

E. coli Miniprep Inoculation

Overview

This protocol covers the inoculation of plasmid containing *E. coli* for miniprep.

Materials

- LB broth
- Labeled, autoclaved glass culture tubes
 - o Each tube should be labeled with construct/assembly name, date, plasmid backbone and inoculation number (typically 3 inoculations per assembly).
 - o Tubes should be labeled with lab tape. Care should be taken to ensure that label is taped all the way around, else label will fall off during incubation.
- Appropriate stock antibiotics
 - o Depending on the backbone either Chloramphenicol (chlor), Ampicillin (Amp), Kanamycin (Kan), or Tetracycline (Tet). The antibiotic needed can be determined by what vector is being used. (E.g. 1C3 is chlor resistant).
 - o Note that Kanamycin and Ampicillin will need to be thawed before use. (You may thaw in hand.)
- 50mL or 15mL Centrifuge Tubes for antibiotic media preparationⁱ
- Sterilized toothpicks

Procedure

1. Determine required volumes of antibiotic containing media. Typically, this is 4mL per tube, and 3 inoculations are performed per assembly.
 - o You will want to round this up to a number that can easily be measured on the centrifuge tube. Generally you will want to have at least 5% extra media to prevent running out.
2. Obtain and label appropriately sized centrifuge tubes.
 - o Minimum labeling is media type, antibiotic and date.
3. Wipe down hood working surface with ethanol as well as your hands and any new items brought into hood.
4. Using good sterile technique, pour the appropriate amount of LB into tubes.
5. Add the appropriate amount of antibiotic to each tube.
 - o Antibiotics are 1000x, so for every 1mL of media you should add 1µl of antibiotic stock. **This is only the case with high copy plasmids** (i.e. 1C3, 1A3, 1T3, 1K3), as low copy plasmids need less antibiotic.
 - o **For low copy plasmids** (e.g. 3K3), different concentrations must be used. For use .7x for Kan (.7µl 1000x stock per 1mL media), .3x for Chlor, .5x for Amp and .7x for Tet.
6. Bring tubes out of hood, throw away trash and wipe down hood with ethanol.

ⁱ Antibiotics are stored at -20C and media is not prepared with them in advance, as they degrade over time.

7. Wipe down a working surface with ethanol and use a P5000 to transfer media to tubes.ⁱⁱ
8. Using a toothpick pick single colonies from the appropriate plates and transfer into appropriate glass tube. Alternatively, if using colony solution, add 5µL to tube.
 - Ensure that only a single colony is taken. Do not pick agar, pick the colony. Try to not take colonies that are obviously defective. (e.g. if a GFP expressing part was assembled from a RFP expressing part, do not pick red colonies).ⁱⁱⁱ
 - Make sure toothpick makes contact with liquid (you will have to tilt the tube). Swish around a little, as long as some number of bacteria make it into the liquid, the inoculation will be fine.
 - Make sure to place cap on loosely to allow for airflow
9. Place tubes in shaking incubator at 250 RPM and 37C degrees^{iv}. Grow overnight, about 14-16 hours before miniprep.

ⁱⁱ Ideally we would do this in the hood as well, but practically it is too difficult as the hood is so small.

ⁱⁱⁱ Be aware of satellite colonies on Amp plates, especially ones that have grown for a long time.

^{iv} In rare cases temperature will be reduced. 30C is often used when working with plasmids that have many inverted repeats, as lower temperature lowers recombination (presumably through slower growth rates).