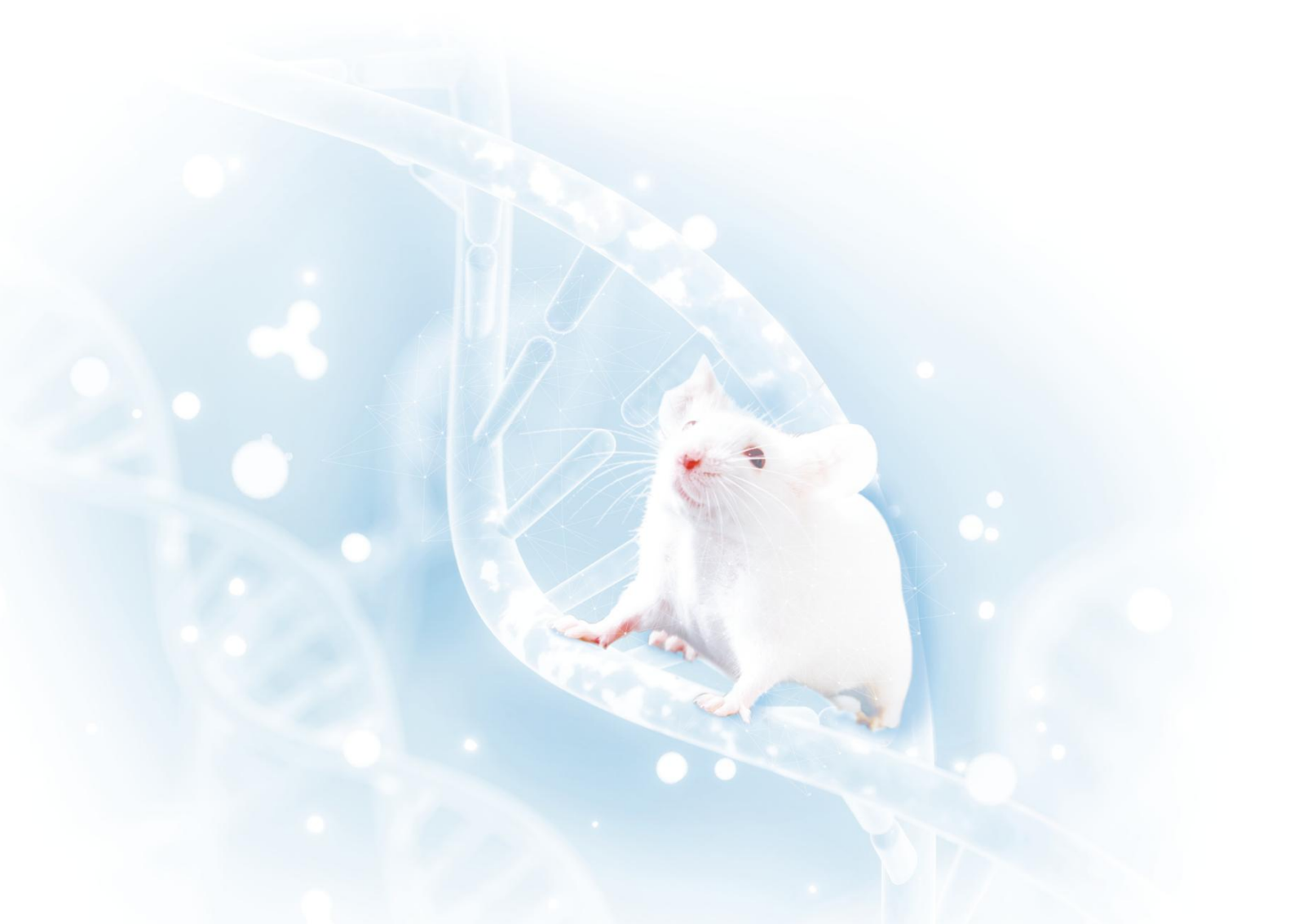


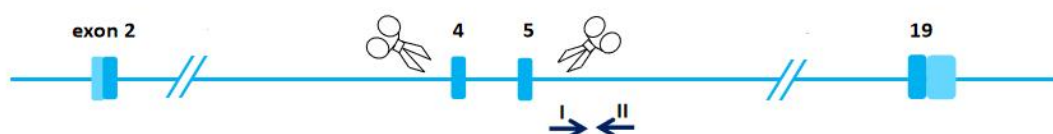
Btk-Flox Genotyping Protocol



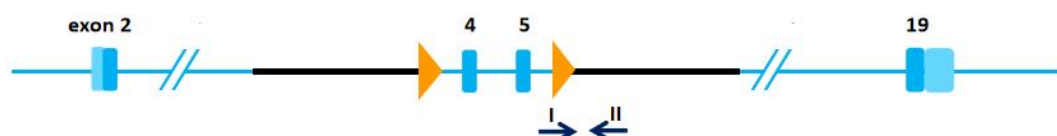
Common Name	Btk-Flox	Cat. NO.	NM-CKO-240595
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



Targeted allele



→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	TGCCTCAACAGATAAAATATTCCACC	Forward
P2	ACAAGTTTCATTCTTGGAAC TCACA	Reverse

Expected results

Results	
Genotype	Wild type: P1P2 =230 bp; Heterozygote: P1P2 =230 bp and 287 bp; Homozygote: P1P2 =287 bp

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)			0.5
	P2(10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2 × Rapid Taq Master Mix from Vazyme(Code Number: P222-O1)			
Cycling	Step	Temp	Time	Note

<i>Reaction</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>	