Transformation Lab

Discussion Questions

1. What is bacterial transformation?
2. What are competent cells and how do they become competent?
3. What is the role of restriction endonuclease or enzymes?
4. What gene does the plasmid in the lab contain?
5. How is a piece of DNA put into a plasmid?
6. What are the steps to allow cells to be transformed?

7. Discuss the differences in growth on the different Petri dishes for each of the plasmids and provide a brief explanation for why we saw the results we did (which plasmid bacteria grew on which plates and why)
    a. LB plate without plasmid
    b. LB plate with plasmid
    c. LB/Amp without plasmid
    d. LB/Amp with plasmid

8. Calculate the transformation efficiency (include units)

    a. **Mass of plasmid** used: _____ µL of plasmid X 0.005 µg/µL = _____

    b. **Total volume of the cell suspension** prepared: _____ µL of $CaCl_2$ + _____ µL of LB + _____ µL of plasmid = _____

    c. **Fraction of transformation volume spread**: _____ µL spread on plate / _____ µL total volume of cell suspension = _____

    d. **Total mass of the plasmid in the transformation volume** (total mass of the plasmid in the cell suspension spread): _____ µg of plasmid mass X _____ fraction of transformation volume = _____

    e. **Transformation efficiency**: # of colonies observed / _____ µg total mass of plasmid in transformation volume = _____ colonies/ µg

9. Biotechnologists generally agree that the transformation protocol that you have just completed has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA.
    a. How does your transformation efficiency compare?
    b. What factors/errors could explain a transformation efficiency that was greater or less than predicted?