7/24/17 G.A. Trial #1

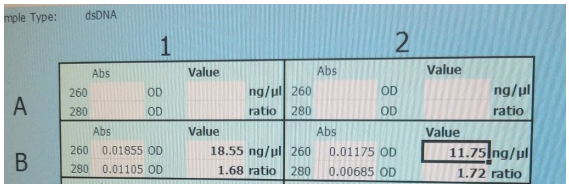
Monday, July 24, 2017 11:36 AM

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

GIBSON ASSEMBLY (G.A.) POSITIVE CONTROL:
-Precipitation protocol is being continued from 7/20/17 with JOE and YCP. Protocol may be found in hard copy lab notebook (written by Tyler) **or under " Protocols"**. Similar protocol may also be found here under the "Eppendorf protocol": http://www.openwetware.org/wiki/Ethanol_precipitation_of_nucleic_acids

Concentrations yielded from first DNA Precipitation:

B1 is JOE and B2 is YCP.



Tyler said make another batch because concentrations were very low.
X for DNA precipitation was 40uL
Used the ratios written in hard copy notebook to add appropriate ethanol and sodium acetate
Put in –20 box and labeled as precipitate 7/24/17

Tomorrow (Tuesday 7/25/17) need to continue the DNA Precipitation protocol. Where the samples are removed from overnight –20 deg incubation.

GIBSON ASSEMBLY WAS PERFORMED. See Gibson Assembly protocol.
Calculations for determining the composition of the reaction:

YCP--> 714 base pairs, obtained from well 6K in Kit Plate 4.
JOE--> 2290 base pairs, obtained from well 9J in Kit Plate 3.

Number of pmols of each fragment:

JOE: 50 ng * 1000 / (2290 bp * 650 Da/bp) = 0.0359 pmol

YCP: we want 3 times as much Insert as Vector
3*0.0359 pmol = 0.10773 pmol of insert.

Xng *1000 / (714 bp*650 Da/bp)= 0.10773 pmol

X= 49.9 ng of insert.

Volumes of Insert and Vector (using plate reader measurements):

50 ng/ (18.55 ng/uL) = 2.695 uL, Rounded up to 2.7 uL of JOE VECTOR.

49.9 ng/ (11.75 ng/uL) = 4.228 uL, Rounded up to 4.3 uL of YCP INSERT.

	YCP+JOE Assembly	PUC19 Gibson Assembly Control
Total Amount of Fragments	0.10773uL (insert) + 0.0359 (vector) = 6.923 uL	10 uL of pUC19 DNA
Gibson Assembly Master Mix	10 uL	10 uL
Deionized H20	10-6.923= 3.077 *uL	
Total Volume	20 uL	20 uL

*Rounded to 3.0 uL

After Gibson Assembly was complete, the assembled products were transformed into DH5alpha cells. For 40 uL of DH5alpha cells, we added 2 uL of DNA.

Test Tube 1: YCP + JOE positive control (as in positive control for our entire project), plated on CAM plate.
Test Tube 2: pUC19 Gibson Assembly Control, plated on AMP plate.

A couple issues. When we made the cells chemically competent today, we didn't mix it well enough and so the pellet didn't form properly. Also, recovery broth was not added after heat shocking cells. We still have plenty of Gibson Assembly product in the 4 degrees fridge (big one), however. We'll just have to transform a new batch of cells tomorrow (7/25/2017).