Measurement of pRSET-A DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, May 31, 2023

Protocol:

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

Results: The DNA concentration of the pREST vector was measured to be 5.6 ng/µL.

Conclusion: We will transform E. coli with pRSET-A in order

mScarlet is used for defluorinating enzymes

pRSET – amp resistance can't have kan; for beta oxidation genes

Transformation of E. coli with pRSET

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Protocol:

1. Thawed NEB 10-beta Competent E. coli cells on ice for 10 minutes
2. Added 25 µL E. coli cells to a microfuge tube
3. Added 2 µL pRSET to the tube
4. Flicked tube to mix and briefly centrifuged the samples down
5. Placed tube 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

Did transformation twice because for the first time, they used LB-amp instead of outgrowth medium; redid

Transformation of E. coli with pREST-iGluSnFR

You used this because pRSET A wasn't a high enough transformation???

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Results: N/A

Conclusion: N/A

Plating G1 Transformed E. coli

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Wednesday, May 31, 2023

Protocol:

1. Warmed an LB-agar ampicillin plate in a 37 °C incubator
2. Pipetted 50 µL E. coli cells onto each plate
3. Spread cells across plate using 3 glass beads for each one
4. Incubated at 37 °C overnight

Results: N/A

Conclusion: The colonies growing on the plate contain the pRSET vector because they were able to survive in the presence of ampicillin, and the pRSET vector confers ampicillin resistance.