Continuation of pRH DNA Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Friday, June 23, 2023

Protocol:

Did you follow a company's protocol?

1. Centrifuged sample at # for 10 minutes at 0°C and removed supernatant
2. Added # µL 70% ethanol to the sample
3. Centrifuged at # for 2 minutes at 4°C and removed supernatant
4. Incubated sample on bench top with the tube open **for how long?**
5. Dissolved DNA pellet with 10 µL Elution Buffer

Results: N/A

Conclusion: N/A

Measurement of pRH DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, June 26, 2023

Protocol:

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

Results: The pRH DNA concentration was found to be 17.6 ng/µL. The 260/280 ratio was 1.85, and the 260/230 ratio was 1.44.

Conclusion: This is fine? Just a very low concentration

G4.1, G4.2, and G4.3 HiFi DNA Assembly

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, June 26, 2023

Protocol:

Note: these steps follow those outlined in the Barrick Lab protocol???.

1. Prepared a 20 µL HiFi assembly reaction for each of the three inserts: G4.1, G4.2, and G4.3 with the following components:

Component	Volume
0.056 pmols insert DNA (G4.1, G4.2, G4.3)	5 µL
NEBuilder HiFi DNA Assembly Master Mix	10 µL
Nuclease-free water	5 µL
Total	**20 µL**

2. Prepared a 20 µL control reaction with the following components:

Component	Volume
0.022 pmols pRSET **(is the backbone pRSET or pRH?)**	5 µL
NEBuilder HiFi DNA Assembly Master Mix	10 µL
Nuclease-free water	5 µL
Total	**20 µL**

3. **Did you amplify the DNA? What does 60 min 50°C mean?**

Step	Temperature	Time	Number of cycles
Initial denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	30
Annealing	60 °C	30 sec	30
Extension	72 °C	12 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: N/A

pRH Gel Purification

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, June 26, 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. 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 10 µL of 1 kb DNA ladder into the first well
    b. Added 2 µL gel loading dye to the digested pRH
    c. Loaded 2 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:

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Conclusion:

Inconclusive?

Took HiFi mix and ran it through a gel – ran too little of the HiFi and couldn't visualize anything

No follow up. Transformation does the same thing

Transformation of E. coli with HiFi

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, June 26, 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 pRH DNA??
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

Plating ?? Transformed E. coli

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Monday, June 26, 2023

Protocol:

1. Warmed a LB-agar ampicillin plate in a 37 °C incubator
2. Pipetted 100 µL E. coli cells onto the plate
3. Spread cells across plate using 3 glass beads
4. Incubated at 37 °C overnight

Results: ???

Are there pictures??

Conclusion: ???

We did not transform a control so the danger is that gel purification is not perfect and contaminant whole plasmid has a much higher transfection efficiency that colonized things. We need to do colony PCR to determine if our insert is present.