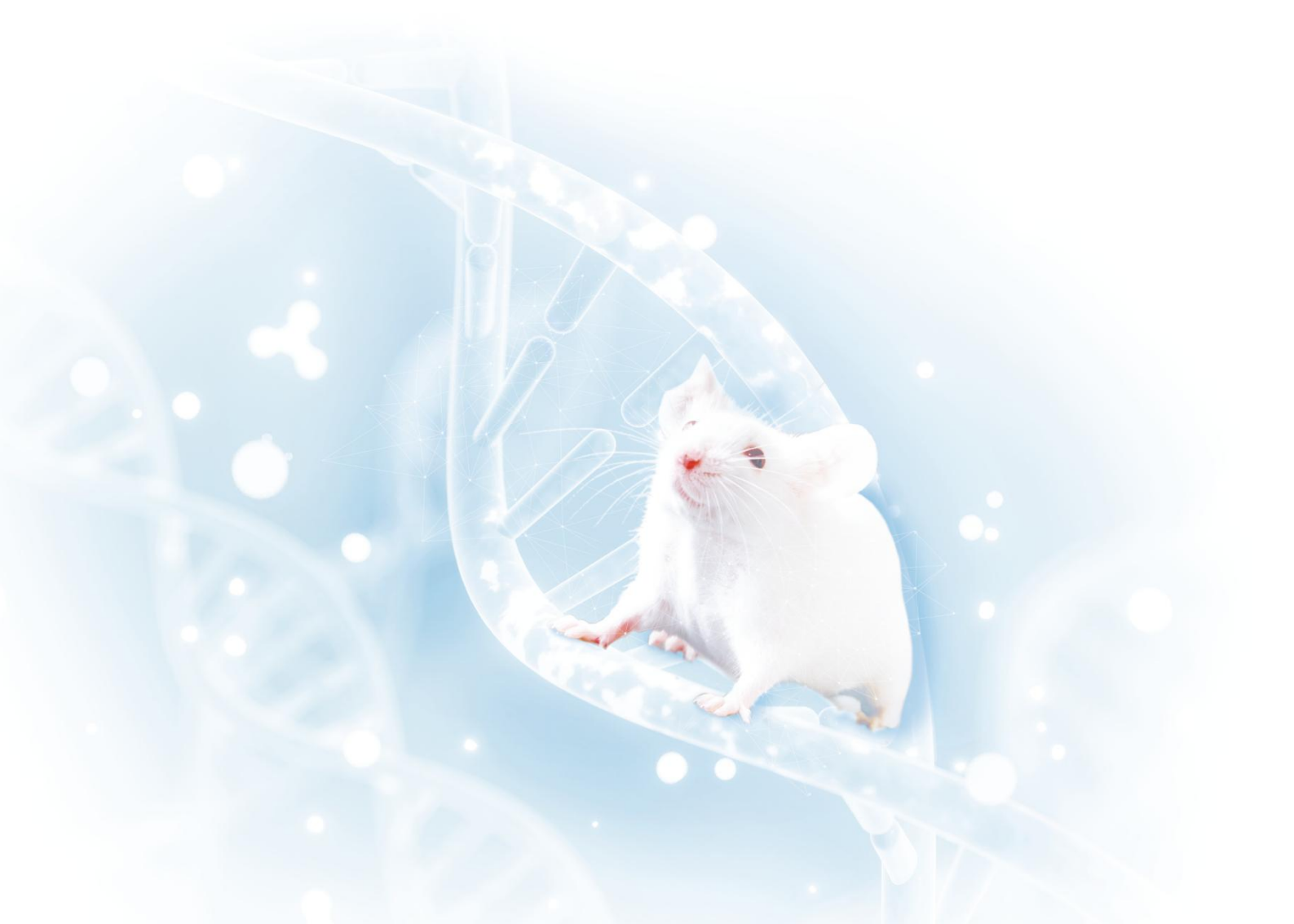
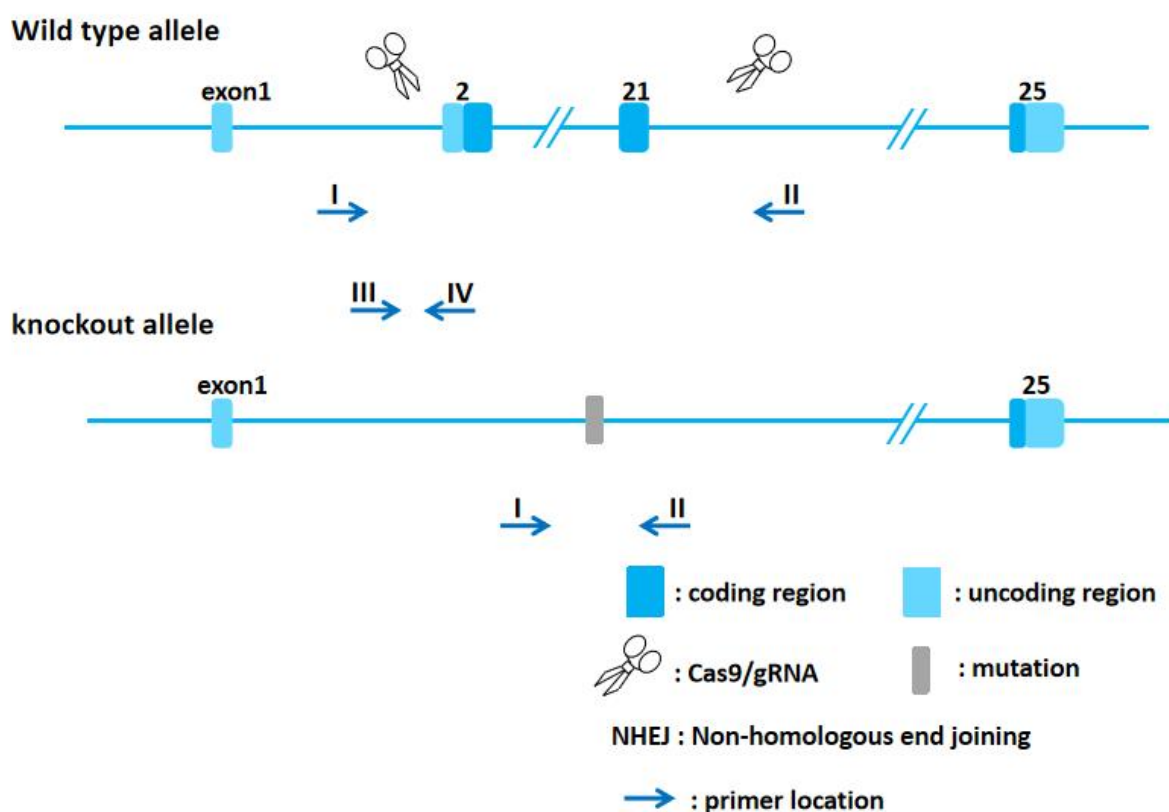


Zmym3-KO Genotyping Protocol



Common Name	Zmym3-KO	Cat. NO.	NM-KO-240623
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGGGAGGGAAGGAGCTACGAG	Forward
P2	TGAAAGAGGCAGAGGCTTGG	Reverse
P3	CGGGTTAGAGACCAACTGCC	Forward

P4	ACTCCTCCAGGGTCCTTCTC	Reverse
----	----------------------	---------

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% TopView™ LE-GO Agarose (#R0491) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -9851+1bp Wild type: P1P2 =10464 bp; P3P4 =478 bp Heterozygote: P1P2 =10464 bp and 614 bp; P3P4 =478 bp Homozygote: P1P2 =614 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

	<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>	