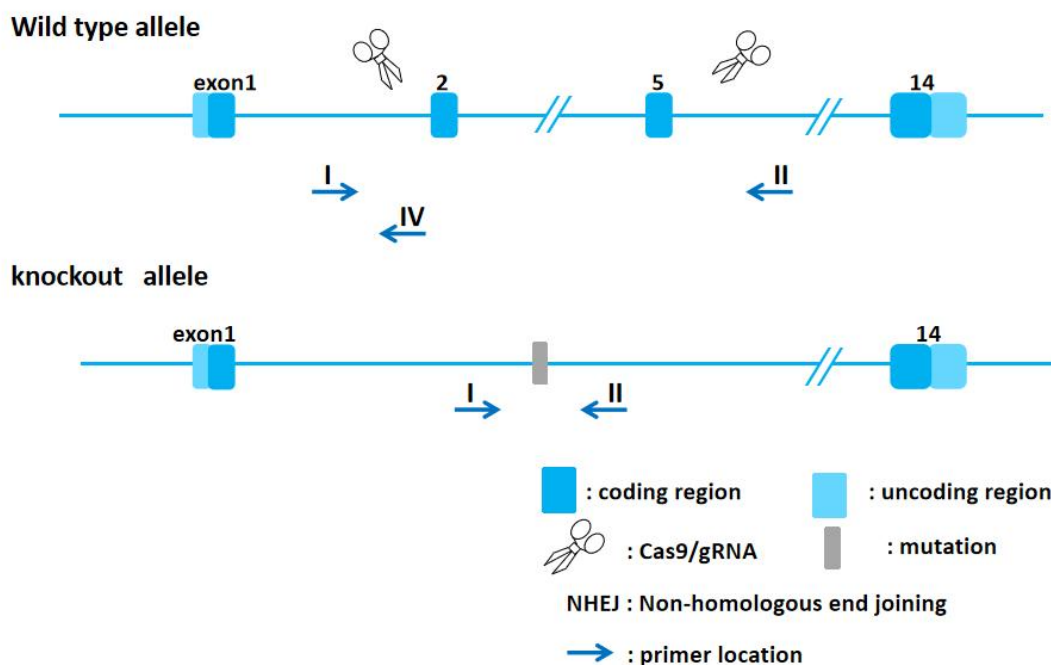


Ttyh3-K0 Genotyping Protocol



Common Name	Ttyh3-KO	Cat. NO.	NM-KO-234834
Strain of Origin	C57BL/6J	Version	V1

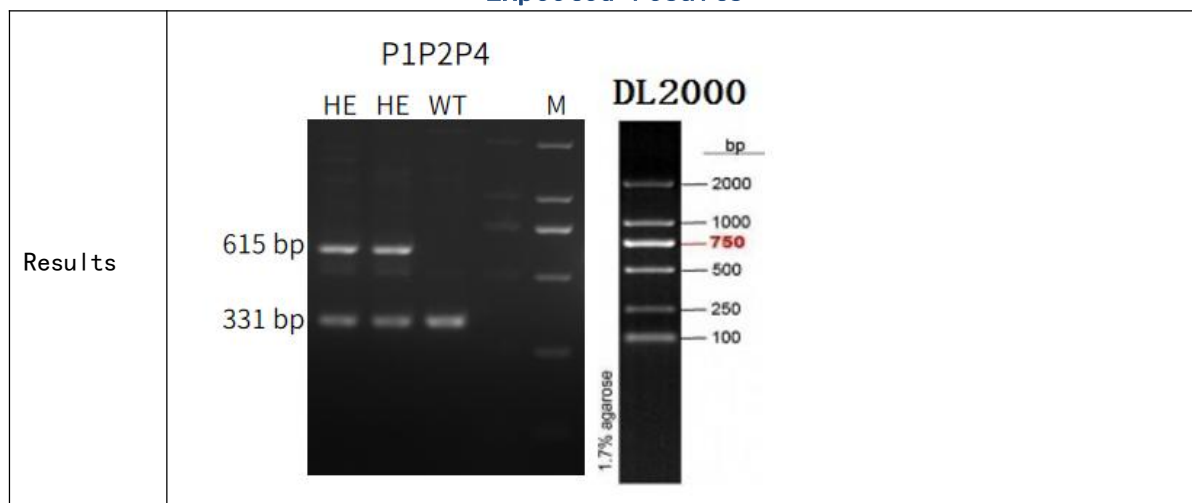
Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CTTTGTTGAACCTTGTCCCTT	Forward
P2	CGCCATCAGCACCAGTTAGTC	Reverse
P4	CATGTTAGCCCCTCTTCCCAGT	Reverse

Expected results



Genotype	Knockout type: -1898bp
	Wild type: P1P4=331 bp
	Heterozygote: P1P2 =615 bp; P1P4=331 bp
	Homozygote: P1P2 =615 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			7.5
	2×Taq Plus Master Mix			10.0
	P1 (10 pmol/μl)			0.5
	P2 (10 pmol/μl)			0.5
	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	