

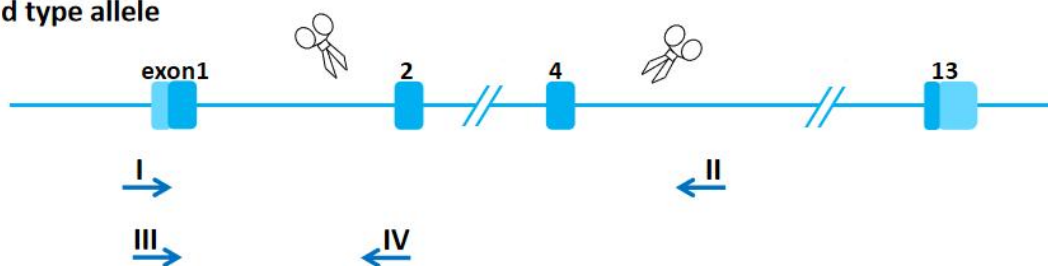
Aldh1a7-KO Genotyping Protocol



Common Name	Aldh1a7-KO	Cat. NO.	NM-KO-240656
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



- : coding region
 : uncoding region
 : Cas9/gRNA
 : mutation
NHEJ : Non-homologous end joining
→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	GCAAGTGCCCTTTCAGAACC	Forward
P2	CACACTGGTGTAAGTTGGGTTG	Reverse
P3	CCAGCAATGTCTTCACCTGC	Forward

P4	TCTTGCTGCTCACCGAATCA	Reverse
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Expected results

Results	0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -8123bp Wild type: P1P2 =8766 bp; P3P4 =499 bp Heterozygote: P1P2 =8766 bp and 643bp; P3P4 =499bp Homozygote: P1P2 =643bp

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

	<i>P1(10 pmol/μl) or P3(10 pmol/μl)</i>			<i>0.5</i>
	<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>	