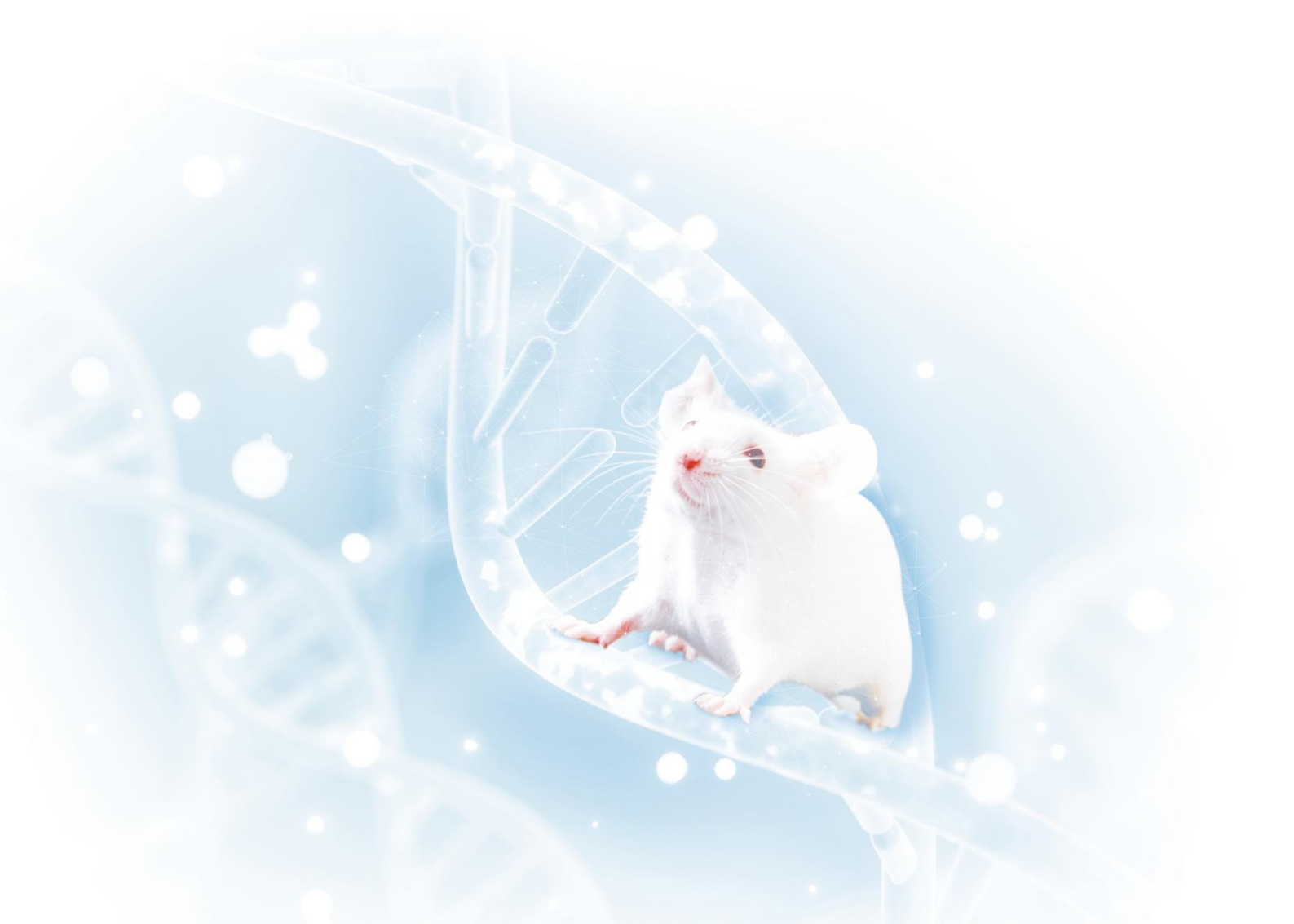


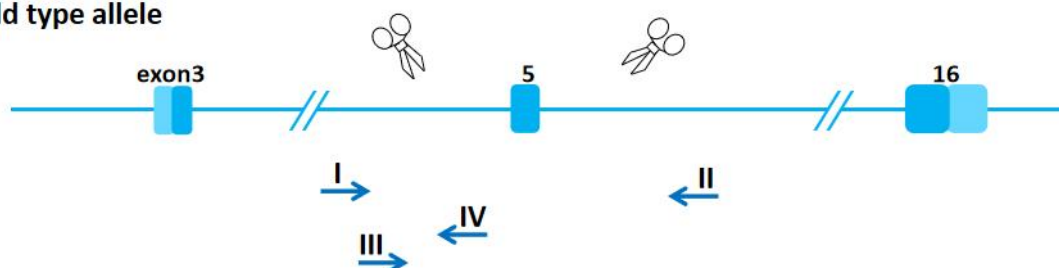
Acs14-K0 (2) Genotyping Protocol



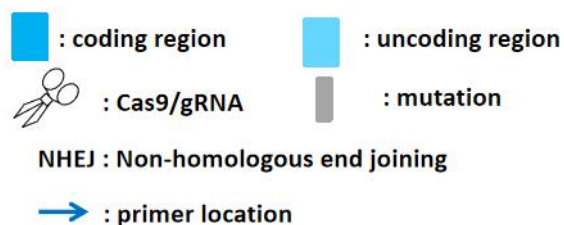
Common Name	Acs14-K0 (2)	Cat. NO.	NM-K0-232916
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	AGCCACCAAGCCTCTACAAC	Forward
P2	CTGGGGAGATGGCCAGT	Reverse
P3	AGTTAGCAGAGGGAGGCTGA	Forward

P4	GGCTTGCTTGTGGCACATTA	Reverse
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Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% Tris/Glycerol LE G0 Agarose #R0421 0.5 µg/lane, 8 cm length gel, 1XTAE, 7 V/cm, 45 min P1/P2 HE WT M H 2110bp 521bp P3/P4 HE M 318bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -1589 bp Wild type: P1P2 =2110 bp; P3P4 =318 bp Heterozygote: P1P2 =2110 bp and 521 bp; P3P4 =318 bp Homozygote: P1P2 =521 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	