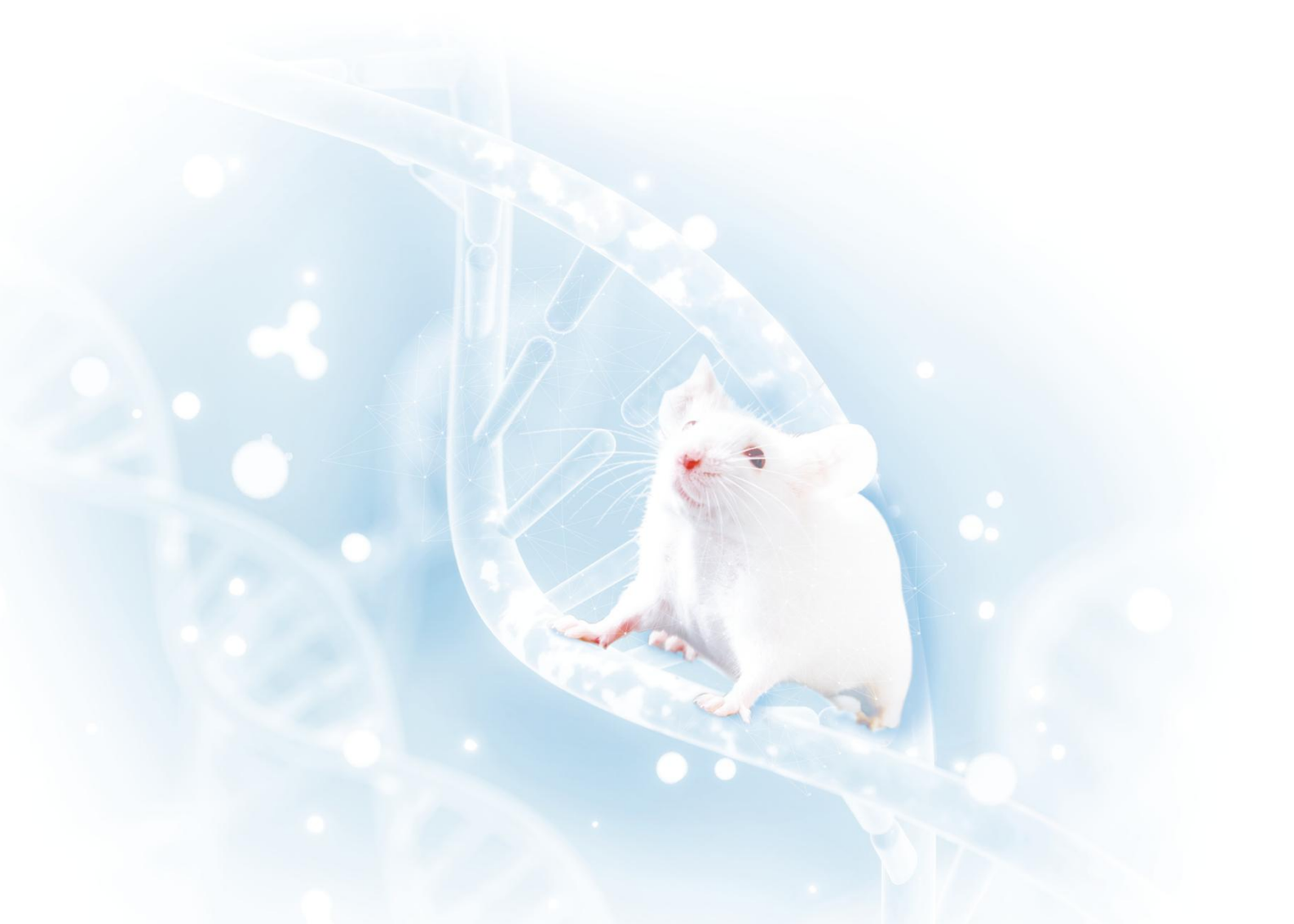


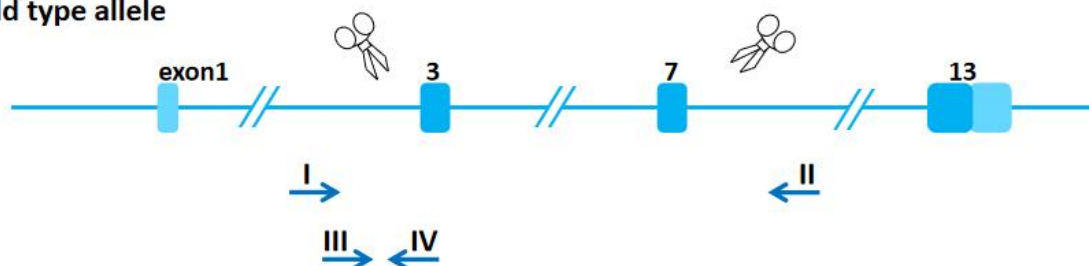
Vrk2-K0 Genotyping Protocol



Common Name	Vrk2-K0	Cat. NO.	NM-K0-234715
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	AACATGGCTCATTTGGGCAAC	Forward
P2	GCTTCCTGCTCAGCCATTTC	Reverse
P3	ACCTTCCCTATACTTGGATGATG	Forward
P4	TTTGTGGGGAAAGCTGAAGG	Reverse

Expected results

Results	
Genotype	Knockout type: -16073bp Wild type: P3P4=337 bp Heterozygote: P1P2 =473 bp; P3P4=337 bp Homozygote: P1P2 =473 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	ddH2O	8.0
	2×Taq Plus Master Mix	10.0
	P1 (10 pmol/μl) or P3 (10 pmol/μl)	0.5

	P2 (10 pmol/μl) or P4 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
Cycling Reaction	Step	Temp	Time	Note
	1	95° C	5 min	
	2	95° C	20 sec	
	3	60° C	20 sec	
	4	72° C	20 sec	35 repeats to 2
	5	72° C	5 min	
	6	12° C	Hold	