

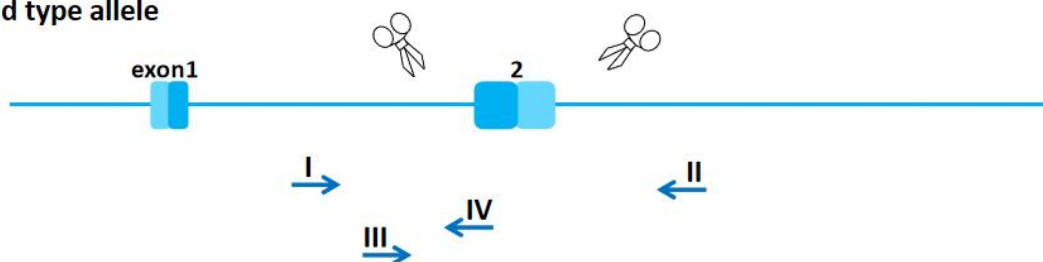
Bmp15-K0 Genotyping Protocol



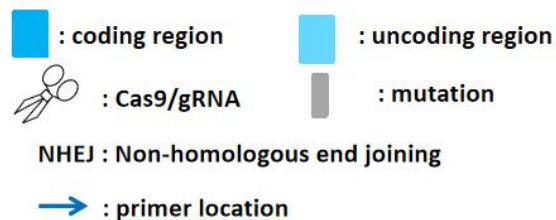
Common Name	Bmp15-KO	Cat. NO.	NM-KO-240454
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



Primers

Primer	Sequence (5' → 3')	Primer type
P1	ATCATAGCAGGTGCTGGCTG	Forward
P2	ACACCTAACATCGTCCTGCC	Reverse
P3	ATCATAGCAGGTGCTGGCTG	Forward

P4	TGCTAGAGGAAAGTCCAGGGT	Reverse
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Expected results

Results	
Genotype	Knockout type: -2627 bp Wild type: P1P2 =3093 bp; P3P4 =395 bp Heterozygote: P1P2 =3093 bp and 466 bp; P3P4 =395 bp Homozygote: P1P2 =466 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 K0 band.

Reaction &Cycling

PCR	Reaction Component	Volume (μl)
Reaction	ddH2O	8.0
System	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	