Measurement of pRD DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL pRD DNA

Results: The pRD DNA concentration was found to be 174.3 ng/µL. The 260/280 ratio was 1.69, and the 260/230 ratio was 1.85.

Conclusion:

DNA concentration went up which is not possible. Relized Before precipitation, someone added the HiFi assembly. Because we thought that we were going to do the assembly

We did an ethanol precipitation to try and salvage some DNA and fix the contamination issue. It was all for naught because the failed HiFi assembly was mistakenly added to what was ethanol precipitated, which means there was much more than just pRD in the precipitation and therefore it is useless.

pRH Digestion

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

1. Prepared 2 50 µL digestion reaction with the following components: **(what do you mean by 2 digests?)**

Component	Volume
3566.4 ng pRH	11.7 µL
HindIII-HF	1 µL
NdeI	1 µL
10X rCutSmart buffer	5 µL
Nuclease-free water	31.3 µL
Total	**50 µL**

Results: N/A

Conclusion: N/A

pRH Gel Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

1. Prepared a 1% agarose running gel:
    a. Added 0.2 g of agarose to 20 mL 1X TBE in an Erlenmeyer flask
    b. Heated flask in a microwave in 10 second increments until fully dissolved
    c. Cooled flask until it was manageable to touch
    d. Added 2.5 µL SYBR Safe and swirled the flask to mix
    e. Modified comb with tape to enlarge well
    f. Poured solution into a gel mold with the modified comb and left to solidify for 20 minutes
    g. Removed comb and covered gel cast completely with 1X TBE
2. Loaded gel:
    a. Loaded 15 µL of 1 kb DNA ladder into the first well
    b. Added 6 µL gel loading dye to the digested pRE
    c. Loaded 112 µL of pRH DNA into the second well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:

Is there no photo of the results?

Conclusion:

We were successful??? We expected bands at 2752 bp for the backbone and 1677 bp for the section between the cut sites.

Gel Extraction of pRH (what are you trying to extract?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

Note: these steps follow those outlined in the Monarch® DNA Gel Extraction Kit Protocol (NEB #T1020) by New England Biolabs.

1. Excised pRH DNA at 3 kb from the agarose gel and placed them in a microfuge tube. The weights of each agar gel piece are listed in the table below.

Test tube #	1	2	3	4
Mass	76.9 mg	61.4 mg	36.5 mg	101.2 mg
Volume of ethanol	307.6 µL	295.6 µL	146 µL	404.8 µL

1. Ethanol was added to each agar gel piece in the volumes listed in the table above. The volume of ethanol was calculated to be four times the mass of the agar piece.
2. Incubated agar at 50 °C and shook at 400 rpm until the gel completely dissolved
3. Inserted a spin column into a 4 collection tubes and loaded each sample into their respective column
4. Centrifuged at 13,000 rpm for 1 minute and discarded flow-through
5. Added 200 µL DNA Wash Buffer 1 to each column and centrifuged the column at 13,000 rpm for 1 minute
6. Added another 200 µL DNA Wash Buffer 1 to the column and centrifuged it at 13,000 rpm for 1 minute
7. Transferred each spin column to a clean microfuge tube
8. Added 10 µL DNA Elution Buffer to each column
9. **Incubated sample at 37°C for 5 minutes**
10. **Centrifuged the tube at 13,000 rpm for 1 minute**

Results: N/A

Conclusion: N/A

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3. Cleaned NanoDrop before loading 2 µL pRH DNA

Results: The pRH DNA concentration was found to be 30.9 ng/µL. The 260/280 ratio was 1.82, and the 260/230 ratio was 0.37.

The pRH DNA concentration was found to be 29.7 ng/µL. The 260/280 ratio was 1.87, and the 260/230 ratio was 0.29.

Combined all 4 into 1, and nanodropped twice because thought 260/230 ratio couldn't be right

The NanoDrop also reported guanidine contamination?

Conclusion: We expected the 260/230 ratio to be a value between 2.0 and 2.2. Since the 260/230 ratio for our DNA was so much lower than expected, it may indicate that there are contaminants present in our sample. What did you do about this?

pRH DNA Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

Did you follow a company's protocol?

1. Estimated the DNA solution to have a volume of 7.1 µL
2. Added 1.5 µL sodium acetate to the microfuge tube with pRH **(what solution is this?)**
3. Precipitated DNA with 17.2 µL 70% ethanol
4. Incubated on ice overnight

Got up to overnight on the benchtop

Performed ethanol precipitation – steal entry

Results: N/A

Conclusion: We will continue pRH DNA purification tomorrow.