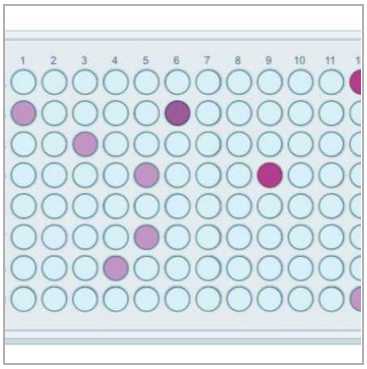

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OPEN ACCESS

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Protocol status: In development
We are still developing and optimizing this protocol

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Preparing Biolog Growth Plates

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ABSTRACT

Overview and Goals
Your bacterial isolate has been grown on agar plates. You practiced pipetting. Now, let's learn about how your organism grows! What nutrients help your bacterium grow? We will use plates with ninety-six wells with different conditions to examine the growth and metabolic capacity of your isolate. For this, we will use special Biolog plates and your Tryptic Soy Agar plates with your organism. Biolog plates allow researchers to test and visually (colorimetrically!) analyze the growth of bacteria in numerous conditions… up to 30 conditions in one plate! Biolog plates work by having powder chemicals in the wells along with an inoculating fluid and a dye that changes color when an organism is growing ("metabolic dye").

After completing this lab you will gain the following lab skills:

- Lab safety and proper personal protective equipment (PPE)
- Proper use of Biolog plates and multichannel pipettors
- Proper use of a turbidimeter to adjust bacterial densities
- Analysis of growth data of bacteria in 96-well plates

MATERIALS

Activity 1:

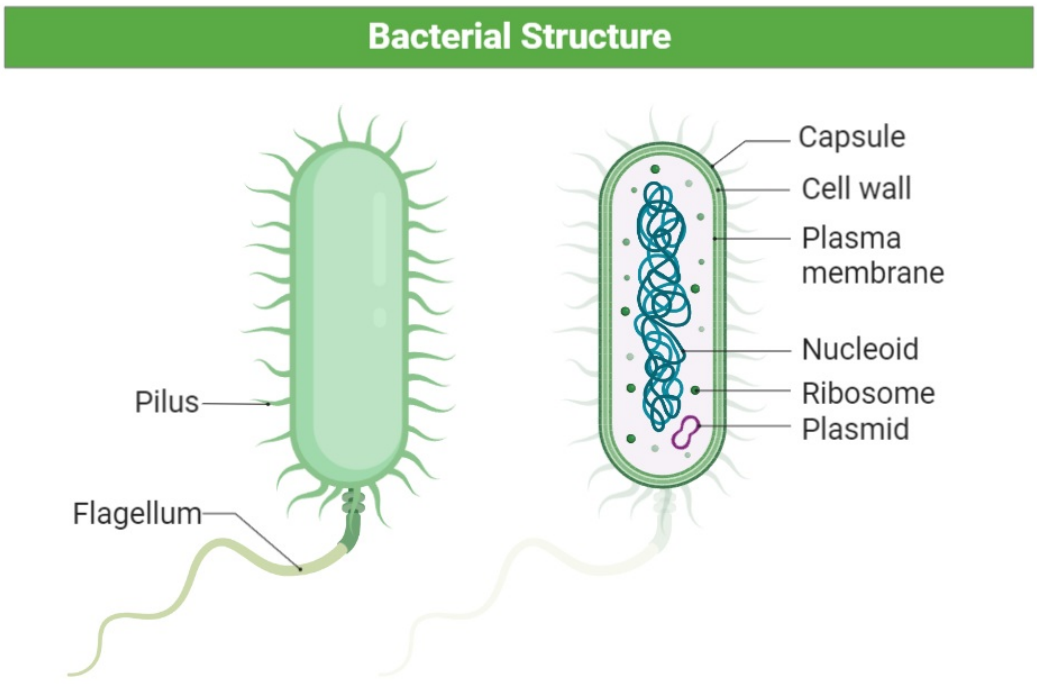
- One 200 µl multichannel micropipette (p200)
- Micropipette tips for p200
- One 96-well plate
- Chem wipes
- One container with water with food coloring (yellow)
- One container with water with food coloring (green)
- Tip disposal container

Activity 2:

- One Inoculatorz™ swab
- IF-A inoculating fluid
- Tryptic Soy Agar (TSA) plate with bacteria
- Biolog GEN3 plate
- One 200 µl multichannel micropipette (p200)
- Micropipette tips for p200
- Chem wipes
- Tip disposal container

