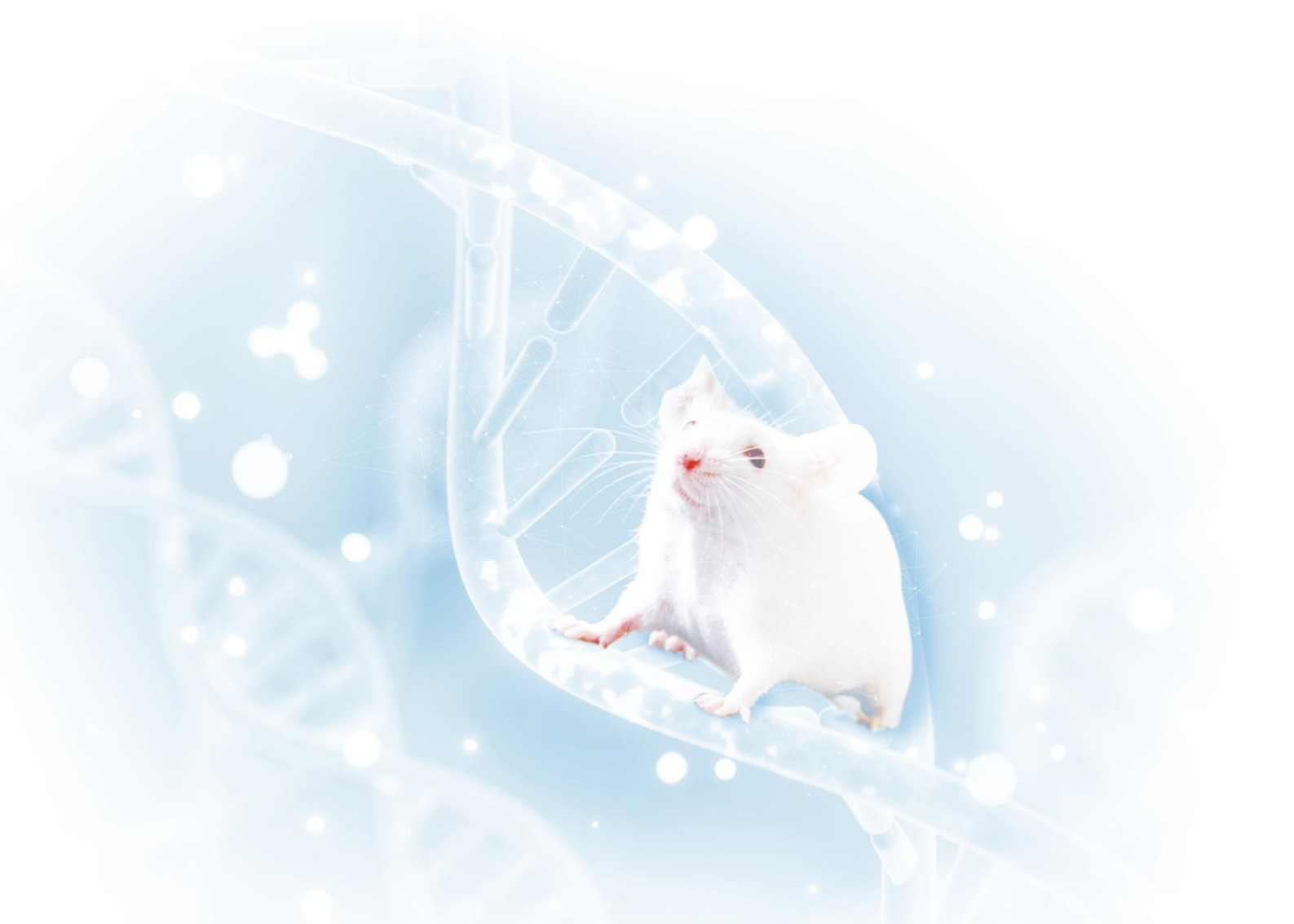


Gpx4-KO Genotyping Protocol

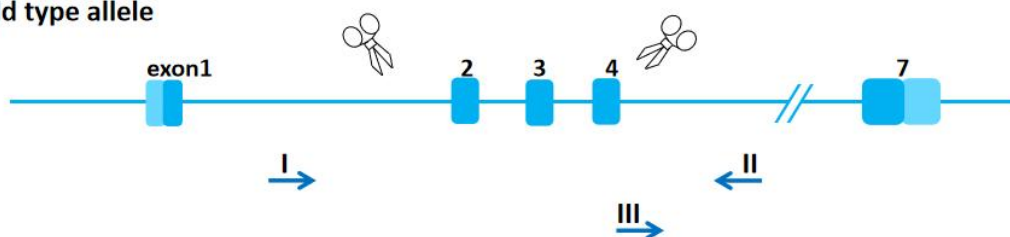


Common Name	Gpx4-KO	Cat. NO.	NM-KO-233589
Strain of Origin	C57BL/6J	Version	V1

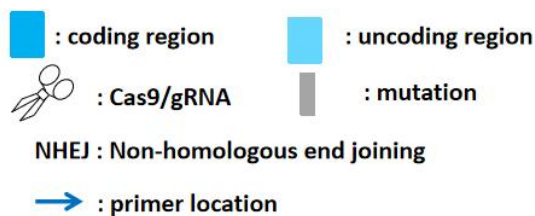
Genotyping strategy

Type 1:

Wild type allele



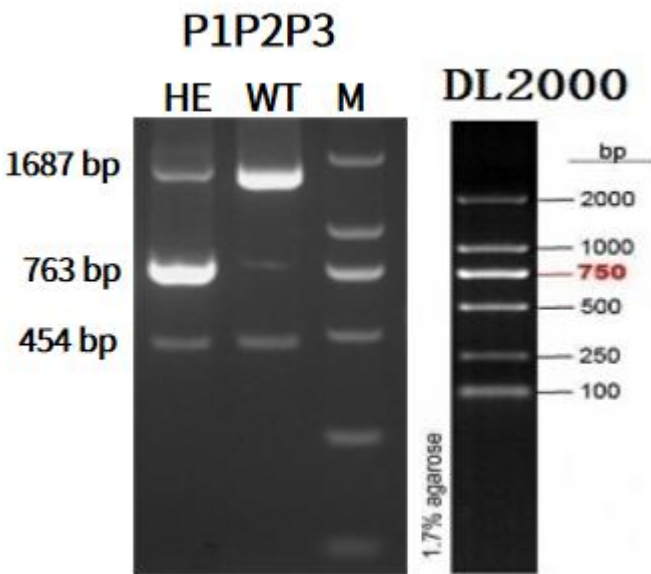
knockout allele



Primers

Primer	Sequence (5' →3')	Primer type
P1	AGGTGGGAAAGCGGAGCCTGATA	Forward
P2	CTGGCTGTCCTTAAAGAGCACTCAA	Reverse
P3	ACCCACTGTGGAAATGGATGAAA	Forward

Expected results

Results	
Genotype	Wild type: P1P2 =1687 bp; P3P2=454 bp Heterozygote: P1P2 =1687 bp and 763 bp; P3P2=454 bp Homozygote: P1P2 =763 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
	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	