

PCR for neo

PCR REACTION:

10X PCR buffer	2λ
MgCL ₂ 25 mM	2 λ
dNTP'S 2.5 mM	2λ
Primer A	5 pmol per reaction (1λ)
Primer B	5 pmol per reaction (1λ)
Taq	0.4 λ
DNA	2λ
H ₂ O	Up to volume (20λ) 9.6 λ

Primer A (sense): 5'-CTGAATGAACTGCAGGACGA-3'

Primer B (antisense): 5'-ATACTTTCTCGGCAGGAGCA-3'

PCR CONDITIONS:

1. 94⁰C for 5 min.
2. 94⁰C for 1 min.
3. 57⁰C for 1 min.
4. 72⁰C for 1 min.
5. Steps 2-4 for 5 more cycles.
6. 94⁰C for 30 sec.
7. 55⁰C for 40 sec.
8. 72⁰C for 1:30 min.
9. Steps 6-8 for 25 more cycles.
10. 72⁰C for 10 min.
11. 20⁰C for 1 min.
12. End

PCR PRODUCT: Neo band: 172 bp

PCR FOR LacZ

PCR REACTION:

10X PCR buffer	2λ
MgCL ₂ 25 mM	2λ
dNTP'S 2,5 mM	2λ
Primer A	10 pmol per reaction 2λ
Primer B	10 pmol per reaction 2λ
DNA	2λ
Taq	0,4λ
dd.H ₂ O	Up to volume (20λ) 7.6λ

Primer A (antisense): 5'-GATCCCGTCGTTTTACAACGTCGT-3'

Primer B (sense): 5'-GAACTTCAGCCTCCAGTACAGCGC-3'

PCR CONDITIONS:

1. 95⁰C for 5 min.
2. 95⁰C for 30 sec.
3. 60,2⁰C for 40 sec.
4. 72⁰C for 1min.
5. Steps 2-4 for 30 more cycles.
6. 72⁰C for 5 min.
7. End.

PCR PRODUCT: Transgene band: 715bp.