

NKX.3.1 PCR PROTOCOLOligonucleotide Sequence: 5' \diamond 3'

(188) M β Cx7 For	GATGTGCTCCAGGCTAAAGTT
(189) M β Cx7 Rev	AGAAACGGAATGTTGTGGAGT
3.1 For	CTCTCTTTGCAACACACATCCAGGATCC
3.1 Rev	GGCTGAACTTCCTCTCCAACCTCAATCACC
Cre Rev	GCCGCATAACCAGTGAAACAGCATTGC

PCR Mix:

Component	Volume (μ L)
Tail DNA	1.00
10x Taq Buffer	5.00
DMSO	2.50
10 mM dNTP Mix	1.50
25 mM MgCl	7.50
MbCx7 For	1.25
MbCx7 Rev	1.25
3.1 For	1.00
3.1 Rev	0.50
Cre Rev	0.50
Taq Polymerase	0.50
ddH ₂ O	30.00
Total	50.00

Thermocycler Profile: File 58-30

94 C for 5 min (initial cycle only)

94 C for 45 sec

58 C for 45 sec

72 C for 2 min

----- \diamond x 29 Cycles

72 C for 8 min

4 C for 5 min

NKX.3.1 PCR Screening

Gel Analysis:

1% agarose gel
15 μ l per lane

Expected Results:

M β Casein Cntrl	500 bp
Knockout	439 bp
Wildtype	697 bp