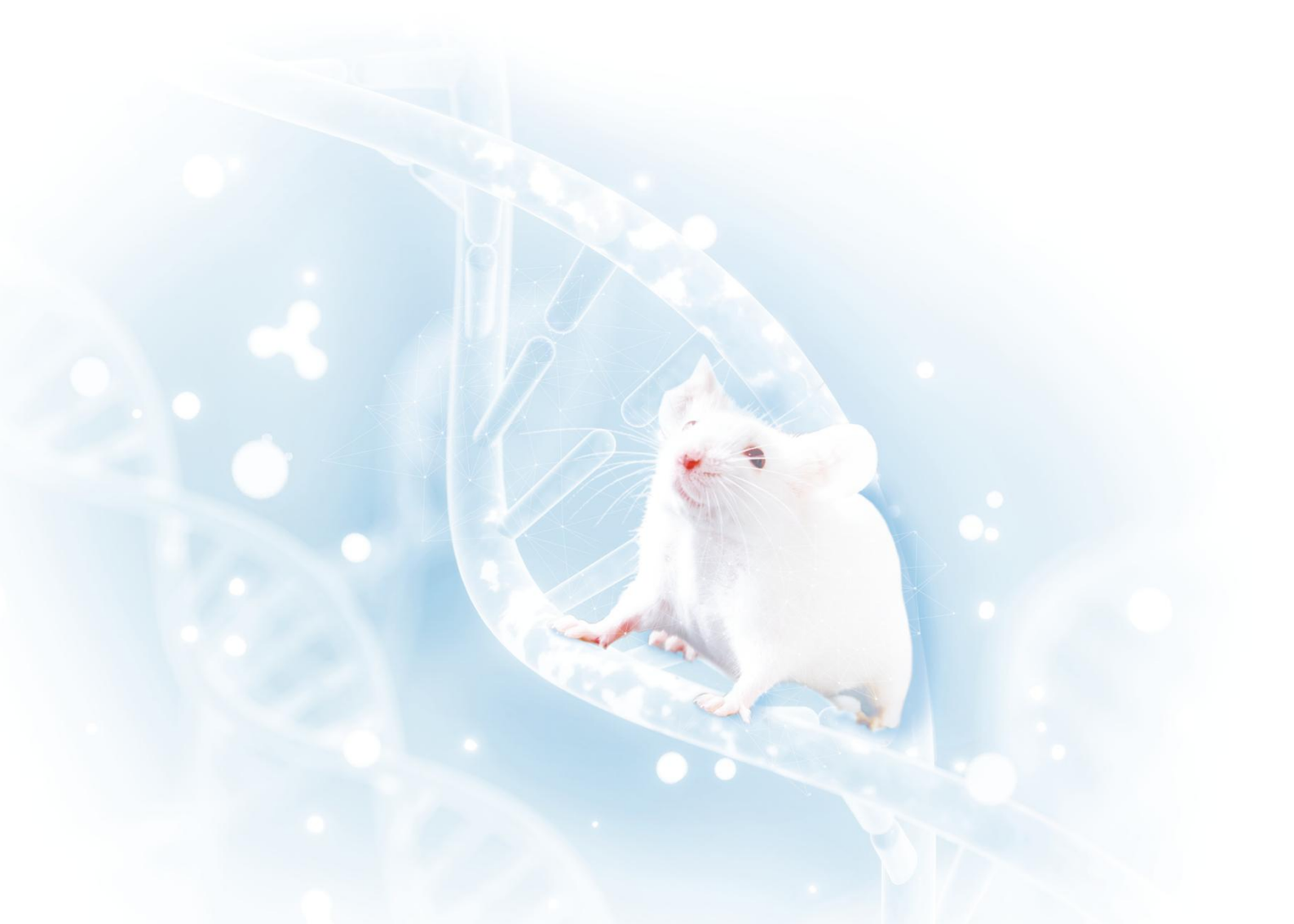
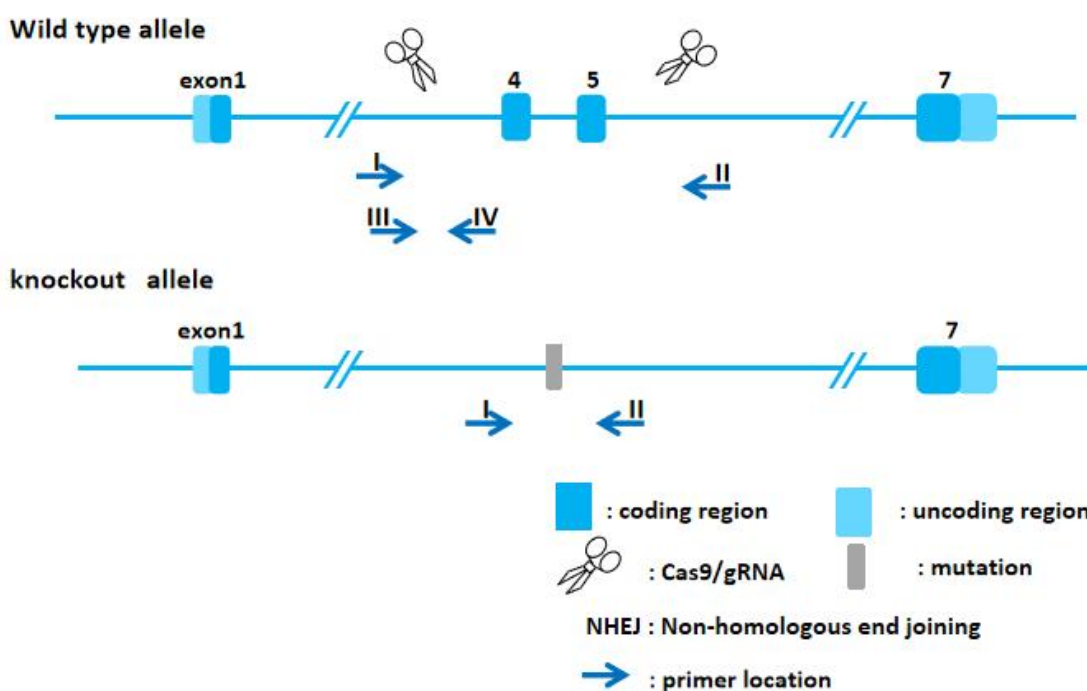


Tgfb3-K0 Genotyping Protocol



Common Name	Tgfb3-KO	Cat. NO.	NM-KO-233439
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GTCTCCATGGTGATGGTGA	Forward
P2	AATGTGAGCAGCTACTTCAAGC	Reverse
P3	CTTGTTGCTCAGAGGTTTCTCAGTT	Forward
P4	AATAGTGGGCATTGTGGAGGGT	Reverse

Expected results

Results	P1/P2 HE HE WT M 2919bp 400bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1% Tris/Viskri™ LE GO Agarose #R0491 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT M 223bp DL2000 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -2519 bp Wild type: P1P2 =2919 bp; P3P4 =223 bp Heterozygote: P1P2 =2919 bp and 400 bp; P3P4 =223 bp Homozygote: P1P2 =400 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

	Reaction Component	Volume (µl)
PCR	ddH2O	8.0
Reaction	2 × Rapid Taq Master Mix	10.0
System	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	