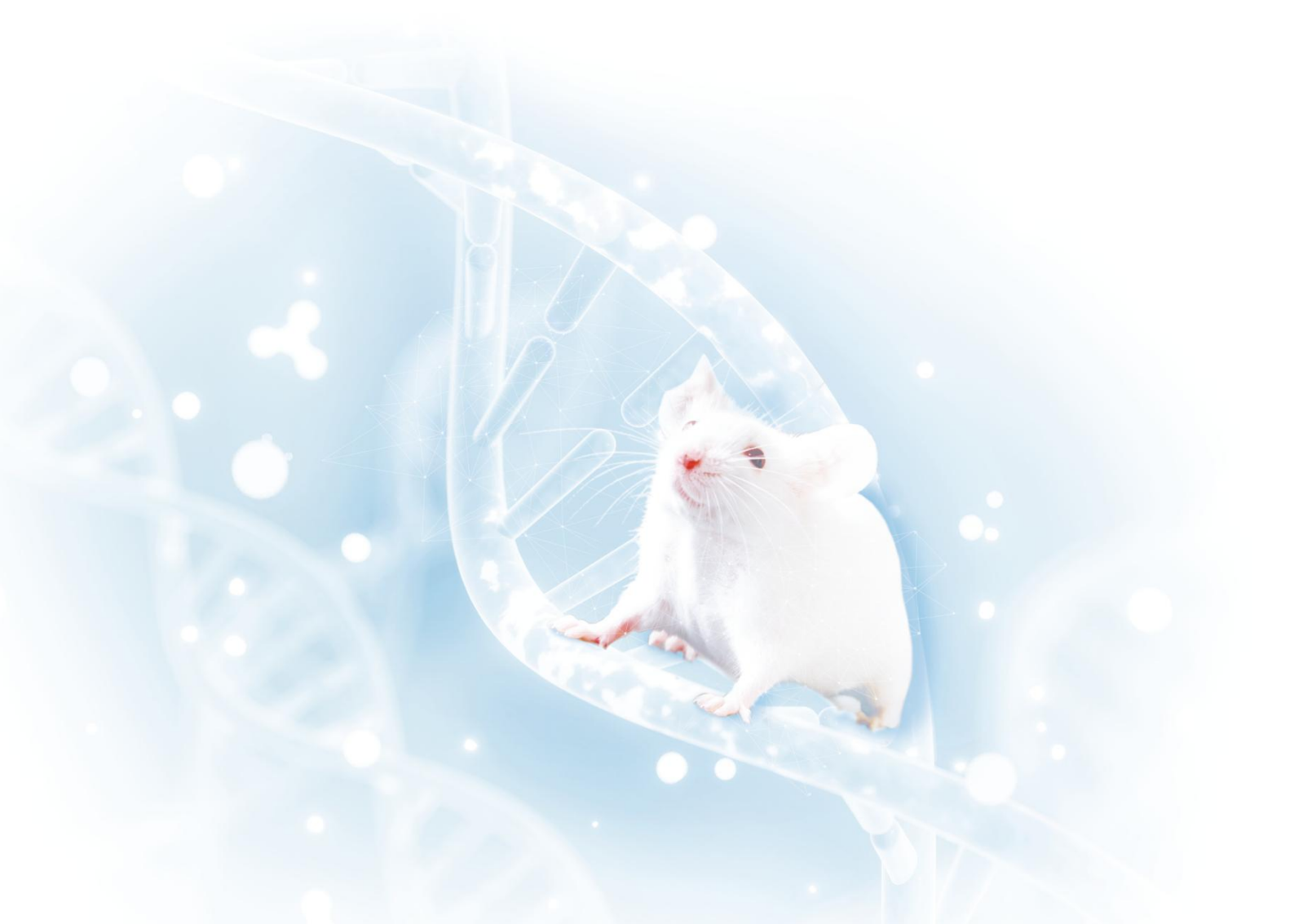
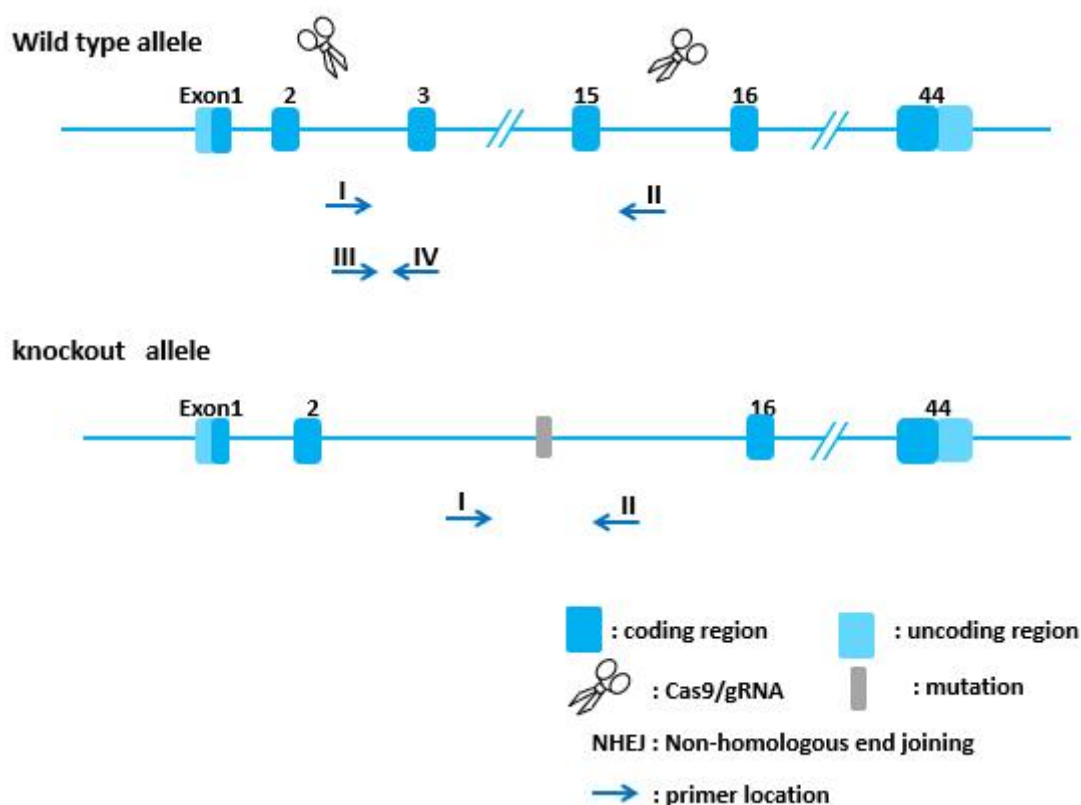


Cad-KO Genotyping Protocol



Common Name	Cad-KO	Cat. NO.	NM-KO-240169
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGTGGGGGTGTTTCAGAGAGA	Forward
P2	AACACCCGCTTCCAGATGAC	Reverse

P3	TCAGAGAGAGGAAAGCCCCA	Forward
P4	CAGTGCTTGCTCTCGTACCT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 1.7% agarose
Genotype	Knockout type: -4689bp Wild type: P1P2 =5179 bp; P3P4 =373 bp Heterozygote: P1P2 =5179 bp and 490 bp; P3P4 =373 bp Homozygote: P1P2 =490 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 Component	Volume (µl)
Reaction	ddH ₂ O	8.0

System	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	60 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	