DpnI Digestion

Participants: Rori Hoover, Patrick Jiang

Date: Wednesday, May 17, 2023

Protocol:

1. Added 0.5 µL DpnI to the PCR product
2. Incubated the mixture for 2 hours at room temperature

Results: N/A

Conclusion: N/A

Transformation of E. coli with G1 with mScarlet Megaprimer

Participants: Rori Hoover, Patrick Jiang

Date: Wednesday, May 17, 2023

Protocol:

1. Thawed NEB Stable Competent E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to a microfuge tube
3. Added 2 µL G1 with mScarlet megaprimer to the tube
4. Flicked the tube to mix and briefly centrifuged the sample down
5. Incubated the tube on ice for 30 minutes
6. Heat shocked the E. coli in a 42 °C water bath for 30 seconds
7. Incubated the tube on ice for 5 minutes
8. Added 475 µL outgrowth medium to the tube
9. Incubated the E. coli at 37 °C while shaking at 400 rpm for 1 hour

Results: N/A

Conclusion: N/A

Plating G1 with mScarlet Megaprimer Transformed E. coli

Participants: Rori Hoover, Patrick Jiang

Date: Wednesday, May 17, 2023

Protocol:

1. Warmed an LB-agar kanamycin plate in a 37 °C incubator
2. Pipetted 50 µL E. coli cells from the microfuge tube onto the plate
3. Placed 3 plating glass beads on the plate and moved the plate back and forth to spread the cells
4. Incubated the plate at 37 °C overnight

Results: No colonies grew on the plate.

Conclusion:

We suspect that none of the E. coli was able to survive because instead of accepting the G1 with mScarlet megaprimer DNA, they took up the self-primerization DNA, and the primers do not confer resistance to kanamycin. Approximately 80% of the DNA in the tube is the result self-primerization, so it is more likely to be transformed by this DNA than G1 with mScarlet megaprimer. In addition, the self-primerization DNA was not digested by DpnI because it is synthetic DNA from the MEGAWHOP reaction.

We need to dilute our primers and restart the process of generating G1 with mScarlet megaprimer again.