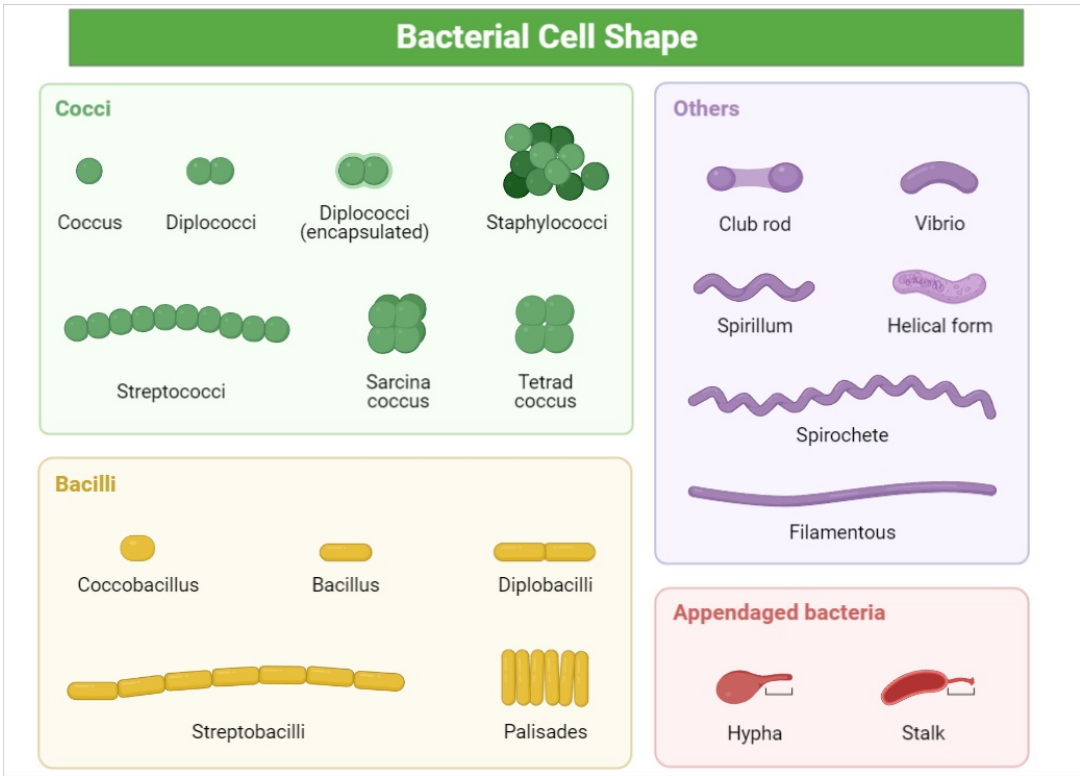
BEFORE START INSTRUCTIONS

Review the figures below to learn about bacterial cell structure, shape, and growth.



Bacterial structures:

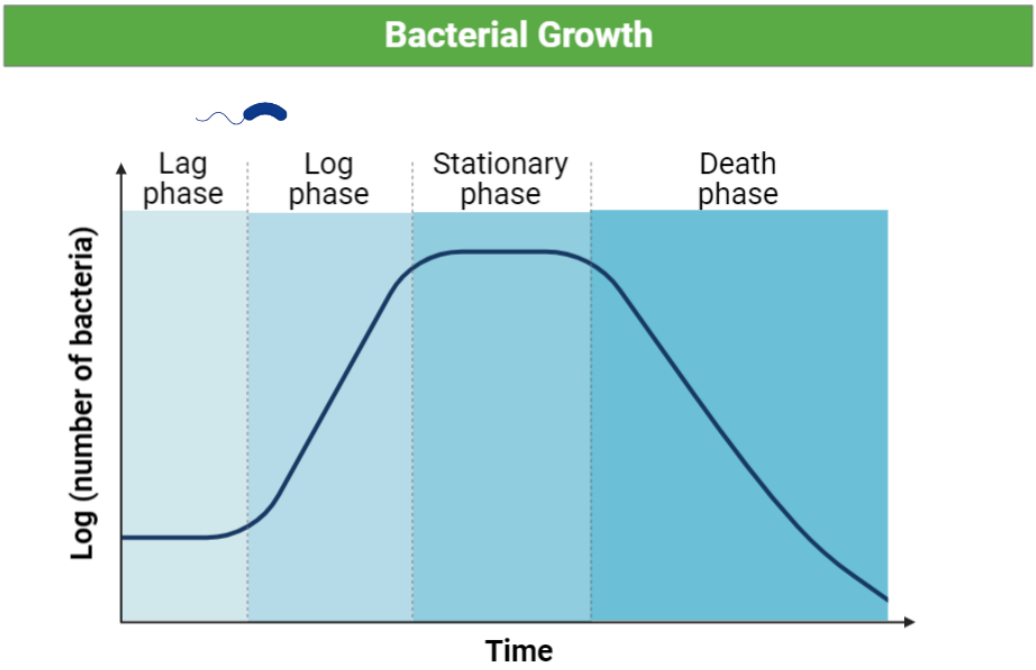
- Pilus
- Flagellum
- Capsule
- Cell wall
- Plasma membrane
- Nucleoid
- Ribosome
- Plasmid



