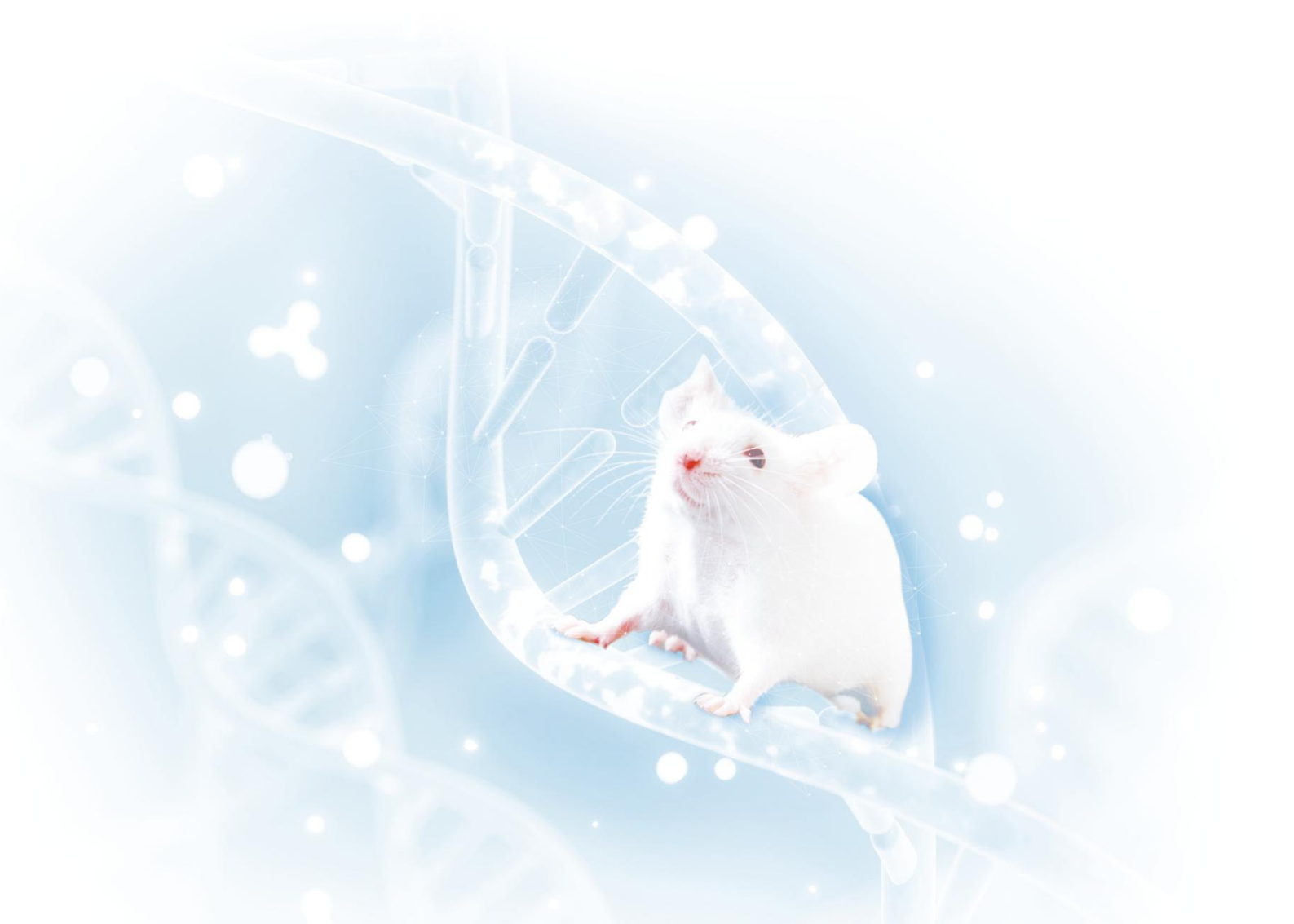


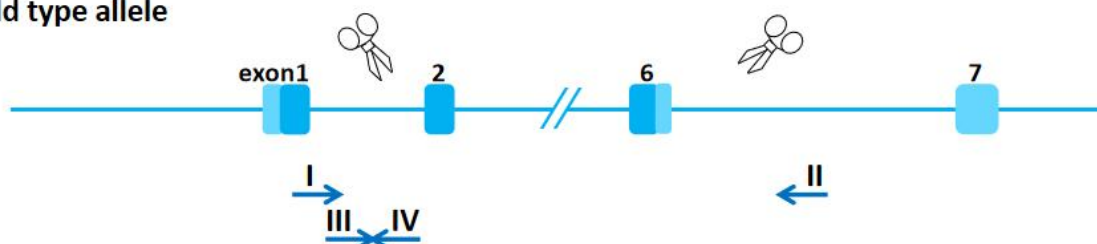
Vegfb-K0 (2) Genotyping Protocol



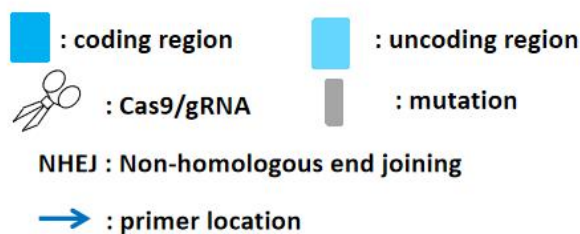
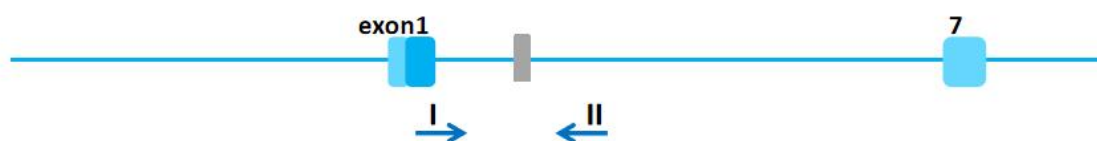
Common Name	Vegfb-K0 (2)	Cat. NO.	NM-K0-240950
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



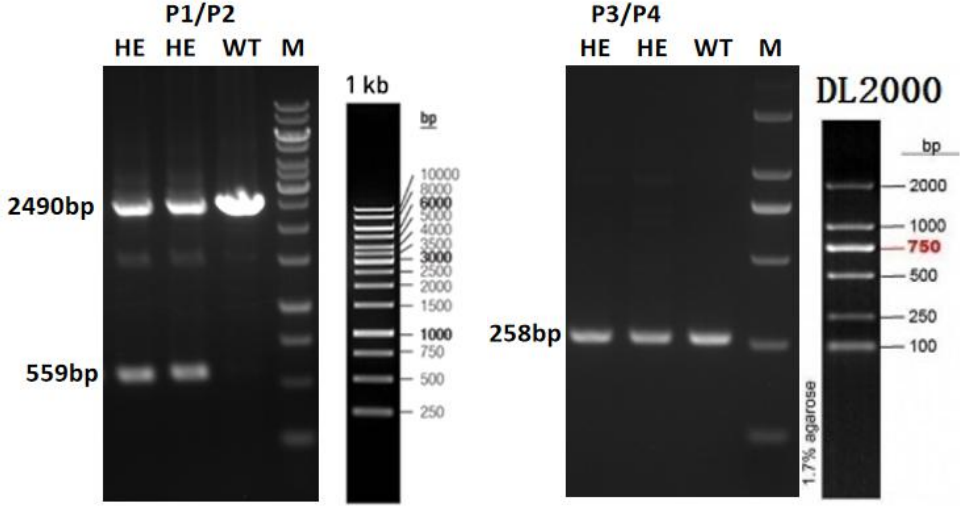
knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCTGCTGCTTGTGCACTG	Forward
P2	GCCCATAAGTCATCCCCTGG	Reverse
P3	TCTGTCGAGGGGATCCTCAG	Forward
P4	CAAGACCCACCGGCATACT	Reverse

Expected results

Results	 P1/P2 HE HE WT M 2490bp 559bp P3/P4 HE HE WT M 258bp DL2000 bp 2000 1000 750 500 250 100 1 kb bp 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1.7% agarose
Genotype	Knockout type: -1931bp Wild type: P1P2 =2490 bp; P3P4=258 bp Heterozygote: P1P2 =2490 bp and 559 bp; P3P4=258 bp Homozygote: P1P2 =559 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	ddH2O	8.0
System	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	