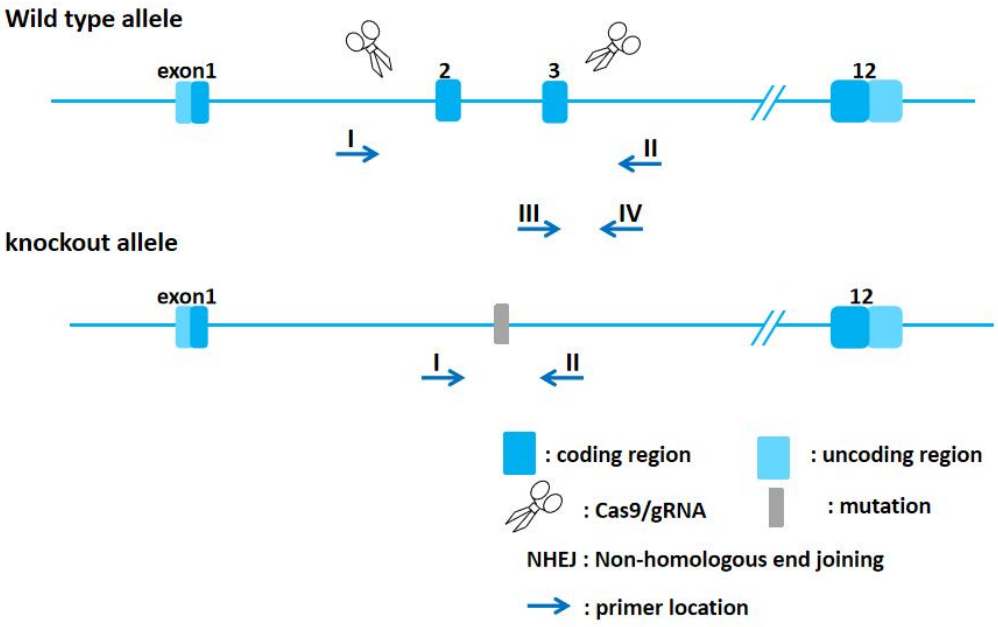
SHANGHAI MODEL ORGANISMS

Editing Genes, Decoding the Mystery of Life

Bnip5-KO Genotyping Protocol

Common Name	Bnip5-KO	Cat. NO.	NM-KO-231641
Strain of Origin	C57BL/6J	Version	V1

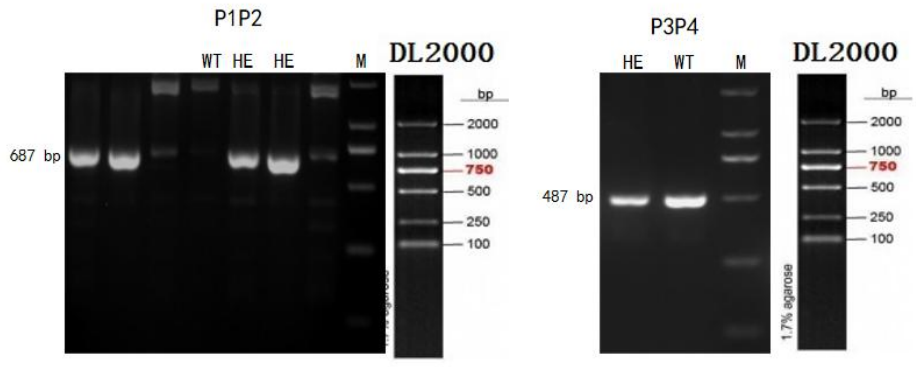
Genotyping strategy



Primers

Primer	Sequence (5' →3')	Primer type
P1	CAGGGCAATGGGTGGGTATT	Forward
P2	CCATCTCTCCAGCCCCAATG	Reverse
P3	TTCTGATTTGGGGTGAGGTTTAG	Forward
P4	AAAGAAATTGAAAGCAAAGCCAC	Reverse

Expected results

Results	

Genotype	Knockout type: -6722 (2220-8941) bp Wild type: P3P4=487 bp Heterozygote: P1P2 =687 bp; P3P4=487 bp Homozygote: P1P2 =687 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	