You transformed bacteria? With what DNA?

Results: Transformation failure!! – no growth on either

Conclusion: there might have been something wrong with the sequence of pRC, so did pRD digest

Troubleshooting: we sequence so there should be no issues with pRD

Transformation of E. coli with

The resut of HIFI assembly and control

G1 and Control

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 20, 2023

Protocol:

1. Thawed NEB 10-beta Competent E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to two microfuge tubes
3. Added 2 µL G1 (pDusk plasmid with pDusk megaprimer from the MEGAWHOP reaction) to one tube and 2 µL DNA from the control reaction to the other
4. Flicked tube to mix and briefly centrifuged the samples down
5. Placed tubes on ice for 30 minutes
6. Heat shocked E. coli in a 42 °C water bath for 30 seconds
7. Placed tubes on ice for 5 minutes
8. Added 475 µL outgrowth medium to each tube
9. Incubated E. coli at 37 °C and shook at 400 rpm for 1 hour

Results: N/A

Conclusion: N/A

pRD Digestion

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 20, 2023

Protocol:

1. Prepared a 28.7 µL digestion reaction with the following components:

Component	Volume
3551.04 ng pRD	21.6 µL
HindIII-HF	1 µL
NdeI	1 µL
10X rCutSmart buffer	2.5 µL
Total	**26.1 µL**

Results: N/A

Conclusion: N/A

Get rid of ng from pRE, pRC, and prD

pRD Gel Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 20, 2023

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 pRE
    c. Loaded 32.1 µL of pRE DNA into the second and sixth well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:

Insert photo from scan (page 25)

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 pRD (what are you trying to extract?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 20, 2023

Protocol:

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

1. Excised pRD DNA at 3 kb from the agarose gel and placed it in a microfuge tube.
2. Added # µL Gel Dissolving Buffer to the agar gel
3. Incubated agar at 50 °C and shook at 400 rpm for 7 minutes **(did you vortex until the gel was dissolved instead?)**
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. Incubated sample at 37°C for 5 minutes
11. Centrifuged the tube at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

0.1282 g **512.8**

0.0922 **368.8**

Measurement of pRD DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, June 20, 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 38.4 ng/µL. The 260/280 ratio was 1.8, and the 260/230 ratio was 0.69.

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?

Did contaminant washing step first the next day –