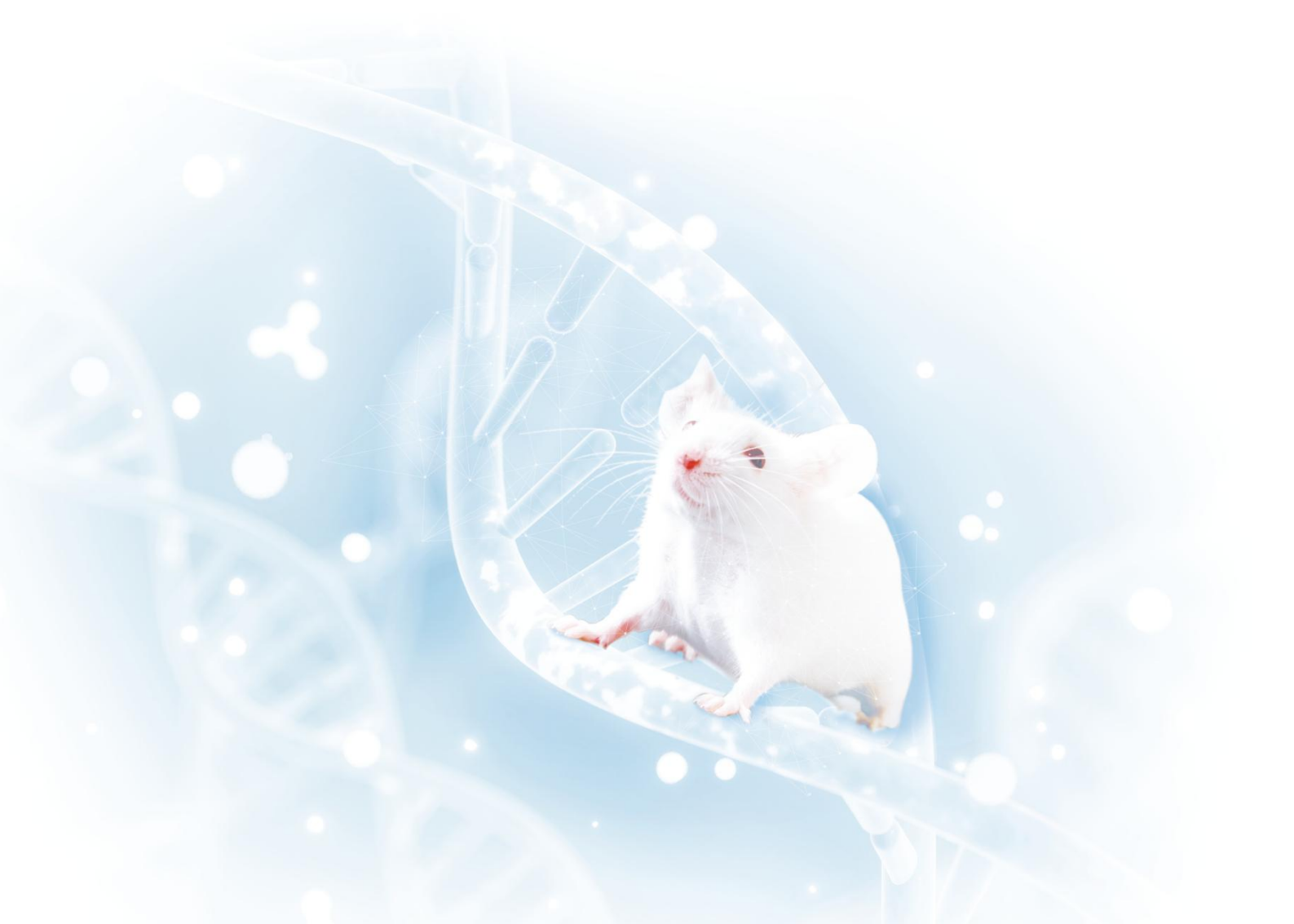


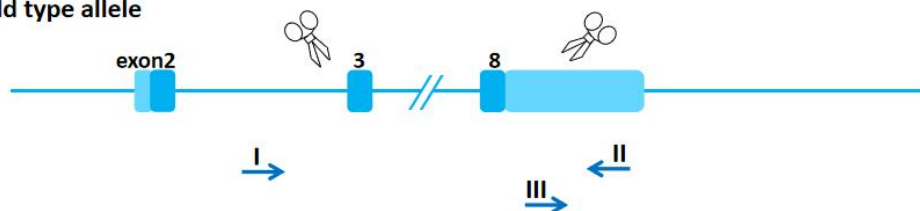
Tspan9-KO Genotyping Protocol



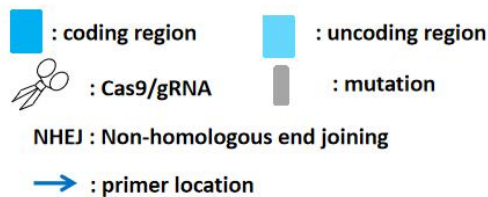
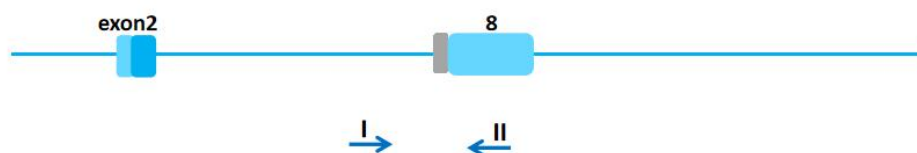
Common Name	Tspan9-K0	Cat. NO.	NM-K0-233166
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGTCAGCTCAATGGAGCATCAAA	Forward
P2	CCACAGGAAAAGGAACTCAAACC	Reverse
P3	CTTAGACCTCATGGAGGGCAGAG	Forward

Expected results

Results	
Genotype	Knockout type: -3845 bp Wild type: P2P3 =458 bp Heterozygote: P1P2 =658 bp; P2P3=458 bp Homozygote: P1P2 =658 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 K0 band

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH2O			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	P3 (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	