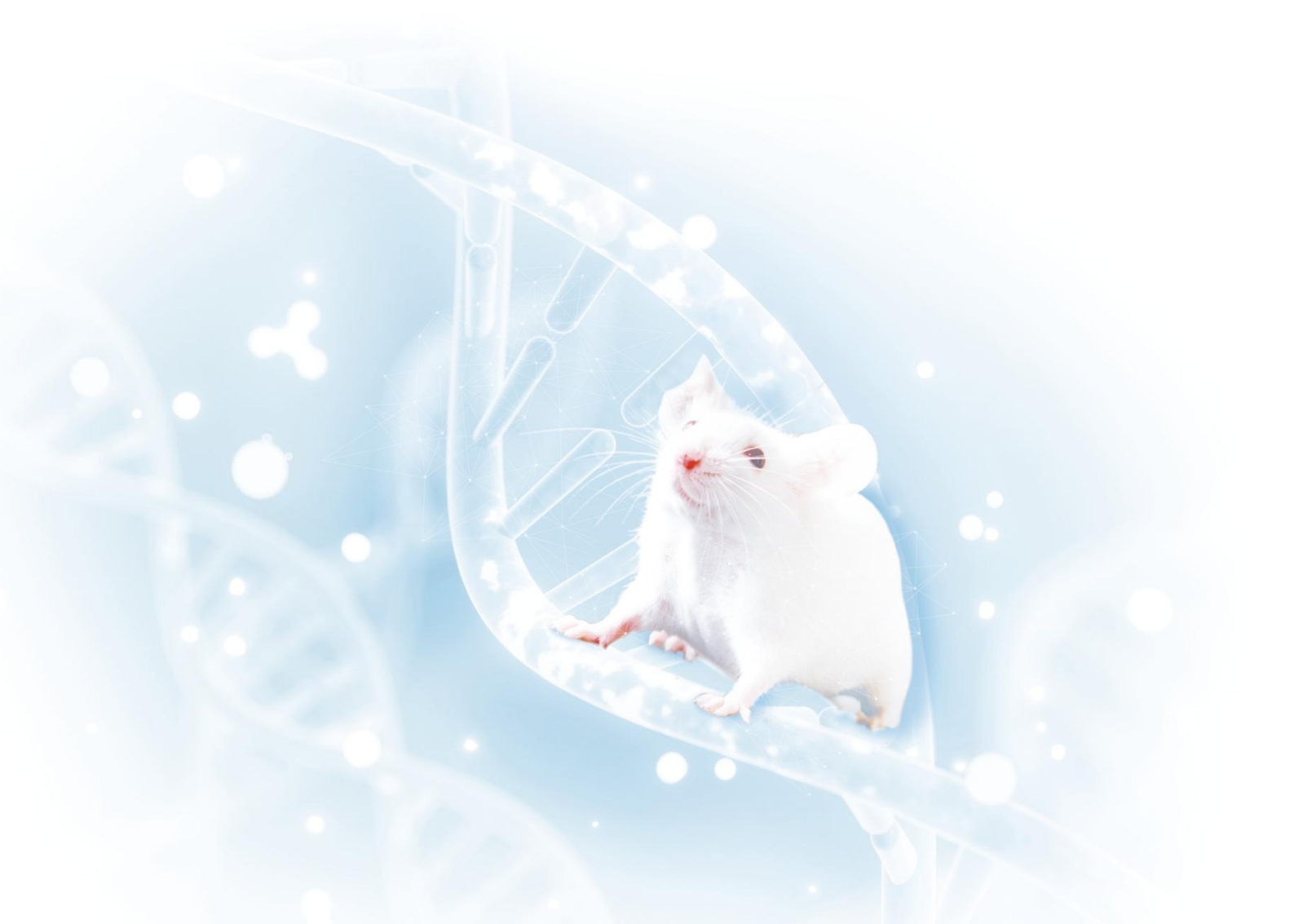
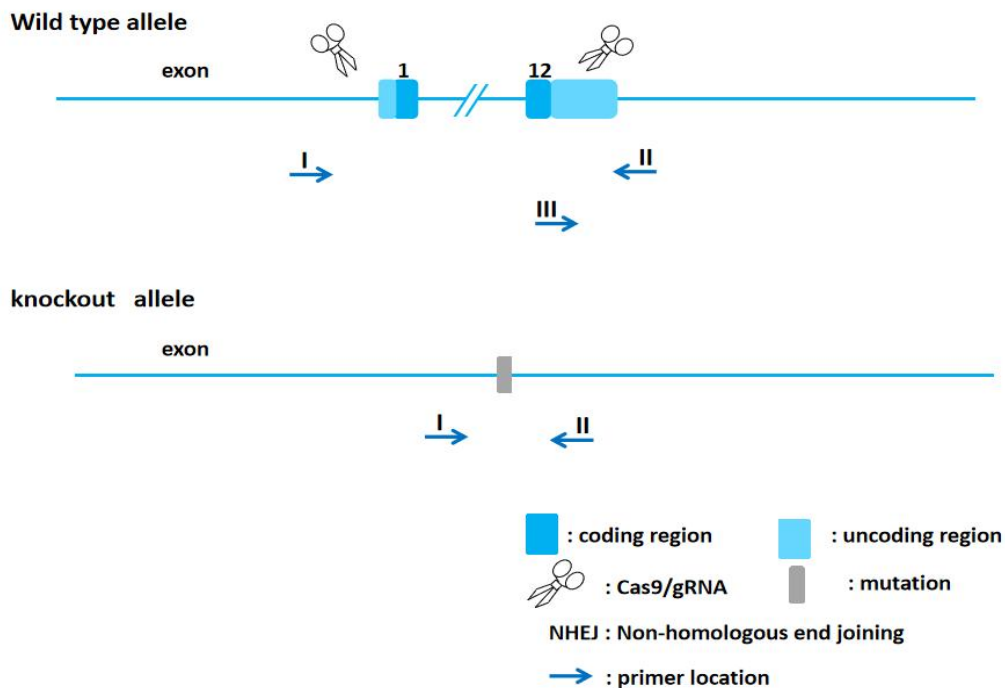


Ttc14-K0 Genotyping Protocol



Common Name	Ttc14-K0	Cat. NO.	NM-K0-232690
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GGGCAGACAGGACTGAGGAAA	Forward
P2	CATCTGCATCAAGCCTTAGACATT	Reverse
P3	GAGCAGCCAGACAGCAGAGTTAA	Forward

Expected results

Results	P1P2P3 HE WT H₂O M DL2000
Genotype	Knockout type: -9470 bp Wild type: P2P3 =598 bp Heterozygote: P1P2 =685 bp; P2P3=598 bp Homozygote: P1P2 =685 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	