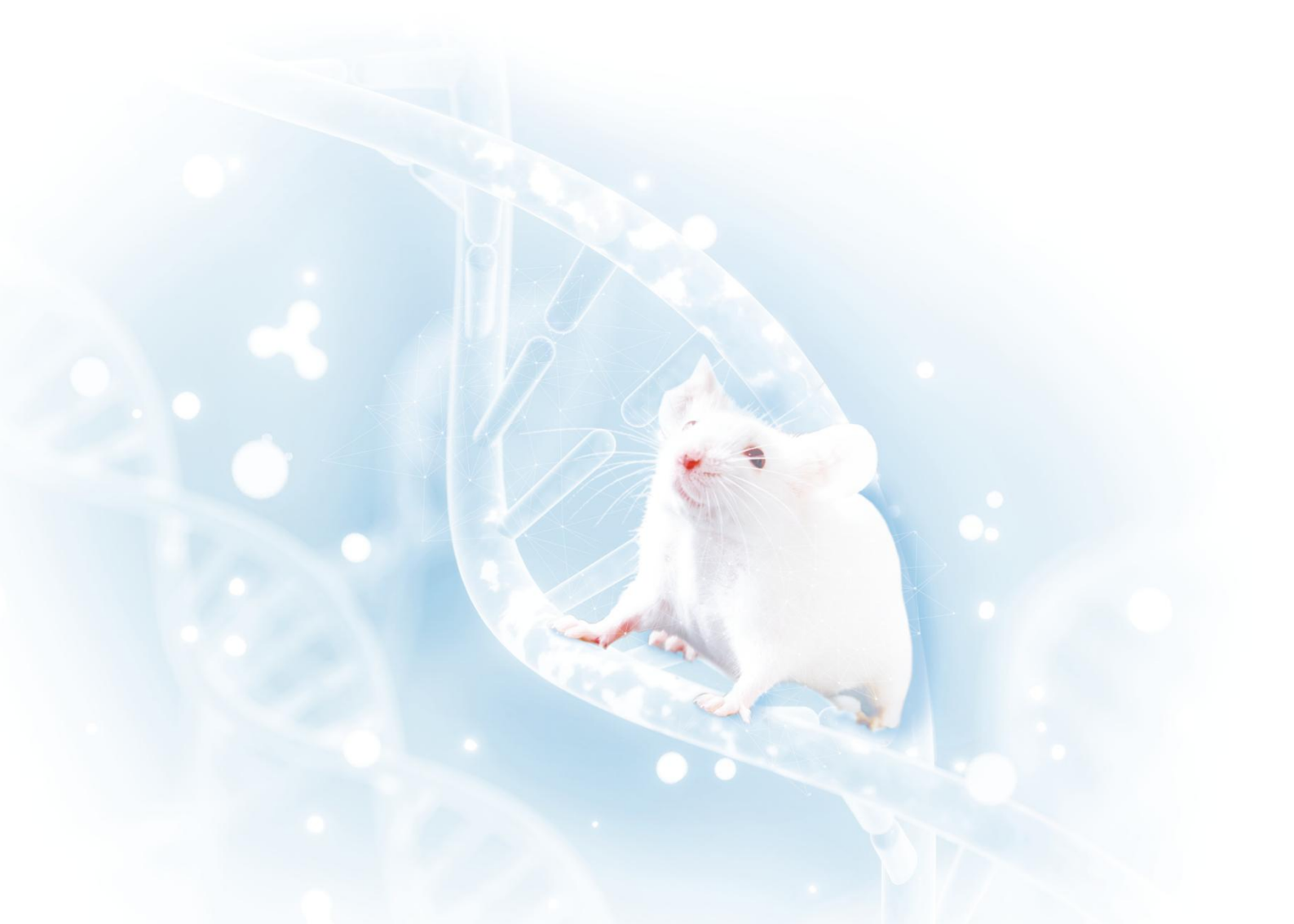
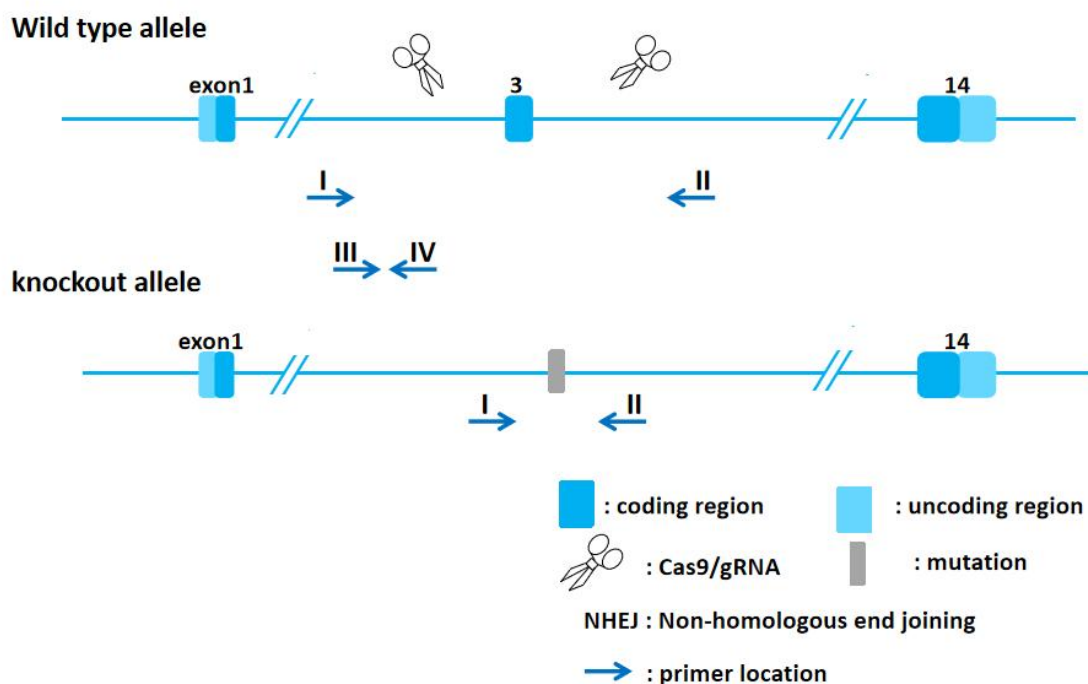


