

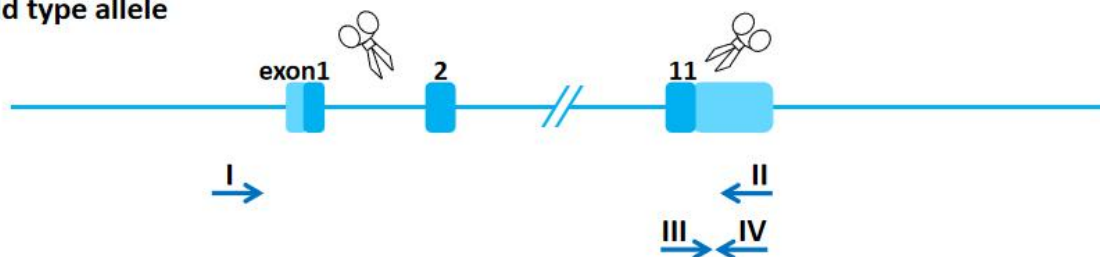
C1s2-K0 (C1s1-K0) Genotyping Protocol



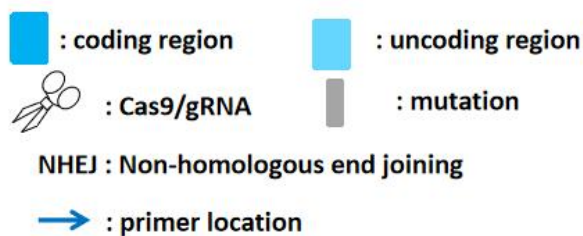
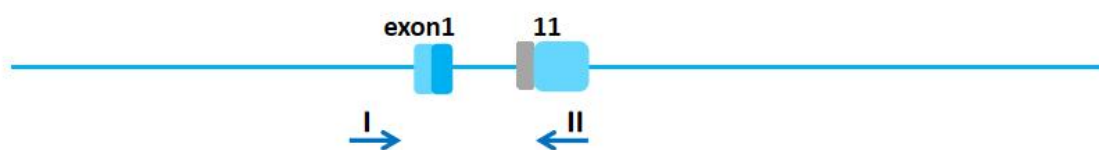
Common Name	C1s2-K0 (C1s1-K0)	Cat. NO.	NM-K0-233484
Strain of Origin	C1s1-K0, C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

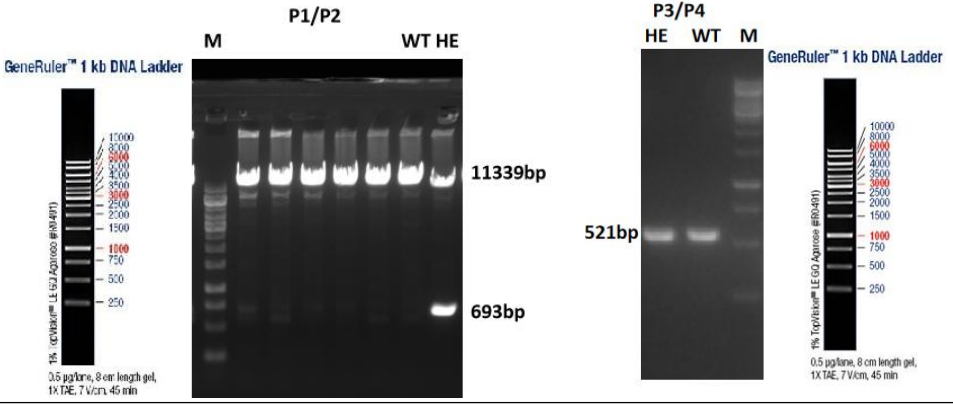


Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGCAGGCACCTGTACTTGTC	Forward
P2	CCATGATGCTATGCCCCAA	Reverse
P3	ATCTGTGCTGGTGAGAAGGG	Forward

P4	AGCATGCTTTGCTGCAAGTT	Reverse
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Expected results

Results	 GeneRuler™ 1 kb DNA Ladder 1% agarose, 1.5x TAE, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 11339bp 693bp 521bp GeneRuler™ 1 kb DNA Ladder 1% agarose, 1.5x TAE, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -10646bp Wild type: P1P2 =11339 bp; P3P4=521 bp Heterozygote: P1P2 =11339 bp and 693 bp; P3P4=521 bp Homozygote: P1P2 =693 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	