

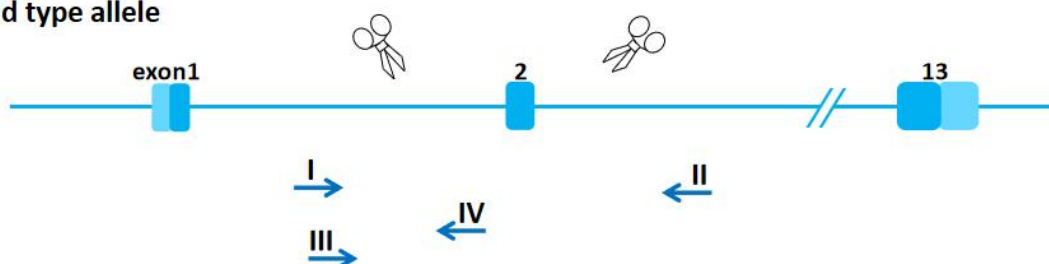
Crhr1-K0 Genotyping Protocol



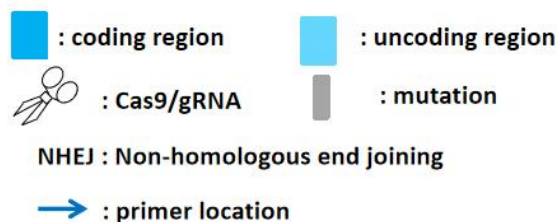
Common Name	Crhr1-K0	Cat. NO.	NM-K0-225111
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTCAGAGTGACACAGAGCCC	Forward
P2	CTCCTGCCAAACTTACCCGT	Reverse
P3	GTCAGAGTGACACAGAGCCC	Forward

P4	GCACCCAACCTTCTACCTCC	Reverse
----	----------------------	---------

Expected results

Results	P3/P4 HE WT M 204bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose P1/P2 HE HE WT M 1899bp 278bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -1621 bp Wild type: P1P2 =1899 bp; P3P4=204 bp Heterozygote: P1P2 =1899 bp and 278 bp; P3P4=204 bp Homozygote: P1P2 =278 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	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	