

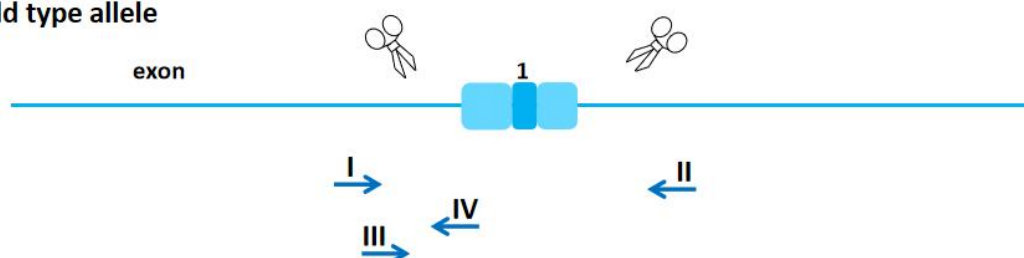
Car1r-K0 Genotyping Protocol



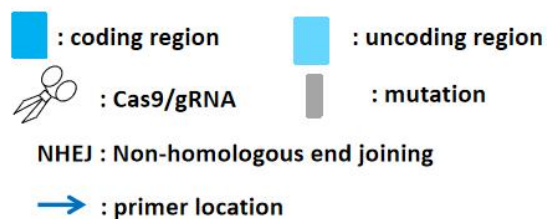
Common Name	Carlr-KO	Cat. NO.	NM-KO-240166
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

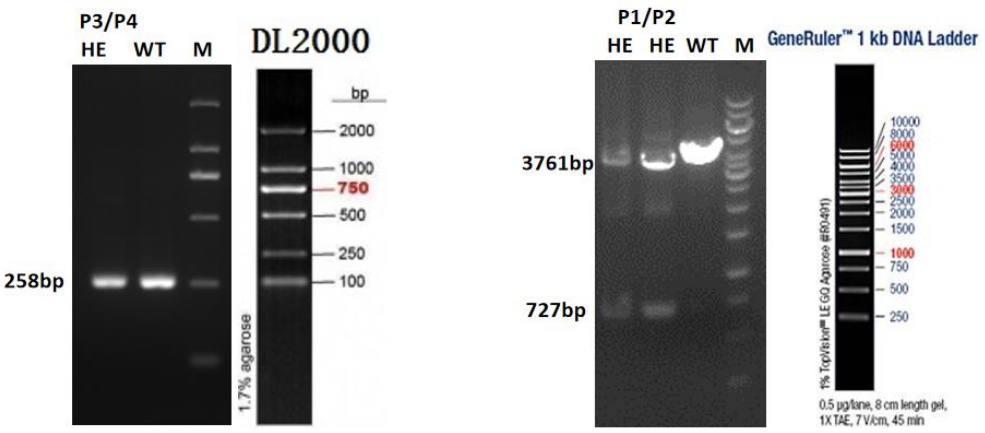


Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGGTTGGGCTTTGGGAACT	Forward
P2	ACGTAGGTGGACTCACTGGT	Reverse
P3	TTAGGCAGCTGTTTGTGGGT	Forward

P4	CTAGCACCTTAGCTCTGCGG	Reverse
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Expected results

Results	 The figure shows two gel electrophoresis results. The left gel is for P3/P4 primers, showing a single band at 258bp for HE and WT lanes, and no band for the M lane. The right gel is for P1/P2 primers, showing a single band at 3761bp for HE and WT lanes, and a band at 727bp for the M lane. A GeneRuler 1 kb DNA Ladder is shown next to each gel.
Genotype	Knockout type: -3034 bp Wild type: P1P2 =3761 bp; P3P4 =258 bp Heterozygote: P1P2 =3761 bp and 727 bp; P3P4 =258 bp Homozygote: P1P2 =727 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	