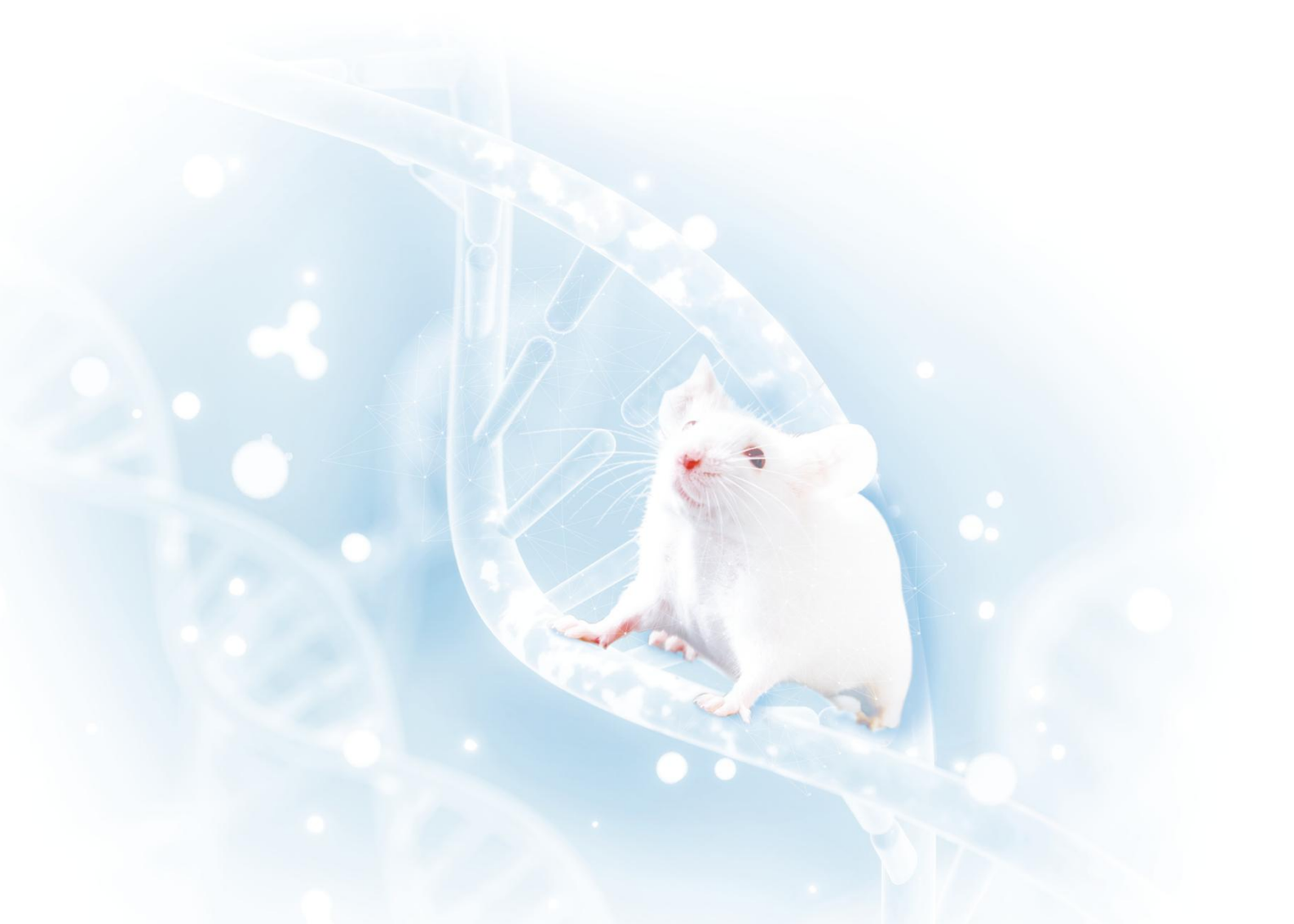


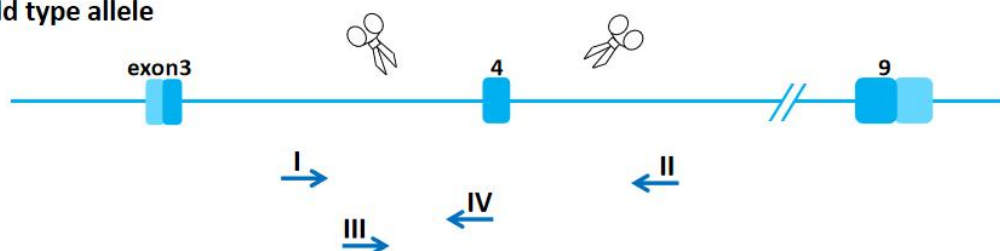
Srr-K0 Genotyping Protocol



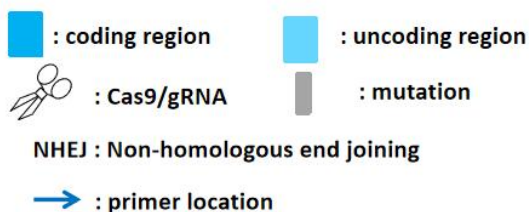
Common Name	Srr-K0	Cat. NO.	NM-K0-240379
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GAGAATCCAGCAGGTTCCCA	Forward
P2	GGCATGTCCTATCAAGCCTGG	Reverse
P3	GGAGAAAAATGTGCTGTGTAATACC	Forward

P4	GCACCTCGAATCTGGACGAG	Reverse
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Expected results

Results	P1/P2 HE HE WT M H 877bp 523bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose P3/P4 HE WT H M 275bp
Genotype	Knockout type: -354 bp Wild type: P1P2 =877 bp; P3P4 =275 bp Heterozygote: P1P2 =877 bp and 523 bp; P3P4 =275 bp Homozygote: P1P2 =523 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	