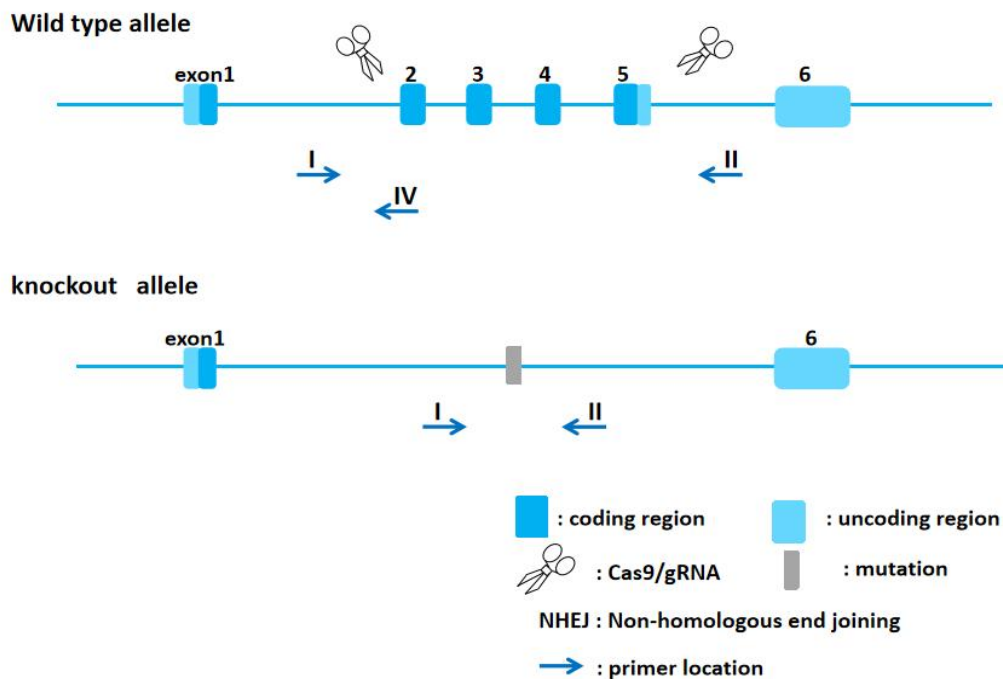


Btc-K0 Genotyping Protocol



Common Name	Btc-K0	Cat. NO.	NM-K0-232138
Strain of Origin	C57BL/6J	Version	V1

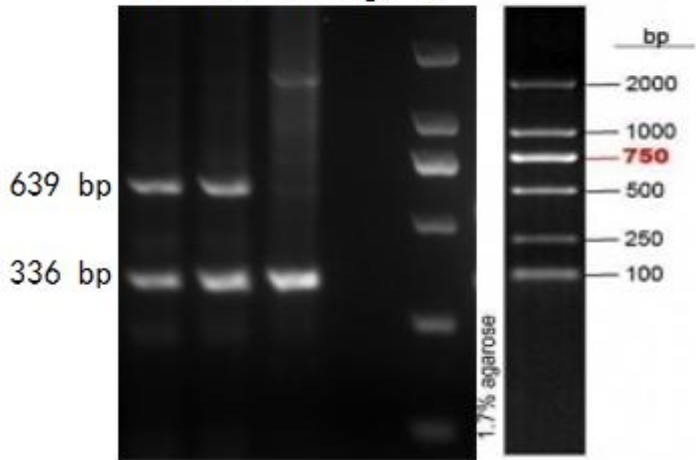
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GGGGTGGTAACCTAGGCAGGAG	Forward
P2	ACTACCATCATCATCCATTGCT	Reverse
P4	GAGCCATTGGTTTCTGGTGTTT	Reverse

Expected results

Results	P1P2P4 HE HE WT H₂O M DL2000 
Genotype	Knockout type: -16341 bp Wild type: P1P4 =336 bp Heterozygote: P1P2 =639 bp; P1P4=336 bp Homozygote: P1P2 =639 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			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	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	