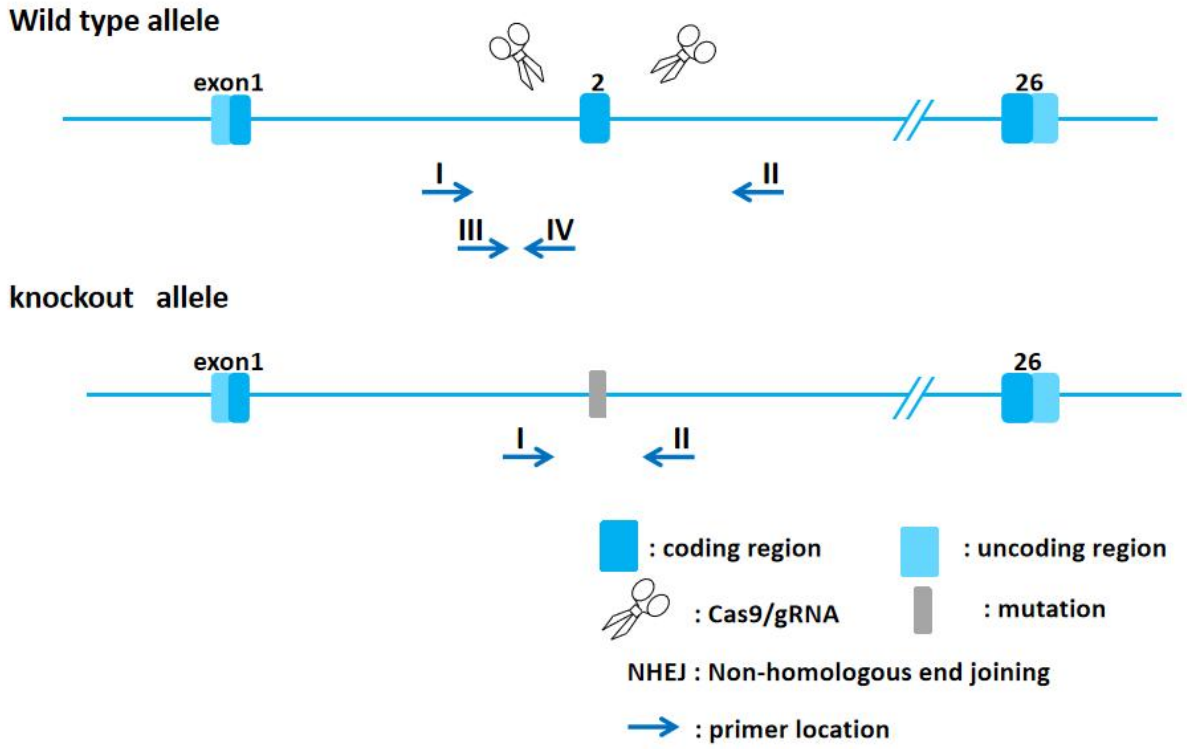
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Adam23-KO Genotyping Protocol

Common Name	Adam23-KO	Cat. NO.	NM-KO-240433
Strain of Origin	C57BL/6J	Version	V1

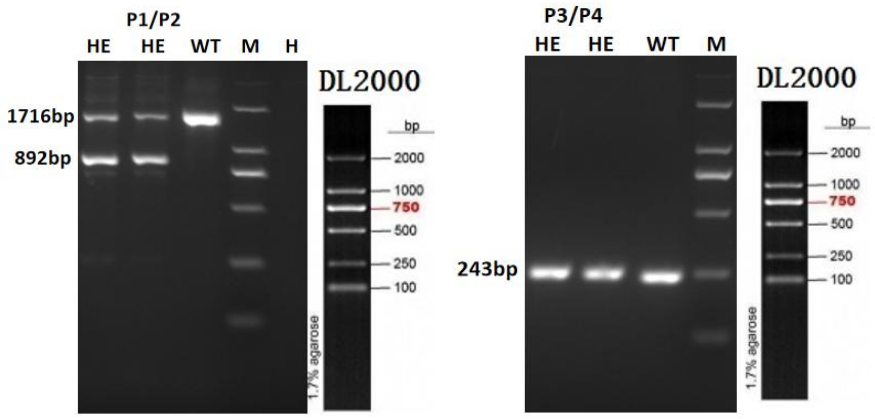
Genotyping strategy



Primers

Primer	Sequence (5’→3’)	Primer type
P1	GCTGGCCTTGGCAGTAAAAA	Forward
P2	CCGCACTGTGTAGACTTGCT	Reverse
P3	TGTCTGGAGTGTTCTTGGCTC	Forward
P4	AGTGGTTTGGCCTGGATAGG	Reverse

Expected results

Results	
Genotype	Knockout type: -824bp Wild type: P1P2 =1716 bp; P3P4=243 bp Heterozygote: P1P2 =1716 bp and 892 bp; P3P4=243 bp Homozygote: P1P2 =892 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×Taq Plus 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×Taq Plus Master Mix from Vazyme(Code Number: P222-1)			
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	