

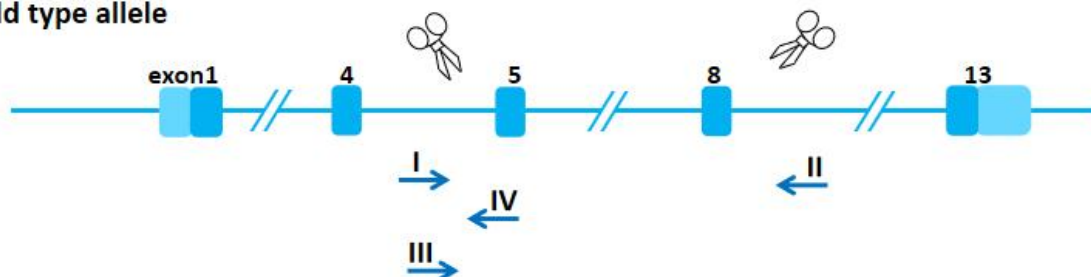
Trpc1-KO Genotyping Protocol



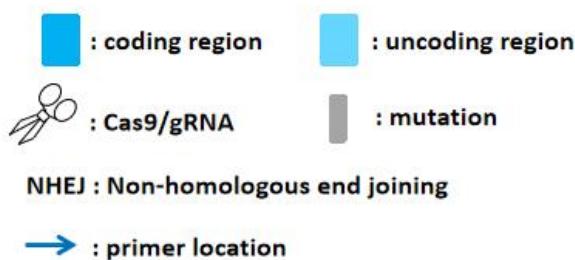
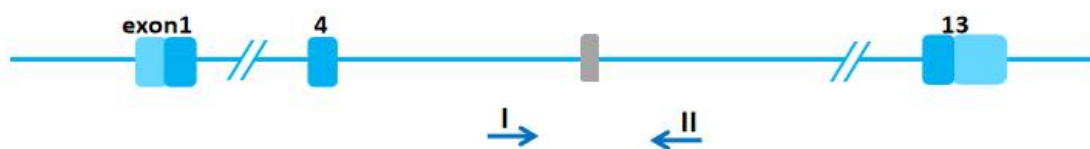
Common Name	Trpc1-KO	Cat. NO.	NM-KO-241474
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	GACCCCTCCAGCCATATGAA	Forward
P2	CATGCTGCACACTTTTGACC	Reverse
P3	ACCCAATCCTGTCTCAGCTA	Forward
P4	ACCTCCACAAGGCTGAGTTC	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder 10025bp 719bp P1/P2 HE HE WT M H 410bp P3/P4 HE HE WT H M DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1XTAE, 7 V/cm, 45 min
Genotype	Knockout type: -9306bp Wild type: P1P2 =10025 bp; P3P4 =410 bp Heterozygote: P1P2 =10025 bp and 719 bp; P3P4 =410 bp Homozygote: P1P2 =719 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	