BIOCHEMISTRY

Colony PCR protocol

Day 1

1. Prepare PCR tubes with 5uL of H2O
2. Pick a single colony with a yellow tip and put the tip into the PCR tube with the 5 uL of H2O
3. Resuspend the colony with the pipet
4. use the tip to replicate the cells into a new plate (this will allow you grow the cells upon confirmation of the positives)
5. Prepare PCR reaction mix
    - 6uL of Taq master mix (purple mix)
    - 4uL of H2O
    - 0.1uL primer F (10uM)
    - 0.1uL primer R (10uM)
    - 2uL of your resuspended colony (step 3)
6. Run PCR program called colony PCR

When running colony PCR, try to use primers that give an amplicon not higher than 2Kb. Ideally, you want one primer flanking the plasmid (i.e. 35S_F) and one primer flanking the insert

7. Run PCR produc in a gel.
8. Confirm size of PCR
9. Grow 2 positive colonies in a 5mL culture with antibiotics.

Day 2

1. Continue with plasmid DNA extraction and confirmation vias sequencing.