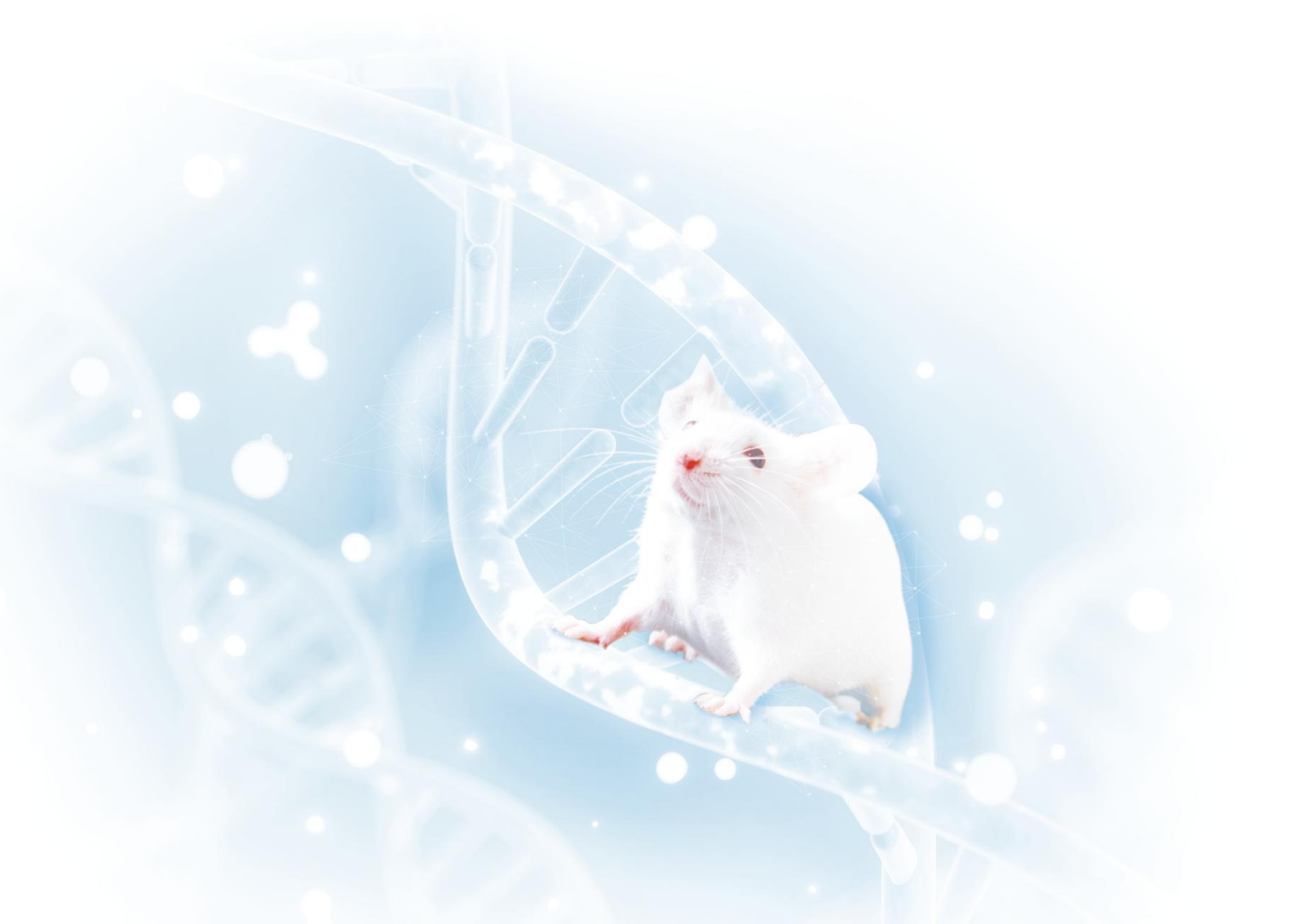


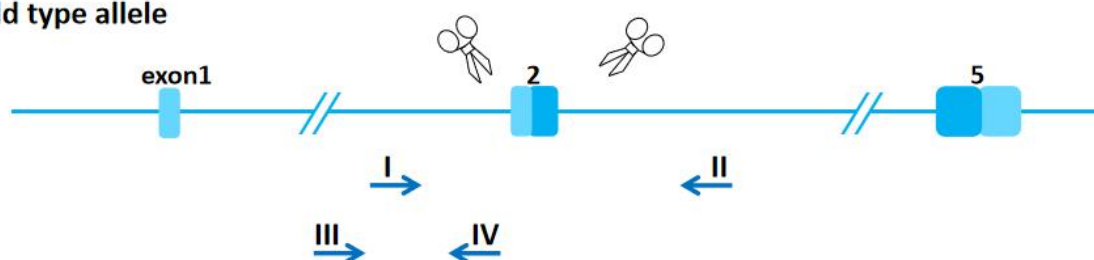
Tcf20-KO Genotyping Protocol



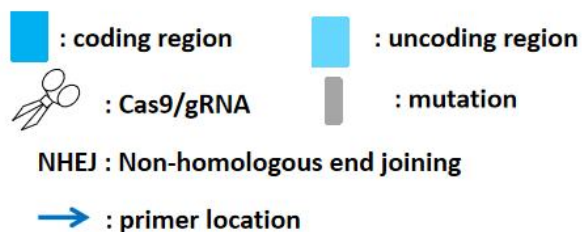
Common Name	Tcf20-KO	Cat. NO.	NM-KO-230134
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



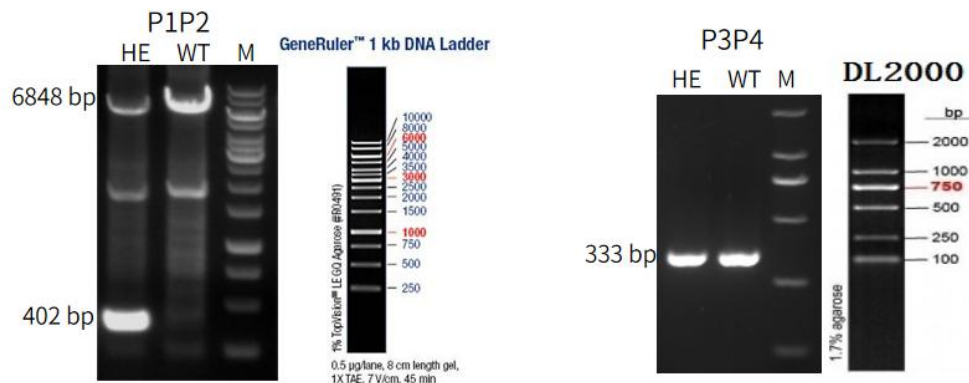
knockout allele



Primers

Primer	Sequence (5' →3')	Primer type
P1	GTCTCTTGCTCTTGGTGGCT	Forward
P2	TCAGCCTGGTCCACAGAGTA	Reverse
P3	GGCTGGAGAGAACAGTCCAC	Forward
P4	CGGAGCAGTTGTTTCAGGAG	Reverse

Expected results

Results	 P1P2 HE WT M 6848 bp 402 bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3P4 HE WT M 333 bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Wild type: P1P2 =6848 bp; P3P4=333 bp Heterozygote: P1P2 =6848 bp and 402 bp; P3P4=333 bp Homozygote: P1P2 =402 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (µl)
	ddH2O			8.0
	2×Taq Plus 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×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	