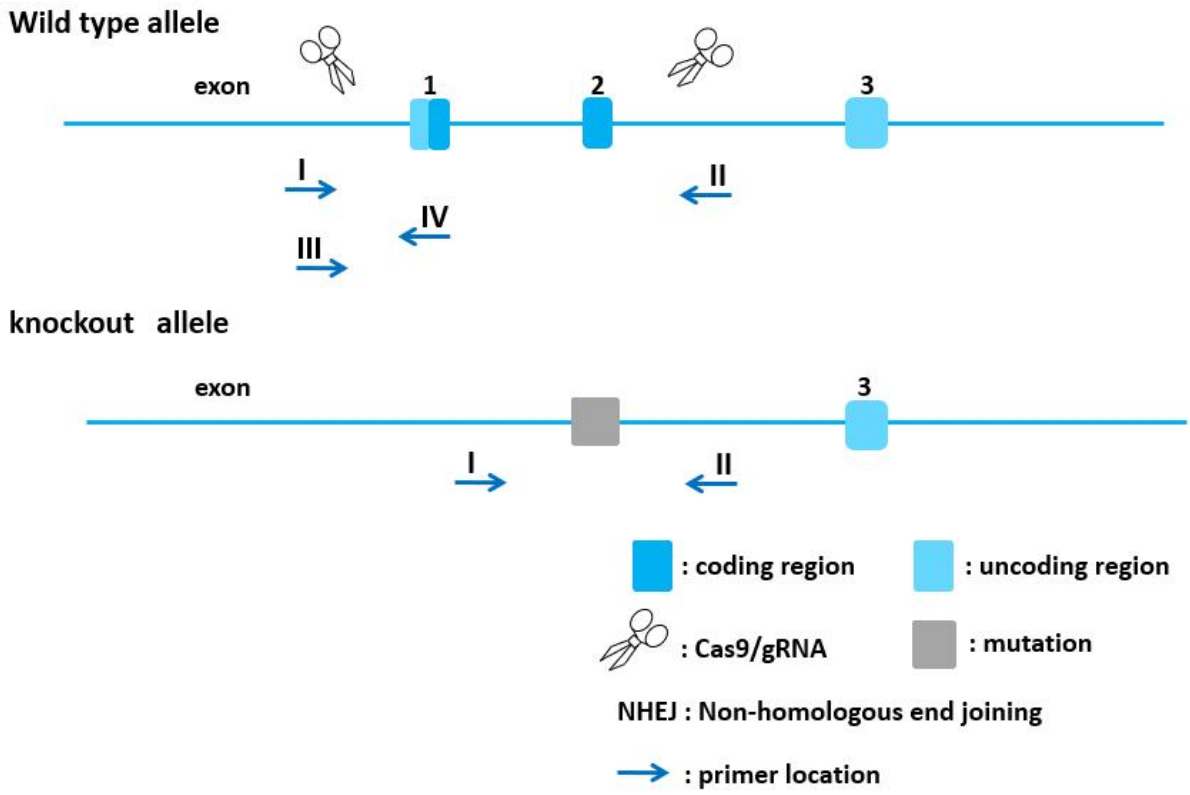
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Wfdc17-KO Genotyping Protocol

Common Name	Wfdc17-KO	Cat. NO.	NM-KO-240671
Strain of Origin	C57BL/6J	Version	V1

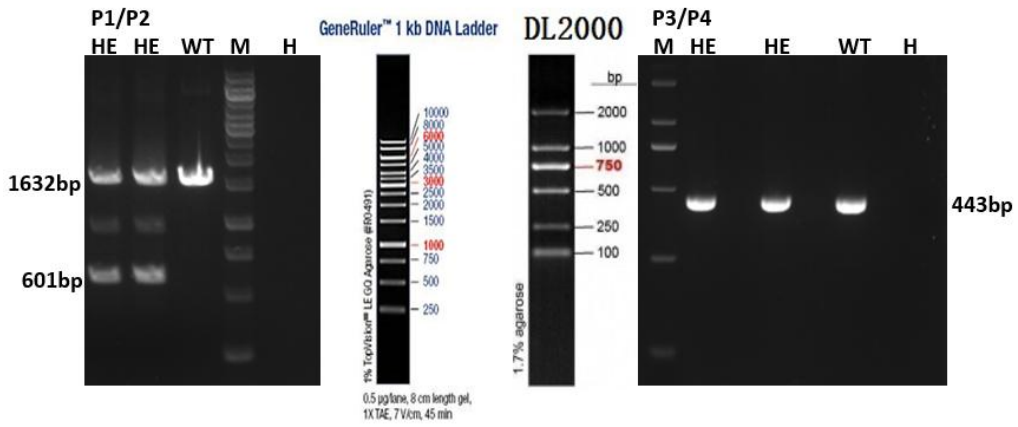
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TACTGGCTGTCTCTGCTCCA	Forward
P2	CCAAGGAAAGTCCCCTGGAAA	Reverse
P3	GCCCTGACACTCCTTACTGG	Forward
P4	ACCTTTGGGGCAAGACACTA	Reverse

Expected results

Results	
Genotype	Knockout type：-1031bp Wild type：P1P2 =1632 bp；P3P4 =443 bp Heterozygote：P1P2 =1632 bp and 601 bp；P3P4 =443 bp Homozygote：P1P2 =601 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			8.0
	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℃	5 min	
	2	95℃	20 sec	
	3	60℃	20 sec	
	4	72℃	20 sec	35 repeats to 2
	5	72℃	5 min	
	6	12℃	Hold	