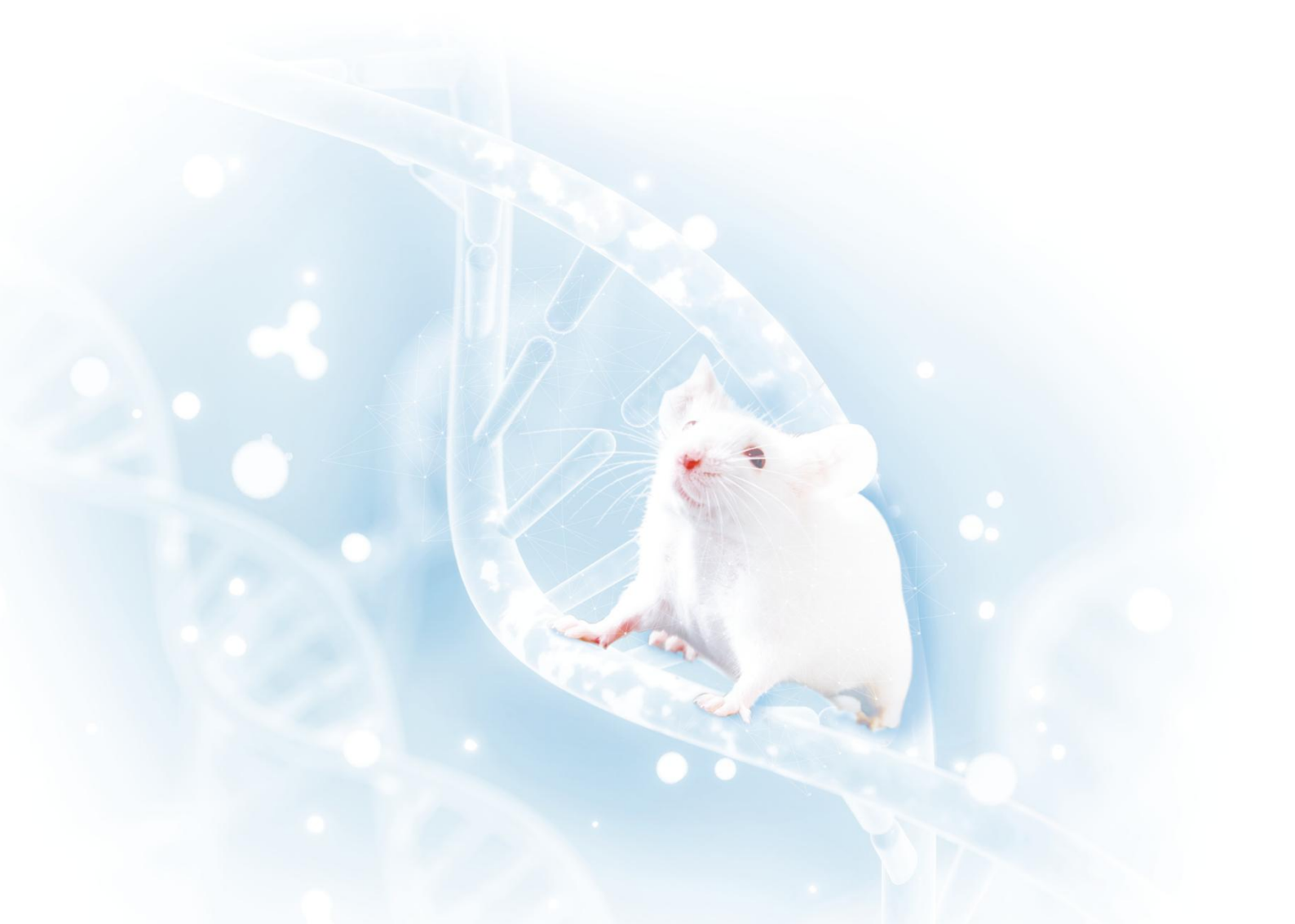
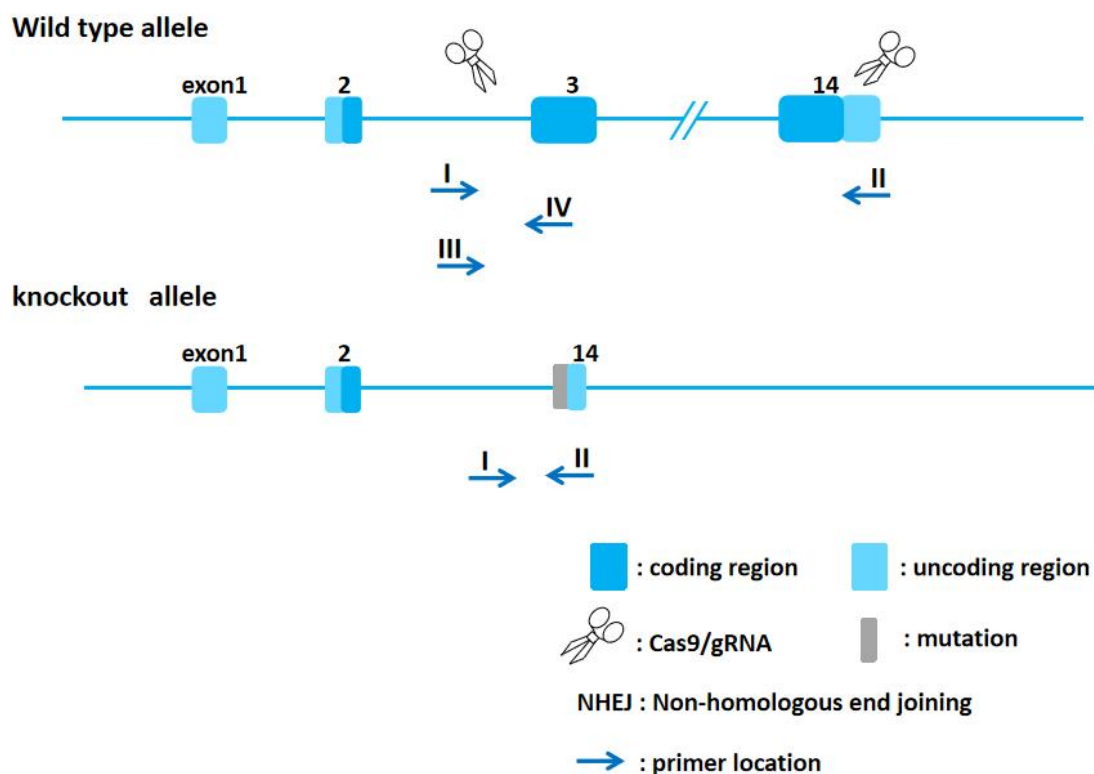


Znfx1-KO Genotyping Protocol



Common Name	Znfx1-KO	Cat. NO.	NM-KO-240673
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	ACAGCCAGGGCTACATACTAA	Forward
P2	TATGCAAGTTAGGGCCTGGAC	Reverse
P3	TGTGTTATGGGCACGTGTGT	Forward
P4	CGGAAAGGAGCAGGATGGTT	Reverse

Expected results

Results	P1/P2 H M WT HE 533bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1800 1600 1500 1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 bp 413bp P3/P4 HE HE WT H M DL2000 2000 1000 750 500 250 100 bp 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -20408bp Wild type: P3P4 =413 bp Heterozygote: P1P2 =533 bp; P3P4 =413 bp Homozygote: P1P2 =533 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	