[iGEM 2017] Inoculation

Introduction

Transfering colonies from a plate to liquid culture.

Typically we grow three replicates from each plate for minipreps. If we are doing measurements the number of replicates will be variable.

The typical cloning pipeline is:
PCR -> Gel -> DpnI -> PCR Purification -> Gibson Assembly -> Transformation -> Colony PCR -> **Inoculation** -> Miniprep

Materials

- Sterile Hood
  - *Make sure you review the Sterile Hood usage protocol prior to performing Inoculations!*
- 10 mL Glass Culture Tubes
- Liquid Growth Media
  - Use LB (high growth media) for minipreps
  - Use M9 (low growth media) for experiments. *(If using Arabinose induction, this needs to be M9 with Glycerol instead of Glucose). (If using 5-alpha E. coli, this needs to be M9+Thiamine). (If using 10-beta E. coli, this needs to be M9+Leucine).*
- Plates with Colonies
- Antibiotics
- Falcon Tubes (15 mL or 50 mL)
- Shaking Incubator

Procedure

Prepare Liquid Growth Media

1. Determine the volume of media needed. We usually use around 3-4 mL media per culture tube, so multiply that by the number of tubes you'll need, and add some more volume as a safety buffer so you don't run out.

2. In the hood, in an appropirately-sized falcon tube, pour in the appropirate volume of media.

   Precision is not super important here.

3. In the hood, add antibiotics according to the following table. For every 1 mL of media, add:

Table1

	A	B	C
1		**High growth media:**	**Low growth media:**
2	**AMPICILLIN**	1 uL	0.5 uL
3	**CHLORAMPHENICOL**	1 uL	0.3 uL
4	**KANAMYCIN**	1 uL	0.8 uL
5	**TETRACYCLIN**	1 uL	1 uL

4. In the hood, invert the falcon tube a few times to homogenize the antibiotic within the solution.

5. Pour 3-4 mL of antiobitic+media into each glass culture tube.

Add Colonies to Media

6. Ethanol down the bench space.

7. Unwrap parafilm from plate.

8. Identify your target colony.

   The colony should be relatively small, so that there has been less time for mutation to accumulate.
   The colony should be spatially isolated, so that you can easily get the colony without accidentally touching other colonies on the plate, ensuring that we have a clonal population of cells in each culture tube.
   If you can rely on an expected visual phenotype (GFP or RFP under strong expression is visible with the naked eye), make sure your colony satsifies this phenotype.

9. Using a new pipette tip, gently scrape off cells from your colony from the surface of the agar.

   Use tips which don't get used often in other procedures. P100s are a good choice.
   Move quickly to minimize the time that the plate is exposed to the open air.
   You don't have to press strongly against the agar-- just a gentle brushing of the surface is sufficient to get the cells off the agar and onto your tip.

10. Swirl around the pipette tip in the liquid media in a culture tube.

    You will have to tilt the tube so the tip can reach the liquid.

11. Set the tube aside and proceed to the next colony.

Put the Culture Tubes in the Shaking Incubator

12. Once you have added colonies to all of your culture tubes, place them into the shaking incubator.

    Make sure the incuabor is set to the right settings- for minipreps we use 250 rpm at 37C. If you are doing an experiment or using another strain, it may be different.
    Make sure that, one in the incuabor, the caps on the tubes are relatively loose so that air can flow easily into the tube.

13. It takes ~14 hours for the cultures to be ready for miniprep.

    If you miniprep too soon then your yield will be low. If you miniprep too late you risk mutation accumulation.