

## Yeast colony PCR

Gabe Zentner

1. Pick part of a colony into a PCR tube using a sterile pipet tip or toothpick. Mark the location of the picked colony with a dot and number on the bottom of the plate so you can go back and pick the rest of it to grow up for a glycerol stock if it is correct
2. Microwave picked colonies on high for 1 min
3. For each PCR, prepare the following reaction mix:
  - 5  $\mu\text{L}$  10X Standard Taq buffer (NEB M0273L)
  - 1  $\mu\text{L}$  10 mM dNTPs (2.5 mM each dNTP)
  - 1  $\mu\text{L}$  10  $\mu\text{M}$  forward primer
  - 1  $\mu\text{L}$  10  $\mu\text{M}$  reverse primer
  - 0.5  $\mu\text{L}$  Taq polymerase (NEB M0273L)
  - 41.5  $\mu\text{L}$   $\text{H}_2\text{O}$
4. Add 50  $\mu\text{L}$  PCR mix to each microwaved colony
5. Cycle using the following parameters:
  - 95°C, 5'
  - 35 cycles of:
    - 95°C, 30"
    - 55°C, 30"
    - 68°C, 1'/kb
  - 68°C, 5'
  - Hold at 8°C
6. Add 10  $\mu\text{L}$  6X orange loading dye (Lifetech R0631), pipet up and down to mix, and load 10  $\mu\text{L}$  on an agarose gel