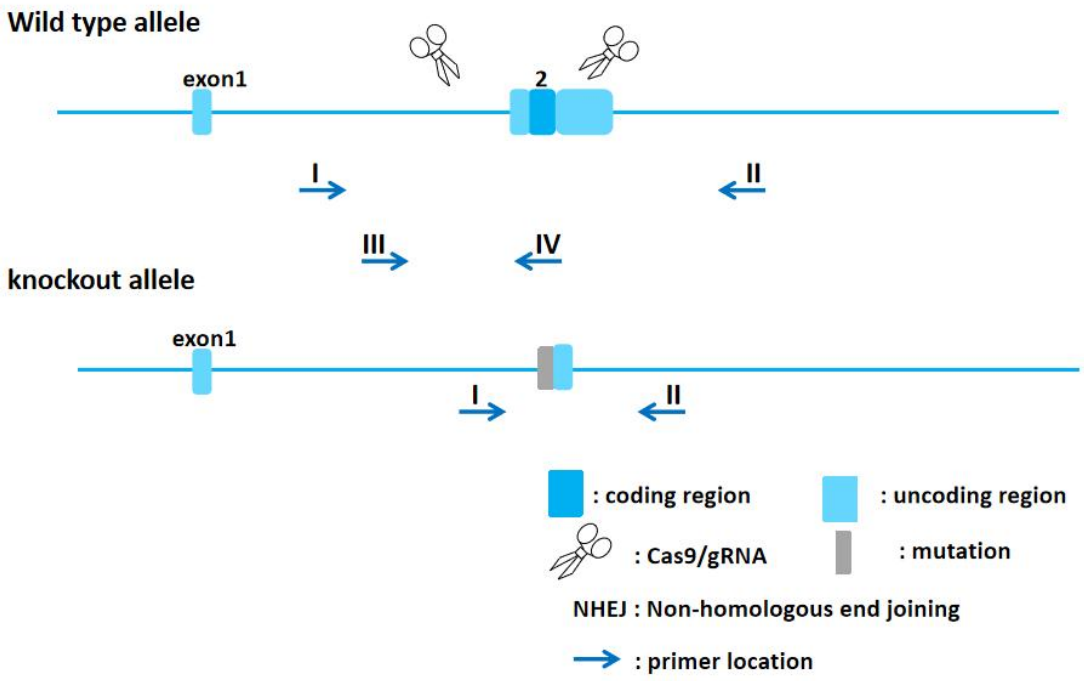
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Ang2-KO Genotyping Protocol

Common Name	Ang2-KO	**Cat. NO.**	NM-KO-240420
Strain of Origin	C57BL/6J	**Version**	V1

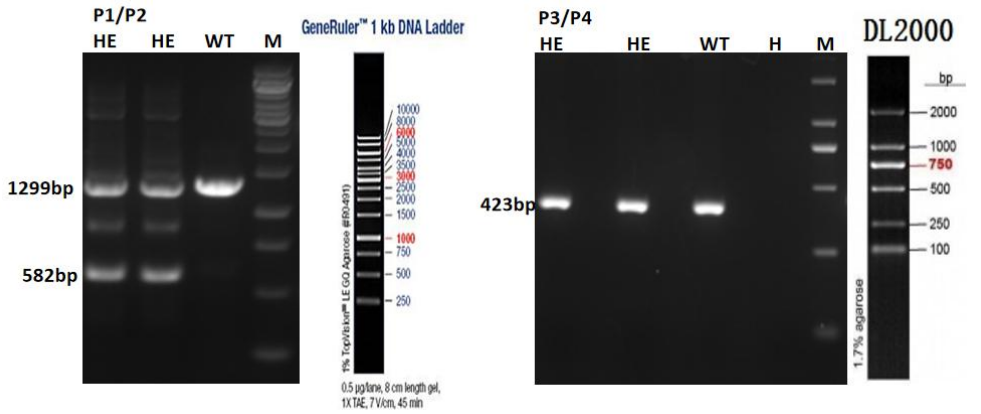
Genotyping strategy



Primers

Primer	Sequence (5’→3’)	Primer type
P1	CATCTTGCCTCAGGAACCCC	Forward
P2	CCCTGACTAGCAGGTAACGA	Reverse
P3	ACACATACTGCACCTGTCCTT	Forward
P4	TCACAACCAGACCCAACAGG	Reverse

Expected results

Results	
Genotype	Knockout type: -717 bp Wild type: P1P2 =1299 bp; P3P4 =423 bp Heterozygote: P1P2 =1299 bp and 582 bp; P3P4 =423 bp Homozygote: P1P2 =582 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

	Reaction Component	Volume (μl)
PCR Reaction System	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	