

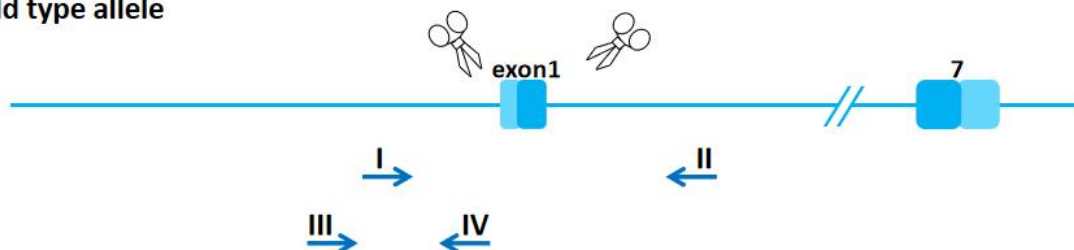
Vsir-K0 (BALB/c) Genotyping Protocol



Common Name	Vsir-K0 (BALB/c)	Cat. NO.	NM-K0-200776
Strain of Origin	Balb/c	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGTGTTAGCCGGTGGACAT	Forward
P2	TGTCCCTGCCGAGGAGTTAT	Reverse
P3	TGAGCTACAAGGCAGCATCC	Forward
P4	GAAGGGCTGGAGTTGCTGAT	Reverse

Expected results

Results	
Genotype	Knockout type: -641bp Wild type: P1P2 =1288 bp; P3P4=621 bp Heterozygote: P1P2 =1288 bp and 647 bp; P3P4=621 bp Homozygote: P1P2 =647 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	