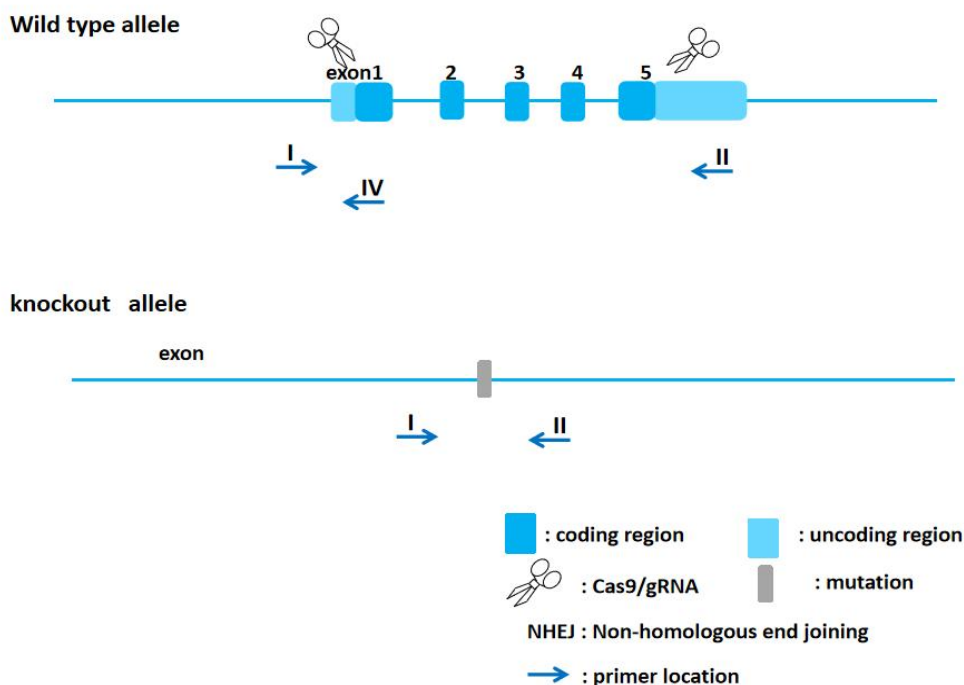


Tacr3-K0 (2) Genotyping Protocol



Common Name	Tacr3-K0 (2)	Cat. NO.	NM-K0-232216
Strain of Origin	C57BL/6J	Version	V1

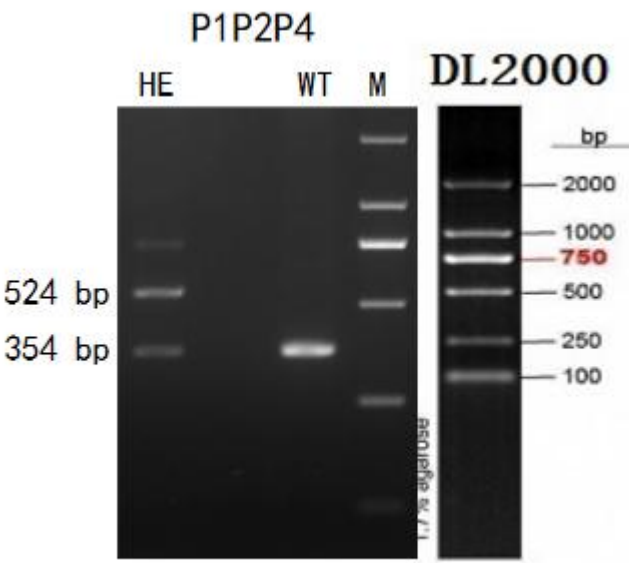
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CTGGGGACTCCTGGAGACTA	Forward
P2	AAAACCCCTCTGCTGTTTCGG	Reverse
P4	GTCCCGTCCGTCCAGTTTTC	Reverse

Expected results

Results	 P1P2P4 HE WT M DL2000 524 bp 354 bp bp 2000 1000 750 500 250 100
Genotype	Knockout type: -103272 bp Wild type: P1P4 =354 bp Heterozygote: P1P2 =524 bp; P1P4=354 bp Homozygote: P1P2 =524 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 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	