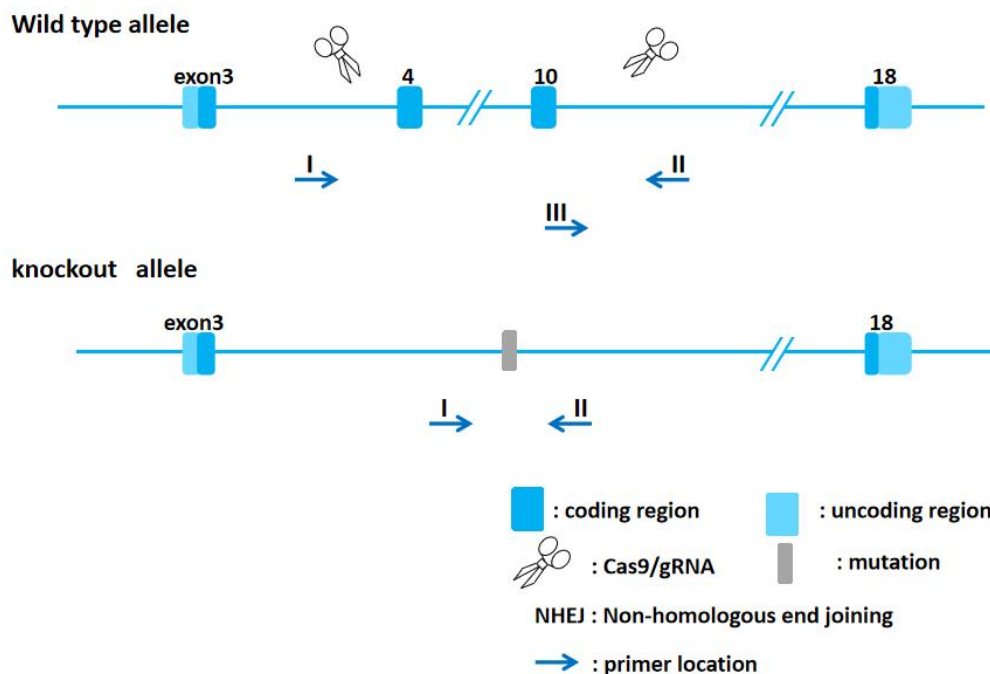


Rasgrp3-K0 (2) Genotyping Protocol



Common Name	Rasgrp3-K0 (2)	Cat. NO.	NM-K0-232694
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCTGATGGGCTGAGTTTGATTG	Forward
P2	GCTCCTTGTGGGTGTAAGTTTCTG	Reverse
P3	CCTTTGCTGACTGTGATGGCT	Forward

Expected results

Results	P1P2P3 DL2000
Genotype	Knockout type: -10319 bp Wild type: P2P3 =413 bp Heterozygote: P1P2 =647 bp; P2P3=413 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 System	Reaction Component			Volume (μl)
	ddH2O			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P3 (10 pmol/μl)			0.5
	Genomic DNA			1.0
	Total			20
Cycling Reaction	2×Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
	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	