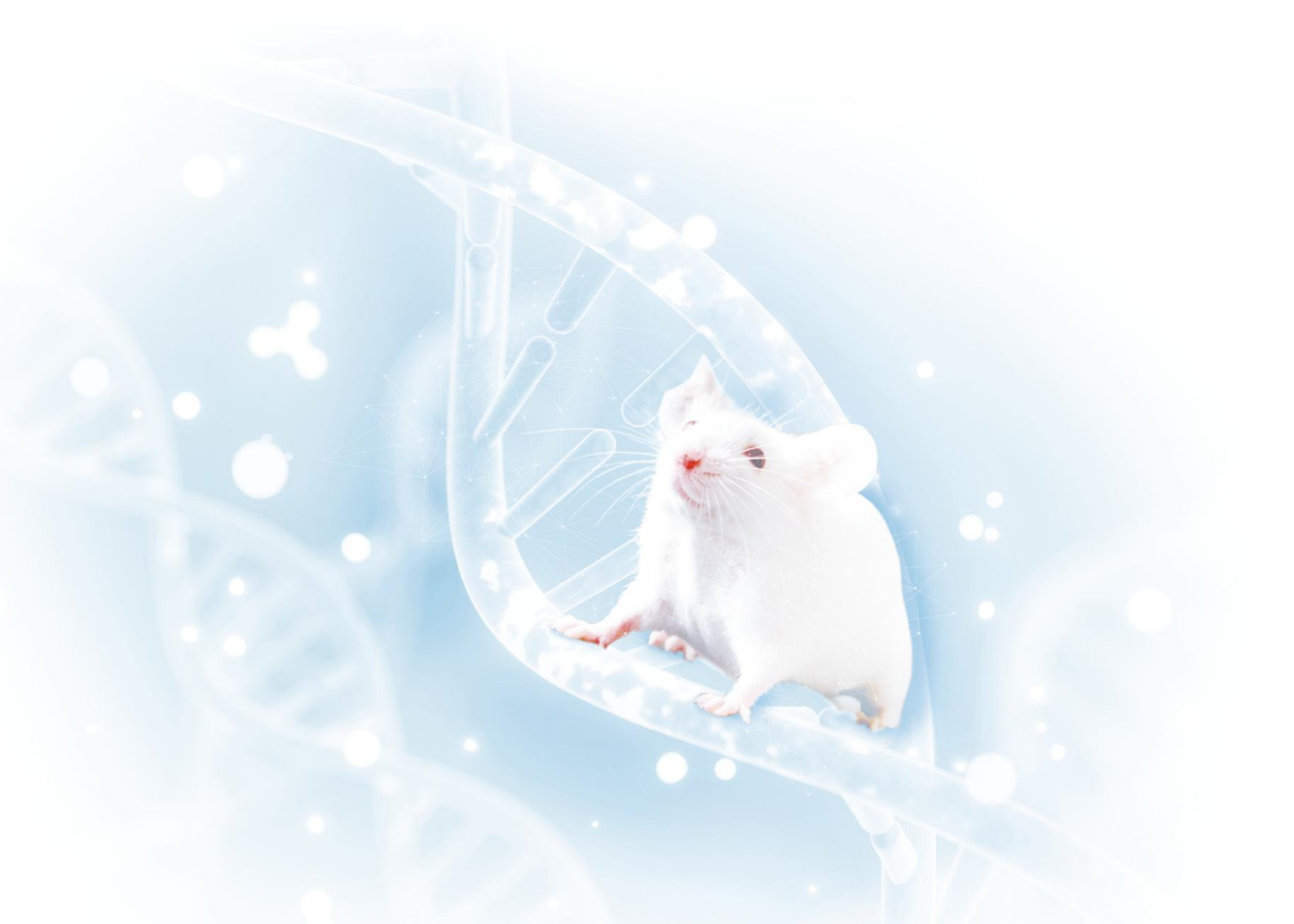


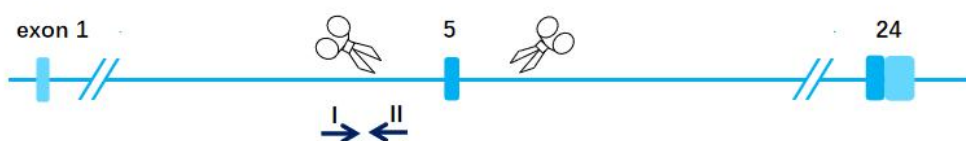
Tmem63b-Flox Genotyping Protocol



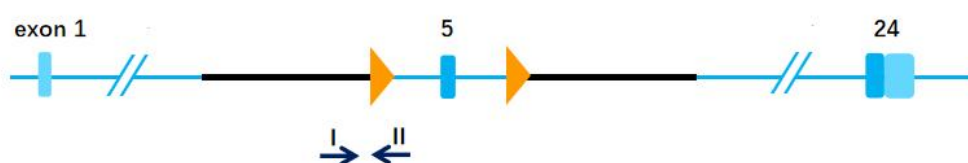
Common Name	<i>Tmem63b-Flox</i>	Cat. NO.	NM-CKO-240518
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



Targeted allele



→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	GTTGGAGCCGTGACAGCTAA	Forward
P2	AGGGTGTCTTCATCACTGCC	Reverse

Expected results

Results	HE HE WT M H 314bp 252bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Wild type: P1P2 =252 bp Heterozygote: P1P2 =252 bp and 314 bp Homozygote: P1P2 =314 bp

Reaction &Cycling

	Reaction Component	Volume (μ l)
PCR Reaction System	ddH ₂ O	8.0
	2×Rapid Taq Master Mix	10.0
	P1(10 pmol/ μ l)	0.5
	P2(10 pmol/ μ l)	0.5
	Genomic DNA	1.0
	Total	20

	<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>	