Testing Bacteria for Competency

1. Want plasmid solutions @ 1ng/5ul (.2ng/ul)
   10ng/5ul (2ng/ul)
   100ng/5ul (20ng/ul)

   Choose a 3-5kb plasmid for the test.

2. Plasmid comes @ x ug/ul concentration

   \[ \frac{x \text{ug/ul}}{20 \text{ ng/ul}} = x \text{ dilution factor} \]

3. Add 1ul plasmid into as many ul solution as the above dilution factor. Mix. Label this “Tube1 (00ng/ul)”

4. Transfer 1 ul from Tube 1 into another tube labeled “Tube 2 (10ng/ul) that contains 99ul ddH2O. Mix.

5. Transfer 1 ul from Tube 2 into another tube labeled “Tube 3 (1ng/ul) that contains 99ul ddH2O. Mix.

6. Transform 100ul of the bacteria in question with 5ul from each tube.

7. Plate 100ul of each onto an amp plate. Let absorb and then incubate upside down O/N at 37C.

8. Count colonies – Choose a plate on which you can count the colonies. Divide plate into 4 sections. Count the colonies on the most “average” section and multiply by 4. This is the # of colonies on the plate (duh). You plated 1/11 of the transformation so

   \[ \text{# colonies on the plate} \times 11 = \text{# colonies in the transformation}. \]

   Then

   \[ \frac{\text{# colonies in transformation}}{\text{amount of DNA converted to ug}} = \text{# colonies/ug DNA} \]

   (refer to # written on the corresponding tube)

   Hope for 10^6 colonies/ug of DNA