6/06/17

Tuesday, June 6, 2017 9:17 AM

Who was in lab today: Jeff, Ayesha, Qingxi, Ana, Salma,
Martin

- Followed Miniprep protocol to purify DNA from overnight cultures of cjBlue and pet28 (plasmids)
  - Used the elution buffer/DNA liquid mix from one of the samples in place of elution buffer for the next sample
- Prepped another digest (will run Gel electrophoresis tomorrow)
- Plated ligated DNA

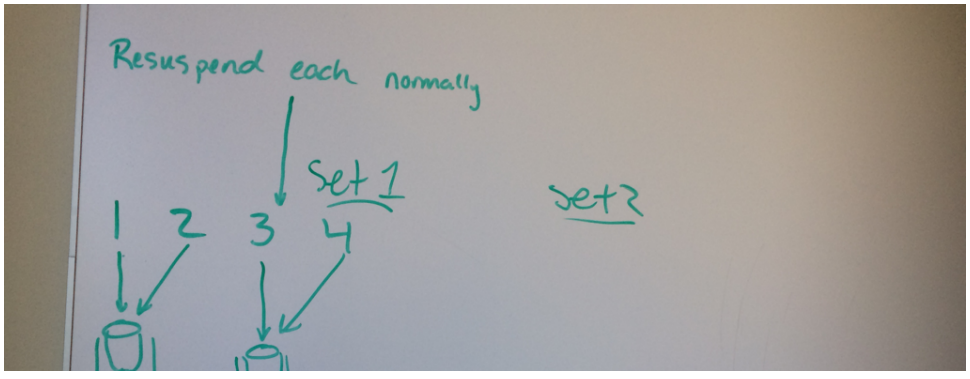
Plating the Ligated DNA:

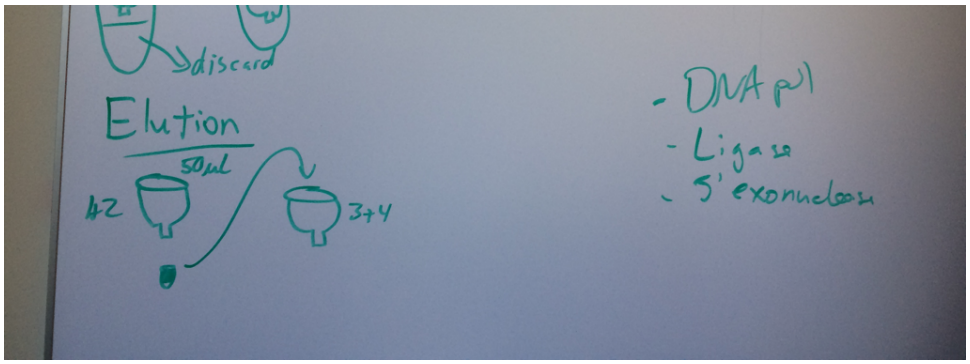
1. See Protocol for **Making chemically competent cells.**
2. See Protocol for **Chemical transformation.**
3. The petri dish with Kanamyacin was used because PET28B is the vector plasmid with Kan resistance.
4. See Protocol for **Plating transformed bacteria.**

We transformed DH5alpha with the ligated plasmid FIRST because we wanted clones. After that we will isolate the plasmid from DH5alpha and transform in to BL21. When the transformed BL21 is plated we should see BLUE colonies.

Getting Max. Amount of Plasmid DNA:

1. 40 uL of total cell culture is used. This can be separated in tubes/flasks
2. Follow **Miniprep Protocol**
3. Distribute cells into 8 centrifuge tubes.
4. Lyse, Neutralize, and Spin
5. Add 2 tubes worth of supernatant to one spin column
6. Now have 4 tubes
7. Wash as described in normal protocol
8. When eluting, use 75 uL elution buffer (in place of 50 uL EB) and only place on one spin column.
9. Take this elution from one spin column, and add it to the second spin column.
10. Spin again
11. Add second eluent to third column, and this third eluent to fourth column.





Follow Miniprep protocol to purify plasmid

1 2 3 4

Spin column, centrifuge, discard flow through

50 uL Elution buffer, collect liquid

Apply 1,2 liquid to 3,4

Find T5 exonuclease sequence (from original Gibson paper)