Yeast Colony PCR

(Hahn lab) 9/17/02

This method is more reliable than the old method of adding yeast directly to PCR tubes. No more than 1 microliter of the crude DNA should be added to the 50 microliter PCR reaction because the SDS in the DNA prep can inhibit the PCR reaction.

**Note:** These elongation times and annealing temperatures work well for most applications where the product is less than 1.0 kb. These parameters may have to be adjusted for your specific application.

Platinum Taq (or other hot start Taq) gives more reliable and consistent results than regular Taq for this assay.

1. **Prepare Yeast DNA (this works best with fresh yeast plates)**

Use a pipetman tip to transfer the equivalent of a medium size yeast colony to 30 microliters of 0.2% SDS

Vortex ~15 seconds

Heat in hot block for 4 min at 90 deg. (important for consistent DNA extraction and PCR results)

Spin in microfuge 1 min. Remove supernatant to a new tube. The crude DNA can be stored at -20 degrees.

2. **PCR Reaction**

Combine the following components on ice:

5 microliters 10x colony PCR buffer
1.5 microliters 50 mM MgCl2
1 microliter 10 mM mix of dNTPs
10 pmoles of each primer (~100 ng of a 25 mer oligo)
2 microliters 25% Triton X-100
0.3 microliter Platinum Taq Polymerase [Invitrogen] (5 u/microliter)
H2O to a final volume of 49 microliters

Add mix to PCR tubes on ice containing 1 microliter of the crude DNA prep from above
3. PCR cycle profile:

95 deg 1 min
95 deg 30 sec
54 deg 1 min (optimum annealing temp varies according to primers)
72 deg 1 min
repeat steps 2 through 4 for a total of 35 cycles
72 deg 6 min
hold at 4 deg
Load 5-10 microliters to Agarose gel for assay.

For DNA sequencing analysis of product, purify using QIAquick PCR purification kit (Qiagen), eluting the product in 30 microliters. Use ~6 microliters for DNA sequencing analysis.

MATERIALS:

0.2% SDS

10X Colony PCR buffer:
0.13 M Tris-HCl pH 8.5
0.56 M KCl
50 mM MgCl2
10 mM dNTP mix

25% Triton X-100

PCR primers ~25 bases in length specific for the region of interest with annealing temp 55-60 deg.