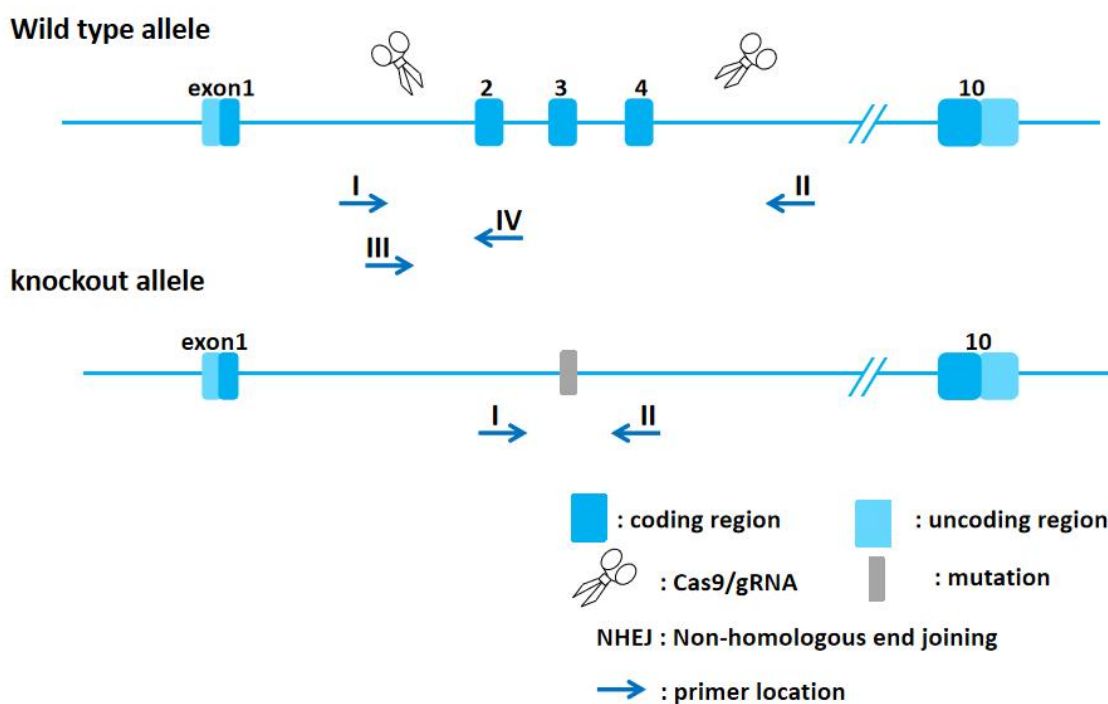


Aida-KO Genotyping Protocol



Common Name	Aida-KO	Cat. NO.	NM-KO-240400
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGCACAGCTATTGGTGCTCA	Forward
P2	CAGGCTACAGTTCCATGCCA	Reverse
P3	TCTGTTAGACCCTCCCCAGC	Forward
P4	GGGAGGAACACACCAACTGA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE G0 Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1XTAE, 7 V/cm, 45 min DL2000 1.7% agarose
Genotype	Knockout type: -2421 bp Wild type: P1P2 =2918 bp; P3P4 =425 bp Heterozygote: P1P2 =2918 bp and 497 bp; P3P4 =425 bp Homozygote: P1P2 =497 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>	