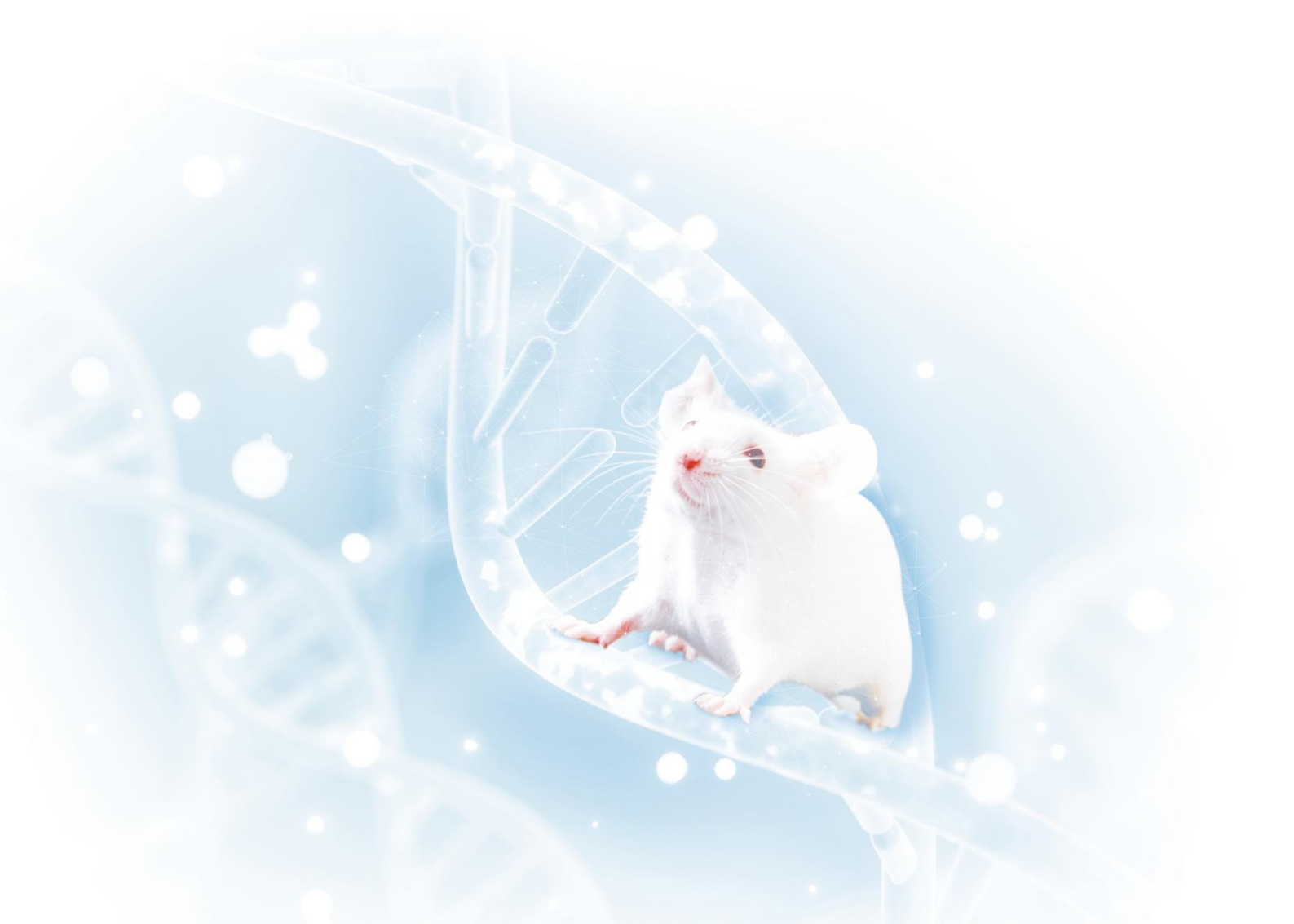
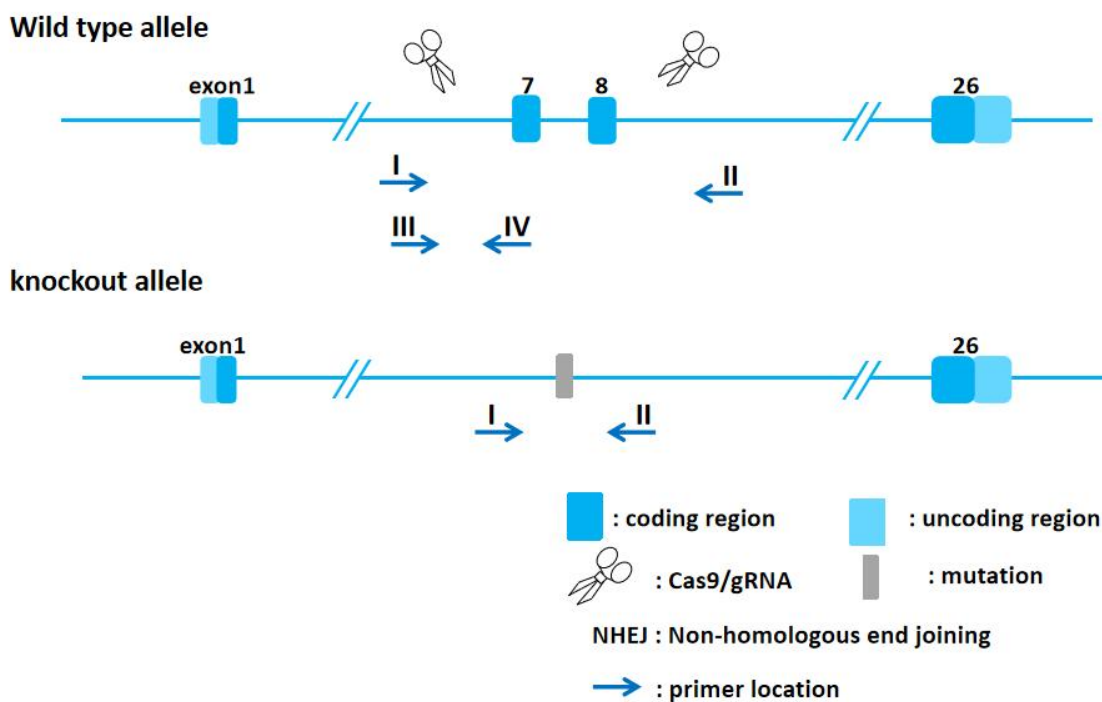


