[iGEM 2017] Chemical Transformation

Introduction

This is how you insert your plasmid(s) into cells. Please be sure you know **which strain** you are using and you know the **appropriate amount of time** to heat shock your **specific strain**.

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

Materials

- Competent Cells (Stored at -80)
- Plasmid DNA (either from gibson, or from storage)
  - Plasmid should be on the order of 1-10 ng/µl (~1:30 dilution for minipreps, or existing concentration for gibson).
- SOC media or 10-Beta media
- Ice
- 1.7 mL Eppendorf Tubes
- Pre-Warmed Plates (with the correct antibiotic)

Procedure

Thaw Cells

1. Take out cells from -80 degrees C freezer so that you have at least 10 µL of cells per type of plasmid you are trying to transform. (There are 50 µL of competent cells per NEB tube of cells.)

   Note, we have one box of 10-Beta cells which have 200µl instead of 50µl.

2. Place cells on ICE for ~10 minutes (until thawed)

3. Transfer appropriate amount of cells to appropriately labelled Eppendorf tube (I would suggest using the same key as you used for the gibson assemblies).

   This also needs to be done on ice

Transform

4. Add 2 uL of the appropriate plasmid DNA to each aliquot of cells.

   Plasmid DNA should be on the order of 1ng/µl or less. Use the 1:30 diluted box when transforming from miniprep. Gibson reaction DNA is already at the right concentration, so 2µl straight from the reaction can be used.

5. Incubate on ice for 30 minutes. Pre-warm heatblock to 42 degrees C.

   CRITICAL Remember to hit not only the heat block power switch, but also the heating element switch (on the front right of the heat block). Make sure the block is set to 42C

6. I would strongly recommend that you take this time to pre-label your plates and place them in the incubator to pre-warm. Plates need to be at least room temperature for plating. Labeling cold plates is not advised. (Remember to place plates upside down in incubator).

    CRITICAL Plates should be labeled with cell type if 5-alpha is not being used

Heat Shock

7. Heat shock cells for appropriate amount of time. This varies based on which strain you are using.

    BL21 gets heat shocked for 10 seconds
    10-Beta and 5-alpha get heat shocked for 30 seconds

8. Incubate on ice for five minutes.

9. Pipette in SOC or 10-Beta media (10-Beta cells only) to each tube based on the amount of cells you used. 50 uL of cells get 950 uL of SOC, for reference.

    19µl of SOC per 1µl of cells

Outgrowth

10. Place in shaking incubator 250 rpm 37C for 1 hour (chlor, amp, or tet) or 2 hours (kan or double transformations).

11. Remove bacteria from shaking incubator.

12. **INVERT EVERY TUBE 4-6 TIMES. IF YOU DONT DO THIS YOU WILL NOT HAVE A SUCCESSFUL TRANSFORMATION.**

Plate

13. Plate out 30µl for gibson transformations on 1C3. For 3K3 plate between 50µl of parts. For experiment transformations plate out 15µl.

    To plate, pour 4-6 sterile glass beads onto the plate in a sterile environment and spread culture evenly by gently shaking/tilting the plate. Dispose of glass beads into ethanol.

14. Put plates in incubator upside down (agar side up, lid down). Let grow overnight.

    Do not be alarmed if you do not see colonies for up to 18 hours.