

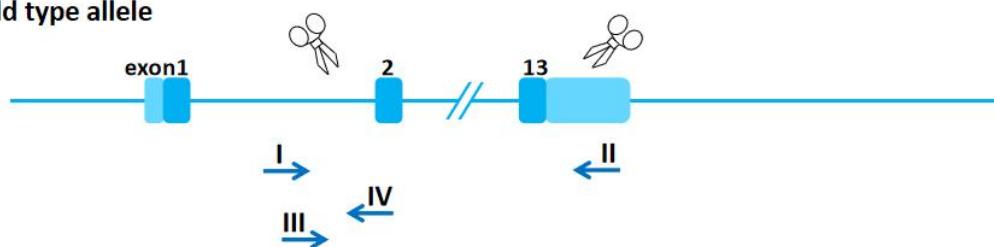
Vasp-K0 Genotyping Protocol



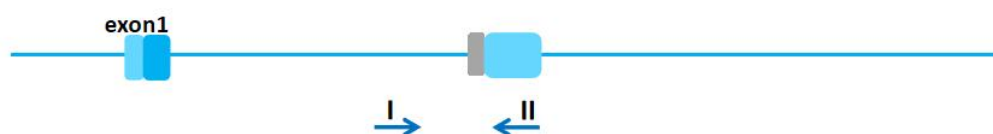
Common Name	Vasp-K0	Cat. NO.	NM-K0-240372
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

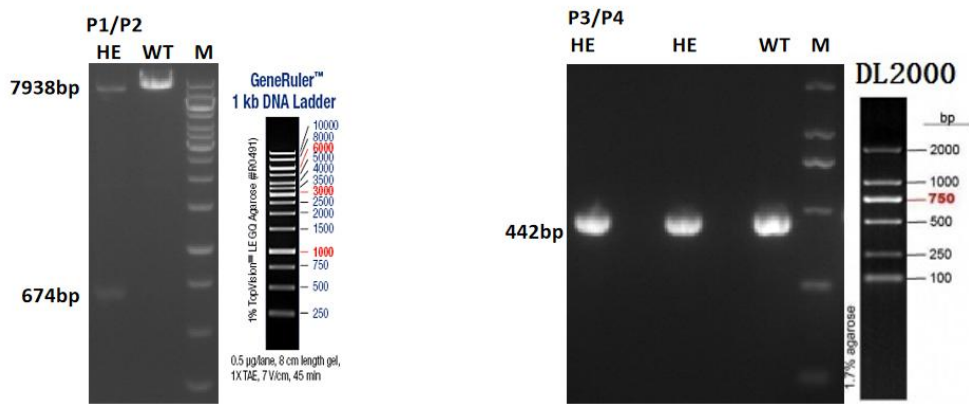


- : coding region
 : uncoding region
 : Cas9/gRNA
 : mutation
 NHEJ : Non-homologous end joining
 : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGTGGCCTGCCTATCTGTTC	Forward
P2	CCCCACCTAGTGGGATACCT	Reverse
P3	GTGTGGCCTGCCTATCTGTT	Forward
P4	CGGAAGGAGTTAGCAGTGGG	Reverse

Expected results

Results	 P1/P2 HE WT M 7938bp 674bp GeneRuler™ 1 kb DNA Ladder P3/P4 HE HE WT M 442bp DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -7264 bp Wild type: P1P2 =7938 bp; P3P4 =442 bp Heterozygote: P1P2 =7938 bp and 674 bp; P3P4 =442 bp Homozygote: P1P2 =674 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)
	ddH2O	8.0
	2 × Rapid Taq 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 × Rapid Taq Master Mix from Vazyme (Code Number: P222-01)			
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	