Usp10-KO Genotyping Protocol



Common Name	Usp10-KO	Cat. NO.	NM-KO-2103907
Strain of Origin	C57BL/6J	Version	V1

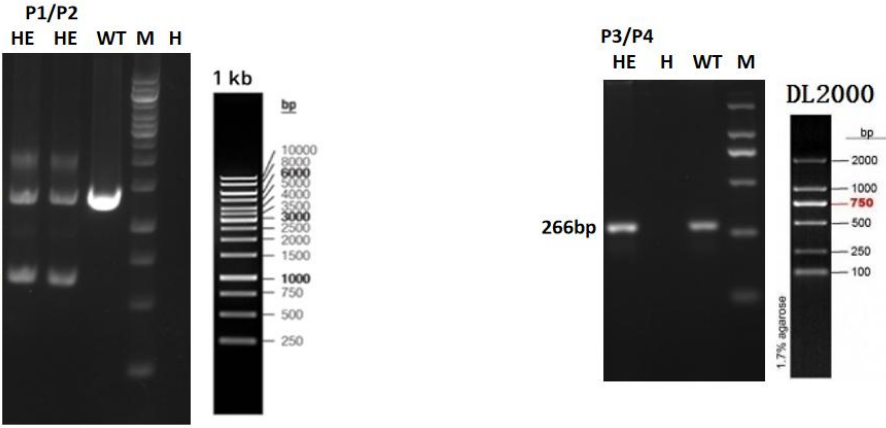
Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	AGCAGACAGTGATTGCCCTG	Forward
P2	ATGAGACCCTCCTCACTGCT	Reverse
P3	TAACTCATCTGCATGGCGCA	Forward
P4	CACGTTCCCAGGAAGACACA	Reverse

Expected results

Results	
Genotype	Knockout type: -667 bp Wild type: P1P2 =1280 bp; P3P4 =266 bp Heterozygote: P1P2 =1280 bp and 613 bp; P3P4 =266 bp Homozygote: P1P2 =613 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	