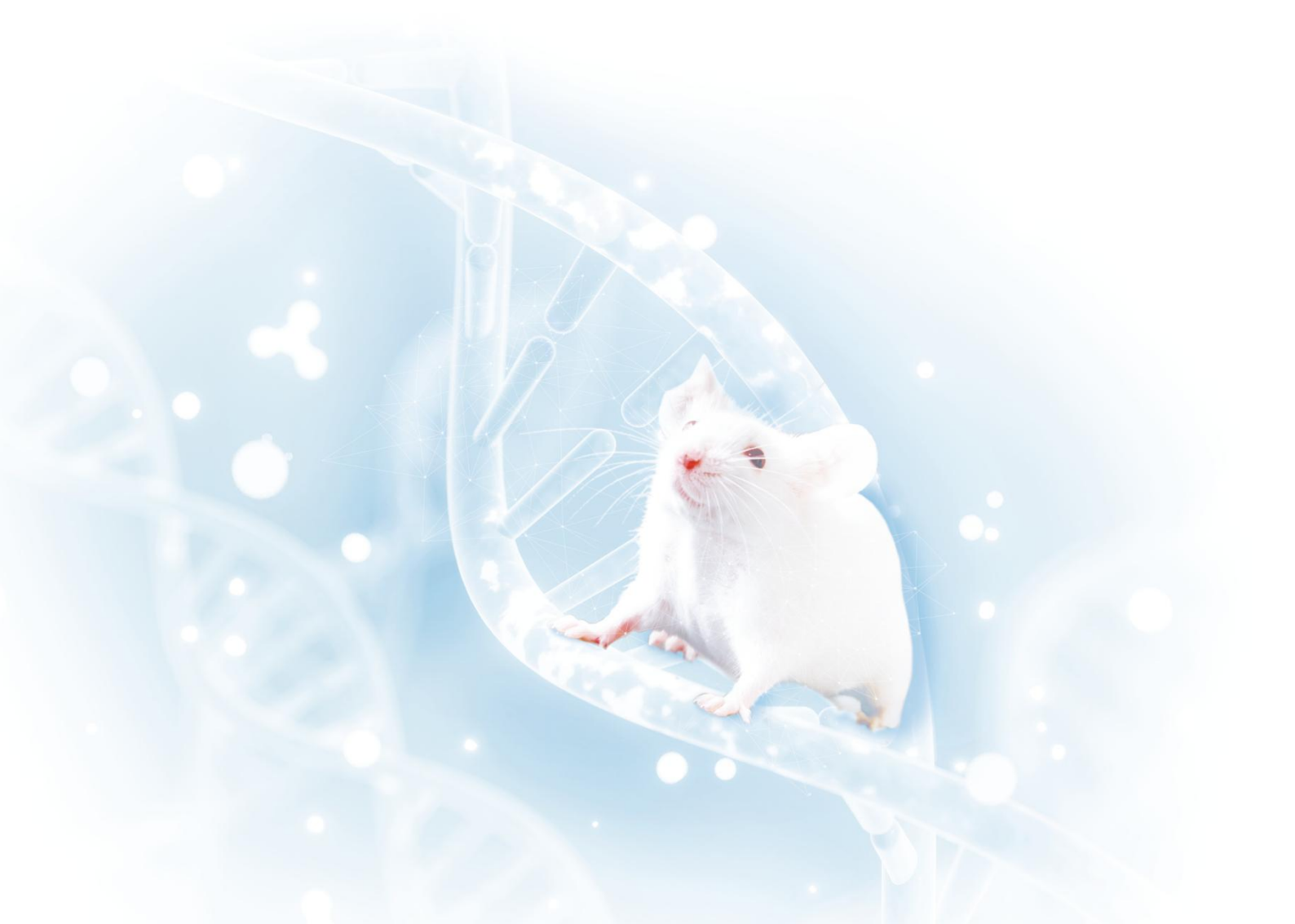


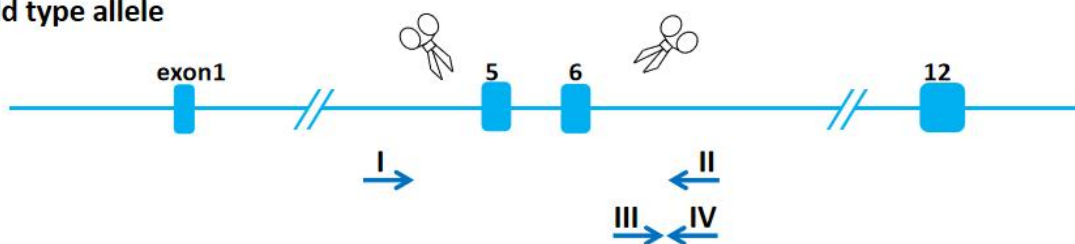
Ab12-KO Genotyping Protocol



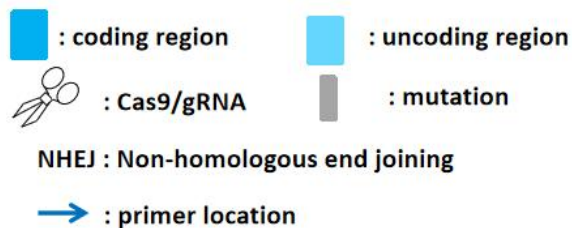
Common Name	Ab12-K0	Cat. NO.	NM-K0-240967
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	GAGTCTTTGGAGTGCTGGCT	Forward
P2	CTGCAGCGCAGCCATTTATC	Reverse
P3	GTCCTTGGAGACCAAAGGAGG	Forward
P4	AGCGCAGCCATTTATCTCTTG	Reverse

Expected results

Results	<p>The figure displays two agarose gel electrophoresis images. The left image, labeled 'P1/P2', shows four lanes: HE, HE, WT, and M. The first three lanes show a prominent band at 3016bp and a smaller band at 388bp. The M lane is a DNA ladder with markers from 250 to 10000 bp. The right image, labeled 'P3/P4', shows four lanes: He, He, WT, and M. All lanes show a band at 252bp. The M lane is a DNA ladder with markers from 100 to 2000 bp. The gels are run on 1.7% agarose.</p>
Genotype	<p>Knockout type: -2635+7bp</p> <p>Wild type: P1P2 =3016 bp; P3P4=252 bp</p> <p>Heterozygote: P1P2 =3016 bp and 388 bp; P3P4=252 bp</p> <p>Homozygote: P1P2 =388 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

	Reaction Component	Volume (μ l)
PCR Reaction System	ddH ₂ O	8.0
	2 \times Taq Plus 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 Taq Plus Master Mix from Vazyme (Code Number: P222-1)			
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	