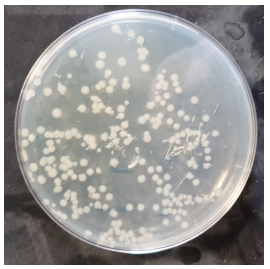
May 30, 2019

Bacterial Plating Quantification



Forked from E. coli Plating Quantification

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Kenneth Schackart[1], Kattika Kaarj[1]

[1]University of Arizona

Yoon Lab

Kenneth Schackart
University of Arizona

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes how to serially dilute and plate cultured bacteria in suspension for quantification.

Guidelines

Labcoat and gloves must be worn at all times.
Always wipe entire work area and all tools with 70% ethanol or 10% bleach as part of clean-up.

Materials

- Gloves
- Stock bacteria culture
- Pipette and tips
- 2 mL centrifuge tubes
- DI water
- Agar plate (see separate protocol for how to make agar plates)
- Inoculation loop
- Parafilm
- 70% ethanol (EtOH) or 10% bleach solution

Troubleshooting

Safety warnings

When working with dangerous or pathogenic bacterial strains such as *E. coli* O157:H7, be extra cautious to avoid spreading the bacteria or getting it on your skin.

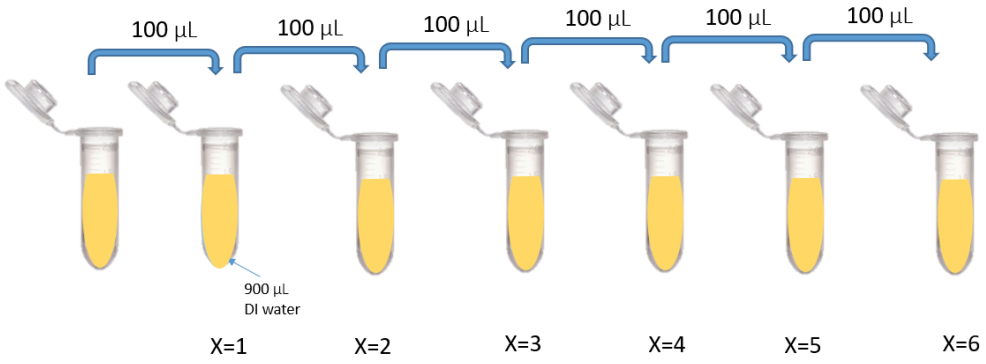
Prepare Serial Dilutions

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 since this is for quantification, not identification. 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:
- Bacterial strain
- Date
- Dilution used (10^x)
- Initials

Incubate

10 Incubate at 37 °C over night.

11 Take image of incubated plate.

Quantify Concentration

12 Count the number of colonies formed. You can use the image of the plate. 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