

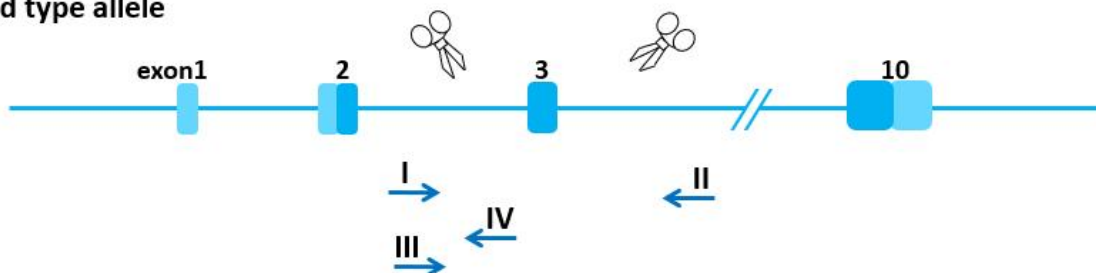
Carns1-KO Genotyping Protocol



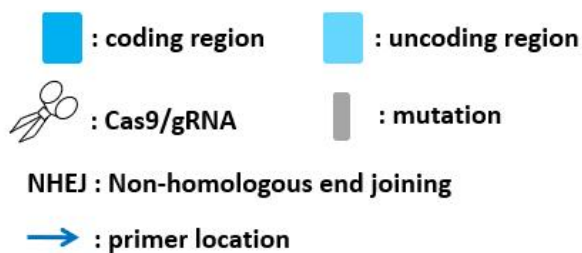
Common Name	Carns1-KO	Cat. NO.	NM-KO-231442
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	ACATCAGGGTGATGAGGAGGA	Forward
P2	GCCTTGAGACTGGCTCTGTT	Reverse
P3	GTCCCAGAGAGAACAGGGCTA	Forward
P4	CCGTCCGGGTCAAGTTCAAA	Reverse

Expected results

Results	P1/P2 HE HE WT M 1296bp 548bp GeneRuler™ 1 kb DNA Ladder 1% TBE/1% LE GEL Agarose #80491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 322bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -748bp Wild type: P1P2 =1296 bp; P3P4 =322 bp Heterozygote: P1P2 =1296 bp and 548 bp; P3P4 =322 bp Homozygote: P1P2 =548 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)
	ddH ₂ O			8.0
	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	