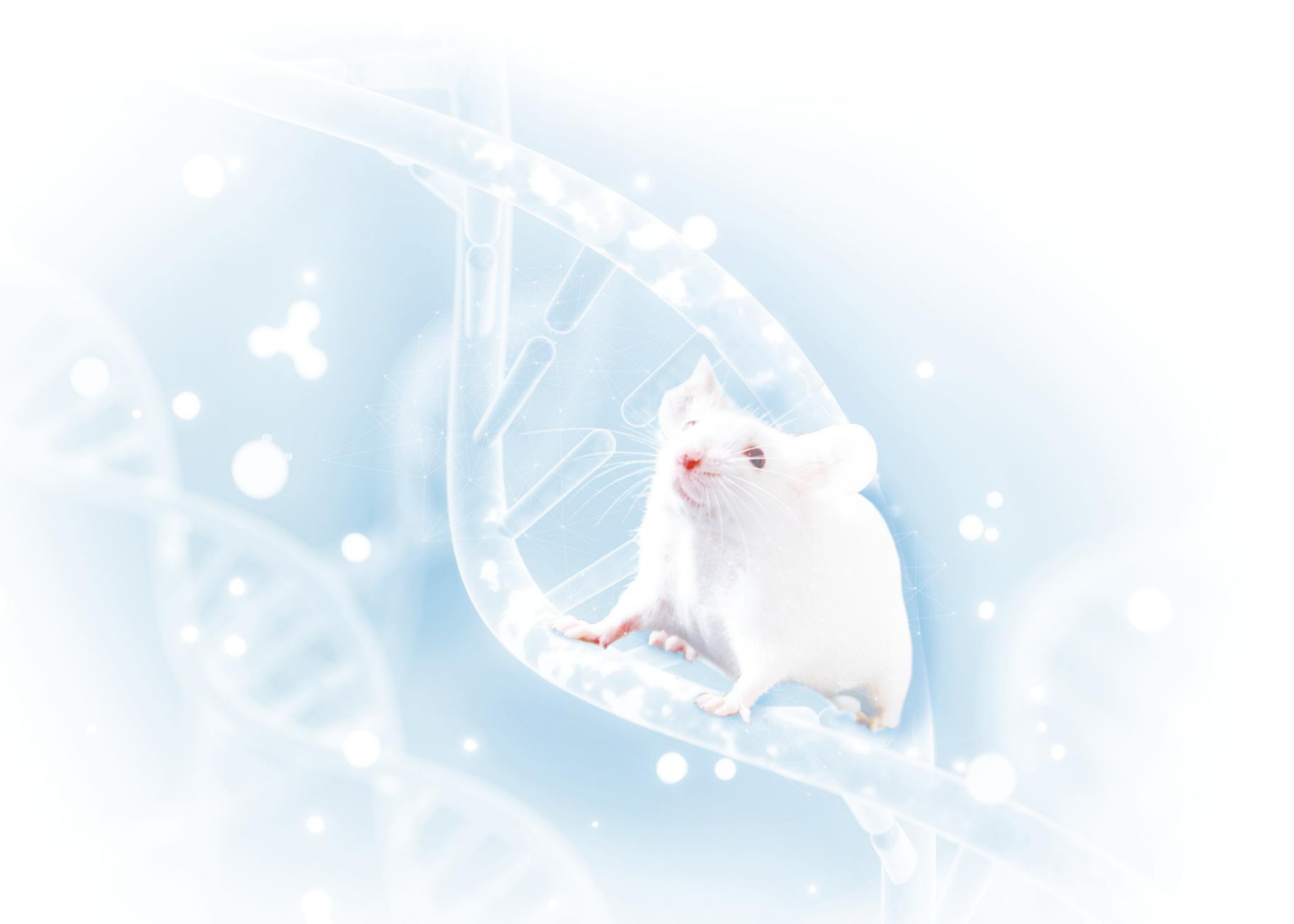


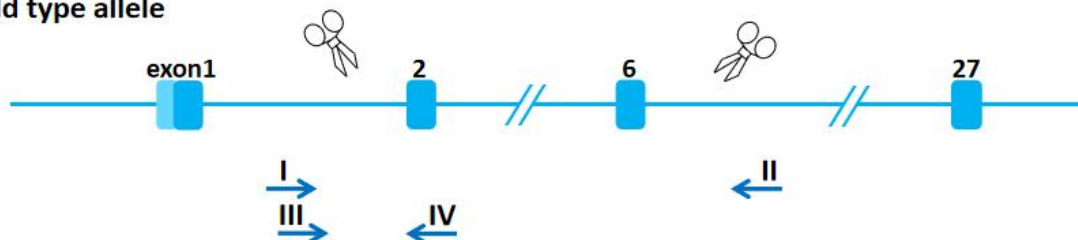
Ankrd36-KO Genotyping Protocol



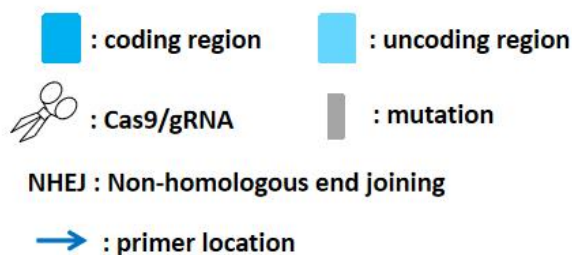
Common Name	Ankrd36-KO	Cat. NO.	NM-KO-240669
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	CACTGGTGGCCTTCACATCT	Forward
P2	CAGAAGCCAGCAAGGGCTAT	Reverse
P3	GCAGTCAACAACACGTTCCC	Forward
P4	CGGGGGTTGTGCTAGTTCAT	Reverse

Expected results

Results	0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -7662 bp Wild type: P1P2 =8202 bp; P3P4 =367 bp Heterozygote: P1P2 =8202 bp and 540 bp; P3P4 =367 bp Homozygote: P1P2 =540 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	