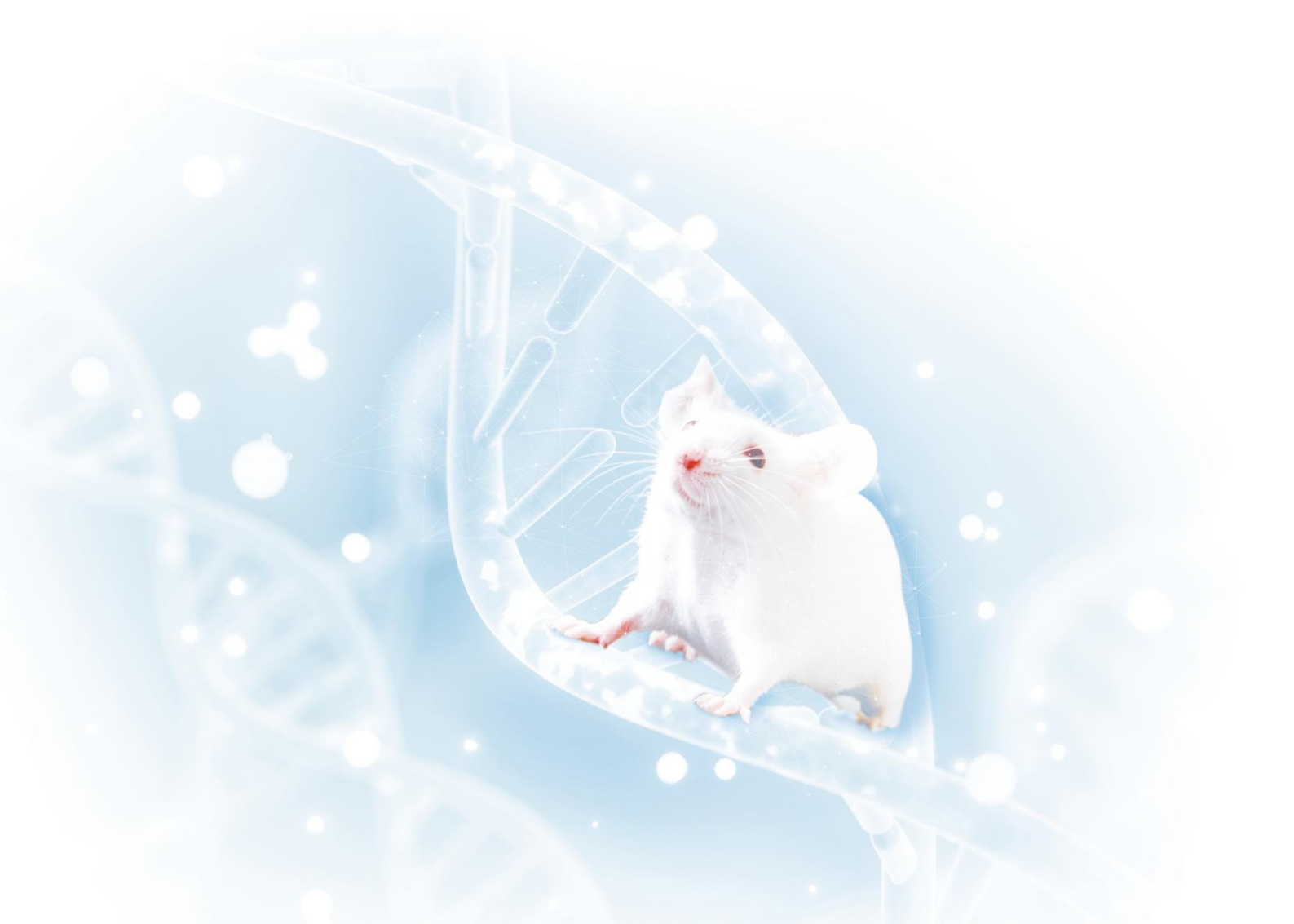


Col9a1-Flox Genotyping Protocol



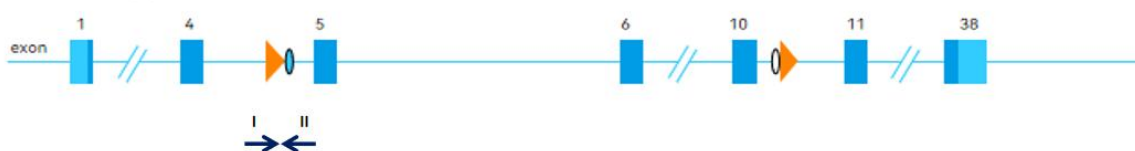
Common Name	Col9a1-Flox	Cat. NO.	NM-CKO-233167
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Col9a1 wild type allele



Col9a1 targeted allele
(Neo and Hygro removed)



→ : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	TGGCTGAGATGGCATTGCTG	Forward
P2	GTCCTGCGGAAAGGCTACAG	Reverse

Expected results

Results	P1+P2
Genotype	Wild type: P1P2 =223 bp Heterozygote: P1P2 =223 bp and 395 bp Homozygote: P1P2 =395 bp

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (μl)
	ddH ₂ O			8.0
	2×Rapid Taq Master Mix			10.0
	P1(10 pmol/μl)			0.5
	P2(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	