May 08, 2019 Version 1

Calibration Protocol - Conversion of OD_{600} to Colony Forming Units (CFUs) - v2 V.1

Forked from Calibration Protocol - Conversion of OD600 to Colony Forming Units (CFUs)

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Abstract

This procedure can be used to calibrate OD_{600} to colony forming unit (CFU) counts, which are directly relatable to the cell concentration of the culture, i.e. viable cell counts per mL.

This protocol assumes that 1 bacterial cell will give rise to 1 colony.

For the CFU protocol, you will need to count colonies for your two Positive Control (BBa_I20270) cultures and your two Negative Control (BBa_R0040) cultures.

Guidelines

Disclaimer: The 2018 InterLab study found that this protocol gave very variable results. We therefore advise teams treat this protocol with some caution, and encourage them to find ways to improve it.

Materials

MATERIALS

- 1.5 mL Eppendorf tubes
- 96 well plate
- Chloramphenicol (25 mg/ml in EtOH)
- LB Broth
- 2.0 mL Eppendorf tubes

Before start

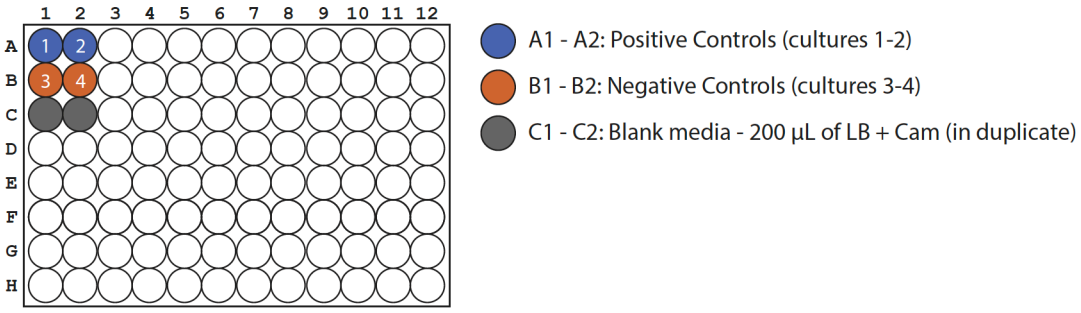
Read through this entire protocol carefully before you start your experiment and prepare any materials you may need. See the "Results" section for an example of a completed data analysis spreadsheet. Please see disclaimer in guidelines section.

Sample Preparation

1 This protocol will result in CFU/mL for 0.1 OD_{600}. Your overnight cultures will have a much higher OD_{600} and so this section of the protocol, called “Sample Preparation”, will give you the “Starting Sample” with a 0.1 OD_{600} measurement.

2 Measure the OD_{600} of your cell cultures, making sure to dilute to the linear detection range of your plate reader.
e.g. Add 25 µL culture to 175 µL LB + Chloramphenicol (Cam) in a well in a black 96-well plate, with a clear, flat bottom

3 Recommended plate setup is below. Each well should have 200 µL



4 Dilute your overnight culture to OD_{600} = 0.1 in 1mL of LB + Cam media. Do this in triplicate for each culture.

Use $(C_1)(V_1) = (C_2)(V_2)$ to calculate your dilutions

C_1 is your starting OD_{600}
C_2 is your target OD_{600} (= 0.1)
V_1 is the unknown volume in µL
V_2 is the final volume (= 1000 µL)

Expected result

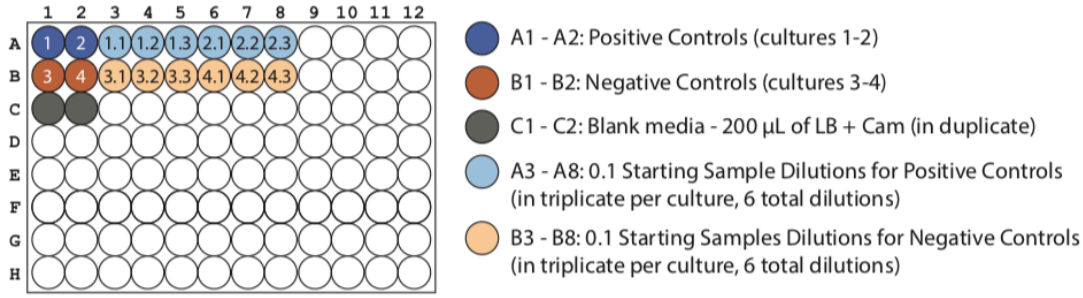
Important:
When calculating C_1, subtract the blank from your reading and multiple by the dilution factor
you used.

