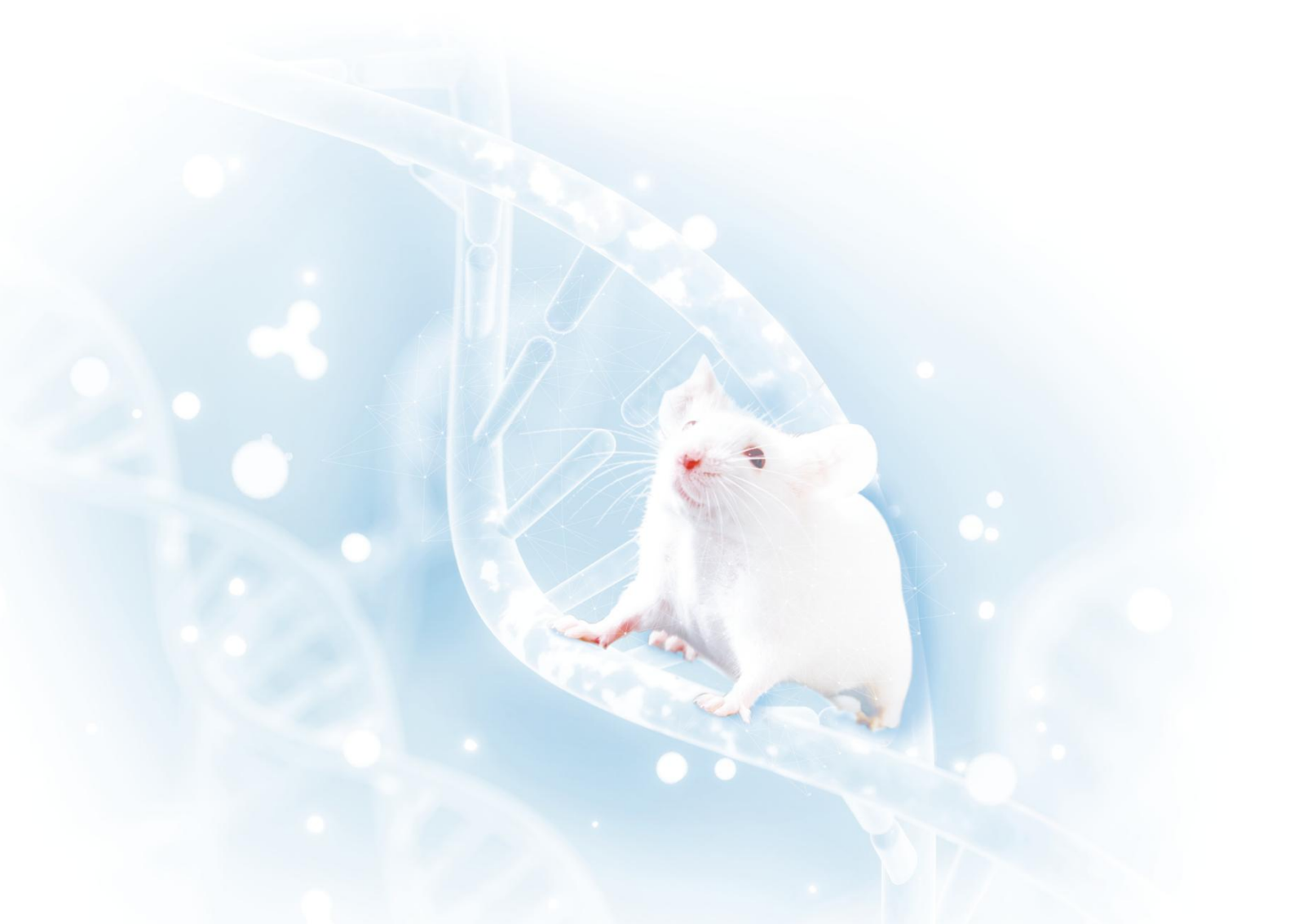


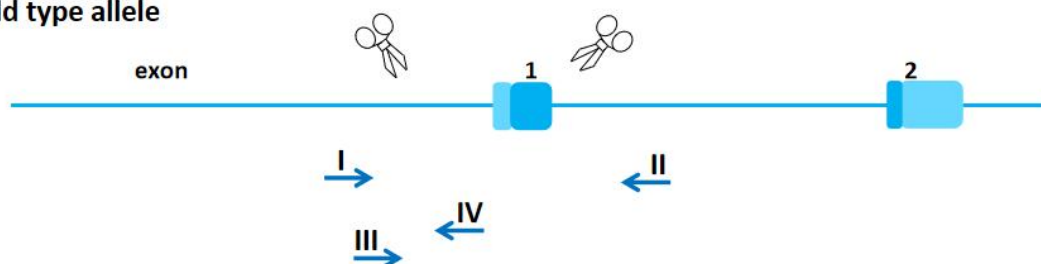
Tst-K0(1) Genotyping Protocol



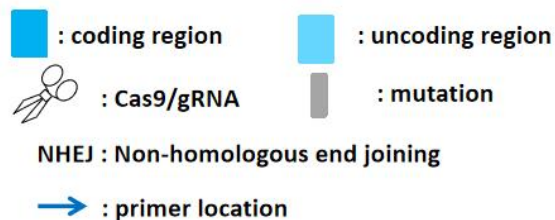
Common Name	Tst-KO (1)	Cat. NO.	NM-KO-240275
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

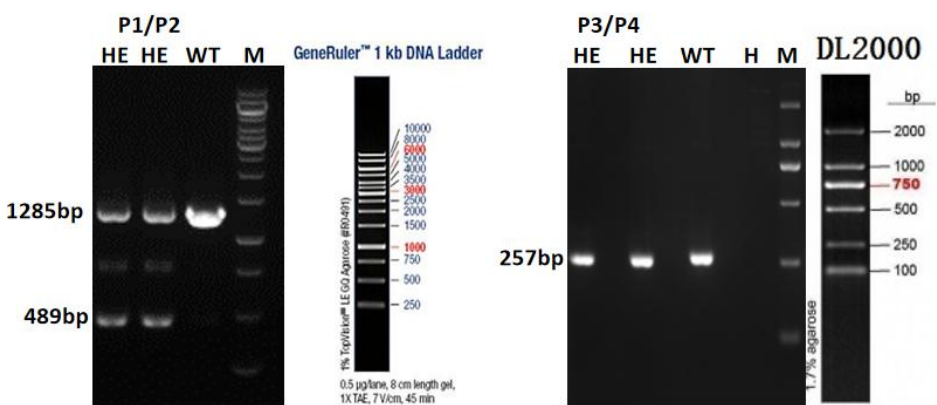


Primers

Primer	Sequence (5' → 3')	Primer type
P1	ACTCTGGCTGCAAGAAACGA	Forward
P2	ATCTACTGCATGTGGGAGCG	Reverse
P3	GAAGACAGAGTCCTGAGCCAG	Forward

P4	TGCGGTGGGCGGAGCCCAG	Reverse
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Expected results

Results	 The image shows two gel electrophoresis results. The left gel is for P1/P2 primers, showing bands at 1285bp and 489bp for HE, WT, and M lanes. The right gel is for P3/P4 primers, showing bands at 257bp for HE, WT, and H lanes. A GeneRuler 1 kb DNA Ladder and a DL2000 marker are also shown.
Genotype	Knockout type: -796 bp Wild type: P1P2 =1285 bp; P3P4 =257 bp Heterozygote: P1P2 =1285 bp and 489 bp; P3P4 =257 bp Homozygote: P1P2 =489 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 Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	