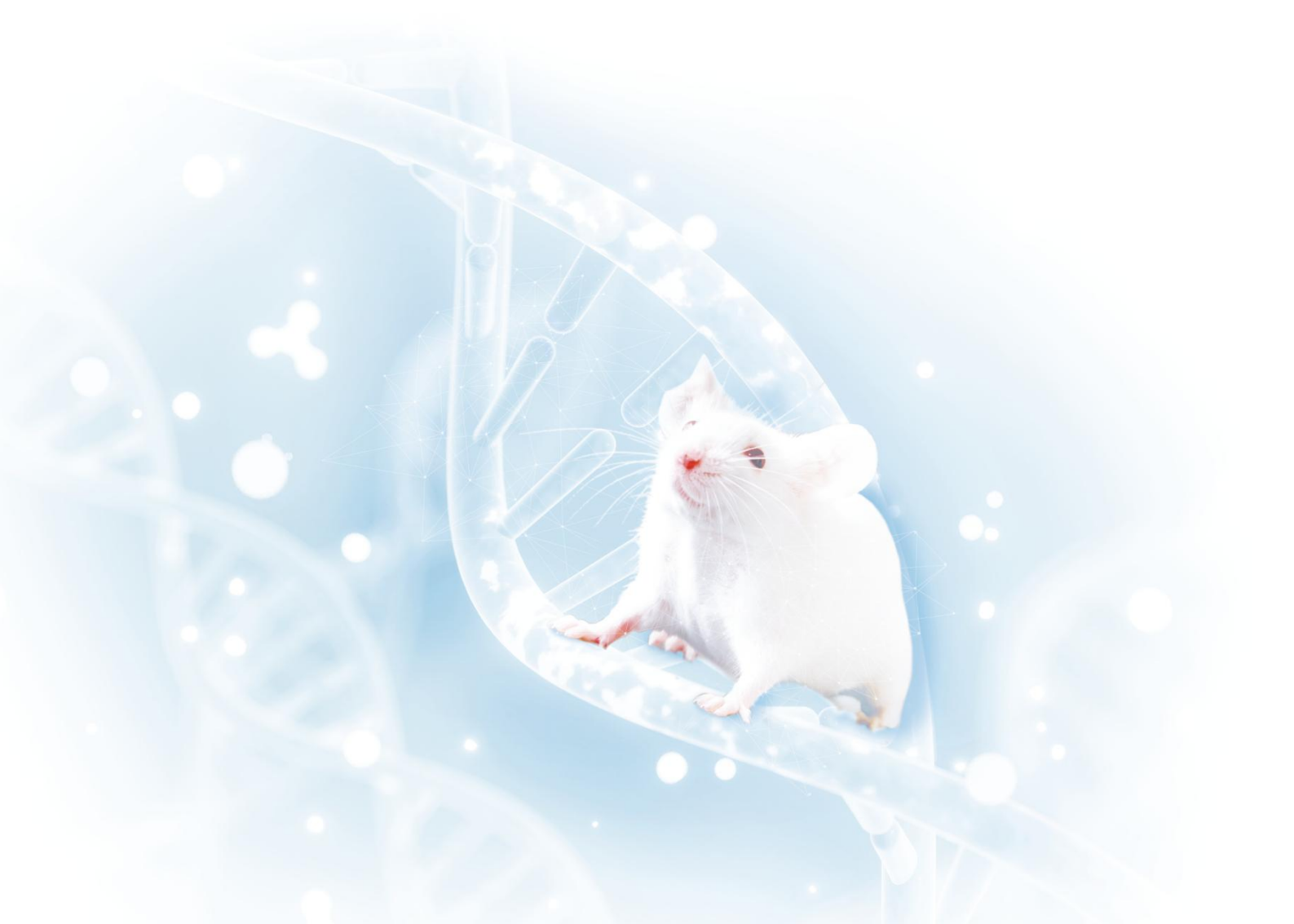
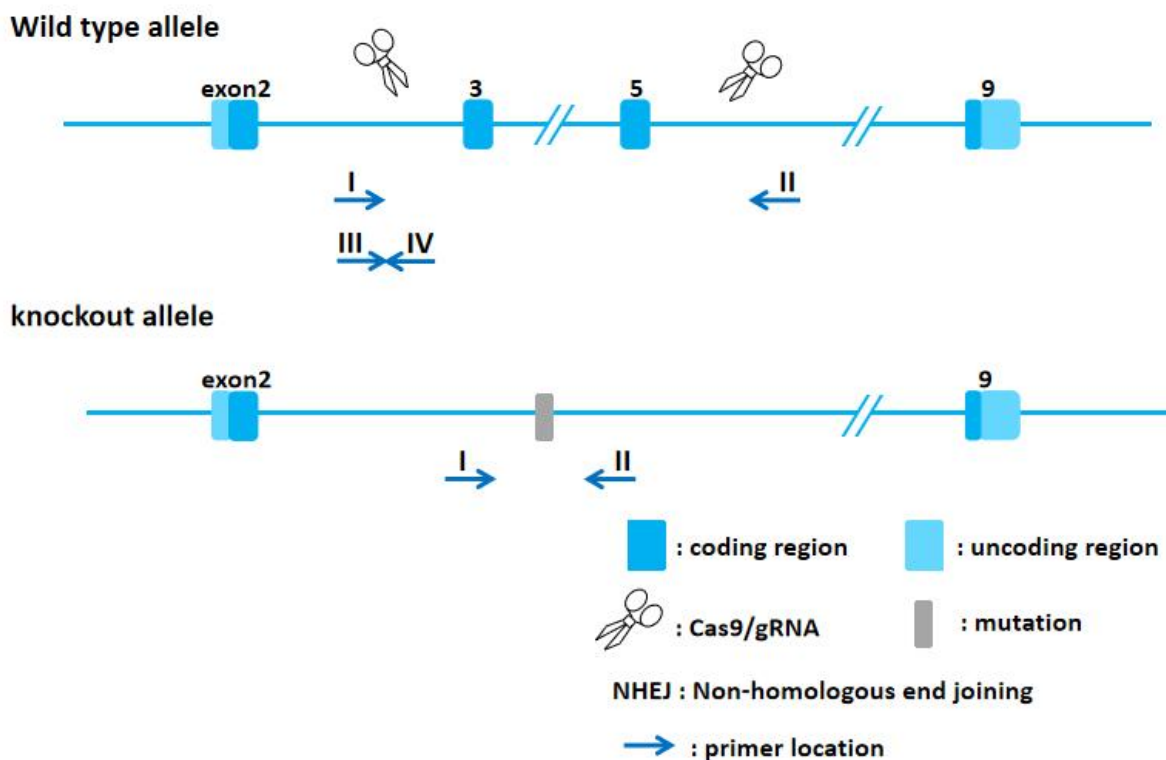


Abhd6-KO Genotyping Protocol



Common Name	Abhd6-KO	Cat. NO.	NM-CKO-240799
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	TGCGGAATAAAGCCAGAGGG	Forward
P2	CCCAGCTCAGGTGCCAATTA	Reverse
P3	TGCGGAATAAAGCCAGAGGG	Forward
P4	GACAGCAGAGAGCAACAGGA	Reverse

Expected results

Results	
Genotype	Knockout type: -3612 bp Wild type: P1P2 =3979 bp; P3P4 =272 bp Heterozygote: P1P2 =3979 bp and 367 bp; P3P4 =272 bp Homozygote: P1P2 =367 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 System	Reaction Component			Volume (µl)
	ddH2O			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	
	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	