Transformation of E. coli

Introduction

Transformation is the process of inducing chemically competent *E. coli* to take up DNA.

Materials

- Dry bath, set to 42°C
  - Fill the wells in the dry bath block 1/2 full with DI water.
- Ice bucket, with ice
  - For thawing competent cells.
- DNA to transform
  - Could be an assembly reaction (LR, Golden Gate, etc) or a miniprepped plasmid.
  - If you removed it from the freezer, make sure it's entirely thawed out.
- pUC19 Transformation Control, 1 pg/μl
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  - The pUC19 control will tell you how efficient your transformations were.
- SOC growth media, at room temperature
  - Check to make sure it's clear and **NOT CLOUDY.**
- Antibiotic plates, one per transformation, plus 1 Amp plate for the pUC19 control
  - Make sure the plates you use match the resistance cassette of the plasmid!
- Competent E. coli, one tube per transformation + one for the pUC19 control
  - These live in the -80 in 235.
  - Thaw on ice 3-4 minutes.
- A timer, set for 30 seconds.

Procedure

Setup

1. Make sure the dry bath is set to 42°C and the wells in the block are 1/2 full of DI water
2. Remove selection plates from the refrigerator. Double-check that they match the selection marker on your plasmid, then place them in the 37° incubator.
3. Retrieve the DNA to transform.

   If frozen: thaw, completely, flick a few times to mix, then pulse down in the microfuge.

4. Fill an ice bucket with ice. Retrieve one tube of competent E. coli per transformation from the -80 and thaw on ice, 3-4 minutes.Shake

00:03:00

5. While the transformation tubes are thawing, label their tops with something descriptive. Record the labels here:

Table1

	A	B	C	D	E	F	G	H
1	puc-19L							
2	puc-19TC							
3	Pdest_mCherry							
4	pDest							

Transformation

6. Add 2 μl DNA from each reaction to a tube of competent cells.

   Immediately after adding the DNA to each tube, stir the cells a few times with the pipette tip.

7. Add 1 μl of the pUC19 transformation control to the positive control tube.

8. Incubate on ice for 30 minutes.

00:30:00

9. Heat shock the cells for **exactly 30 seconds** in the 42° heat block. (Yes, set a timer.)

00:00:30

10. Place back on ice for 2 minutes.

00:02:00

11. Add 250 μl SOC to each tube.

12. Tape the tubes to the platform of a shaker at 37°C and shake at 270 RPM for 60 minutes.

01:00:00

Plating

13. Label the selection plates using the labels you recorded above.
14. Shake ~10 plating beads onto each plate.
15. Pipette 100 μl of each transformation onto the corresponding plates. NOTE: if you are using DNA from a golden gate reaction, see the golden gate protocol for instructions to dilute your sample.
16. Cover the plates and shake the beads around to spread the cells out.
17. Dispose of the beads by tapping them into the waste container.
18. Incubate the plates **upside down** overnight in the 37° incubator.

    Don't incubate for more than 18-24 hours.

If LR was Done using pDEST_mCherry

19. Plate 5 ul of outgrowth + 200 ul water

Compute transformation efficiency

20. Count the colonies on your positive transformation plate.

    If there are many many colonies, then hooray! You had a great transformation. Just estimate.

21. Divide the number of colonies by the fraction of the transformation you plated.

    So, if you resuspended your transformation in a total volume of 300 ul, then plated 100 ul, multiply the number of colonies by 3.

22. Transformation efficiency is expressed in colonies per microgram pUC19. Multiply the number of colonies (from step 22) by the appropriate conversion factor.

    So if you transformed 1 picogram of pUC19 DNA, multiply by 10^6.

23. Record your transformation efficiency in your (daily) lab notebook.