Measurement of G1 DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang, Niam LeStourgeon

Date: Tuesday, May 9, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL miniprepped G1
4. Repeated step 3 for the remaining 7 G1 samples

Results:

Sample	DNA Concentration
1	88.2 ng/µL
2	36.4 ng/µL
3	57.9 ng/µL
4	18.0 ng/µL
5	50.8 ng/µL
6	54.5 ng/µL
7	36.5 ng/µL
8	45.1 ng/µL
Average DNA Concentration	48.4 ng/µL

Conclusion: N/A

BbsI Digestion

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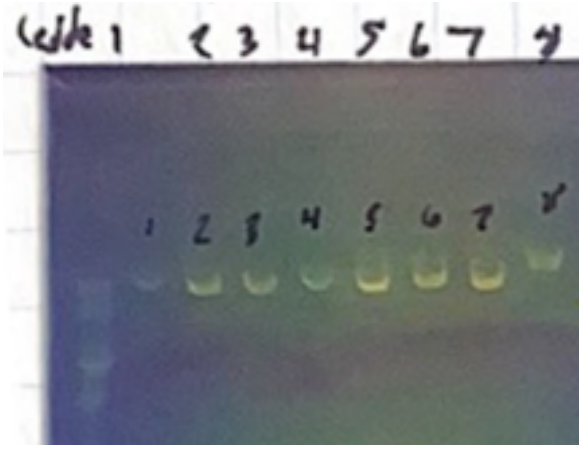
Protocol:

1. Prepared 8 25 µL reaction with the following components:

Component	Sample #							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.5 µg G1	5.67 µL	13.74 µL	8.64 µL	27.78 µL	9.84 µL	9.17 µL	13.70 µL	11.09 µL
10X rCutSmart buffer	2.5 µL							
BbsI	0.5 µL							
Nuclease-free water	16.33 µL	8.26 µL	13.36 µL	-5.78 µL	12.16 µL	12.83 µL	8.30 µL	10.91 µL
Total	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**

2. Prepared a 1% agarose running gel:
    a. Added 0.25 g agarose to 25 mL 1X TBE in an Erlenmeyer flask
    b. Heated the flask in a microwave in 10 second increments until fully dissolved.
    c. Cooled the flask until it was manageable to touch.
    d. Added 2.5 µL SYBR Safe and swirled the flask to mix.
    e. Poured the solution into a gel mold with a comb and left it to solidify for 20 minutes.
    f. Removed the comb and covered the gel cast completely with 1X TBE
3. Loaded gel:
    a. Loaded 5 µL 1 kb DNA ladder into the first well
    b. Added 3 µL gel loading dye to all 8 samples
    c. Loaded 5 µL of each sample into wells 2-9
5. Placed the gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:



Conclusion:

There is only one band present in the gel after gel electrophoresis, which indicates that BbsI did not cut any of our DNA. As such, we can conclude that we were successful in generating G1.

We noted the poor visualization of the 1 kb DNA ladder, but because all we needed to see in this step was whether one band or two bands appeared, we decided to move on and not redo the gel. We will use mScarlet in order to determine whether or not our Golden Gate assembly was successful. We must insert mScarlet into G1, so our next step is to generate mScarlet megaprimer and perform gel extraction of the DNA.

mScarlet Megaprimer Generation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

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Protocol:

1. Prepared a 25 µL reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	1.25 µL
10 µM reverse primer	1.25 µL
10 ng template DNA (R8)	2.1 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	7.9 µL
Total	**25 µL**

2. Amplified the DNA in the 25 µL reaction with a thermocycler, using the following conditions:

Step	Temperature	Time	Number of cycles
Initial denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	30
Annealing	52 °C	30 sec	30
Extension	72 °C	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: We will run a gel to confirm the generation of mScarlet megaprimer the next time we are in the lab.