

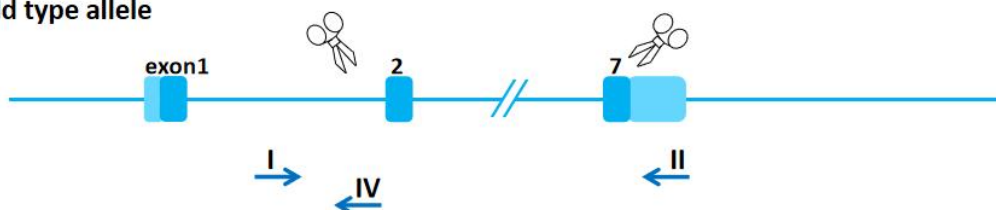
Trim11-KO Genotyping Protocol



Common Name	Trim11-KO	Cat. NO.	NM-KO-233702
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



- : coding region
 : uncoding region
 : Cas9/gRNA
 : mutation
 NHEJ : Non-homologous end joining
→ : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	CATGGGGCTGAGAACCCAAA	Forward
P2	CACGCTACCGTCCTCTTTGT	Reverse
P4	CAGTGACTTCTCCAGCCTCC	Reverse

Expected results

Results	P1P2P4	
	HE HE WT H ₂ O M	DL2000

Genotype	Knockout type: -9633bp
	Wild type: P1P4=301 bp
	Heterozygote: P1P2 =589 bp; P1P4=301 bp
	Homozygote: P1P2 =589 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)
	ddH ₂ O			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	