Bacterial shapes:

- Cocci
- Coccus
- Diplococci
- Diplococci (encapsulated)
- Staphylococci
- Streptococci
- Sarcina coccus
- Tetrad coccus
- Bacilli
- Coccobacillus
- Bacillus
- Diplobacilli
- Streptobacilli

- Palisades
- Appendaged bacteria
- Hypha
- Stalk
- Others
- Club rod
- Vibrio
- Spirillum
- Helical Form
- Spirochete
- Filamentous



Bacterial growth phases over time:

- Lag phase (slow growth)
- Log phase (exponential growth)
- Stationary phase (growth plateau)
- Death phase (decline, depends on the microbe and growth conditions)

Next, read pages 2-5 of this document about the Biolog GEN III MicroPlate™ (text, ~10 min). We will begin by practicing the use of a multi-channel pipette with containers (“reservoirs”) with water and food coloring.

Activity 1-Multichannel pipetting

1 Set your p200 multichannel pipet to 100 µL

2 Load 8 tips onto each gasket of the multichannel pipet.

3 Press the plunger TO the first stop.

4 Submerge all 8 tips into the water with food coloring.

5 With thumb control, release the plunger to take up liquid. Check each tip to make sure each has the same volume of liquid.

> **Note**
>
> Note the height of the liquid in the tips. Is it consistent?

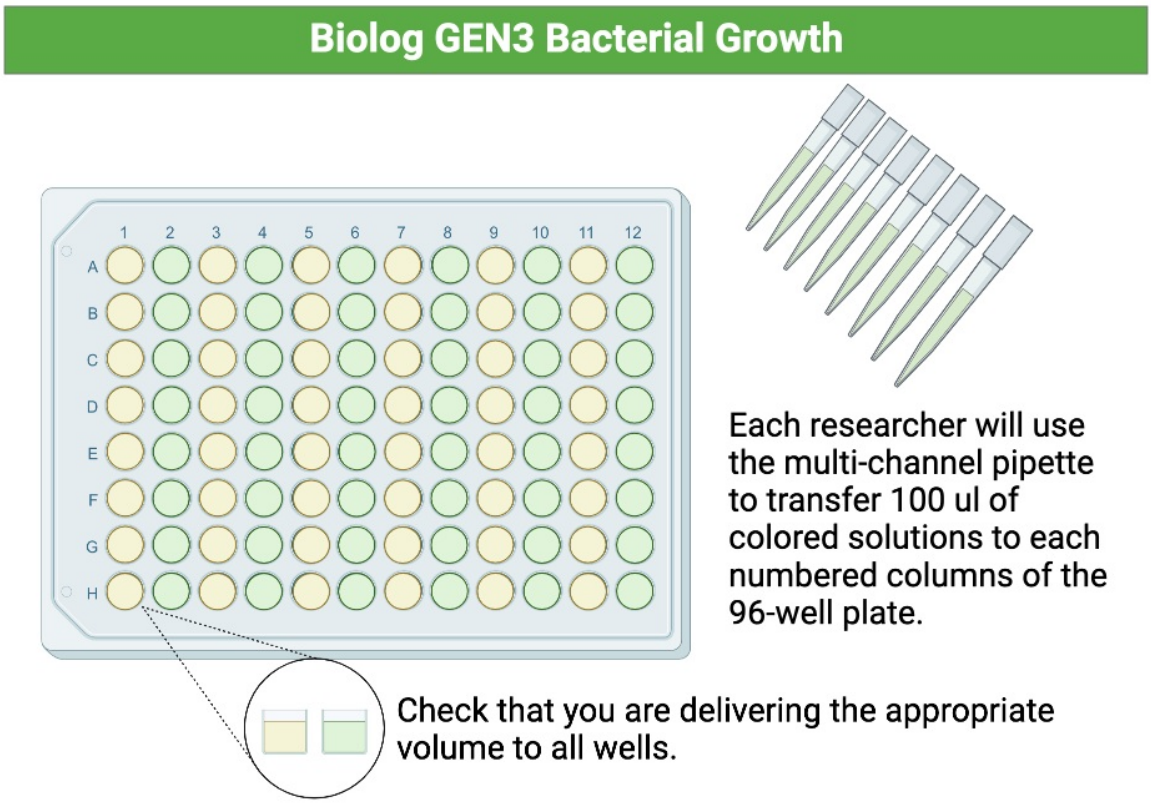
6 Align each tip with one well across one column of the 96-well plate (refer to image or the descriptions below)

6.1 Yellow water should go in odd numbered columns 1, 3, 5, 7, 9, 11.

6.2 Green water should go in the even numbered columns 2, 4, 6, 8, 10, 12.

6.3 If using colors other than yellow and green, make your own pattern.

Reference image:



7 Press the plunger THROUGH the first stop, raise your tips up out of the plate, and then you may release your plunger with thumb control.

