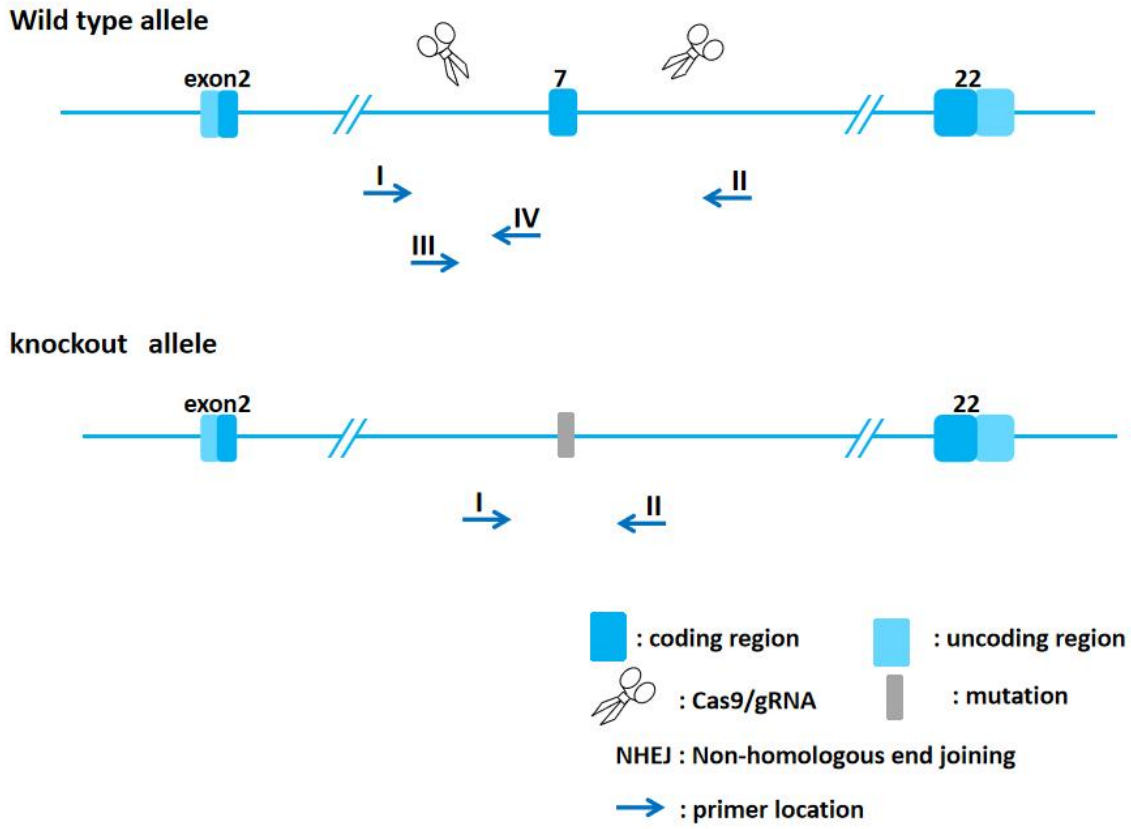
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Thbs1-KO Genotyping Protocol

Common Name	Thbs1-KO	Cat. NO.	NM-KO-226309
Strain of Origin	C57BL/6J	Version	V1

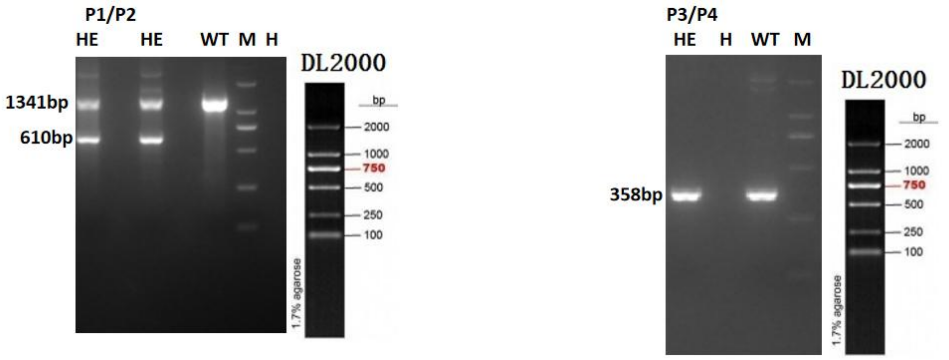
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCTGGCCTGCTACCAAAGTG	Forward
P2	CTGAGTCGTGGCTCATGTGT	Reverse
P3	CGGTTTTGGCCAGCCCTAAT	Forward
P4	AGGAGGGATGTCTCTGCTGG	Reverse

Expected results

Results	
Genotype	Knockout type：-732+1bp Wild type：P1P2 =1341 bp；P3P4 =358 bp Heterozygote：P1P2 =1341 bp and 610 bp；P3P4 =358 bp Homozygote：P1P2 =610 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 × 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℃	5 min	
	2	95℃	20 sec	
	3	60℃	20 sec	
	4	72℃	20 sec	35 repeats to 2
	5	72℃	5 min	
	6	12℃	Hold	