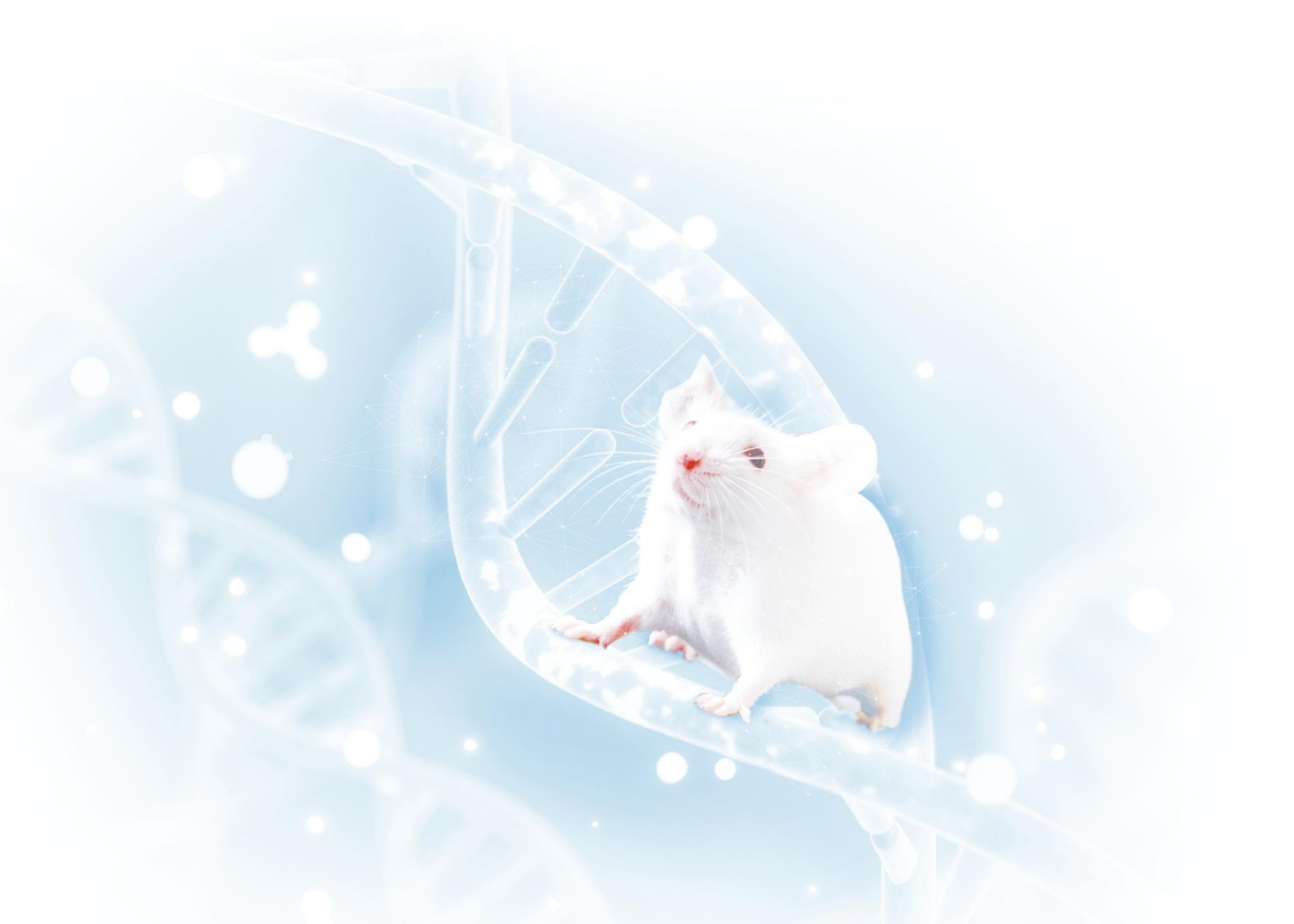


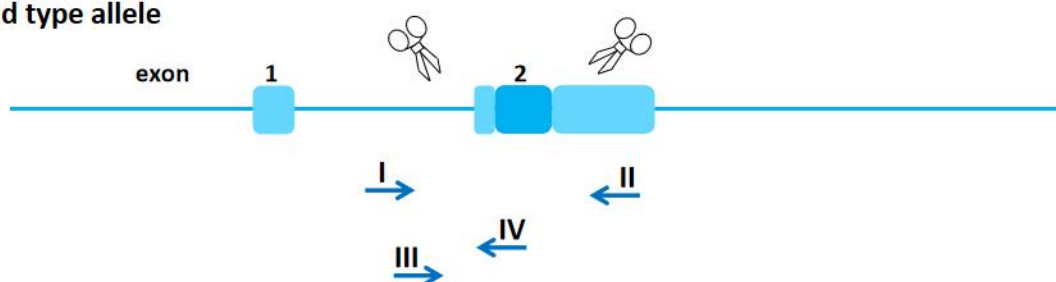
Tor4a-K0 (FVB) Genotyping Protocol



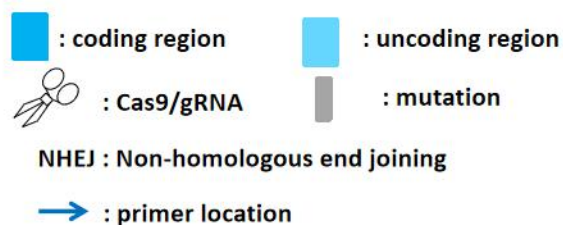
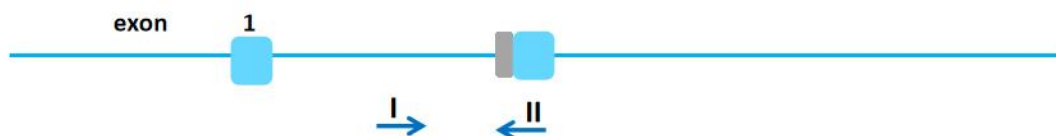
Common Name	Tor4a-K0 (FVB)	Cat. NO.	NM-K0-240034
Strain of Origin	FVB	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GGGTTGGAAGGCCTAGGTTG	Forward
P2	GGACCAGAATCCTACCGCAG	Reverse
P3	TCAGTGACAACGAGCCTTCTG	Forward

P4	CGCTATCACACATGGTCCCT	Reverse
----	----------------------	---------

Expected results

Results	The figure shows two gel electrophoresis results. The left gel is for P3/P4 primers, showing bands at 369bp for HE, WT, and M lanes. The right gel is for P1/P2 primers, showing bands at 2160bp and 660bp for HE, HE, and M lanes. Ladders are shown for both gels.
Genotype	Knockout type: -1500 bp Wild type: P1P2 =2160 bp; P3P4=369 bp Heterozygote: P1P2 =2160 bp and 660 bp; P3P4=369 bp Homozygote: P1P2 =660 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 Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	