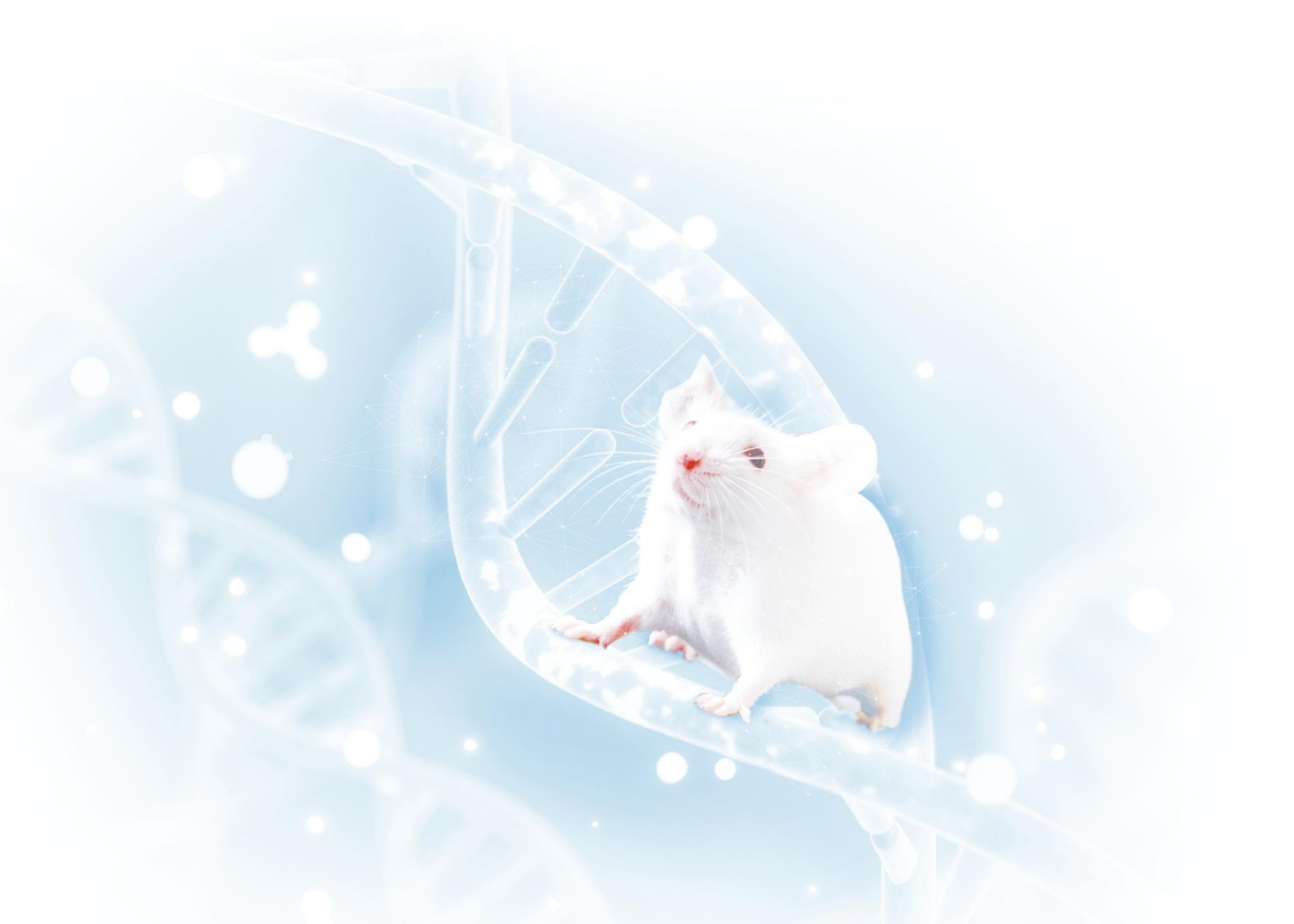


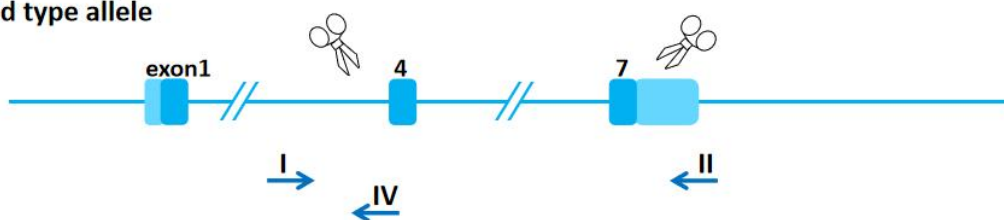
Tox3-KO Genotyping Protocol



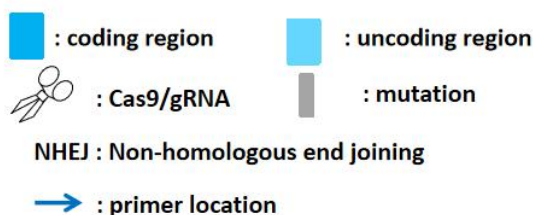
Common Name	Tox3-KO	Cat. NO.	NM-KO-230466
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' →3')	Primer type
P1	CTTTCATAAGGGTTCAACAGAGG	Forward
P2	AGAAGTTCAAATAGCCTGGAAGTGT	Reverse
P4	AGCTGAGACTGGTTGATGGTGGT	Reverse

Expected results

Results	
Genotype	Wild type: P1P4=480 bp Heterozygote: P1P2 =658 bp; P1P4=480 bp Homozygote: P1P2 =658 bp

Reaction & Cycling

PCR Reaction System	Reaction Component	Volume (μl)
	ddH ₂ O	7.5
	2×Taq Plus Master Mix	10.0
	P1(10 pmol/μl)	0.5
	P2(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	