Example: C_1 = (1:8 OD_{600} - blank OD_{600}) x 8 = (0.195 - 0.042) x 8 = 0.153 x 8 = 1.224

Example: $(C_1)(V_1) = (C_2)(V_2)$
(1.224)(x) = (0.1)(1000 µL)
x = 100/1.224 = 82 µL culture
Add 82 µL of culture to 918 µL media for a total volume of 1000 µL

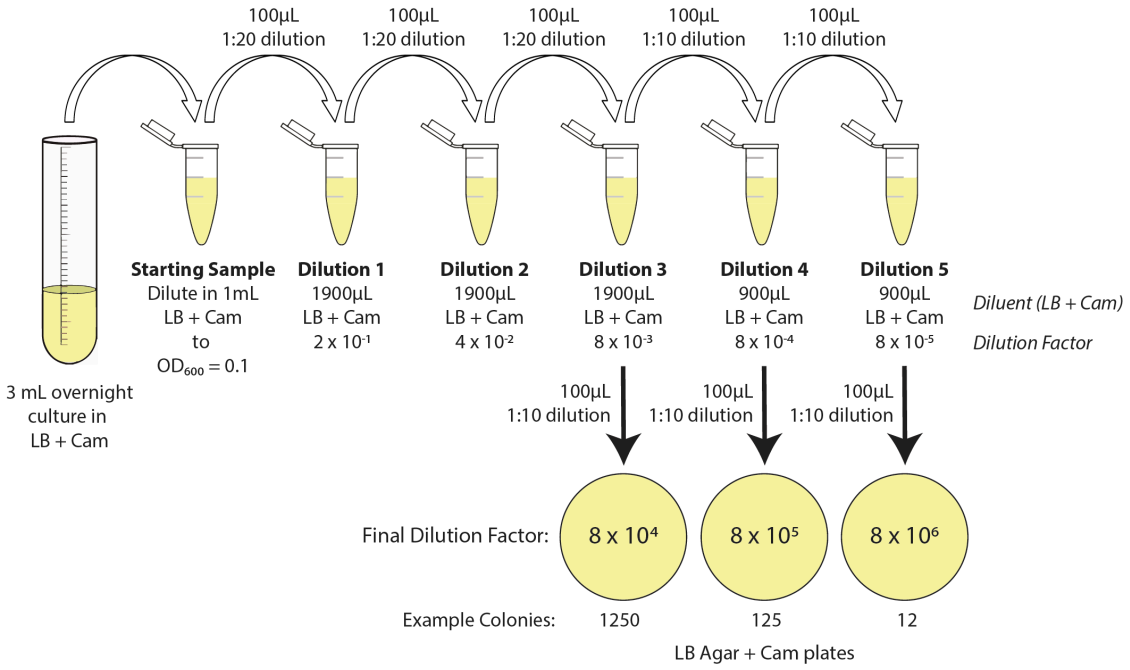
5 Check the OD_{600} and make sure it is 0.1.

Recommended plate setup is below. Each well should have 200 µL.



Dilution Series

6 Do the following serial dilutions for your triplicate Starting Samples you prepared in Step 5. You should have 12 total Starting Samples - 6 for your Positive Controls and 6 for your Negative Controls.



7 You will need 3 LB Agar + Cam plates (36 total)

8 Prepare three 2.0 mL tubes (36 total) with 1900 µL of LB + Cam media for Dilutions 1, 2, and 3

9 Prepare two 1.5 mL tubes (24 total) with 900 µL of LB + Cam media for Dilutions 4 and 5

10 Label each tube according to the figure above (Dilution 1, etc.) for each Starting Sample

11 Pipet 100 µL of Starting Culture into Dilution 1. Discard tip. Do NOT pipette up and down. Vortex tube for 5-10 secs

12 Repeat Step 11 for each dilution through to Dilution 5 as shown above

13 Aseptically spead plate 100 µL on LB + Cam plates for Dilutions 3, 4, and 5

14 Incubate at 37 °C overnight and count colonies after 18-20 hours of growth

CFU/mL/OD Calculation

15 Based on the assumption that 1 bacterial cell gives rise to 1 colony, colony forming units (CFU) per 1mL of an OD_{600} = 0.1 culture can be calculated

16 First, count the colonies on each plate with fewer than 300 colonies

17 Next, multiply the colony count by the Final Dilution Factor on each plate, or use this Excel spreadsheet:

iGEM Data Analysis Template - Plat...

Expected result

Example using Dilution 4 from above:

# colonies	x	Final Dilution Factor	= CFU/mL
125	x	(8×10^5)	= 1×10^8 CFU / mL in Starting Sample (OD_{600} = 0.1)

Congratulations!

18 You have now completed this calibration protocol