Colony PCR

Overview

This protocol covers colony PCR with Q5 High Fidelity DNA Master Mix. Colony PCR is often used to confirm the presence of correct plasmids in transformed bacteria. This protocol relies on and assumes familiarity with the standard PCR protocol.

Materials

- Plate with plasmid containing bacteria
- Toothpicks
- 1.7mL tubes
- .2mL Tubes
- Nuclease Free Water
- 10μM Primers
- Q5 Hot Start 2x High Fidelity 2x Master Mix (MM)
    - Note that we have both hot start and non-hot start versions, make sure you use hot start
    - Do not confuse with 2x HiFi DNA Assembly Master Mix, which is several times more expensive.

Procedure

1. Typically, either 4 or 8 colonies are PCRed. For each colony to be PCRed, set up a keyed .2mL tube containing 10μL of NFW.
2. Use a toothpick to pick a distinct single colony into each tube. Make sure colony makes it into solution.
3. Determine and locate appropriate primers. Retrieve from -20C and wait to thaw.
4. Key a different color of .2mL tubes, with the same key used in step 1. These tubes will be used for the PCR
5. Prepare a DNA master mix for the colonies you are using.
    - Each colony will be used in a 10μL colony PCR. The composition of 1 reaction is: 3 uL NFW, 0.5 uL Forward Primer, 0.5 uL Reverse Primer, 1 uL Colony Solution, 5 uL Q5 Hot Start 2X Hi-Fi Master Mix. It is recommended to upscale the amount of master mix made by ~10% to account for loss at various stages.
    - Example: If I was making a master mix for 8 colonies and using a 10% upscale, I would add the following to a 1.7mL tube: 4.4μL Forward Primer, 4.4μL Reverse Primer, 26.4μL NFW, 44μL Q5 Hot Start.
6. Add 9μL of master mix to each tube.
7. Add 1μL of colony solution to each tube.
8. Spin down PCR tube.

9. Proceed with standard PCR protocol. Remember to set thermocycler to 10µL reaction volume.