Adgrd1-KO Genotyping Protocol



Common Name	Adgrd1-KO	Cat. NO.	NM-KO-190234
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCCGTTACCTTGATGAGCC	Forward
P2	TTCTTCCAACCACTCTGGGC	Reverse
P3	CATAGCTCCGAGCACAAAGCA	Forward
P4	GTAAGGACCTGGGGGAGAGT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 M HE HE HE 3843bp 616bp P3/P4 HE HE WT H M 347bp DL2000 bp 2000 1000 750 500 250 100 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 1.7% agarose
Genotype	Knockout type: -3227 bp Wild type: P1P2 =3843 bp; P3P4 =347 bp Heterozygote: P1P2 =3843 bp and 616 bp; P3P4 =347 bp Homozygote: P1P2 =616 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

	<i>P2(10 pmol/μl) or P4(10 pmol/μl)</i>			<i>0.5</i>
	<i>Genomic DNA</i>			<i>1.0</i>
	<i>Total</i>			<i>20</i>
	<i>2 × Rapid Taq Master Mix from Vazyme(Code Number: P222-01)</i>			
<i>Cycling Reaction</i>	<i>Step</i>	<i>Temp</i>	<i>Time</i>	<i>Note</i>
	<i>1</i>	<i>95°C</i>	<i>5 min</i>	
	<i>2</i>	<i>95°C</i>	<i>20 sec</i>	
	<i>3</i>	<i>60°C</i>	<i>20 sec</i>	
	<i>4</i>	<i>72°C</i>	<i>20 sec</i>	<i>35 repeats to 2</i>
	<i>5</i>	<i>72°C</i>	<i>5 min</i>	
	<i>6</i>	<i>12°C</i>	<i>Hold</i>	