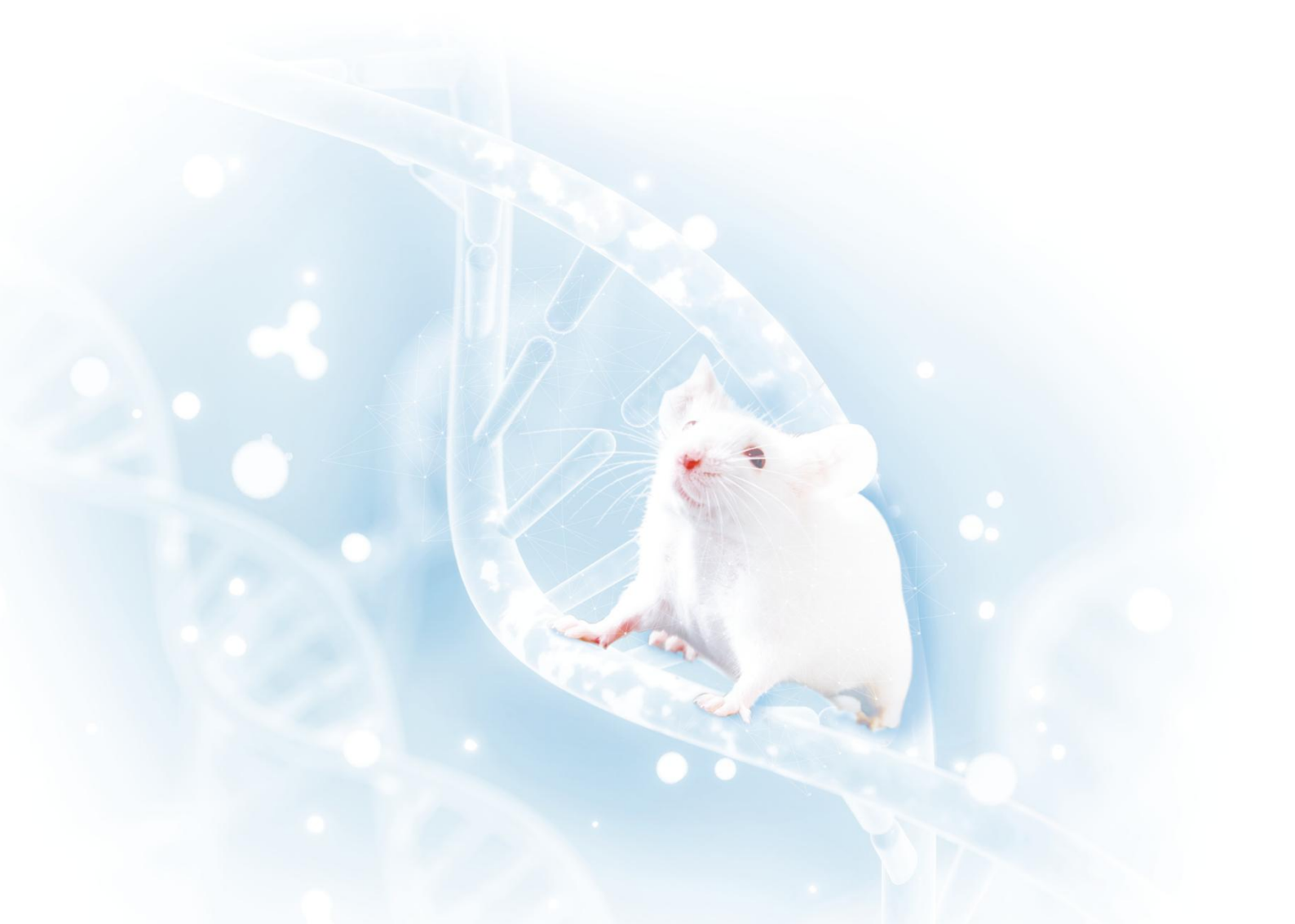


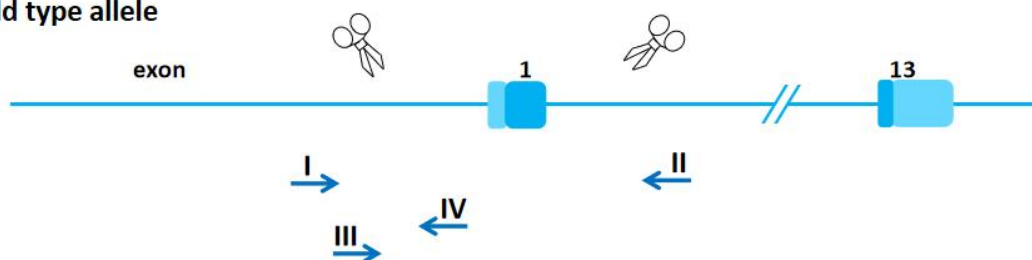
Cybb-KO (M-NSG) Genotyping Protocol



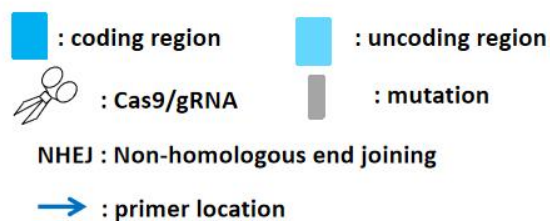
Common Name	Cybb-KO (M-NSG)	Cat. NO.	NM-KO-232204
Strain of Origin	M-NSG	Version	V1

Genotyping strategy

Wild type allele



knockout allele

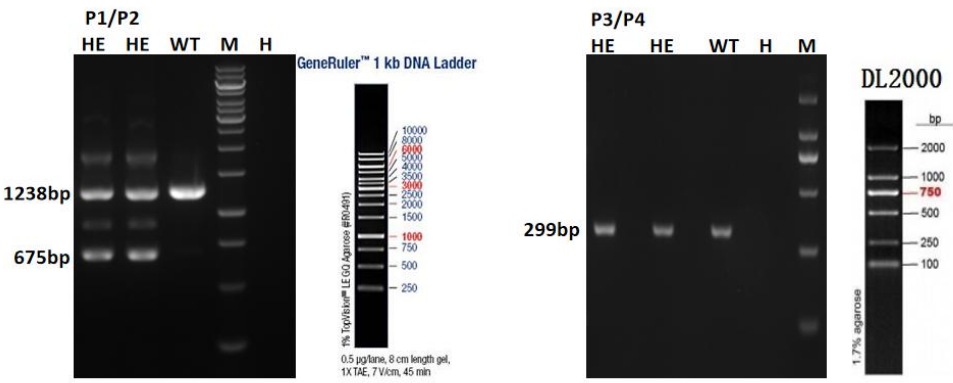


Primers

Primer	Sequence (5' → 3')	Primer type
P1	CAACTGTATTATTCCTTGGCACTGT	Forward
P2	CACTCTGGTAAATGCTGGCTA	Reverse
P3	TGGAAGAGCCTGTGAGAAGAA	Forward

P4	TGAAAGGCTGTTCCCATACA	Reverse
----	----------------------	---------

Expected results

Results	 The figure displays two gel electrophoresis images. The left gel, labeled 'P1/P2', shows lanes for HE, HE, WT, M, and H. The first two HE lanes show bands at 1238bp and 675bp. The WT lane shows a band at 1238bp. The M lane is a DNA ladder. The H lane shows a band at 675bp. The right gel, labeled 'P3/P4', shows lanes for HE, HE, WT, H, and M. The first two HE lanes show a band at 299bp. The WT lane shows a band at 299bp. The H lane shows no band. The M lane is a DNA ladder. A GeneRuler™ 1 kb DNA Ladder is shown between the two gels, and a DL2000 ladder is shown to the right of the P3/P4 gel.
Genotype	Knockout type: -563 bp Wild type: P1P2 =1238 bp; P3P4 =299 bp Heterozygote: P1P2 =1238 bp and 675 bp; P3P4 =299 bp Homozygote: P1P2 =675 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 K0 band.

Reaction &Cycling

PCR	Reaction Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	