Serial Dilutions

Serial dilution is a technique used to accurately and systematically dilute a sample. The technique is typically used for enumeration of organisms within the original sample but can also be used for isolation.

Any dilution factor may be used in the dilution series but typically each step is a ten-fold dilution. If the goal is enumeration, then samples are diluted and then plated on media for growth and colony formation or plated with a host for plaque formation. Plates that contain 30-300 colonies/plaques are counted and the concentration of the original sample can be calculated by using the dilution factor and amount plated. Plates with greater than 300 or fewer than 30 colonies/plaques will not yield statistically significant data.

Equipment needed

1. Sample for dilution
2. Sterile medium for dilution (media / buffer)
3. Sterile vessel (dependent on volume, e.g. microfuge tubes, 96-well plate)
4. Pipettes and tips
5. Tube rack
6. Pen
7. 70% Ethanol spray
8. Paper towels

Steps

This protocol is written assuming that you are using microfuge tubes and a 10-fold serial dilution in a final volume of 1ml up to a dilution of 10^{-6}. See notes below for using alternative dilutions and vessels.

1. Clean work area. If using the hood refer to SOP LC-01-001-018
2. Collect supplies required to complete task
3. Label 6 tubes to be used in dilution
4. Aliquot 900ul of medium/buffer into tubes 1-6 by pipette
5. Pipette 100ul from the sample tube into tube 1
6. Mix tube 1 by vortex or inverting 4-6 times. Tube 1 now contains the first 10-fold dilution of the sample (typically referred to as 10^{-1} since the sample concentration is now the starting concentration $x10^{-1}$).
7. Using a new tip, pipette 100ul from tube 1 into tube 2
8. Mix tube 2 by vortex or inverting 4-6 times
9. Repeat steps 7-8 with tubes 2 and 3 etc. until all tubes have been used
10. If plating out, plate 100-200ul per plate onto appropriate media
11. Clean work area

<u>Using 96-well plates</u>

The same steps as above could be used with a deep-well 96-well plate. In this case mixing should be done by pipetting the mixture up and down or using a suitable vortex mixer after covering the plate. Use the clean pipette tip that will be used to make the next dilution for the mixing rather than the one you

just used for the transfer from the previous well since this one may contain residual concentrated sample on the outside of the tip.

In a regular 96-well plate volumes would need to be adjusted since well volume is smaller. Thus, for 10-fold serial dilutions, use e.g. 180ul medium plus 20ul sample. Alternatively, deep well plates could be used to allow greater sample volumes.

Dilution for isolation

Two-fold serial dilutions in 96-well plates can be used for isolation of organisms in liquid. An example can be viewed here http://www.level.com.tw/html/ezcatfiles/vipweb20/img/img/34963/3-2Single_cell_cloning_protocol.pdf where 200ul of the sample is added to well A1 then diluted 2-fold down column 1. 100ul of buffer/media is then added to column 1 to bring the volume up to 200ul. Column 1 is then 2-fold diluted using multichannel pipette across the plate to column 12. Finally, 100ul of buffer/media is added to all wells so that they all contain 200ul before incubation.

Calculating the concentration of the original sample

Concentration/ml = (Colonies counted x dilution factor)/vol. plated

e.g. if you counted 45 colonies on the plate from plating 0.1ml of the third dilution of a ten-fold serial dilution

Concentration/ml = (Colonies counted x dilution factor)/vol. plated

Concentration/ml = $(45 \times 10^3)/0.1$

$= 45x10^3/0.1$

$=450x10^3$

$=4.5x10^5$/ml

If you used dilution factor other than 10 then you just change the base in the equation. For example, if you used a 2-fold dilution series and counted your third dilution the above equation would become:

Concentration/ml = $(45 \times \mathbf{2}^3)/0.1$

It will not escape you to note that this math could be done in multiple ways (KL/MM!)