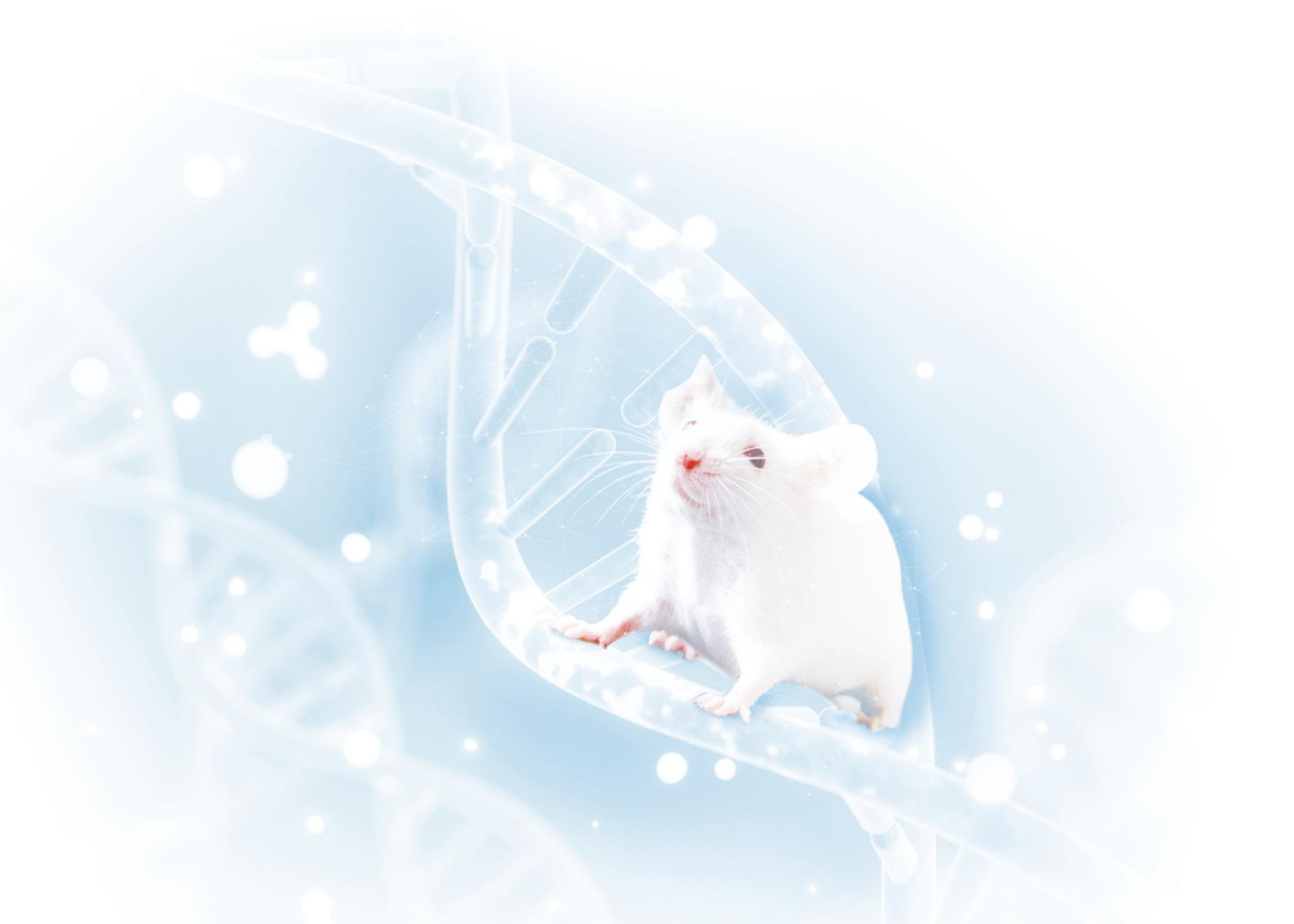


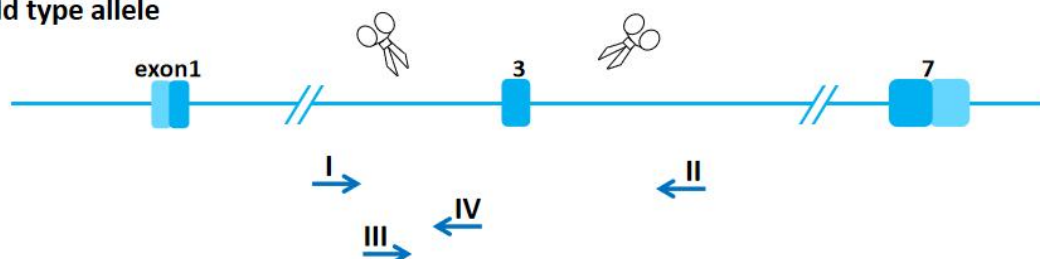
Tes-K0 Genotyping Protocol



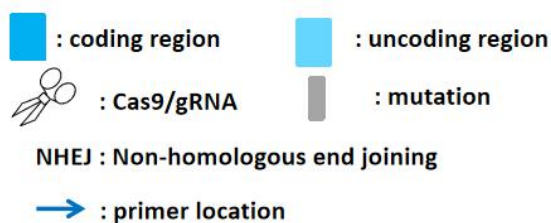
Common Name	Tes-K0	Cat. NO.	NM-K0-240270
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

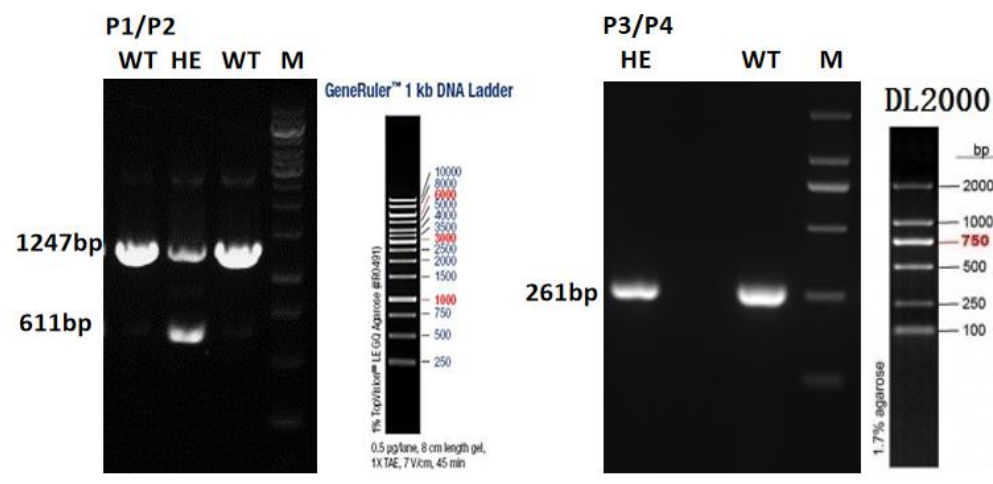


Primers

Primer	Sequence (5' → 3')	Primer type
P1	ACAATGAAGCGGGTGACGAA	Forward
P2	TGGATCAGCGGCTTGTTTCAT	Reverse
P3	GTCCGGGACAAAGACCACAT	Forward

P4	ATGTGCAGTCCTTGGTGGTT	Reverse
----	----------------------	---------

Expected results

Results	 P1/P2 WT HE WT M 1247bp 611bp P3/P4 HE WT M 261bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7V/cm, 45 min DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -636 bp Wild type: P1P2 =1247 bp; P3P4 =261 bp Heterozygote: P1P2 =1247 bp and 611 bp; P3P4 =261 bp Homozygote: P1P2 =611 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 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	