Overnight liquid cultures (picking colonies)

Introduction

Overnight cultures are used to prepare miniprep DNA.

Materials

- The plate from which you are picking colonies
- 15 ml round-bottom polystyrene tubes, one per culture
  - The ones with the snap caps, **NOT** conical tubes with screw caps
- 5 mL LB per culture
- A container that can hold 5ml x the number of cultures
  - For a modest number of minipreps, a 50 ml conical tube works well.
  - For larger minipreps, use a sterile bottle (100 ml is frequently useful.)
- Antibiotic stock, 1000X

Procedure

Materials Setup

1. Warm up the LB to at least room temperature (if it came from the fridge), but not warmer than 37°C
2. Label one round-bottom culture tube for each miniprep. Use "NAME-1, NAME-2, ..."etc for the naming convention, where NAME is a shortened name of the plasmid (eg, "hEF1a:mKate").

   Your impulse is to just use number, or initials and number, but trust me -- you will want to be able to identify this tube in three weeks when you've forgotten what you were doing.
3. Using a sterile pipette, transfer 5 ml of LB to the mixing container for each culture PLUS 5 ML.
4. Add antibiotic stock to a final concentration of 1X (1 μl stock for each 1 ml in the mixing container.)
5. Cap tightly and mix well.

Culture Setup

6. Using a sterile pipette, transfer 5 ml of LB+antibiotic to each round-bottom culture tube.

   If you ar e making cultures with different antibiotics, **take care that the right media goes in each tube.**
7. Squirt ethanol on a pair of foreceps and wipe dry with a Kimwipe.

8. Use the forecеps to pick up a sterile 200μl pipette tip, scrape a colony off of the plate, and drop the pipette tip in the corresponding tube.

   Repeat for each tube.

9. Transfer to an incubating shaker at 37°C and incubate 14-16 hours.

   Don't over-grow too badly, or your yield will suffer.
   If you need to grow longer, you can grow at 30°C instead for 20 hours.