Luria-Delbruck Fluctuation Test

In this experiment we will recapitulate one of the most remarkable experiments in quantitative biology in the last century. We will test the hypothesis that selection acts on pre-existing mutations in a population as opposed to mutations arising after a selection pressure is applied. To do this we will grow many parallel populations of *E. coli* and subject each to an identical environmental stress (antibiotics). The statistics of the number of antibiotic resistant mutants we observe will provide a quantitative test of our hypothesis. WARNING: you will be handling the antibiotic Rifampicin which is TOXIC. You must wear latex gloves at all times when handling Rifampicin plates!

Below is a protocol for performing this experiment. Your objective is to perform this experiment using the wild-type strain of E. coli and again with a mutator strain which has a higher mutation rate.

Consumable materials:
Lysogeny Broth (LB) rich medium (250mL)
Tris Buffered Saline (TBS) (pH 7)
LB plates (10)
LB-rif plates (22)
96-well plate (sterile)
Sterile eppendorf tubes

Step 1:

1. Get an overnight liquid culture of *E. coli MG1655* from your TA Laura. This has been incubated at 30C with shaking for at least 14 hours.
2. The overnight culture will be dense (10^9 cells/mL). In a sterile 96-well plate start 23 cultures, each with a total volume of 200 uL and a starting density of < 5000 cells/mL (by diluting your overnight culture appropriately using LB!). Layout your cultures such that 3 columns of the plate remain unused at this point. Note: to reduce contamination, always create aliquots (smaller portions from the original) of LB and later TBS.

2. Incubate your cultures until the next class period at 30C with shaking.

Step 3:

1. **Measuring N0:** In the 96-well plate add 90 uL of TBS to each well of 3 columns. For 3 of your 23 cultures make serial 10-fold dilutions by adding 10uL to 90uL of TBS. **Use a new sterile pipette tip for each transfer!**

2. Plate the 10^6 and 10^7 fold dilutions of all three of your cultures onto **nonselective** media (LB plates) for all 3 cultures - 6 plates total, incubate at 37C.

3. **Measuring the number of mutants:** For the other 20 cultures in your 96 well plates, plate the entire culture on LB-rifampicin plates (each culture on a separate plate). Incubate at 37C for two days. Note: if doing this for a mutator strain (a strain that mutates faster than the wild-type strain) plate 10^3 dilutions. How will this affect N(0) for the mutator strain?

4. Ensure to **coordinate** with your TA Laura when you will count the colonies on the LB (nonselective) plates the next day and when you will count the colonies on the LB-rifampicin plates.

Step 4:

1. Count the colonies on your LB (nonselective) plates one day after plating. What was the density of your cultures? Be sure to account for the dilution factor and the volume you plated. Report your number in units of cells/mL.

Step 5:

1. Count the colonies on your LB-rifampicin plates. Compute σ^2/μ . Do you think mutations arose in response to the antibiotic or were pre-existing in the population before you plated? Compute the mutation rate using the p0 method discussed in lecture. The formula is: $a = -ln(P(0)/(N(t) - N(0))$ what values should you use for N(t) and N(0)? P(0) is the fraction of plates that have no colonies. Report the mutation rate you calculate!

Extended investigation:

The mutation rate is itself an evolved property of biological systems. Too high and organisms will die due to the negative impacts of random mutations, too low and populations will be unable to adapt to changing environmental conditions. It has been reported that mutation rates for bacteria can differ by several orders of magnitude. Acquire a set of *E. coli* mutants and repeat the experiment above. What is the mutation rate that you found?