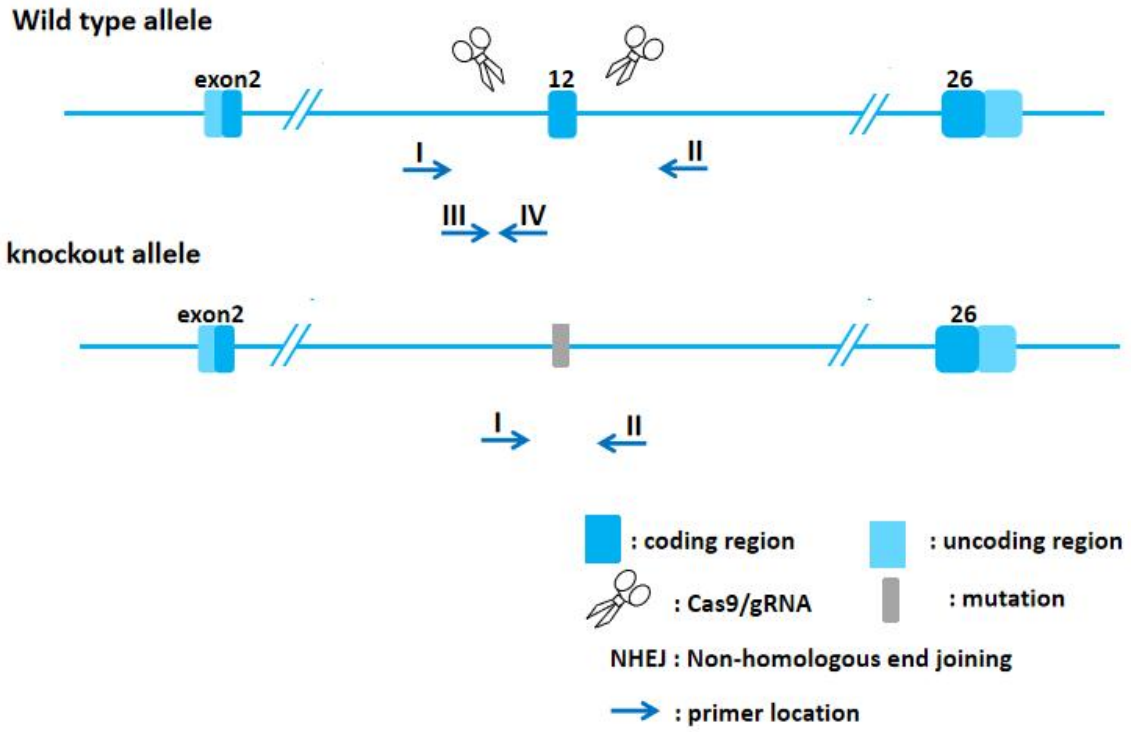
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Ano1-KO Genotyping Protocol

Common Name	Ano1-KO	Cat. NO.	NM-KO-242044
Strain of Origin	C57BL/6J	Version	V1

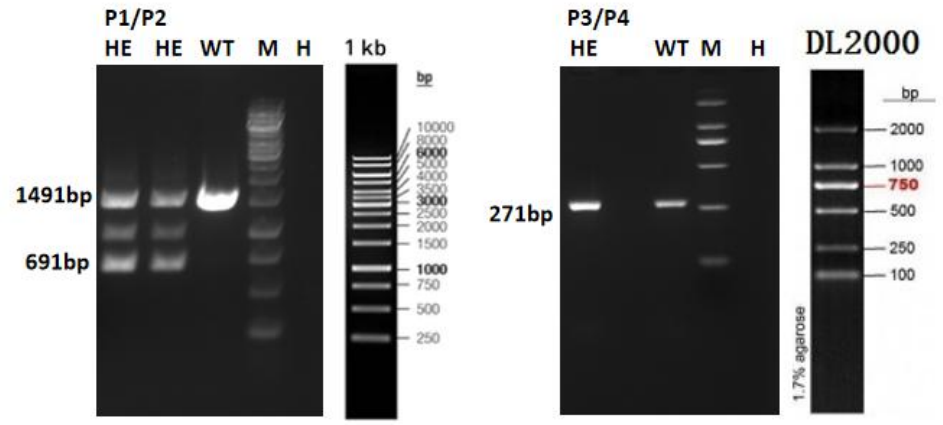
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GAGCCTAGCCACCCAAAGAG	Forward
P2	GGCTCATGTGCACCTCTCTA	Reverse
P3	TCACTCAGCATTGGTCTGGC	Forward
P4	TCCACATTTCTCTTGGGGCA	Reverse

Expected results

Results	
Genotype	Knockout type：-800bp Wild type：P1P2 =1491 bp；P3P4 =271 bp Heterozygote：P1P2 =1491 bp and 691 bp；P3P4 =271 bp Homozygote：P1P2 =691 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			8.2
	2xPhanta Flash Master Mix*			10.0
	P1/P3(10 pmol/μl)			0.4
	P2/P4(10 pmol/μl)			0.4
	Genomic DNA			1.0
	Total			20
	2xPhanta Flash Master Mix(Vazyme,Code No:P510)			
Cycling Reaction	Step	Temp	Time	Note
	1	98℃	3 min	
	2	98℃	10 sec	
	3	60℃	10 sec	
	4	72℃	1 min	32 repeats to 2
	5	72℃	5 min	
	6	12℃	Hold	