E. coli G3 DNA Miniprep

Participants: Brianna Branson, Isabella (Bella) Lirtzman

Date: Thursday, July 27, 2023

Protocol:

Note: these steps follow those outlined in the Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010) General Guidelines by New England Biolabs.

1. Centrifuged all 8 G3 liquid cultures at 13,000 rpm for 30 seconds and discarded supernatant
2. Resuspended pelleted cells in 200 µL Plasmid Resuspension Buffer
3. Added 200 µL Plasmid Lysis Buffer to each tube and gently inverted the tubes until the solution turned dark pink
4. Incubated cells on the bench for 1 minute
5. Added 400 µL Neutralization Buffer to each tube and inverted the tubes until the solution turned yellow and a precipitate formed
6. Incubated cells on the bench for 2 minutes
7. Centrifuged tubes at 13,000 rpm for 5 minutes
8. Inserted a spin column into 8 collection tubes and transferred the supernatant into the column
9. Centrifuged the tubes at 13,000 rpm for 1 minute and discarded flow-through
10. Added 200 µL Plasmid Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
11. Added 400 µL Plasmid Wash Buffer 2 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
12. Transferred each column to a clean 1.5 mL microfuge tube
13. Added 30 µL Elution Buffer to each tube
14. Waited 1 minute before centrifuging all of the tubes at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of G3 DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 27, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL sample
4. Repeated step 3 for all G1 samples

Results:

Sample	DNA Concentration	260/280	230/260
G3-1	79.4 ng/µL	1.83	1.69
G3-2	68.0 ng/µL	1.79	1.38
G3-3	68.6 ng/µL	1.77	1.45
G3-4	73.8 ng/µL	1.76	1.43
G3-5	78.1 ng/µL	1.74	1.37
G3-6	68.7 ng/µL	1.77	1.72
G3-7	57.2 ng/µL	1.82	2.11
G3-8	40.8 ng/µL	1.89	1.29

Conclusion: N/A

G5 PCR Gel Purification (what exactly are you purifying?)

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 27, 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 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 **7 µL 1 kb bp DNA ladder** into the first well
    b. Added **5** µL gel loading dye to 75 µL of each G5 PCR product
    c. Loaded **28** µL of each G5 PCR product in ascending order from left to right starting with the second well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 20 minutes
4. **15 minutes after loading the 100 bp DNA ladder, a 1kb ladder was loaded into the same well**

How did you load the gel? Why are there two layers?

Did you modify the comb to expand the size of the well?

Results:

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Conclusion:

???

Gel Extraction of G5 PCR Products

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 27, 2023

Protocol:

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

1. Excised G5 PCR product band at 1000 bp 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	5	6	7	8
Mass	73.2 mg	115.9 mg	210.6 mg	146.8 mg	101.3 mg	100.0 mg	148.5 mg	118.8 mg
Volume of ethanol	292.8 µL	463.6 µL	842.4 µL	587.2 µL	405.2 µL	400 µL	594 µL	475.2 µL

2. 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.
3. Incubated agar at 50 °C and shook at **400 rpm** for 7 minutes
4. Inserted a spin column into a 8 collection tubes and loaded each sample into their respective 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 all of the columns at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to each column and centrifuged all of the columns at 13,000 rpm for 1 minute
8. Transferred each 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

Measurement of G5 DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 27, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL sample
4. Repeated step 3 for all G5 samples

Results:

Sample	DNA Concentration	260/280	230/260
G5-1	80.1 ng/µL	0.73	1.9
G5-2	88.6 ng/µL	1.89	0.07
G5-3	120.4 ng/µL	1.9	0.14
G5-4	64.1 ng/µL	1.87	0.19
G5-5	84.2 ng/µL	1.85	1.93
G5-6	97.5 ng/µL	1.86	2.16
G5-7	76.9 ng/µL	1.86	1.05
G5-8	95.6 ng/µL	1.86	1.47

The NanoDrop reported that G5-2, G5-3, and G5-4 likely had guanidine HCL contamination.

Conclusion: N/A

G3 Digestion

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, July 27, 2023

Protocol:

1. Transferred 4 µL of each miniprepped G3 DNA sample into a microfuge tube
2. Added 0.5 µL XbaI and BamHI into each tube
3. Incubated samples at 37°C for 15 minutes
4. Prepared a 2% agarose running gel:
    a. Added 0.4 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 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
5. Loaded gel:
    a. Loaded 3 µL 100 bp DNA ladder into the first well
    b. Added 1 µL gel loading dye to each sample
    c. Loaded **??** µL of each sample in ascending order from left to right starting with the second well
6. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:

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Conclusion: ???