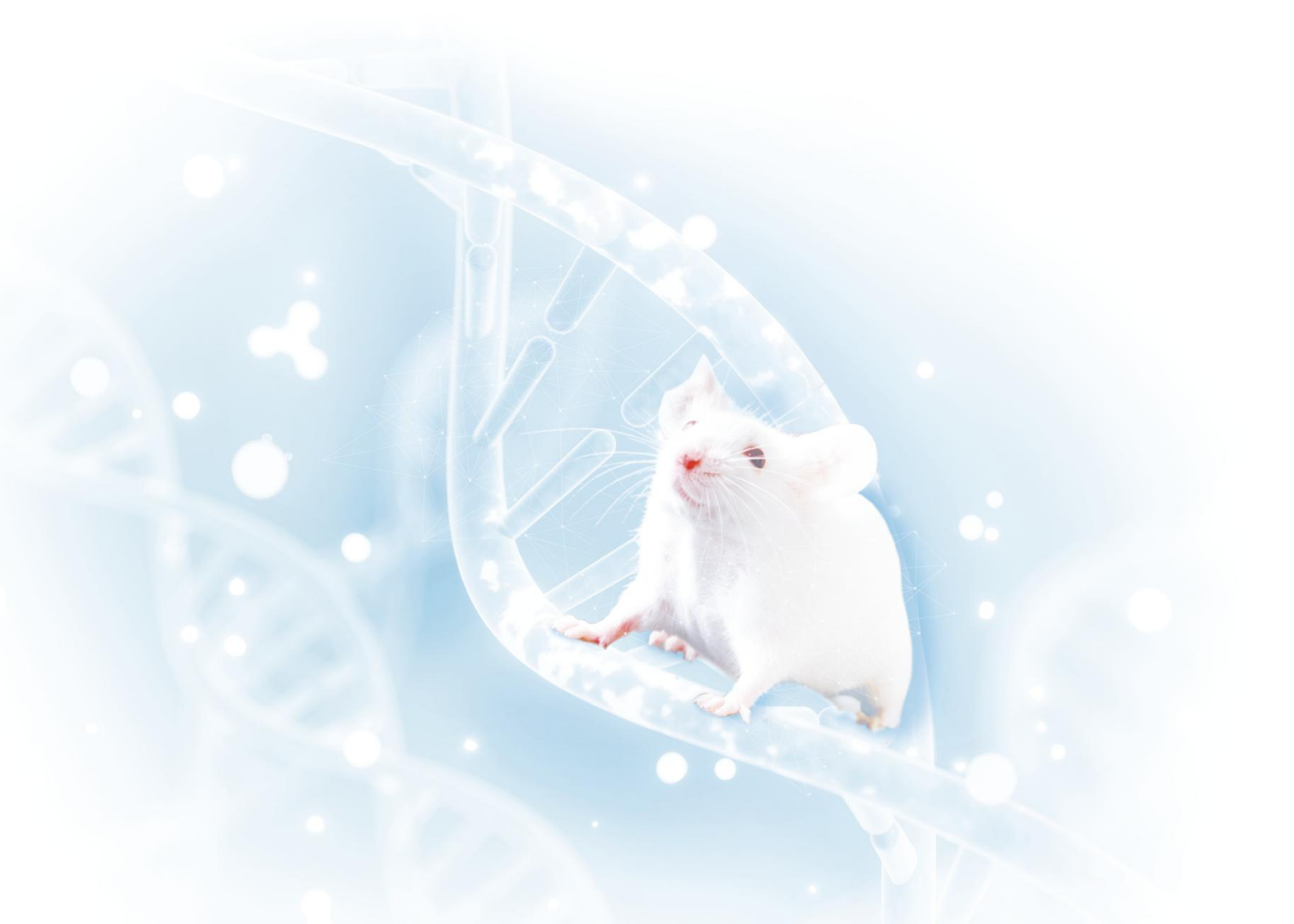


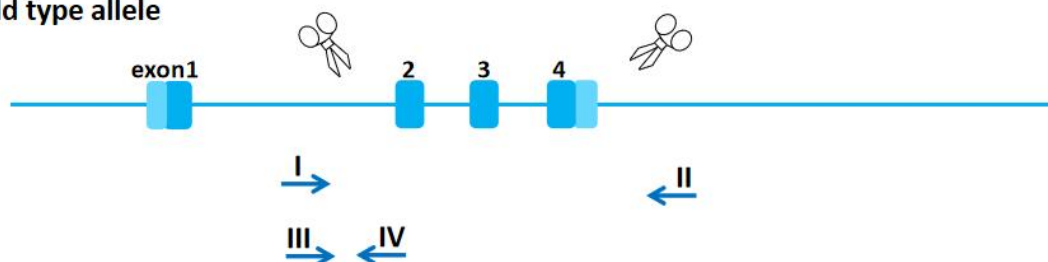
Sva-KO Genotyping Protocol



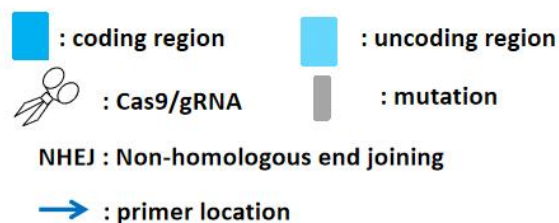
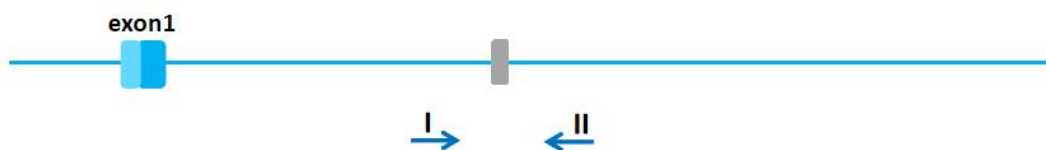
Common Name	Sva-KO	Cat. NO.	NM-KO-240404
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	TCAGACCTAAAGGGCAGTCAG	Forward
P2	TGGAACAGGAGTGGTGTTGA	Reverse
P3	ACCATGAGACCAGGTTGACA	Forward

P4	ACGGGGTTCATTGTGTAAGC	Reverse
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Expected results

Results	GeneRuler™ 1 kb DNA Ladder 1% Tg/Visor™ LE GO Agarose (#R0491) 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min P1/P2 HE HE WT M 3813bp 533bp P3/P4 HE HE WT M 418bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose
Genotype	Knockout type: -3280 bp Wild type: P1P2 =3813 bp; P3P4 =418 bp Heterozygote: P1P2 =3813 bp and 533 bp; P3P4 =418 bp Homozygote: P1P2 =533 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	ddH ₂ O	8.0
System	2 × Rapid Taq Master Mix	10.0

	P1(10 pmol/ μ l) or P3(10 pmol/ μ l)			0.5
	P2(10 pmol/ μ l) or P4(10 pmol/ μ l)			0.5
	Genomic DNA			1.0
	Total			20
	2 $\times$ 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	