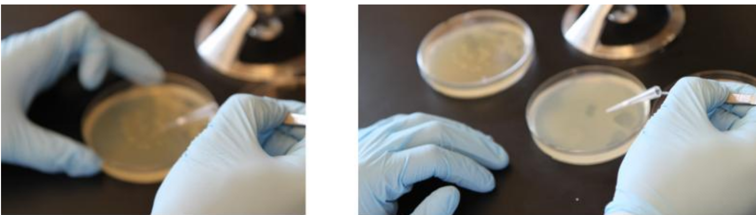
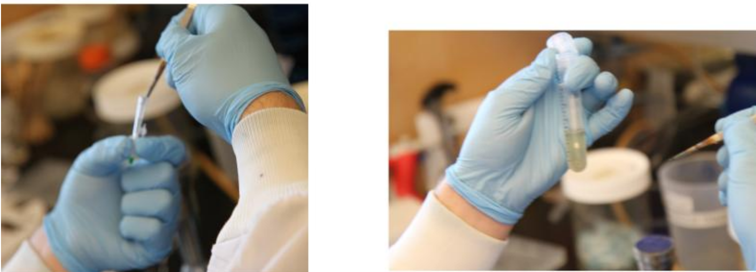
1. Using a pipette aid, carefully add 5ml of liquid media to your 15ml inoculation tube. Repeat for every colony you are inoculating. Place your inoculation tubes into your 37C shaker incubator and allow 15 minutes for the media to warm up. (if you are making a reference plate, place that plate into your incubator at this time)
   Remove tubes from incubator along with your plates





2. Using a sterile pipette tip carefully pick up a single colony from your plate.

   i, If you are making a reference plate, gently touch the tip to a section of the agar on your plate, be careful not to break the agar.

   ii. If you are running colony PCR, gently swirl your tip in the colony PCR reaction tube

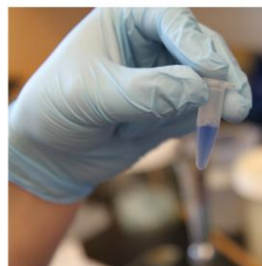
3. Drop the Pipette tip into the inoculation tube and return the tubes to your shaker incubator to grow. (estimate time of growth varies across cell types, usually between 8 and 24 hours)

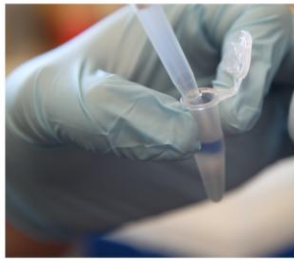
4. Once your liquid culture has finished growing (visual indicator for this being murky media) move your tubes to a large centrifuge machine and spin down the cells for 5 minutes at 3500rpm. This step should result in a very visible cell pellet at the bottom of your tubes

5. Carefully aspirate or pour off the media and the pipette tip.

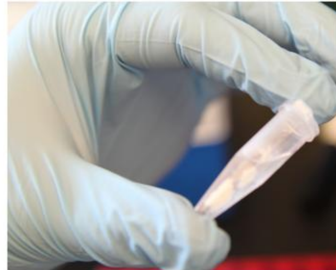
6. Resuspend cell pellet by adding 250ul Qiagen P1 resuspension buffer and vortexing the tube until the pellet disappears. Transfer the contents to 1.5ml microcentriuge tubes.

7. Add 250ul Qiagen P2 Lysis Buffer to your tube. (A color change should occur turning your contents blue) Invert the tubes gently five times and let sit for 5 minutes (it will turn blue if there is a high density of cells)





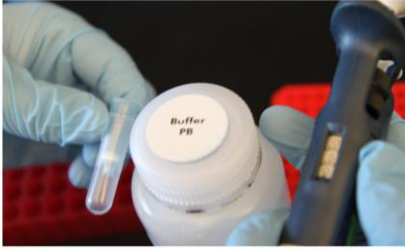
8. Add 350ul Qiagen N3 Neutralization Buffer to your tube and invert until your tube becomes clear again (there should be solid junk floating around)



9. Place your centrifuge tube into your centrifuge and spin for 10 minutes at 13000rpm to separate the cell debris from your desired DNA (after this spin there should be cell mass plastered to your tube

10. Carefully pipette off the supernatant without disturbing the cell debris and transfer it onto a the blue Qiagen miniprep spin column

11. Place the spin columns in their supplied 2ml collection container and spin for 1 minute at 13000rpm. Dump the liquid that collects in the 2ml tube into trash



12. Pipette 500ul of Qiagen PB binding buffer to your spin column and spin for 1minute at 13000rpm. Dump the liquid that collects in the 2ml tube.

13. Pipette 750ul of Qiagen PE wash buffer to your spin column and spin for 1minute at 13000rpm. Dump the liquid that collects in the 2ml tube.

(Optional) repeat step PE wash

Place the spin column back into the centrifuge and spin again for a 1-minute dry run at 13000rpm to completely remove all remaining PE from the filter

14. Remove the 2ml container from the spin columns and place the column into new labeled 1.5ml tubes (not supplied) add 50ul of Qiagen EB Elution Buffer to the spin column, make sure you pipette onto the filter. (Warning: do not touch the filter with you tip as it may break). For a greater concentration, add only 30ul of elution Buffer.

Allow the EB to sit in your tubes for 1 minute before moving the spin column to the centrifuge and spin for 1 minute

Your mini prepped DNA in now in the 50ul (or 30ul) of EB
collected in your 1.5ml tube.