8/1/17 G.A. Trial #2

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SUMMARY:

A) Made 250 mL of SOC media based on the following protocol.
http://www.thelabrat.com/protocols/15.shtml

B) Autoclaved more bottles to hold media and buffers. **Bottles were autoclaved with water in them to remove any detergents as detergents inhibit competent cell growth and transformation**.

- C) Started G.A. Trial #2.
    - This time the control is a gibson assembly with vector but no insert (JOE W/O YCP) and the experimental group is vector with insert (JOE+YCP) to make sure that JOE is being cut for insert to be added.
    - Expected Results: 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 have significantly more colonies.
    - CAM+IPTG dishes will be plated tomorrow once BL21 competent cells have been made.

Calculations for determining the composition of the reaction:

YCP--> 714 base pairs
JOE--> 2290 base pairs

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/ (35.9 ng/uL) = 1.3928 uL, Rounded down to 1.39 uL of JOE VECTOR.

49.9 ng/ (48.0 ng/uL) = 1.0396 uL, Rounded up to 1.04 uL of YCP INSERT.

Volume of experimental group:
10 ul Gibson Assembly Master Mix + 1.39 ul JOE VECTOR + 1.04 ul YCP INSERT + 7.57 ul H2O
Volume of positive control (JOE W/O YCP)
10 ul Gibson Assembly Master Mix + 1.39 ul JOE VECTOR + 8.64 ul H2O

The resulting product is put in the small 4C fridge with labels "G.A. JOE+YCP 8/1/2017" and " (+) control G.A. 8/1/2017"

- **PERFORMED PCR CLEAN UP FOR PCR PRODUCTS FROM 7/27/17**
  Obtained concentration of **102.7 ng/ul** from the TECAN icontrol machine, and **103.8 ng/ul** from the nanodrop machine. Followed procedure for Gel Extraction in the Protocols page, skipping step 1,2,3, and 6. The resulting product is in an eppendorf tube and is located at the –20C freezer in the iGEM 2017 box.

Tomorrow:

-Plate G.A. Trial # 2 products on CAM+IPTG plates using the BL21 competent cells made tomorrow.

-Complete competent cell protocol tomorrow. Check OD of SOB+seed stock AT 9AM. Make sure the OD less than or equal 0.3. OD should be measured at 600nm using NanoDrop.