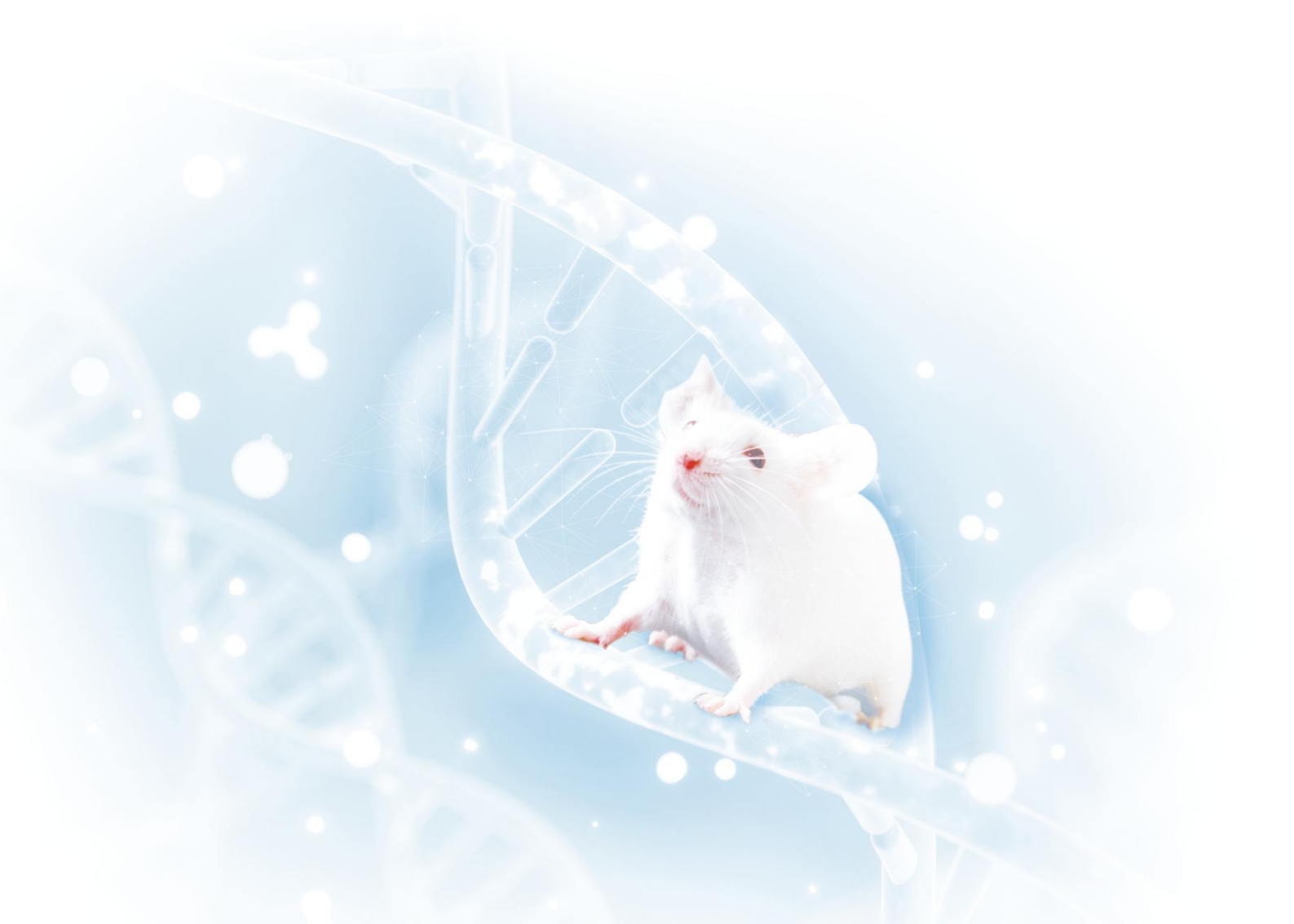


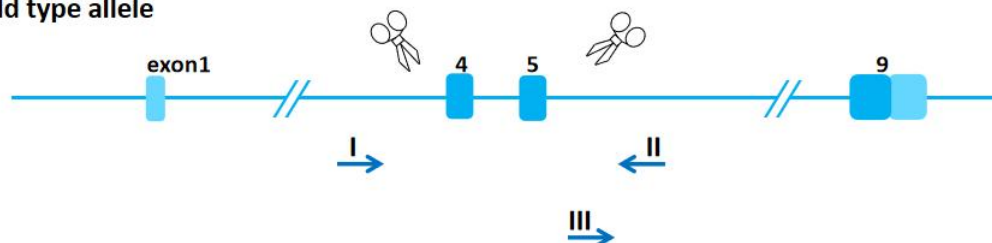
Tnfaip3-K0 Genotyping Protocol



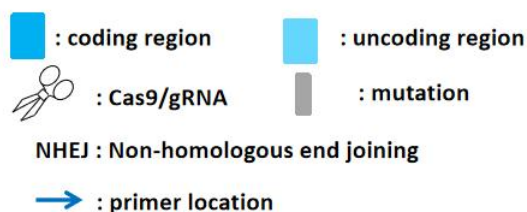
Common Name	Tnfaip3-KO	Cat. NO.	NM-KO-233467
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TTCAGCCACAAGCTGGAAT	Forward
P2	ACCTGCTCTGTGCCAAACAT	Reverse
P3	CGGAGGATTAAATATCCCTTCTGT	Forward

Expected results

Results	P1P2P3 518 bp 345 bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -916bp Wild type: P3P2=345 bp Heterozygote: P1P2 =518 bp; P3P2=345 bp Homozygote: P1P2 =518 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	