

## Colony PCR

1. Pick colony with pipette tip
2. Transfer colony to PCR tube by rubbing tip in tube
  - a. After transferring to tube, put tip in 4 mL LB, place at 37C
3. Add 20  $\mu$ L PCR Supermix to each tube
4. PCR
  - a. Program:
    1. 95°C 2 min
    2. 95°C x 30"
    3. 52°C x 1.5 min
    4. 70°C x 1.5 min
    5. repeat 2-4 25X
    6. 70°C x 5 min
    7. 4°C forever
5. Analyze PCR
  - a. Add 3  $\mu$ L 6X loading buffer
  - b. Load 15  $\mu$ L on gel and run 1.5% agarose gel
  - c. Verify inserts

### Supermix recipe (recipe for 200 reactions. Scale appropriately)

- 100 $\mu$ L 2X FailSafe PCR Buffer D (Epicentre, FSP995D)
- 10 $\mu$ L Forward primer for gene of interest (100pmol/ $\mu$ L stock)
- 10 $\mu$ L Reverse primer for gene of interest (100pmol/ $\mu$ L stock)
- 2 $\mu$ L Taq polymerase (or other cheap polymerase)
- 78 $\mu$ L H<sub>2</sub>O

(note: some people add DMSO to a final concentration of 6% in these reactions. On all of the various genes I've worked on, the above recipe has worked beautifully. However, for some reason you have problems, you may give that a shot).