

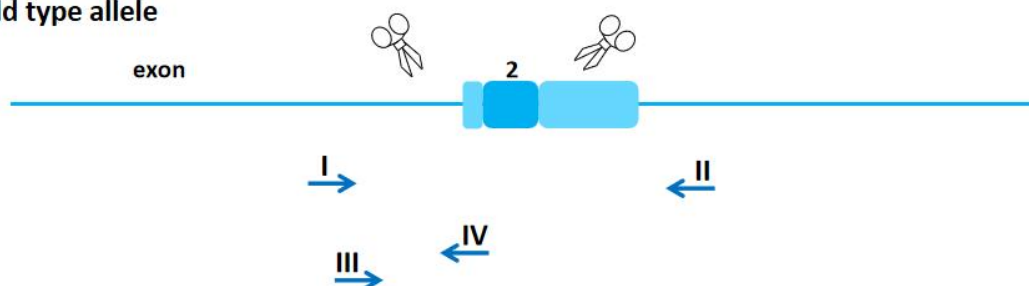
C1qtnf4-K0 Genotyping Protocol



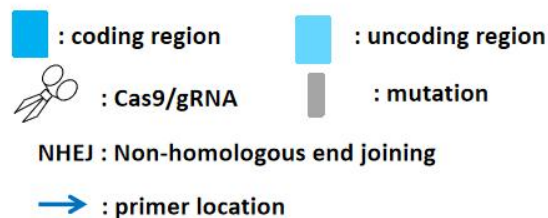
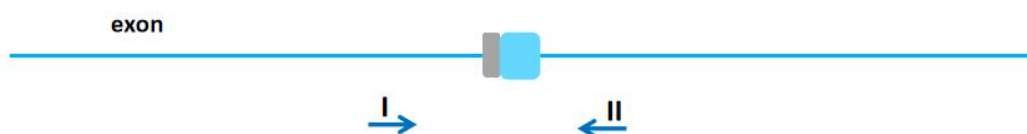
Common Name	C1qtnf4-KO	Cat. NO.	NM-KO-200070
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GCCATCTCTCCAGCCCTAAC	Forward
P2	GCTGTTGTGTTCTAGGGGG	Reverse
P3	AGCTAGTCTAAGCCGGAAGGA	Forward

P4	CACCTTGTCGAAGGTCACCG	Reverse
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Expected results

Results	
Genotype	Knockout type: -1055 bp Wild type: P3P4=288 bp Heterozygote: P1P2 =833 bp; P3P4=288 bp Homozygote: P1P2 =833 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 K0 band.

Reaction &Cycling

PCR	Reaction Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	