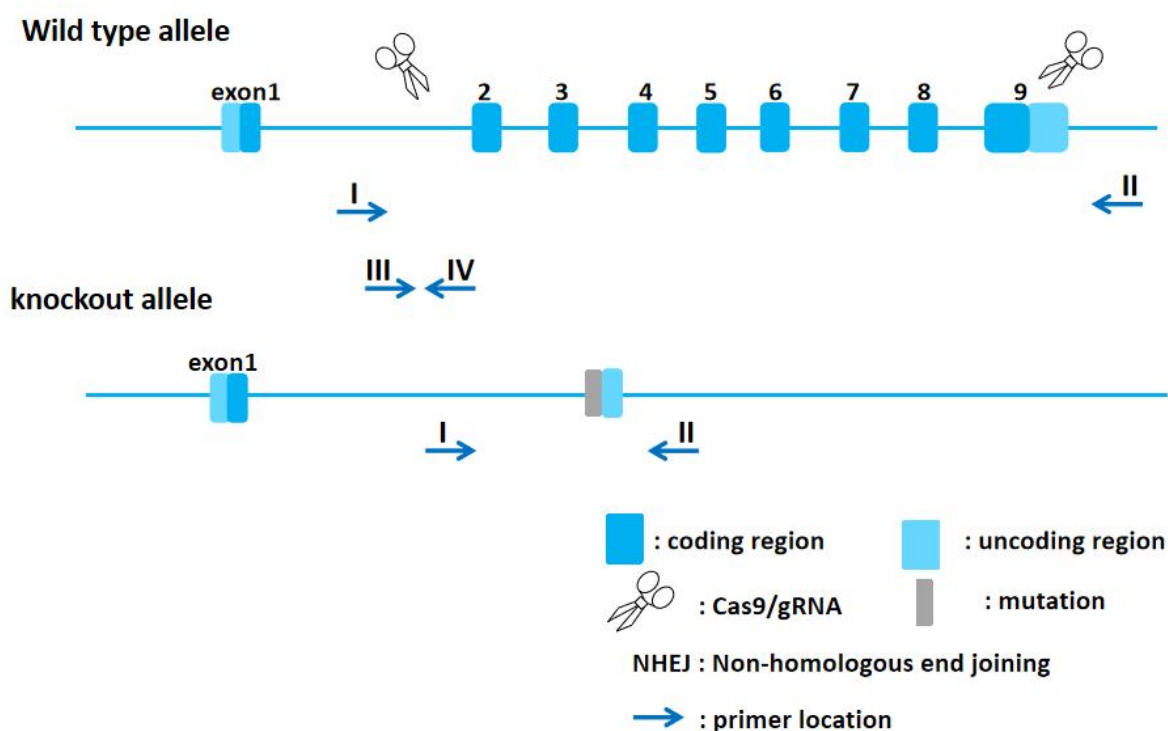


Cyp27b1-K0 Genotyping Protocol



Common Name	Cyp27b1-KO	Cat. NO.	NM-KO-240380
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5' → 3')	Primer type
P1	CCGGCAAAGGGATGTACTGT	Forward
P2	GCTGCCTGGAGTTGGTAGTT	Reverse
P3	TGAAGGAAGTCTCCCCCTA	Forward
P4	AGACCATATTGGCCCGTACC	Reverse

Expected results

Results	P1/P2 HE WT M GeneRuler™ 1 kb DNA Ladder 3771bp 582bp P3/P4 HE HE WT H M DL2000 278bp 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: -3189 bp Wild type: P1P2 =3771 bp; P3P4 =278 bp Heterozygote: P1P2 =3771 bp and 582 bp; P3P4 =278 bp Homozygote: P1P2 =582 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 Component	Volume (µl)
Reaction System	ddH2O	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	