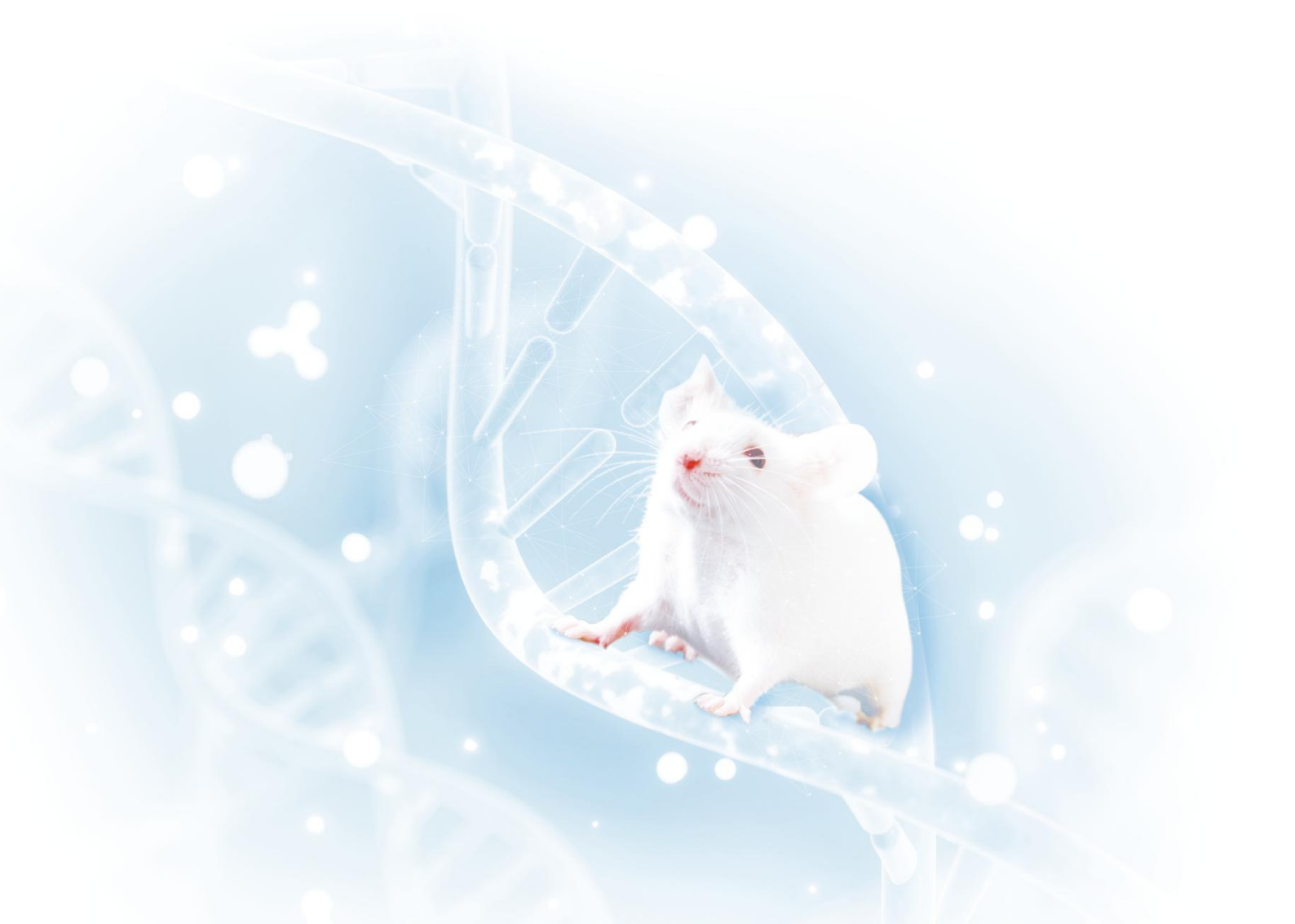


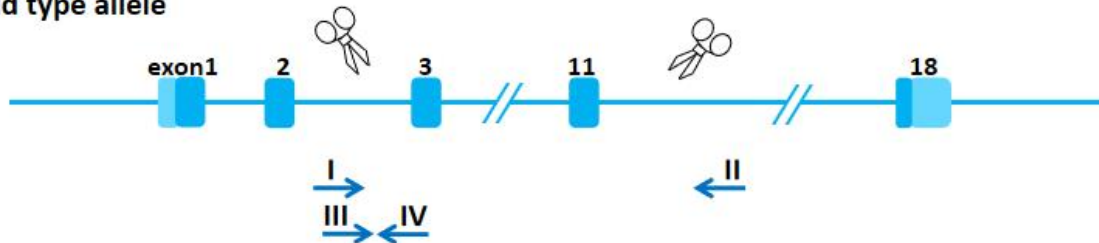
Cfb-KO Genotyping Protocol



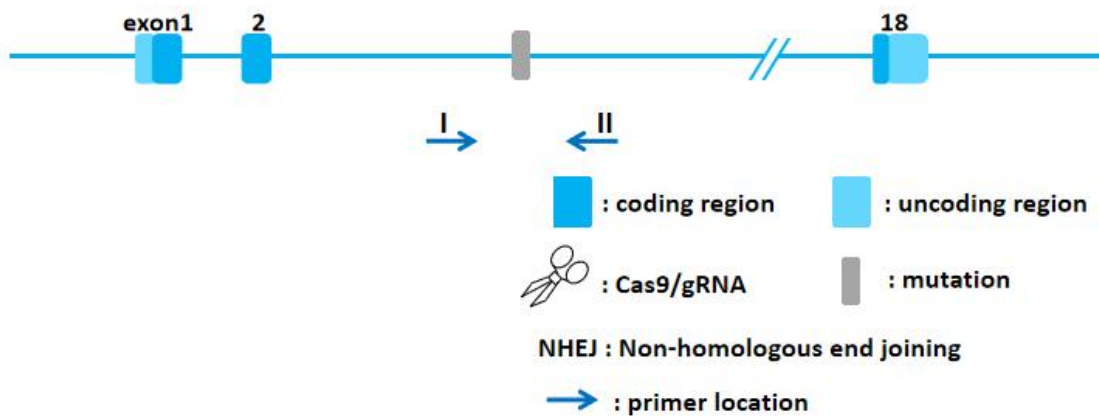
Common Name	Cfb-KO	Cat. NO.	NM-KO-241038
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	CACCTCTGCAGGTGTGTGAT	Forward
P2	TCAGACACAAGGCAACCCTG	Reverse
P3	TCCTCGGGCAGTGAAAACAA	Forward
P4	CCCCGGAGAACGTAACCATC	Reverse

Expected results

Results	
Genotype	<p>Knockout type: -2848 bp</p> <p>Wild type: P1P2 =3378 bp; P3P4 =482 bp</p> <p>Heterozygote: P1P2 =3378 bp and 530 bp; P3P4 =482 bp</p> <p>Homozygote: P1P2 =530 bp</p>

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 System	Reaction Component		Volume (µl)	
	ddH ₂ O		8.0	
	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	35 repeats to 2
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	20 sec	
	5	72°C	5 min	
6	12°C	Hold		