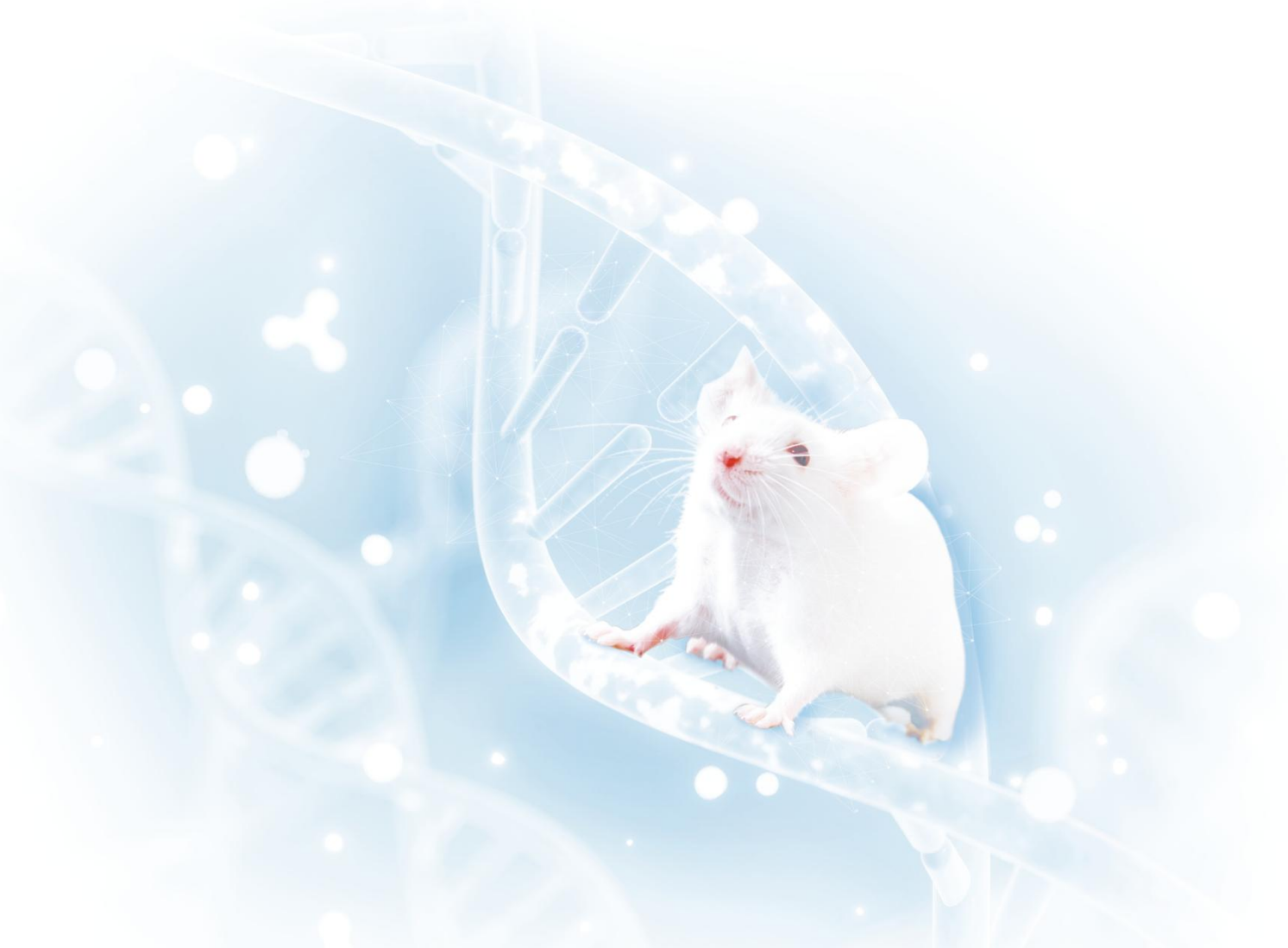
SHANGHAI MODEL ORGANISMS

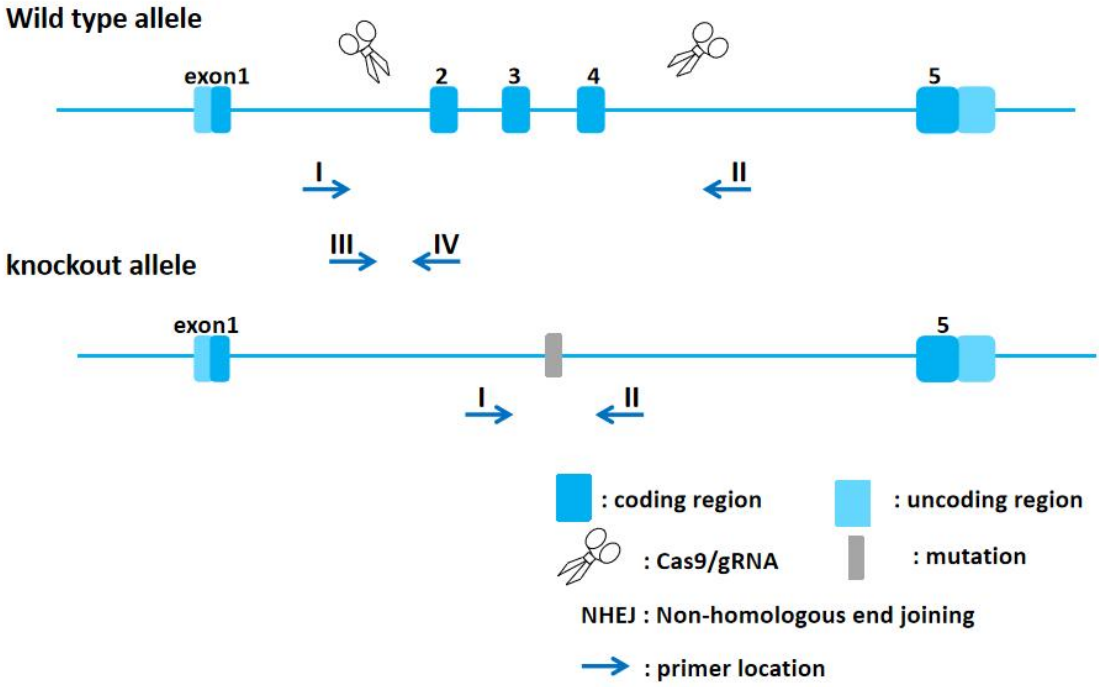
Editing Genes, Decoding the Mystery of Life

Acr-KO Genotyping Protocol



Common Name	Acr-KO	Cat. NO.	NM-KO-240258
Strain of Origin	C57BL/6J	Version	V1

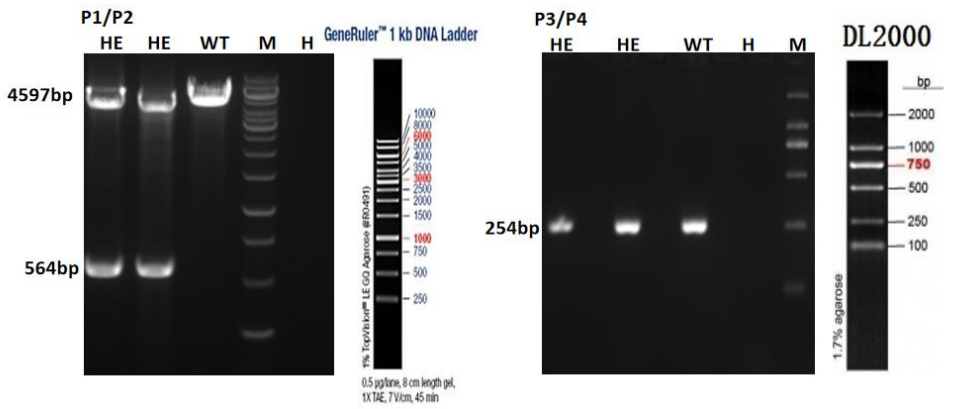
Genotyping strategy



Primers

Primer	Sequence (5’→3’)	Primer type
P1	CACTTGCTTGGGTCTCCCAT	Forward
P2	CCCTGAGGTGCCTTGCAATA	Reverse
P3	GTCCAGGGTTTCCCGTCAAT	Forward
P4	CGGGTACCTGCTTGTGAGTT	Reverse

Expected results

Results	
Genotype	Knockout type: -4033 bp Wild type: P1P2 =4597 bp; P3P4 =254 bp Heterozygote: P1P2 =4597 bp and 564 bp; P3P4 =254 bp Homozygote: P1P2 =564 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	