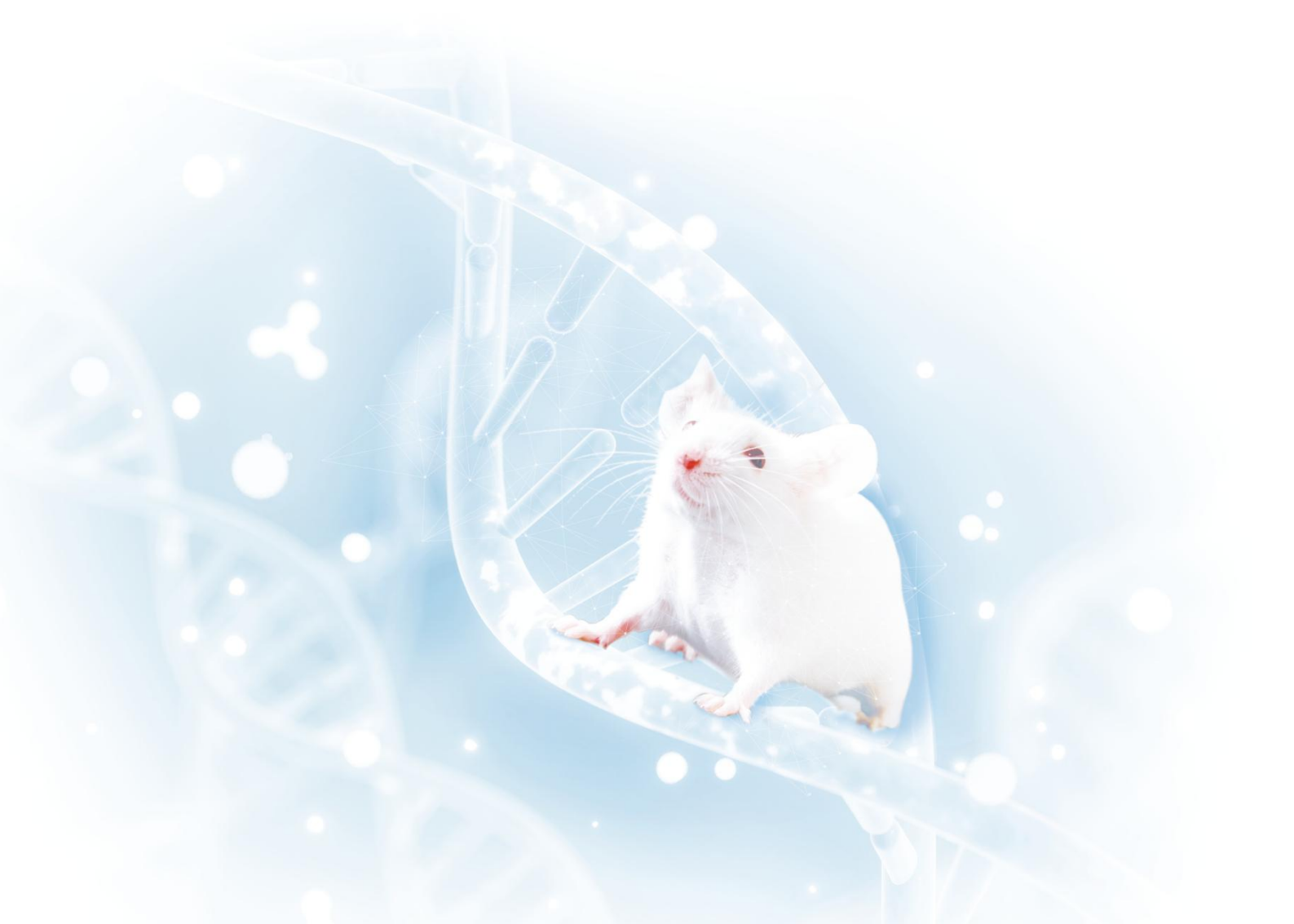


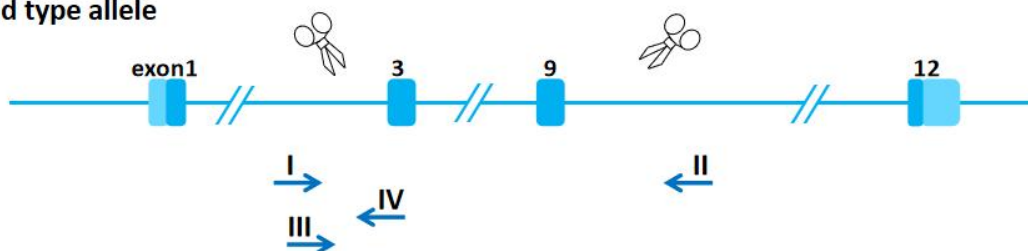
Aup1-K0 Genotyping Protocol



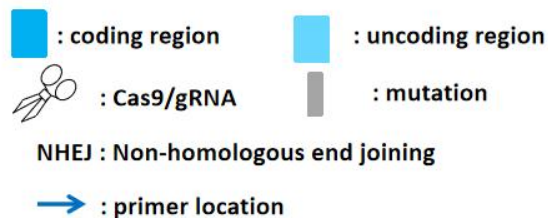
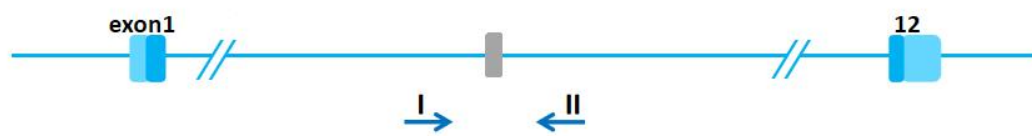
Common Name	Aup1-K0	Cat. NO.	NM-K0-240331
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TCTTTGACTCGCACCGGTAA	Forward
P2	CCCAGACCTGCCCTTAAGTC	Reverse
P3	GCTCGGCTACTGCTCTAC	Forward
P4	ATAAGGACCCTGACGCGGT	Reverse

Expected results

Results	P1/P2 HE HE WT M 2323bp 515bp GeneRuler™ 1 kb DNA Ladder 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P3/P4 HE HE WT H M 284bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -1808 bp Wild type: P1P2 =2323 bp; P3P4 =284 bp Heterozygote: P1P2 =2323 bp and 515 bp; P3P4 =284 bp Homozygote: P1P2 =515 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)
	ddH2O	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	