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

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Thursday, June 22, 2023

Protocol:

Note: these steps follow those outlined in the NEBuilder HiFi DNA Assembly Reaction Protocol by New England Biolabs.

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
0.05 pmols insert DNA (G4.1, G4.2, G4.3)	3.64 µL
NEBuilder HiFi DNA Assembly Master Mix	10 µL
Nuclease-free water	6.36 µL
Total	**20 µL**

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

Component	Volume
0.05 pmols pRSET	1.36 µL
NEBuilder HiFi DNA Assembly Master Mix	10 µL
Nuclease-free water	8.64 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

pRD Ethanol Precipitation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, June 21, 2023

Protocol:

Did you follow a company's protocol?

1. Estimated the DNA solution to have a volume of 15.3 µL
2. Added 1.5 µL sodium acetate to the microfuge tube with pRD **(what solution is this?)**
3. Precipitated DNA with 37.5 µL 70% ethanol
4. Centrifuged at 13,000 rpm for 30 minutes
5. Placed sample on ice
6. Centrifuged at 13,000 rpm for 10 minutes and removed supernatant
7. Added 750 µL 70% ethanol to the sample
8. Centrifuged at 13,000 for 2 minutes and removed supernatant
9. Incubated sample on bench top upside-down on a paper towel
10. Dissolved DNA pellet with 6 µL Elution Buffer

Results: N/A

Conclusion: N/A

Combined HiFi reaction with the rest of pRD and did ethanol precipitation

M9 solution and salt and agar solution and [oured plates