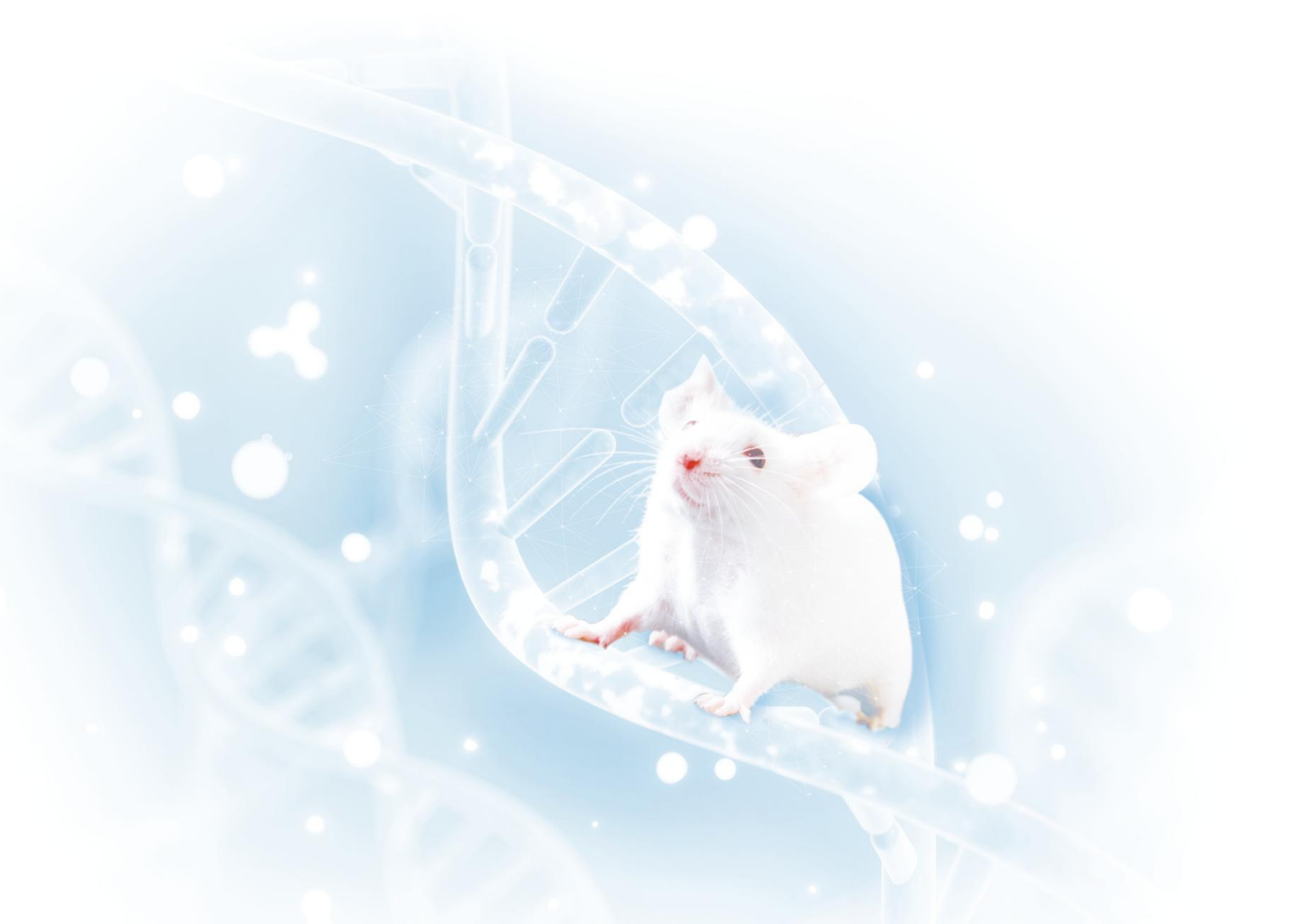


Cd274-K0 Genotyping Protocol

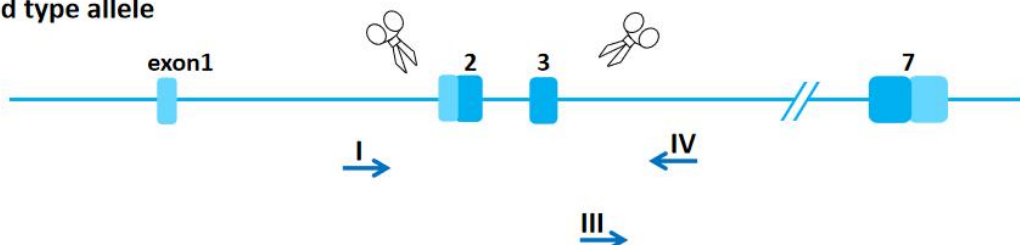


Common Name	Cd274-KO	Cat. NO.	NM-KO-225091
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Type 1:

Wild type allele



knockout allele

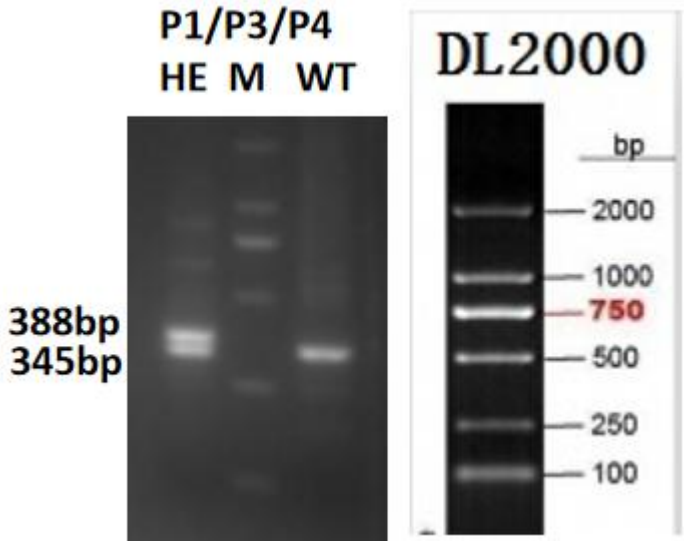


- : coding region
- : uncoding region
- : Cas9/gRNA
- : mutation
- NHEJ : Non-homologous end joining
- : primer location

Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGAATGAGCACACAGGTCAGAGA	Forward
P3	TGCTGCCCTTCAGATCACAG	Forward
P4	ACTGCAAAGCATGGCTGAGT	Reverse

Expected results

Results	
Genotype	Wild type: P3P4=345 bp Heterozygote: P1P4 =388 bp; P3P4=345 bp Homozygote: P1P4 =388 bp

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
	P3 (10 pmol/μl)			0.5
	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	