

IGF1-loxP PCR PROTOCOL

Oligonucleotide Sequence: 5' \diamond 3'

(188) M β Cx7 For	GATGTGCTCCAGGCTAAAGTT
(189) M β Cx7 Rev	AGAAACGGAATGTTGTGGAGT
(522) IA-8	AGTGATAGGTCACAAAGTTCC
(523) IA-6	AAACCACACTGCTCGACATTG
(524) IA-3	CACTAAGGAGTCTGTATTTGGACC

PCR Mix:

Component	Volume (μ L)
Tail DNA	1.00
10x Taq Buffer	5.00
10 mM dNTP Mix	1.00
25 mM MgCl	5.00
Internal Control MbCx7 For Oligo	1.25
Internal Control MbCx7 Rev Oligo	1.25
IA-8	1.25
IA-6	1.25
IA-3	1.25
Taq Polymerase	0.50
ddH ₂ O	31.25
Total	50.00

Thermocycler Profile: IGF/flox

94 C for 1 min (initial cycle only)

94 C for 1 min

56 C for 1 min

72 C for 1 min

----- \diamond x 24 Cycles

72 C for 8 min

4 C for 5 min

IGF1-loxP PCR Screening

Gel Analysis:

1% agarose gel
15 μ l per lane

Expected Results:

M β Casein Cntrl	500 bp
WT IGF-1	350 bp
IGF-1/flox	200 bp