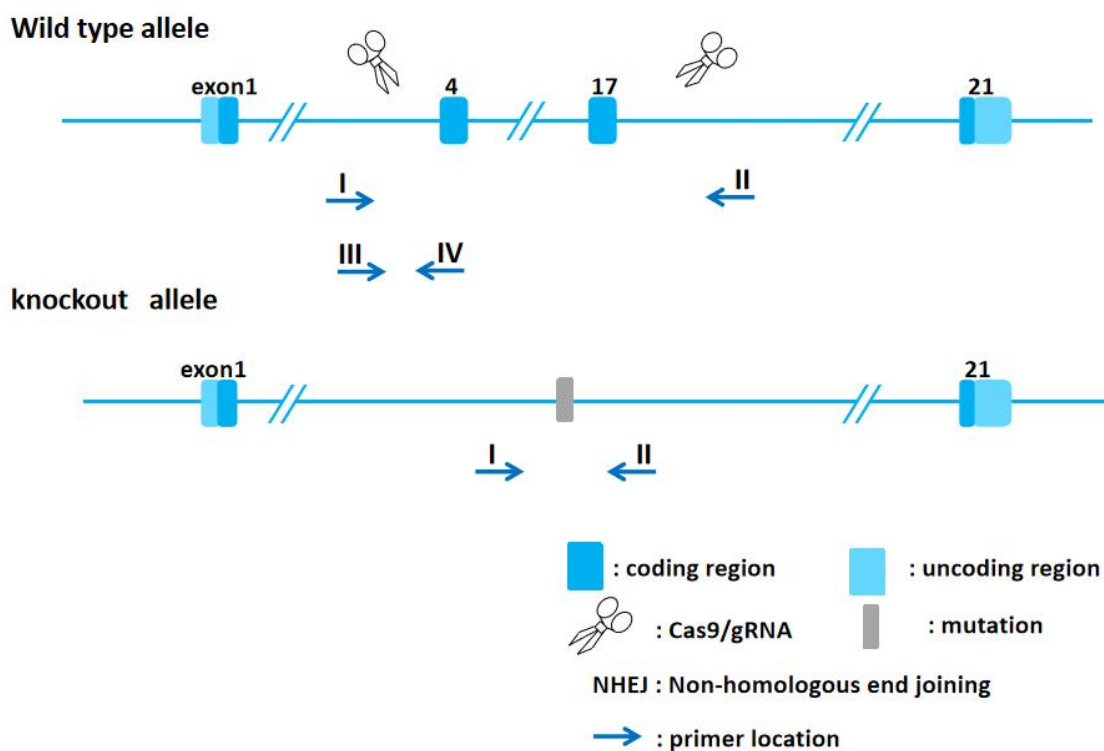


Bltp3b-KO Genotyping Protocol



Common Name	<i>Bltp3b-KO</i>	Cat. NO.	NM-KO-240614
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GTCAGTGTACAGACATTAGATCG	Forward
P2	CCTGTGAGCCCTGCTTGTTA	Reverse
P3	TGGAGTGCTTCCCTCTCTTAC	Forward
P4	ATCGGTGACGGACCGTTAGG	Reverse

Expected results

Results	
Genotype	Knockout type: -34132 bp Wild type: P3P4 =448 bp Heterozygote: P1P2 =588 bp; P3P4 =448 bp Homozygote: P1P2 =588 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>	