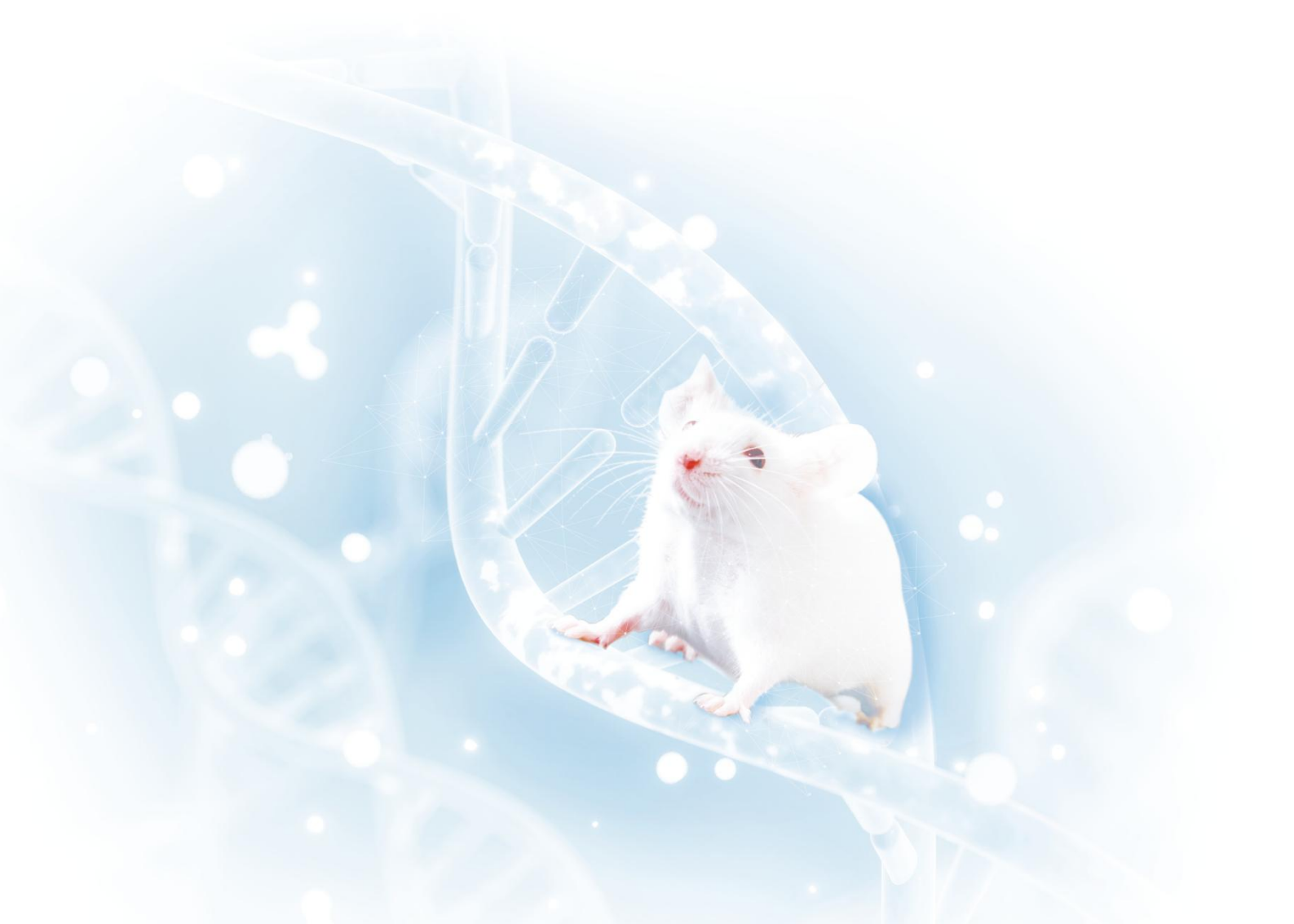


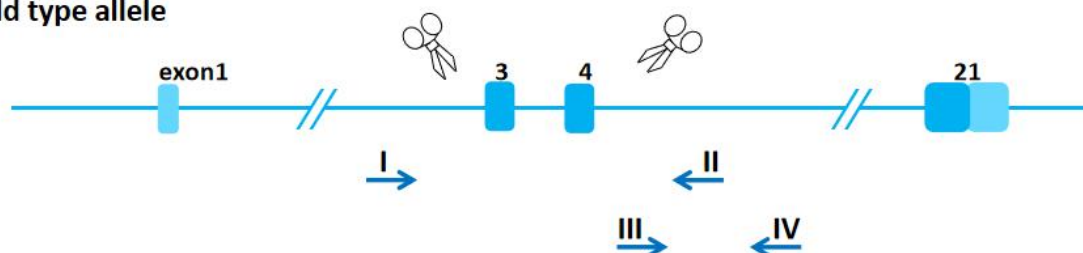
Aars-K0 Genotyping Protocol



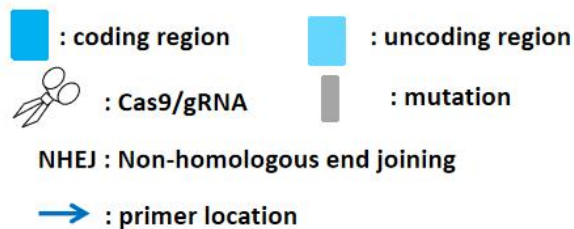
Common Name	Aars-K0	Cat. NO.	NM-K0-231482
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	TGTCATATGAATTCTTCTGGGATGA	Forward
P2	GCCGACAAGAAATCCCTCCA	Reverse
P3	TGCATGCAGATAGAGCACTCA	Forward
P4	CCTCTCTGCCACCAGGGTAT	Reverse

Expected results

Results	
Genotype	Knockout type: -1311bp Wild type: P1P2 =1800 bp; P3P4=257 bp Heterozygote: P1P2 =1800 bp and 489 bp; P3P4=257 bp Homozygote: P1P2 =489 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 Component	Volume (μl)
Reaction	ddH2O	8.0
System	2×Taq Plus 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×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	