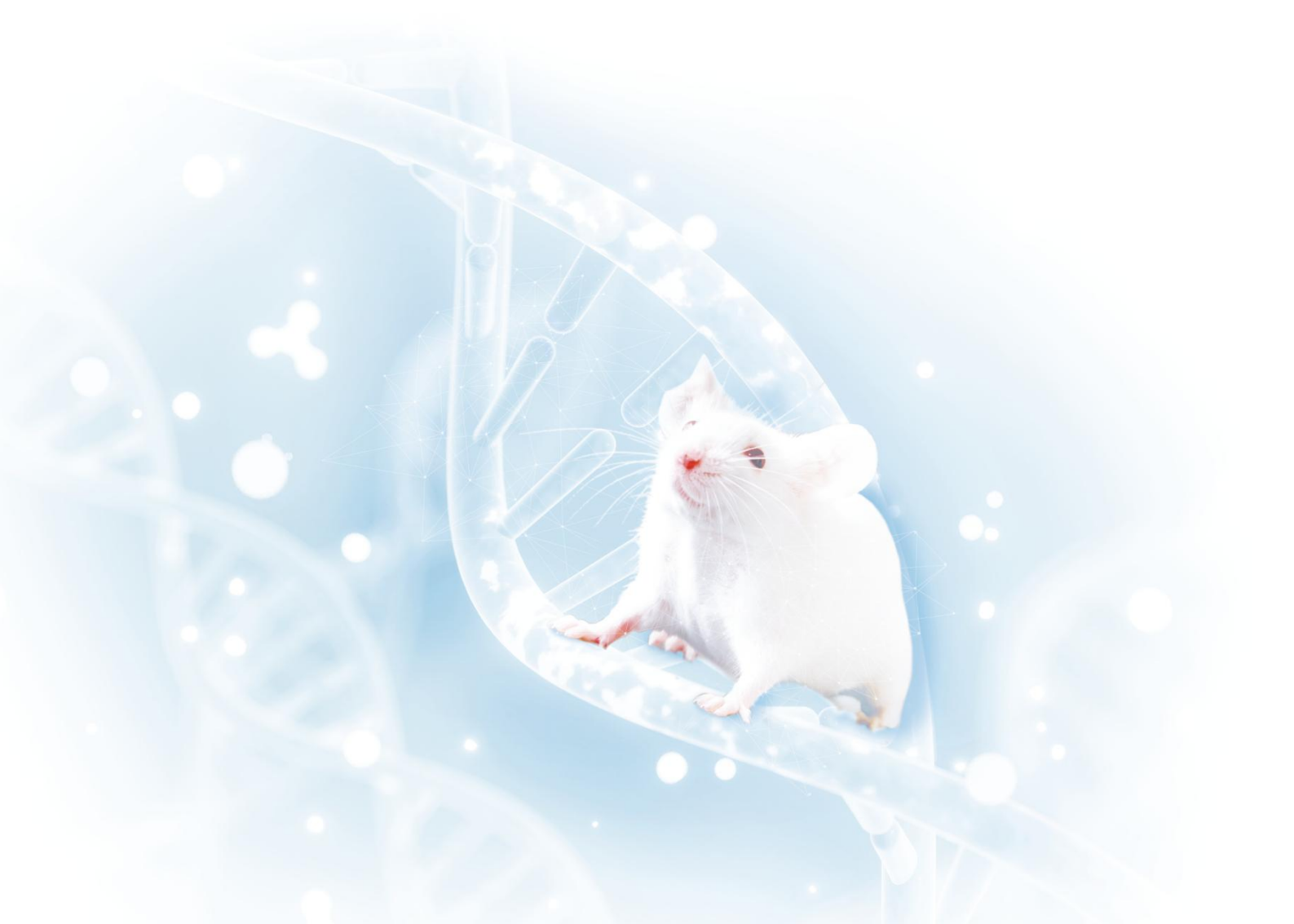


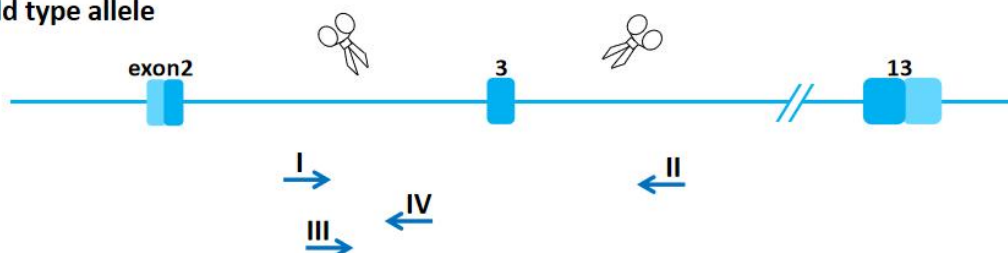
Alpk2-KO Genotyping Protocol



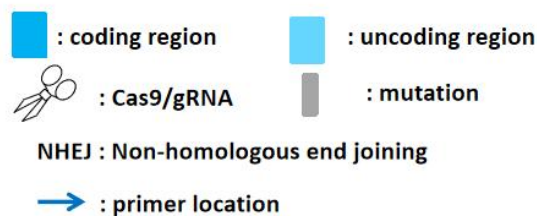
Common Name	Alpk2-K0	Cat. NO.	NM-K0-2101856
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele

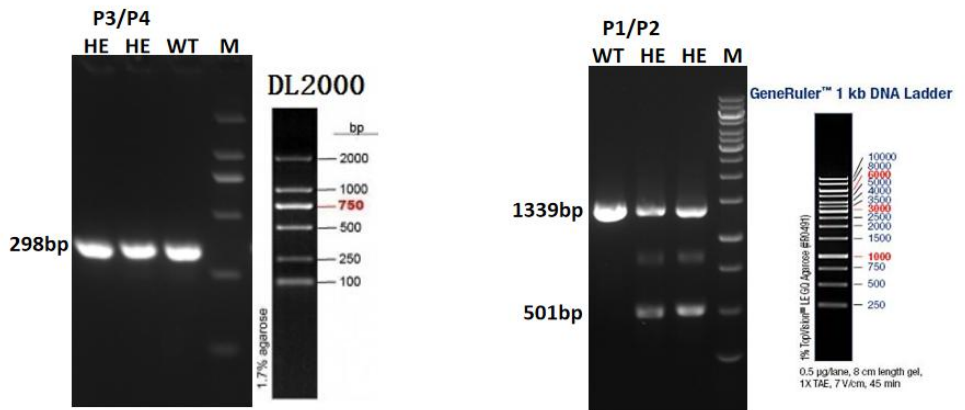


Primers

Primer	Sequence (5' → 3')	Primer type
P1	TTGCATCTCCGTTGGATAAACT	Forward
P2	ACTTGCTGTGGGTTCTGGTC	Reverse
P3	TGACTGACTCCATGTGACACT	Forward

P4	TCCTCCAAAATTAGGGTGGCT	Reverse
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Expected results

Results	
Genotype	Knockout type: -838 bp Wild type: P1P2 =1339 bp; P3P4=298 bp Heterozygote: P1P2 =1339 bp and 501 bp; P3P4=298 bp Homozygote: P1P2 =501 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 × 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	