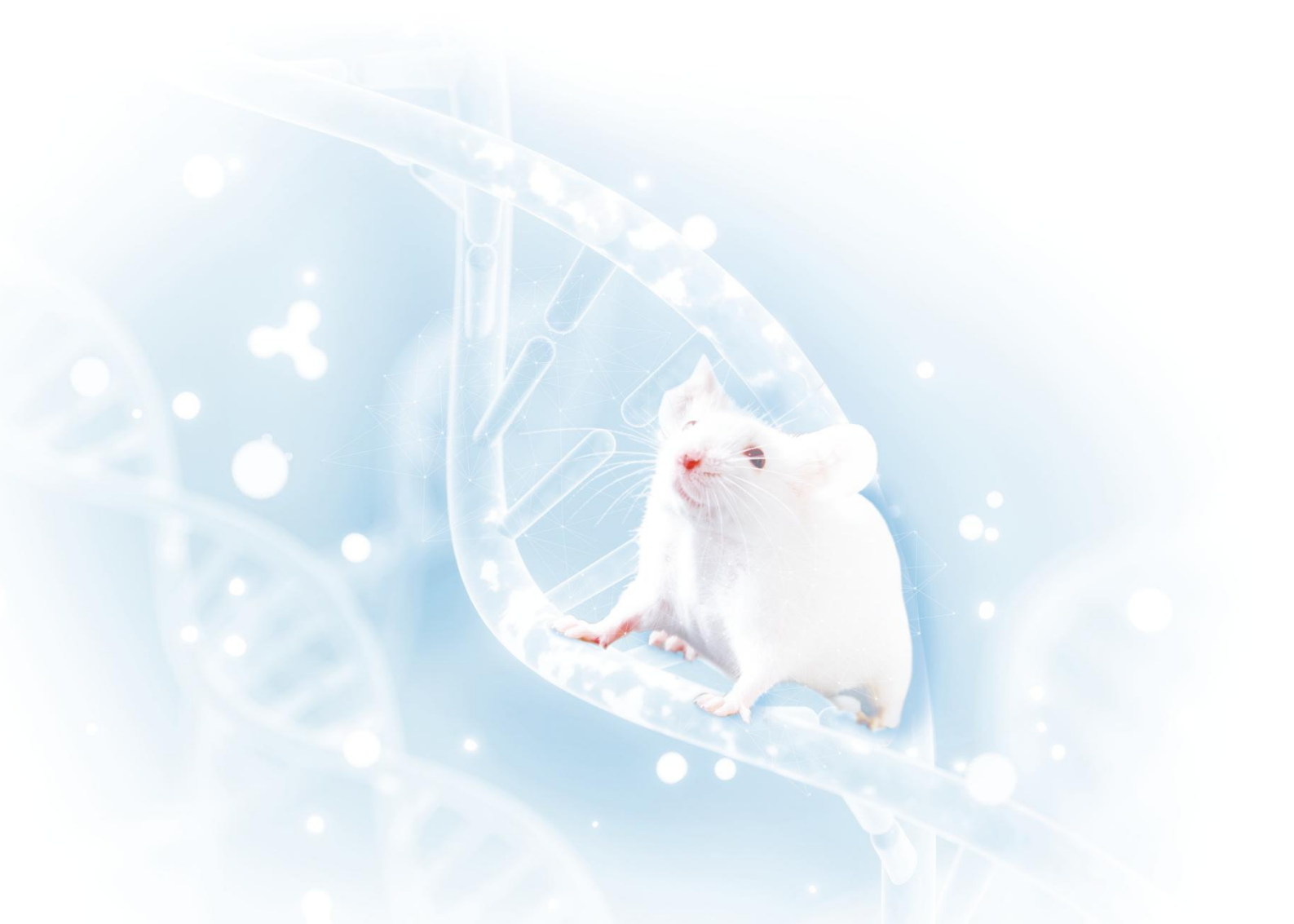


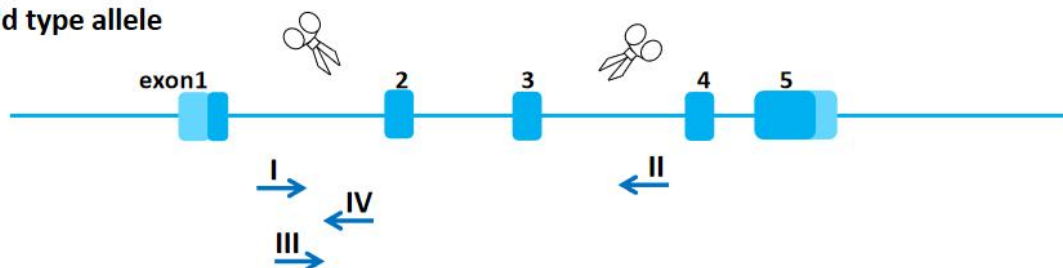
Cgas-KO Genotyping Protocol



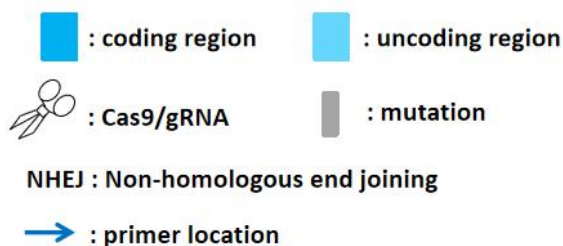
Common Name	Cgas-KO	Cat. NO.	NM-KO-232748
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	CCCAGCCCCATCACAAGGTT	Forward
P2	CCAAAAGCAGTGAACGGATAGACA	Reverse
P3	CCCCCTGTGTTGGAACAGTAT	Forward
P4	CTGGCCTGGAATGTACCACA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE GQ Agarose (#80-491) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2869+1bp Wild type: P1P2 =3527 bp; P3P4 =277 bp Heterozygote: P1P2 =3527 bp and 659 bp; P3P4 =277 bp Homozygote: P1P2 =659 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	