

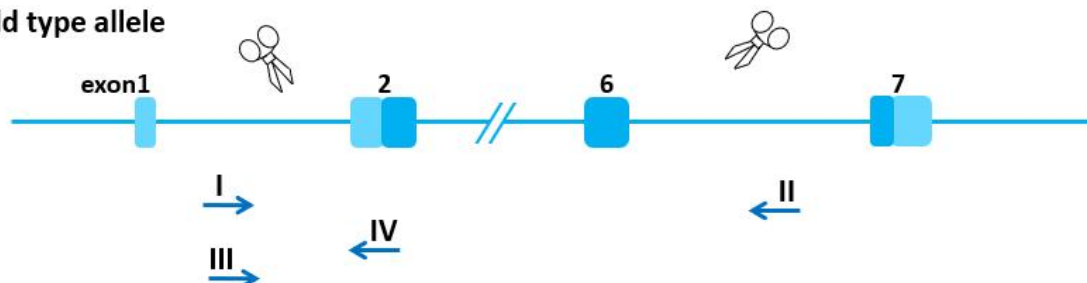
Ucp3-KO Genotyping Protocol



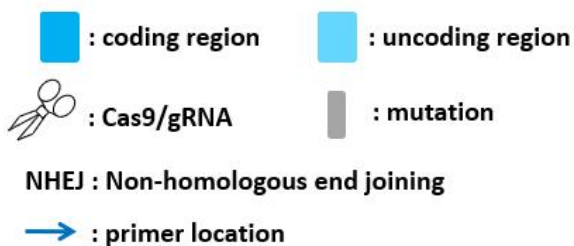
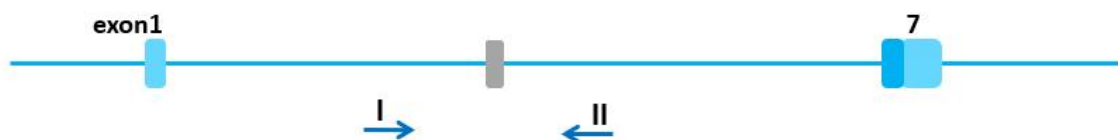
Common Name	Ucp3-KO	Cat. NO.	NM-KO-240554
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5'→3')	Primer type
P1	GGCAGTGTGTACTCAGGCAT	Forward
P2	CTATGGAGTCTCTGCAGCCG	Reverse
P3	ACCTCGGCTCTCTAAGCCAT	Forward
P4	CCCAGGAACCTCACAACCGT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder P1/P2 HE HE WT M 4210bp 698bp P3/P4 HE HE WT H M 413bp DL2000 bp 2000 1000 750 500 250 100 1.7% agarose 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -3512bp Wild type: P1P2 =4210 bp; P3P4 =413 bp Heterozygote: P1P2 =4210 bp and 698 bp; P3P4 =413 bp Homozygote: P1P2 =698 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	