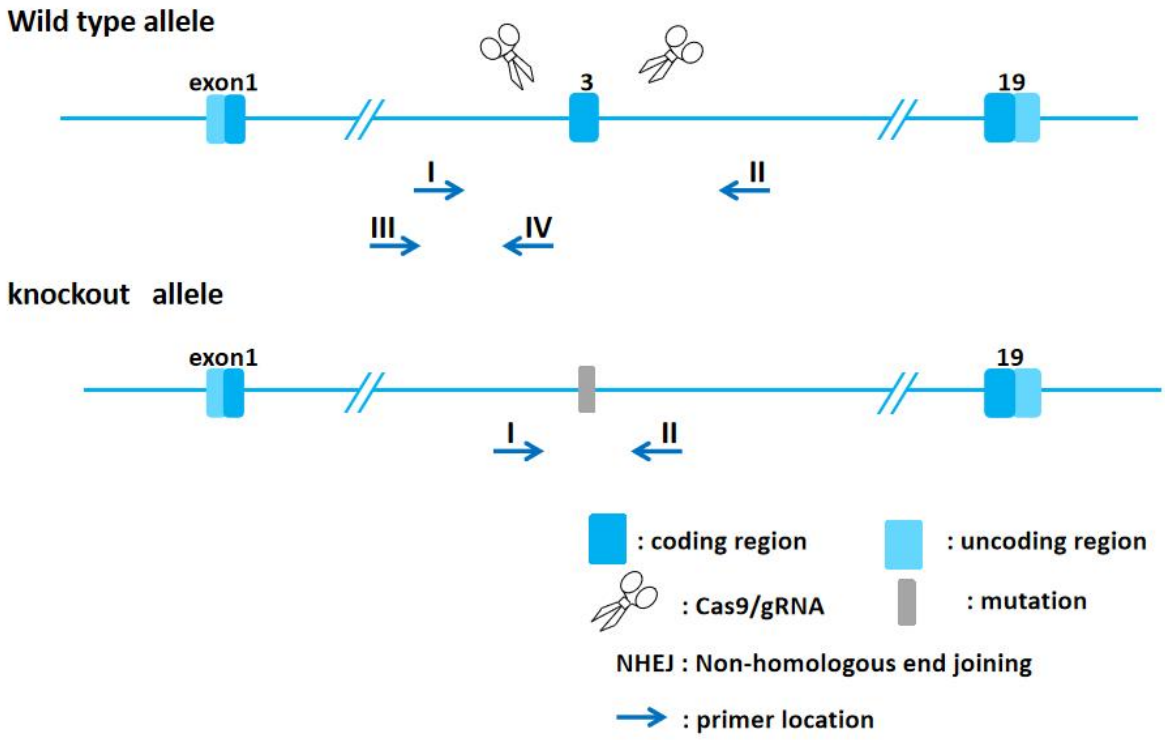
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Cd44-KO Genotyping Protocol

Common Name	Cd44-KO	Cat. NO.	NM-KO-190039
Strain of Origin	C57BL/6J	Version	V1

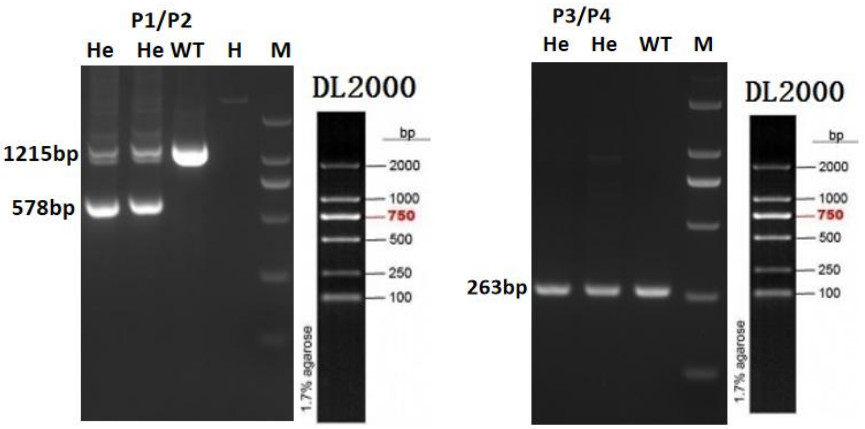
Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	TGGGTGTGGGAGTGGATAGT	Forward
P2	GGTTTCACAGGCCTTCCCAT	Reverse
P3	AACGGAAGGCCAATTCACTT	Forward
P4	AGACGGACAGAGGCAAAACTA	Reverse

Expected results

Results	
Genotype	Knockout type: -637bp Wild type: P1P2 =1215 bp; P3P4=263 bp Heterozygote: P1P2 =1215 bp and 578 bp; P3P4=263 bp Homozygote: P1P2 =578 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	