

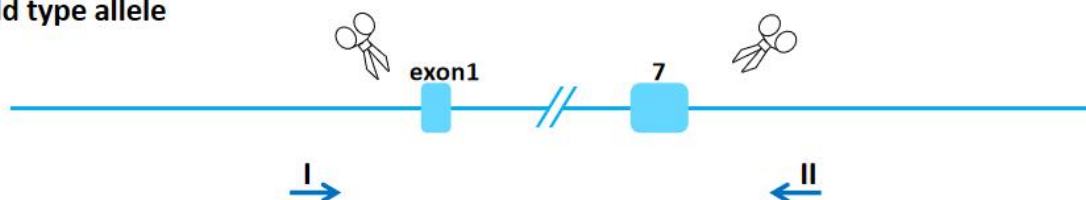
4933409F18Rik-K0 Genotyping Protocol



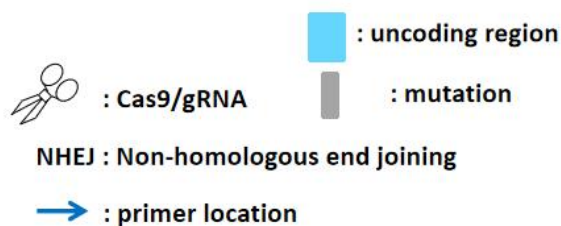
Common Name	4933409F18Rik-KO	Cat. NO.	NM-KO-234687
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGTCAGGGCTTCAGTGCAT	Forward
P2	CCAGCTCATGCCGAATGTTT	Reverse
P3	CCACTGTGGGCCACGAATAA	Forward
P4	GAGAATGCAGAGCAGGGACC	Reverse

Expected results

Results	
Genotype	Knockout type: -20888bp Wild type: P3P4=328 bp Heterozygote: P1P2 =618 bp; P3P4=328 bp Homozygote: P1P2 =618 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×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	