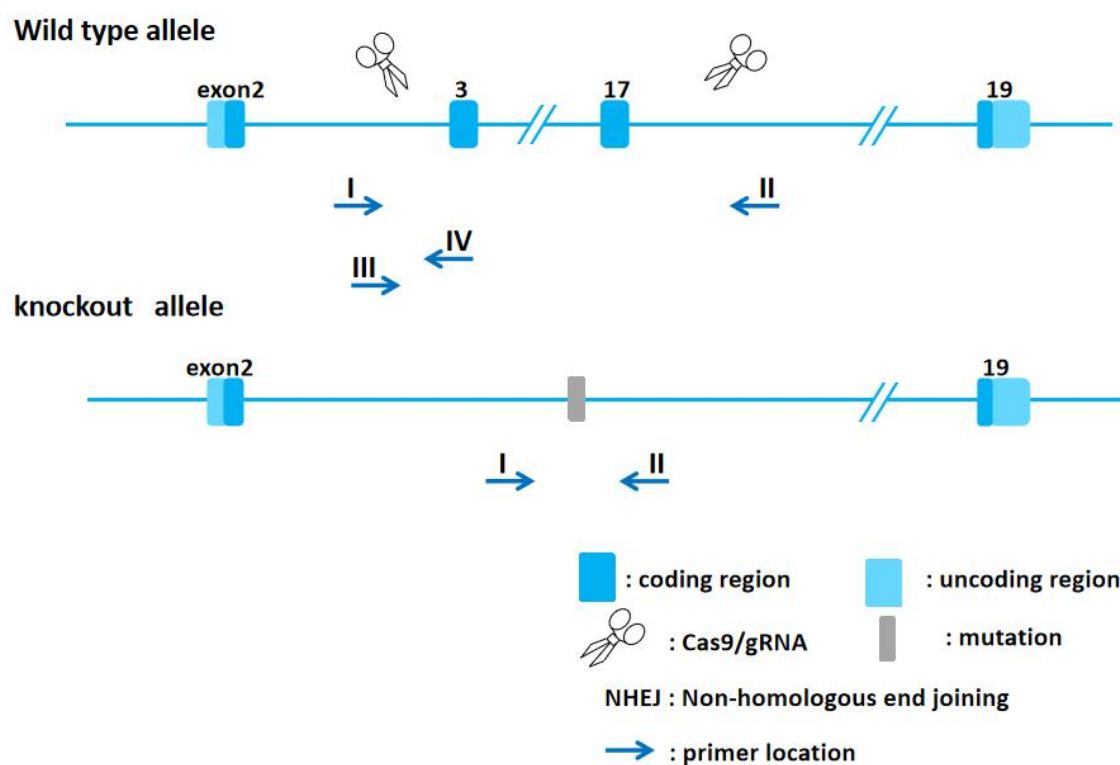


Boc-K0 Genotyping Protocol



Common Name	Boc-K0	Cat. NO.	NM-K0-240287
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TCCAGGTCTGTCCAACCTCCA	Forward
P2	GATGGGGTGGGTTAGTGCAA	Reverse
P3	GCTTGCCTGGGTGCTAAATTC	Forward
P4	CCCAGAGCATCATCAGAGCC	Reverse

Expected results

Results	P1/P2 HE HE WT M 485bp P3/P4 HE HE WT H M 280bp DL2000 bp 2000 1000 750 500 250 100
Genotype	Knockout type: -33359 bp Wild type: P3P4 =280 bp Heterozygote: P1P2 =485 bp; P3P4 =280 bp Homozygote: P1P2 =485 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	