

# 7/31/17

Monday, July 31, 2017 10:28 AM

Who's in Lab: Ayesha, Nathan, Qingxi, Martin, Ana

## SUMMARY:

1) Will start the process for making new competent cells as we suspect this has been the main problem in our experiments.

Today, we will plate out the DH5a and BL21 cells we already have. These are considered our seed stocks.

We will be using the iGEM protocol:

[http://parts.igem.org/Help:Protocols/Competent\\_Cells](http://parts.igem.org/Help:Protocols/Competent_Cells)

2) We received the sequencing results and the results were good. We could not access the sequencing of the LC1853 strain that was supposed to be obtained using the T7 rev primer. We will begin designing primers for these two sequences this week.

Tyler helped find the sequence we can use to design primers. This is what we did to find the sequence:

- a. We blasted the sequence of LC 1853 using the T7 forward primer from UIUC core sequencing facility.
- b. Go to the "Alignments" section and the "Description" section. The description section indicates on the right how identical the sequence you entered is with the genome of the organism the program is returning. We picked the link with 97% ident. The alignments section shows visually how the sequences match up. The occasional spaces indicate a sequencing error so don't be alarmed by those.
- c. Then click on the genome "Accession" number next to the link to the organism's genome. [On right side of the page].
- d. Then go to "edit and resubmit" and you blastx the results to get the protein sequence. From here you scroll down to the "Description" section and choose the link with highest ident. Number. We chose the one with 88%. Copy the protein accession number for this protein sequence.
- e. In part c, we opened a new tab with organisms entire genome. Click on "Nucleotide" from among the "Related Information" options on the right. A new page will open with several links. Click the first link.
- f. Here we will use the control find function and enter the protein accession number. This will lead you to the CDS (Coding sequence of what we are looking for). Click on the CDS and you have found your sequence!!

LC 1853 sequence we will using for designing primers:

[https://www.ncbi.nlm.nih.gov/nucore/NZ\\_CP011107.1?from=1304443&to=1306716&sat=4&sat\\_key=191505528&report=fasta&strand=2](https://www.ncbi.nlm.nih.gov/nucore/NZ_CP011107.1?from=1304443&to=1306716&sat=4&sat_key=191505528&report=fasta&strand=2)

We still need to find the LC1539 sequence.

I [Ayesha] think this may be the sequence [https://www.ncbi.nlm.nih.gov/nucore/NC\\_018092.1?from=27614&to=29941&sat=4&sat\\_key=191360860](https://www.ncbi.nlm.nih.gov/nucore/NC_018092.1?from=27614&to=29941&sat=4&sat_key=191360860)

**Note:** When making primers change the first codon of sequence, which is usually GTG, into ATG because this is more widely recognized and used by E. Coli.

3) we will verify whether our gibson assembly trial #1 actually worked last week by running gels of the YCP + JOE DNA we isolated using Tyler's protocol on 7/28/17

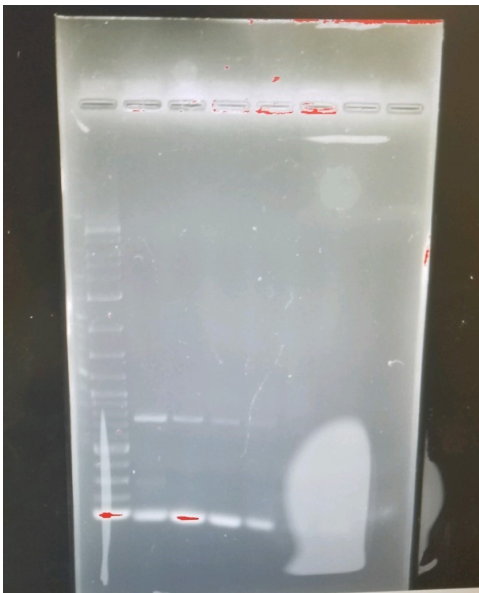
Ayesha's gel:



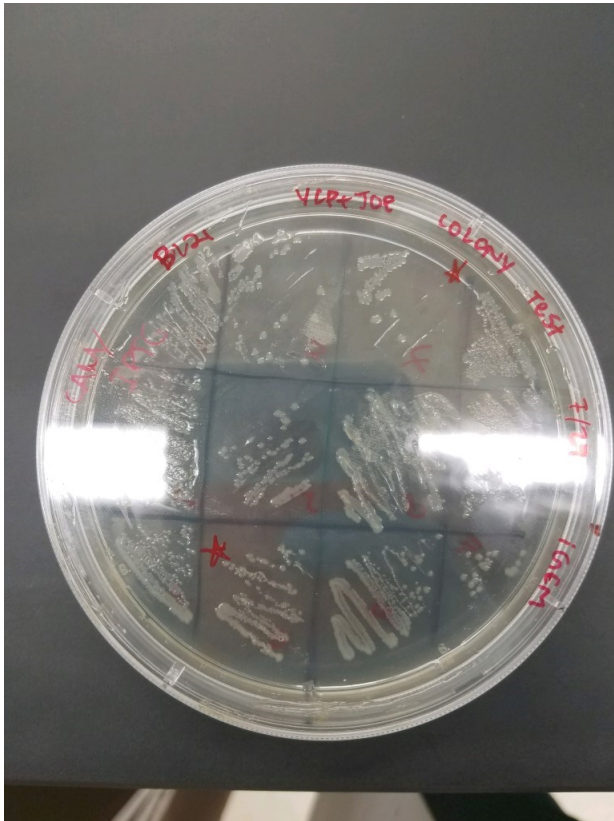
Lane 1: 3 uL of 2kb DNA ladder  
 Lane 2: YCP+JOE from G.A. trial #1  
 Lane 3: JOE plasmid conc. 35.9 ng/uL  
 Lane 4: Colony 6  
 Lane 5: Colony 7  
 Lane 6: Colony 8  
 Lane 7: Colony 9  
 Lane 8: Colony 10

There is no band in lane 3 as expected. This is because we only added the Forward and Reverse Primers for YCP so only YCP could be amplified in the PCR reaction. Lane 3 contained JOE plasmid without YCP insert. Colonies 6, 7, and 10 showed presence of YCP insert in JOE vector based on the bright bands near the 0.7 kb mark.

Martin's Gel:



4) We made 500 mL SOB Media and CCMB80 buffer for competent cells based on iGEM protocol.  
 CCM8B buffer: [http://parts.igem.org/Help:Protocols/Competent\\_Cells](http://parts.igem.org/Help:Protocols/Competent_Cells)  
 SOB: <http://parts.igem.org/Help:Protocols/SOB>



The starred boxes are the ones that showed YCP in the colony PCR results.

#### LABELED TUBES/BOTTLES:

- CMMB80 buffer in 4 deg fridge
- SOB buffer on bench

#### TOMORROW:

- Learn how to use NanoDrop machine in the lab
- Add seed stock to SOB media (5 pm)
- Make more SOB media
- Make SOC media
- Find LC1539 sequence
- Another Gibson Assembly must be done. This time the control would be doing a gibson assembly with vector but no insert (JOE W/O YCP) and the experimental group would be vector with insert (JOE+YCP) to make sure that JOE is being cut for insert to be added. The plate with JOE W/O YCP should not have colonies on it because the mismatched ends (caused by the cutting during G.A.) will not reanneal. Some background may be possible but the plate with JOE+YCP should significantly more colonies.