

## **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  $\geq 3$  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
- H<sub>2</sub>O 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.