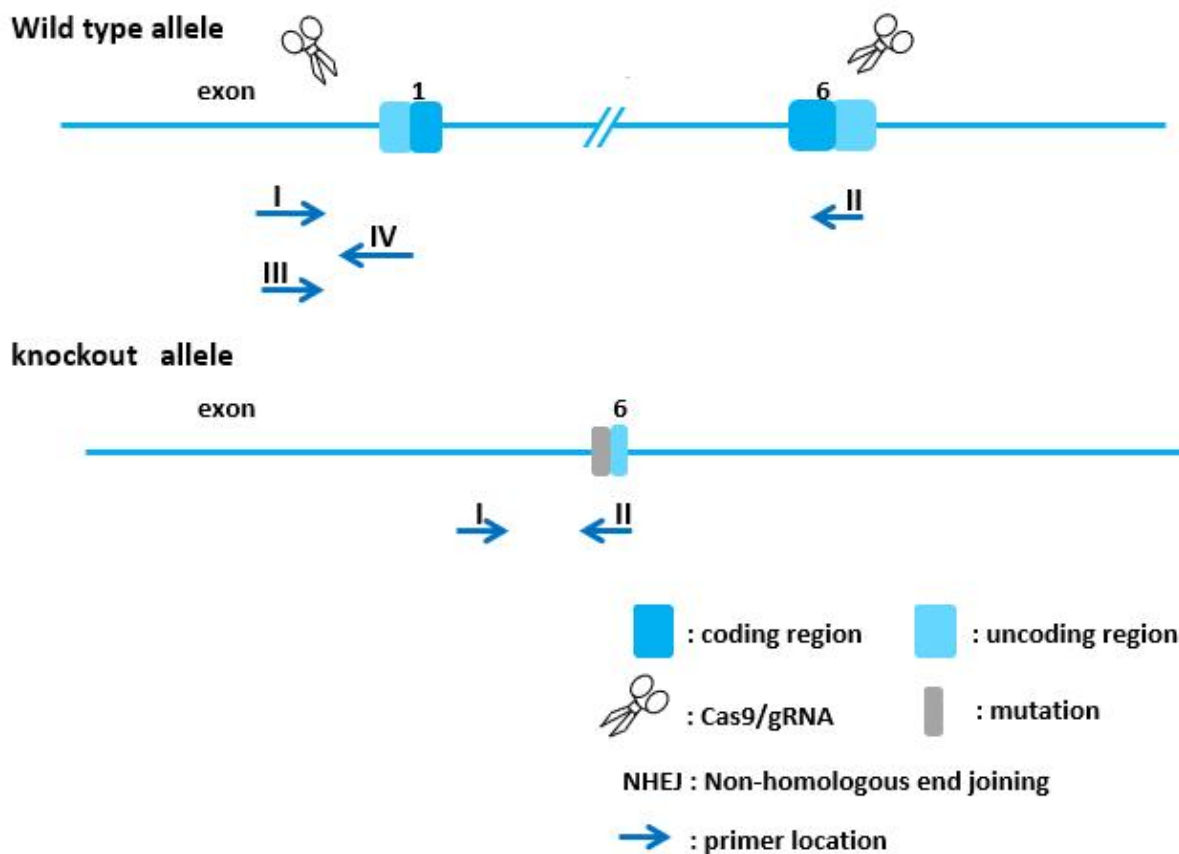


Zfp263-KO Genotyping Protocol



Common Name	Zfp263-KO	Cat. NO.	NM-KO-241016
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	CCCGACATGACACTCTAGC	Forward
P2	GAGCCCAAACCTCTCCTGCTT	Reverse
P3	ACTCTAGCGCCTCTGGACTC	Forward
P4	GGAACCCAGGTCACAATCTCA	Reverse

Expected results

Results	P1/P2 HE HE WT M H 6451bp 555bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT H M 449bp DL2000 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -5896bp Wild type: P1P2 =6451 bp; P3P4 =449 bp Heterozygote: P1P2 =6451 bp and 555 bp; P3P4 =449 bp Homozygote: P1P2 =555 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)
	ddH ₂ O			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	