Yeast Colony PCR

(Hahn lab) 1/8/16

This method is much reliable than our older method that used platinum taq. We have had good luck getting PCR products ≥ 3 kb with this method.

Note: These elongation times and annealing temperatures work well for most applications but parameters may have to be adjusted for your specific application.

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

Use a 20 microliter pipetman tip to transfer the equivalent of a small size yeast colony to 30 microliters of 0.2% SDS

Vortex ~20 seconds

Heat in hot block for 4-5 min at 90 deg.

Vortex 10 sec.

Spin in microfuge 2 min. Remove 20 ul supernatant to a new tube. The crude DNA can be stored at -20 degrees.

2. PCR Reaction

Combine the following components at RT:

- 1 microliter yeast DNA from above
- 15 pmoles of each primer (~150 ng of a 25 mer oligo)
- 10 microliters 2x KOD extreme buffer
- 2.5 microliters 2 mM mix of dNTPs
- H2O to a final volume of 19.2 microliters
- 0.8 microliter KOD extreme

3. generic PCR cycle profile:

- 94 deg 2 min
- 98 deg 10 sec
- 55 deg 30 sec (works well for most primers, but may need to optimize)
- 68 deg 1.1 min/KB

- repeat steps 2 through 4 for a total of 32 cycles
- 68 deg 1.1 min/KB
- hold at 12 deg

analyze products on Agarose gel.

For DNA sequencing analysis of product, purify using QIAquick PCR purification kit (Qiagen), eluting the product in 30 microliters. Use ~5 microliters for DNA sequencing analysis.

MATERIALS:

0.2% SDS

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

KOD extreme (EMD Millipore) contains enzyme, dNTP mix and 2x buffer.