E. coli Plating Quantification

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481b Laboratory

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ABSTRACT

This protocol describes how to serially dilute and plate cultured *E. coli* K-12 in suspension for quantification.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Labcoat and gloves must be worn at all times.

MATERIALS TEXT

- Gloves
- Stock *E. coli* K-12 culture
- Pipette and tips
- 2 mL centrifuge tubes
- DI water
- Agar plate
- Inoculation loop
- Parafilm

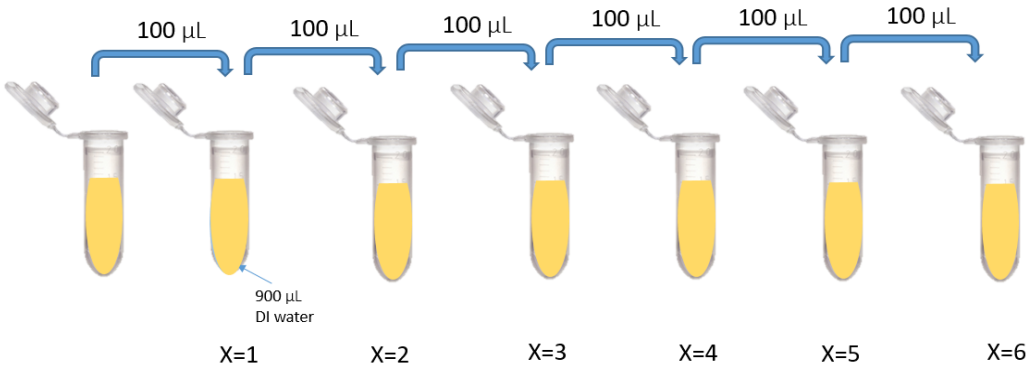
Prepare Serial Dilutions

1 Transfer 100 µl of stock solution to 2 mL centrifuge tube.

2 Add 900 µl DI water to the same centrifuge tube to make a dilution that is one-tenth the concentration of the stock solution.

3 Using 100 µl of the dilution you just made, make another dilution that is one-tenth the concentration of the second solution.

4 Repeat 6 times until you have a solution that is diluted by 10^6.



How to perform serial dilutions. X represents the number of 10-fold dilutions performed at each point.

Plate

5 Choose a dilution that you feel is appropriate and dispense **100 µl** of that dilution on agar plate.

6 Evenly spread the cell suspension over the agarose gel with inoculation loop.

NOTE
This may be different from how you have streaked before. We are wanting the solution evenly spread so that we can count how many colonies are formed after incubation.

7 Wrap the agar plate's edge in parafilm.

NOTE
If you have not worked with Parafilm, ask a TA for help in wrapping your plate.

8 Wait 2 minutes, then flip the plate upside-down for incubation.

9 Label the plate with:
- Group number
- Date
- Dilution used (10^x)

Incubate

10 Incubate at **37 °C** over night.

NOTE
Performed by T.A.

11 Take image of incubated plate.

NOTE
Performed by T.A.

Include this image in the lab report results section.

Quantify Concentration

12 Using the image of your plate, count the number of colonies formed. This can be done by hand or using an image processing software such as ImageJ.

13 Calculate the original *E. Coli* concentration using the following equation:

$$C_0\left[\frac{CFU}{mL}\right] = \frac{colonies\ counted\ [CFU]}{volume\ \ dispensed\ on\ plate\ [mL]} \times \frac{10^x}{1}$$

x represents the number of tenfold dilutions

Include the result of this calculation in the lab report.