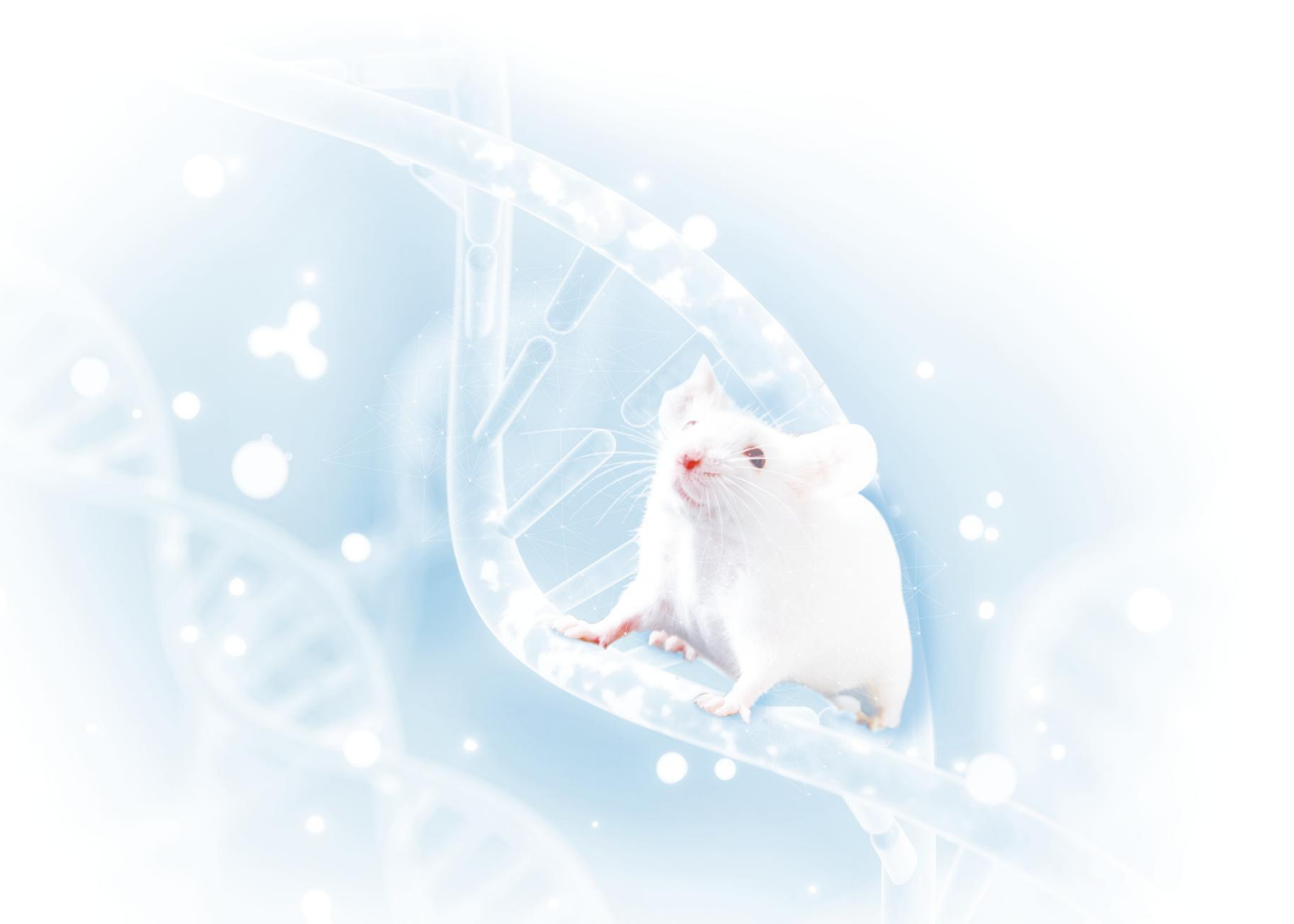


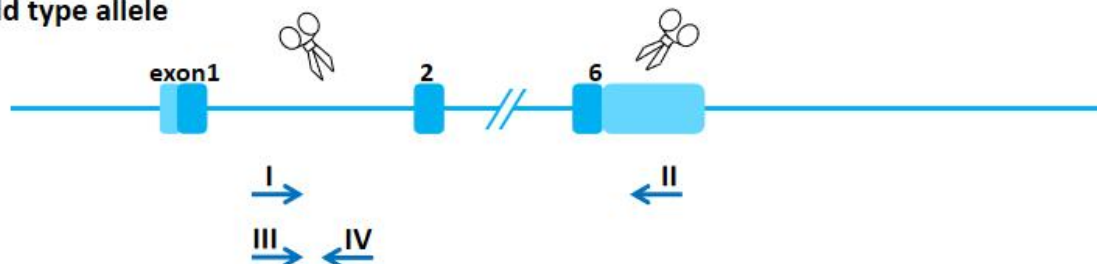
Abitram-KO Genotyping Protocol



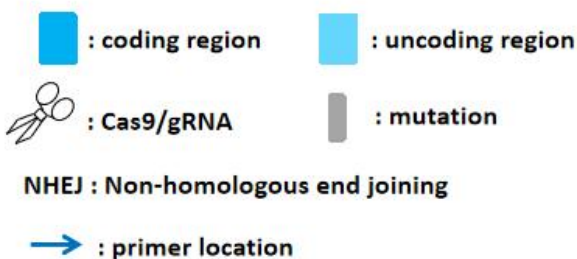
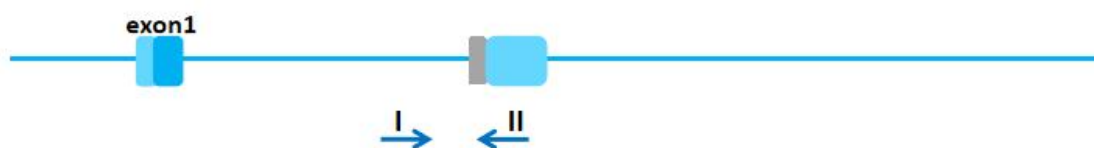
Common Name	Abitram-KO	Cat. NO.	NM-KO-241468
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	CGTGCTCTCCCTGAGCATC	Forward
P2	TTAGCTTCCTGCCCTGGCTA	Reverse
P3	CGTGCTCTCCCTGAGCAT	Forward
P4	TATGTGGGTCTACCGGTTTCC	Reverse

Expected results

Results	
Genotype	Knockout type: -3489 bp Wild type: P1P2 =3972 bp; P3P4 =432 bp Heterozygote: P1P2 =3972 bp and 483 bp; P3P4 =432 bp Homozygote: P1P2 =483 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)
	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	
	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	