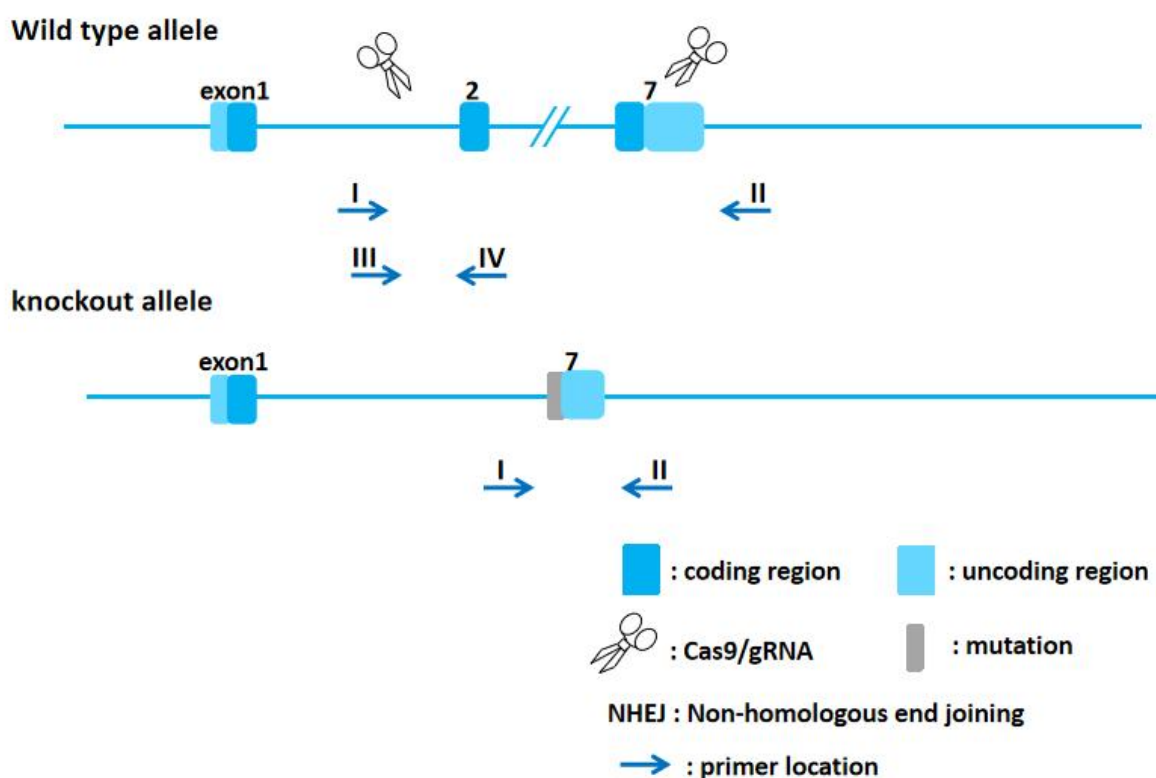


Ung-KO Genotyping Protocol



Common Name	Ung-KO	Cat. NO.	NM-KO-240416
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	GTGGAAGGTCTTGGGTGAGG	Forward
P2	GTGTGTCTCAACAGCCACCT	Reverse
P3	TCAQAGTCAATCGGTGTGCAQ	Forward
P4	TGTCAAGTGTGGCACGGAAA	Reverse

Expected results

Results	0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -7100bp Wild type: P1P2 = 7718 bp; P3P4 = 488 bp Heterozygote: P1P2 = 7718 bp and 618 bp; P3P4 = 488 bp Homozygote: P1P2 = 618 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/ μ l) or P4(10 pmol/ μ l)			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	