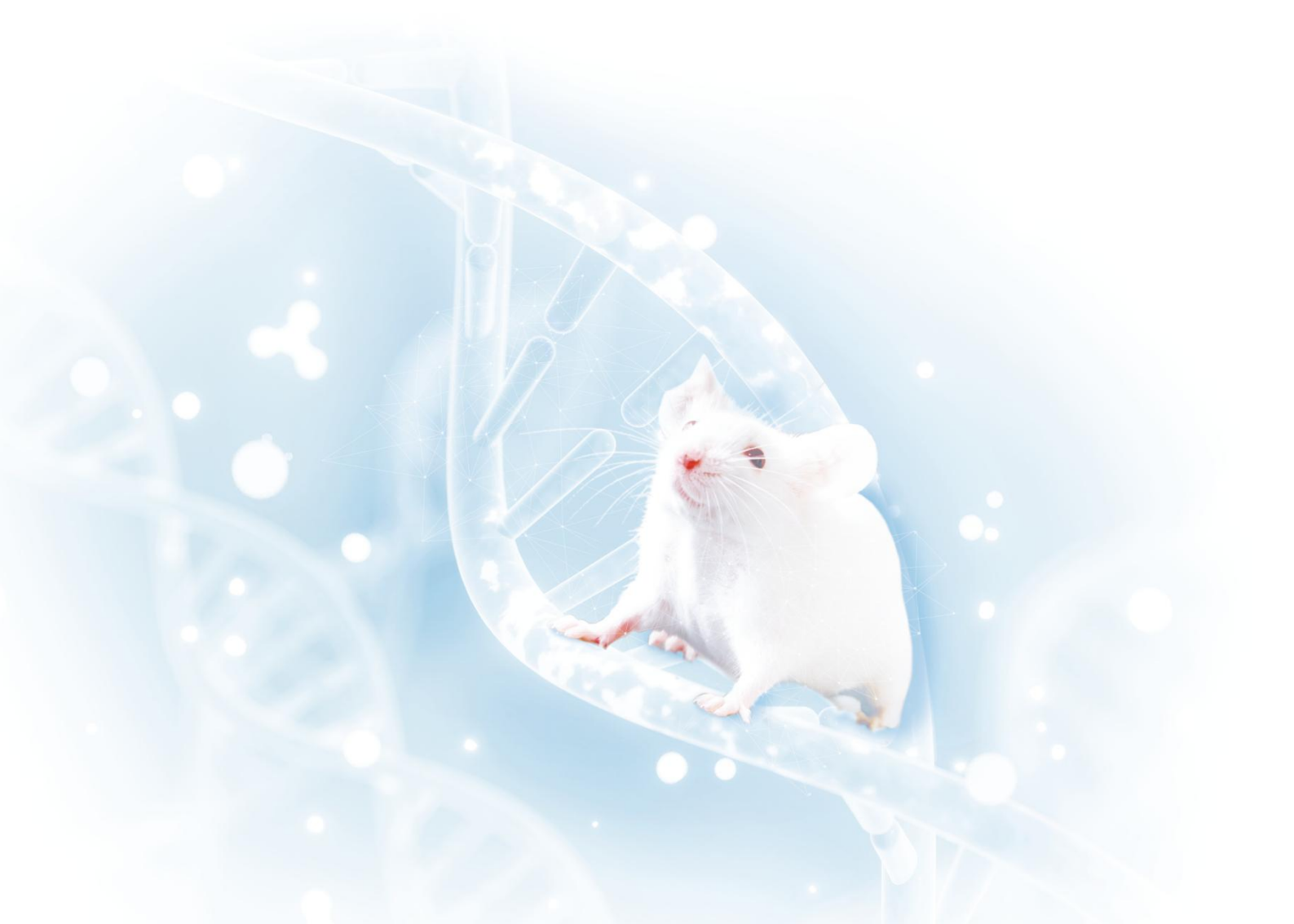


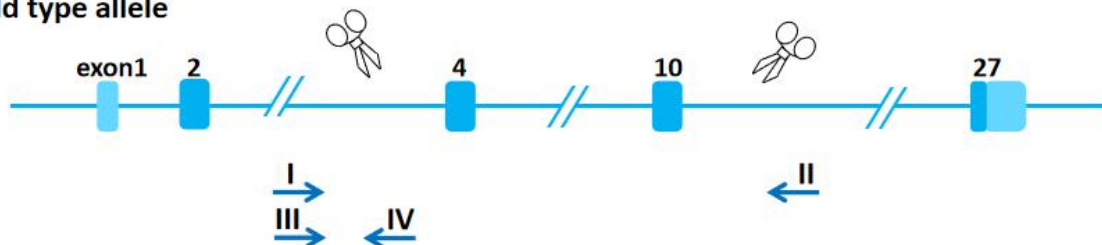
Pold1-KO Genotyping Protocol



Common Name	Pold1-KO	Cat. NO.	NM-KO-2106548
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



: coding region : uncoding region

: Cas9/gRNA : mutation

NHEJ : Non-homologous end joining

→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	TATGTGGGTGAGTTGGCAGG	Forward
P2	CCCAGGACACAATGGTAGGG	Reverse
P3	TATGTGGGTGAGTTGGCAGG	Forward
P4	GGCCCACTGAAGGATGTCA	Reverse

Expected results

Results	0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -2410 bp Wild type: P1P2 =2932 bp; P3P4 =484 bp Heterozygote: P1P2 =2932 bp and 522 bp; P3P4 =484 bp Homozygote: P1P2 =522 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 System	Reaction Component			Volume (µl)
	ddH ₂ O			8.0
	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	