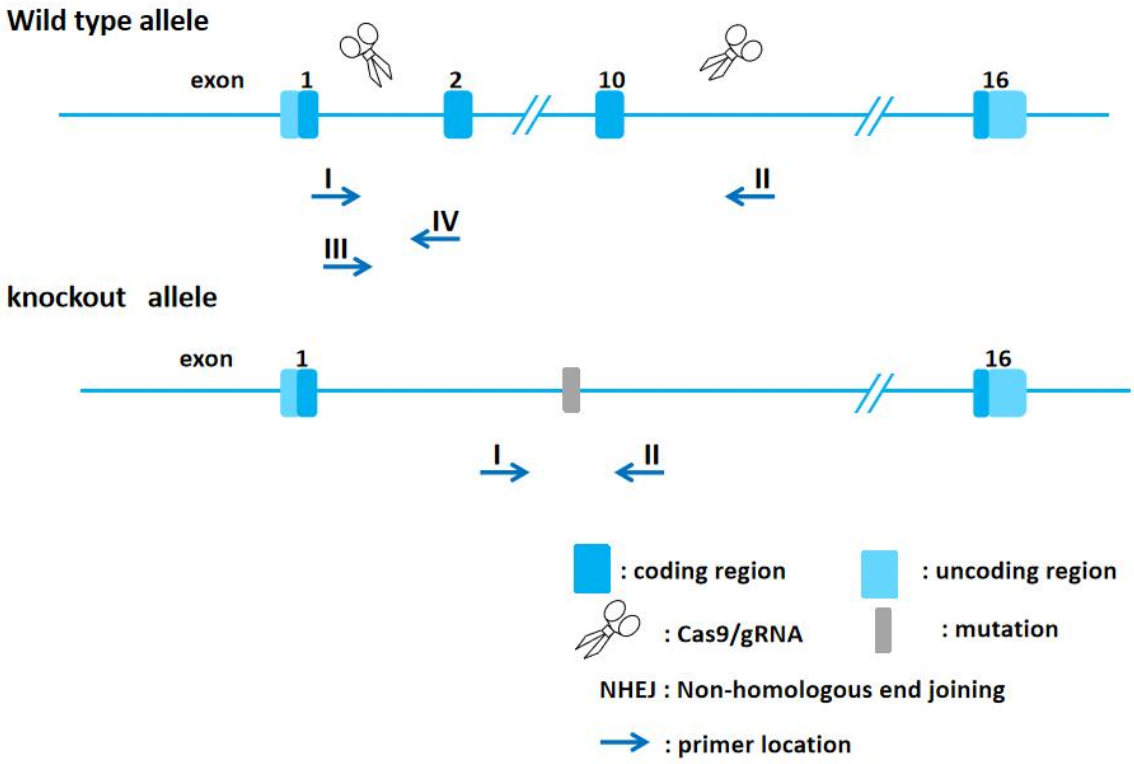
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Tert-KO Genotyping Protocol

Common Name	Tert-KO	**Cat. NO.**	NM-KO-240173
Strain of Origin	C57BL/6J	**Version**	V1

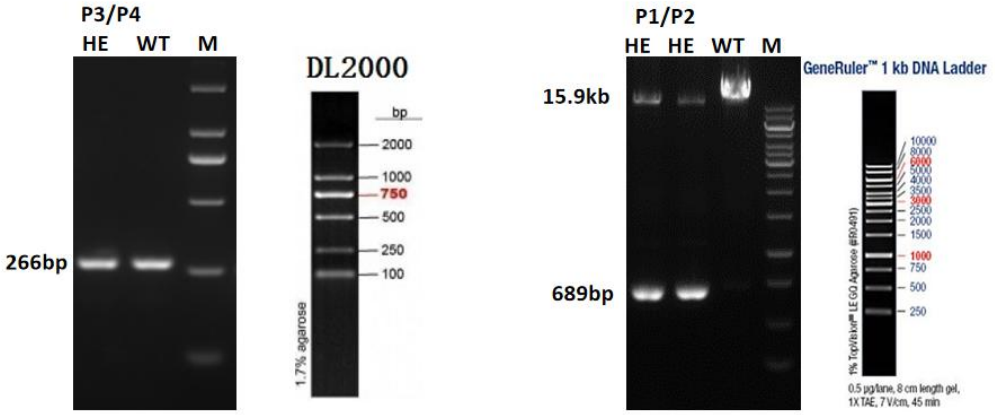
Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	CCTGGGTCCTGGCTGTTTTC	Forward
P2	GCCTTGGCAAATAGCCCTTC	Reverse
P3	TTGGTTGCCCAATGCCTAGT	Forward
P4	AGCAGCTCAAAGCCAAAAGC	Reverse

Expected results

Results	
Genotype	Knockout type: -15197 bp Wild type: P1P2 =15886 bp; P3P4 =266 bp Heterozygote: P1P2 =15886 bp and 689 bp; P3P4 =266 bp Homozygote: P1P2 =689 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)
	ddH20	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	