Jeffrey Chuong 4/9 - 4/13

SATURDAY, 4/9/2022

https://barricklab.org/twiki/bin/view/Lab/ProtocolsAcinetobacterGoldenTransformation

Purified PCR products from Dean's repeated experiment
In final step, let elution sit for one minute, Ran elution twice through the same column

PCR product concentration = 3.78 ng/uL

Set up two Golden Gate reactions in PCR strip tubes

- First reaction uses 3 uL of Leia's 5' flank product, 5 uL of Vivek's 3' flank product, 5 uL of Jeffrey's pBTK622 plasmid
- Second reaction is scaled down to approximately 100 ng 5' flank, 100 ng 3' flank, 150 ng plasmid

Inoculated 1 ADP1-ISx culture without antibiotics, and 1 negative control without ADP1-ISx in 5 mL LB media

- Incubated overnight at 30 C

SUNDAY, 4/10/2022

Made LB and LB+Kan agar plates
Transformed 6 liquid cell culture tubes overnight, duplicates (tdk/kan, +DNA control with kan, -DNA control)

MONDAY, 4/11/2022

Diluted each transformation 10^{-2} and further made serial dilution of 10^{-3}, total 10^{-5}

- Dilutions in sterile saline

Plated 6 plates of tdk/kan, +DNA control, -DNA control duplicates diluted 10^{-2} on LB+Kan
Plated 6 plates of tdk/kan, +DNA control, -DNA control duplicates diluted 10^{-5} on LB
Plated 2 accident plates of tdk/kan duplicates diluted 10^{-2} on LB

Serial Dilution Calculation
Cell density approximately $9.6*10^8$ CFUs/mL. Transformation culture total volume is approximately 500 uL.
First dilution: $9.6*10^8 / 10^2 = 9.6*10^6$ CFUs/mL
Second dilution: $9.6*10^6 / 10^3 = 9.6*10^3$ CFUs/mL

50 uL of the second dilution = $9.6*10^3$ CFUs/mL * .05 mL = approximately 480 CFUs on LB plates