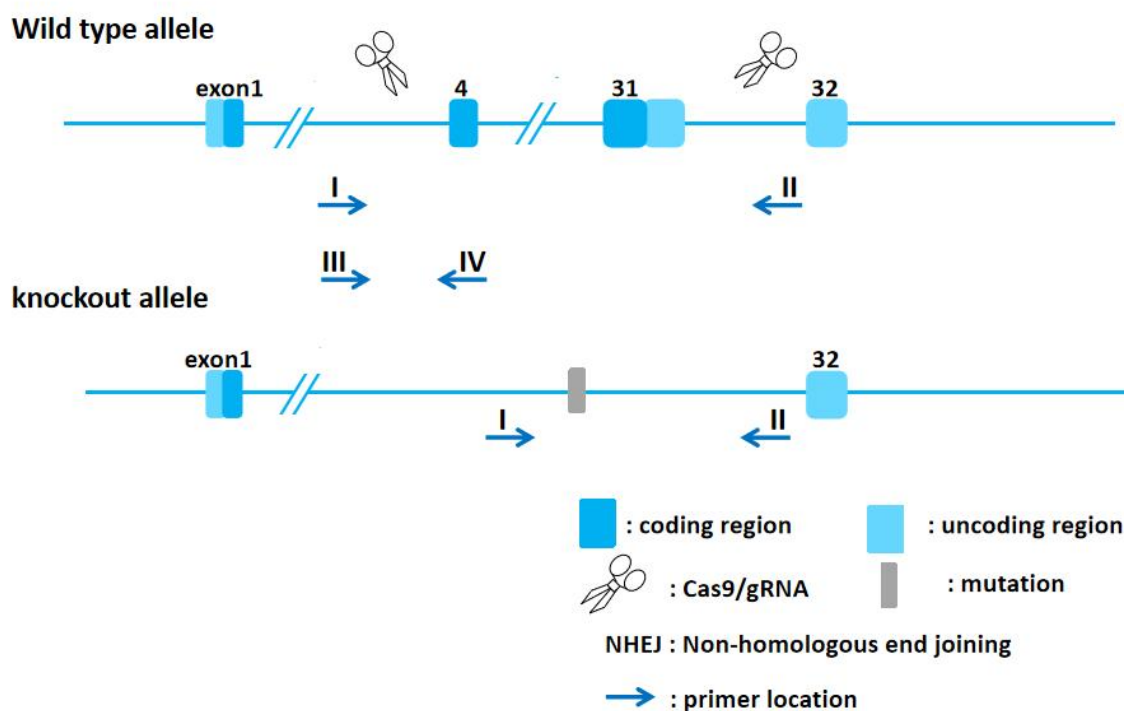


Cast-KO Genotyping Protocol



Common Name	Cast-K0	Cat. NO.	NM-K0-240175
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGGCTCGGATCTAAACAAAGT	Forward
P2	CTGCCTTCCTCTGCCTTTGA	Reverse
P3	ACATGGCTCGGATCTAAACAA	Forward
P4	CTGTGGTACTCATGCTGGGG	Reverse

Expected results

Results	
Genotype	Knockout type: -58688 bp Wild type: P3P4 =461 bp Heterozygote: P1P2 =469 bp; P3P4 =461 bp Homozygote: P1P2 =469 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	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	