Sample Preparation for Sequencing

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 13, 2023

Protocol:

1. Added 1.845 µL 164.4 ng/µL pRD to a microfuge tube
2. Diluted DNA with 8.155 µL nuclease-free water
3. Shipped sample to Plasmidosaurus for sequencing

Results: N/A

Conclusion: N/A

pRC digest

pRC Gel Purification

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Protocol:

1. Prepared a 1% agarose running gel:
    a. Added 0.25 g of agarose to 25 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 3 µL SYBR Safe and swirled the flask to mix
    e. Poured solution into a gel mold with a comb and left to solidify for 20 minutes
    f. 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 pRC
    c. Loaded 25 µL of pRC DNA into the second and third well
    d. What volumes did you use for pDusk with HinDIII-HF + Bg1 and pDusk with NdeI + Bg1?
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

pDusk is a separate plasmid that we have – has cut site of HinDIII-HF, NdeI, and BgI – want to test whether

Ran BgI because at a far enough bp that it would be visible – wanted to see it

The one band showed that HindIII and NdeI work because pRC worked

pRC

Results:

Insert photo from scan (page 23)

Conclusion:

What was your conclusion?

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

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Protocol:

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

Excised backbone at ~2kb

1. Excised pRC DNA from the agarose gel and placed it in a microfuge tube.
2. Added 1187.2 µL Gel Dissolving Buffer to the agar gel
3. Incubated agar at 50 °C and shook at 400 rpm for 7 minutes
4. Inserted a spin column into a collection tube and loaded the sample into the column
5. Centrifuged at 13,000 rpm for 1 minute and discarded flow-through
6. Added 200 µL DNA Wash Buffer 1 to each column and centrifuged the column at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to the column and centrifuged it at 13,000 rpm for 1 minute
8. Transferred the spin column to a clean microfuge tube
9. Added 6 µL DNA Elution Buffer
10. **Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute**

Results: N/A

Conclusion: N/A

103.9 175.2 – masses of each agar – added different volumes

2 1

Measurement of pRC DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 13, 2023

Protocol:

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

Results: The G2 DNA concentration was found to be 65.8 ng/µL.

Conclusion: tomorrow, we will perform Hi-fi assembly? How did you come to this conclusion?

Error samp: 258.2 ng/µL – when nanodropped the first time, got this value, but 260/230 was negative, so did it with new DNA