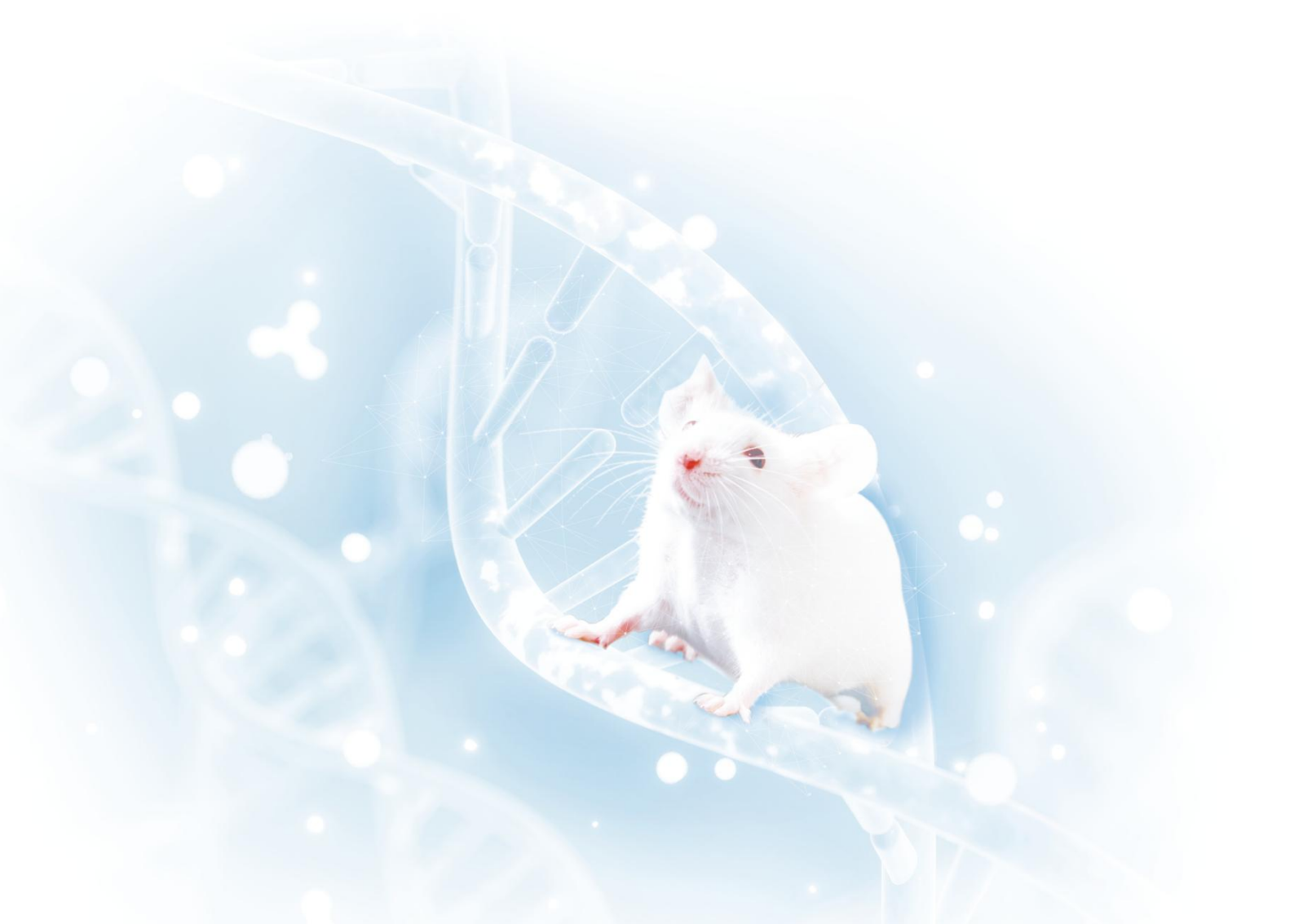


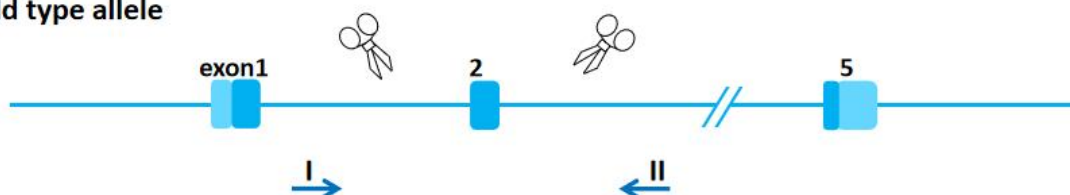
Taco1-KO Genotyping Protocol



Common Name	Taco1-KO	Cat. NO.	NM-KO-241529
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



: coding region : uncoding region

: Cas9/gRNA : mutation

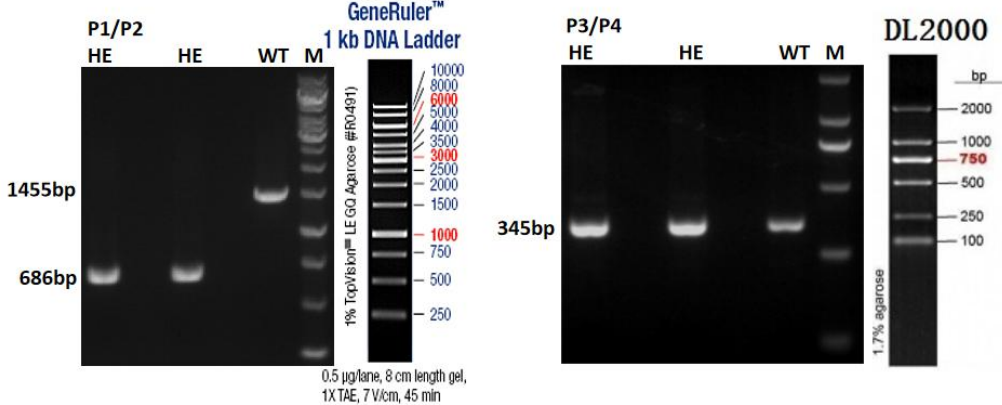
NHEJ : Non-homologous end joining

→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	GCCACAGCACACATGGAAAT	Forward
P2	TCCGGAGAAAAGACGTGTTGG	Reverse
P3	AGGTCAGTAGGCTTGACACAT	Forward
P4	TGAGGTGTGTAAAGTCCTGGC	Reverse

Expected results

Results	 GeneRuler™ 1 kb DNA Ladder 1% Tris/Bis agarose (#R0401) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min DL2000 1.7% agarose
Genotype	Knockout type: -769bp Wild type: P1P2 =1455 bp; P3P4 =345 bp Heterozygote: P1P2 =1455 bp and 686 bp; P3P4 =345 bp Homozygote: P1P2 =686 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 System	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>	