

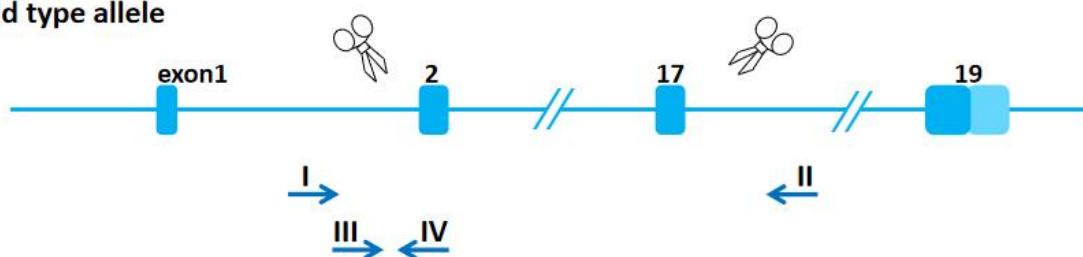
Cecr2-KO Genotyping Protocol



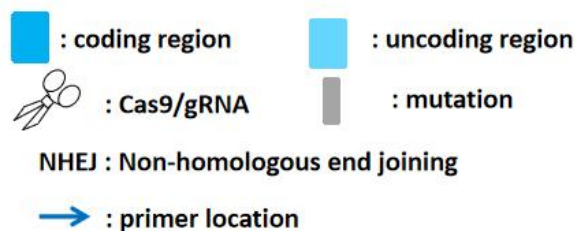
Common Name	Cecr2-KO	Cat. NO.	NM-KO-234844
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TTAGGACCTGCTTGCTCTGG	Forward
P2	GAGTTTGAGATCAGCAGGGGT	Reverse
P3	CCACCATTTGGTTCCTGGGAT	Forward
P4	TCTTCGCTGATAGCAGCCTT	Reverse

Expected results

Results	
Genotype	Knockout type: -41860bp Wild type: P3P4=289 bp Heterozygote: P1P2=639 bp; P3P4=289 bp Homozygote: P1P2=639 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	