

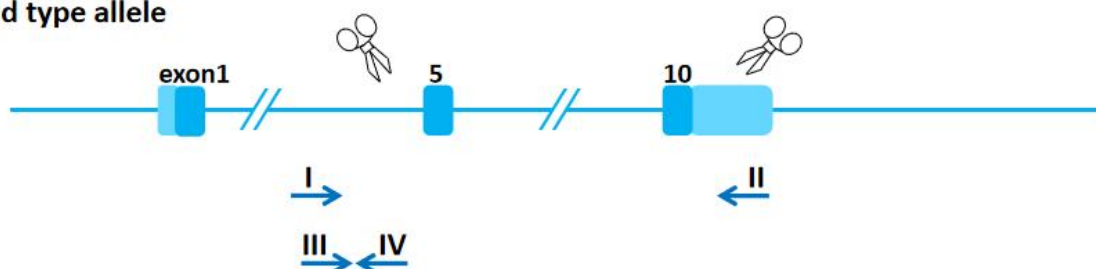
Taz-KO Genotyping Protocol



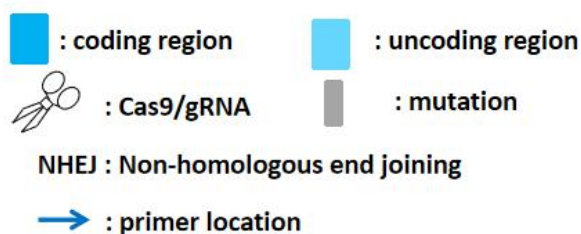
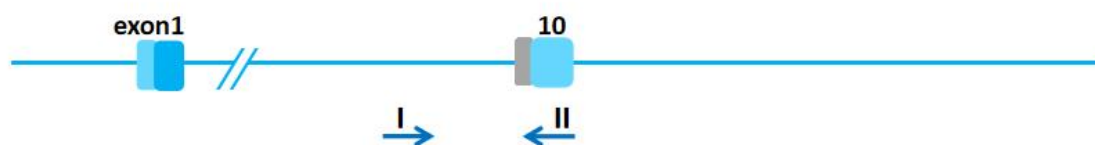
Common Name	Taz-K0	Cat. NO.	NM-K0-200577
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

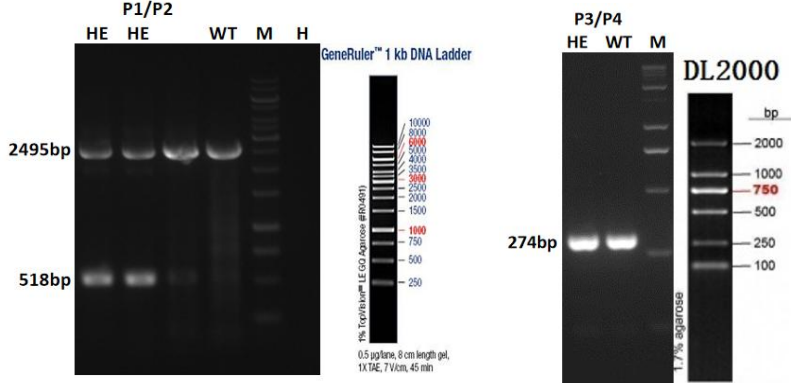


Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTGGAAGCTCTGGAGCTGATTT	Forward
P2	GCTATCCCTGATGAGGTTTGTGA	Reverse
P3	AGGGAGGTTGAGTTGCCATGA	Forward

P4	GGAAGATTGCTCTGAGCTTGAGG	Reverse
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Expected results

Results	 The figure displays two gel electrophoresis images. The left image, labeled 'P1/P2', shows lanes for HE, HE, WT, M, and H. Bands are visible at 2495bp and 518bp. The right image, labeled 'P3/P4', shows lanes for HE, WT, and M. A band is visible at 274bp. A GeneRuler 1 kb DNA Ladder and a DL2000 marker are also shown.
Genotype	Knockout type: -1977bp Wild type: P1P2 =2495 bp; P3P4=274 bp Heterozygote: P1P2 =2495 bp and 518 bp; P3P4=274 bp Homozygote: P1P2 =518 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	8.0
	2×Taq Plus 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×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	