8 Have each researcher do two to three columns of the plate to practice.

Activity 2- Innoculation

9 Calibrate the turbidimeter to a "blank" sample of inoculation fluid in a tube

9.1 Using the Biolog Turbidimeter, blank the turbidimeter with a clean tube containing uninoculated IF-A.

Note

-Wipe the tube clean of dirt and fingerprints
-Because the tubes used are not optically uniform, they should be blanked individually.

9.2 Set the 100% transmittance adjustment knob so that the meter reads **100%.**

9.3 Record reading.

10 Use Turbidimeter with bacterial sample in the inoculation fluid in a tube

10.1 Collect bacteria from your streak plate by touching the Inoculatorz™ swab to the bacteria

Safety information

Be gentle: do not push the swab through the agar media

10.2 Open your Inoculating Fluid tube (IF) tube and submerge the swab into the liquid. Swirl the swab around in the fluid for 00:00:15 to ensure inoculation. 15s

Safety information

Do not leave the liquid uncapped longer than necessary to prevent contamination. The tubes are glass: work carefully.

10.3 Cap the tube and gently invert the three (3) times to ensure thorough mixing.

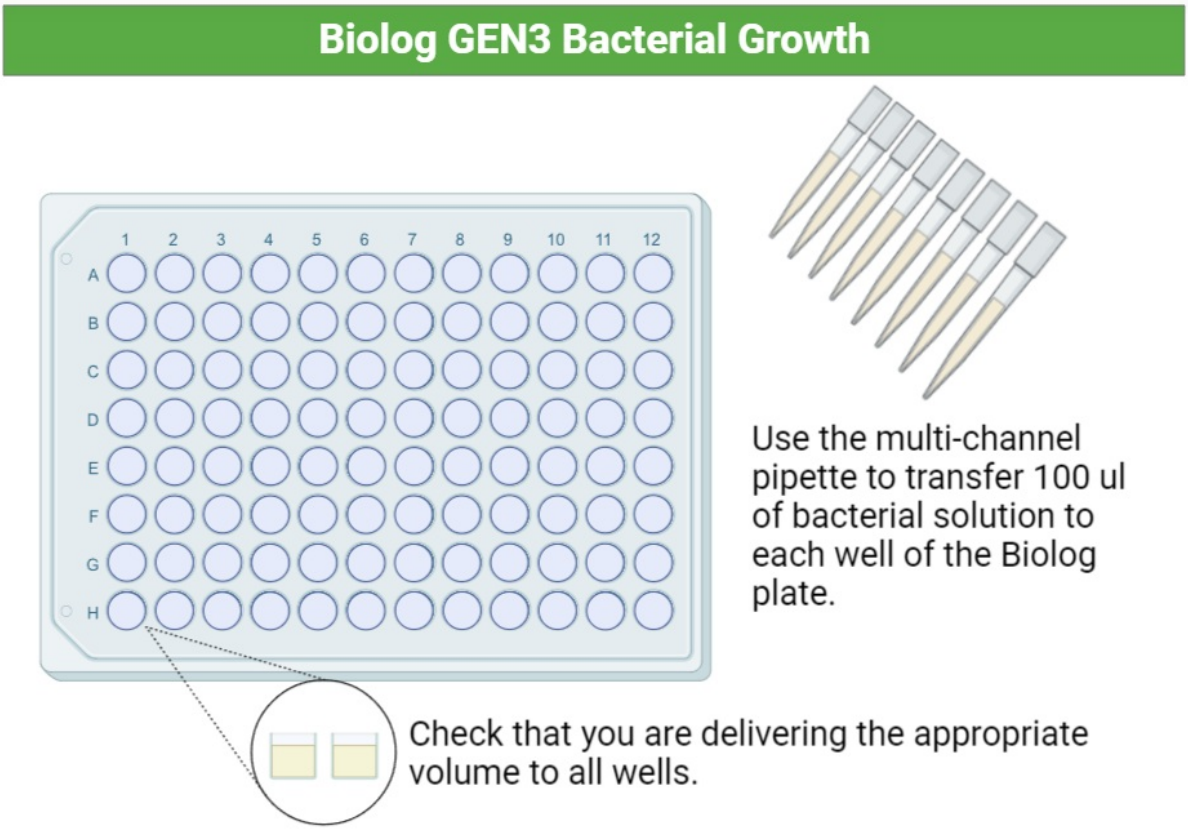
10.4 The target cell density is **95%T** for our protocol.

1. Add more bacteria with the swab if necessary to get as close to this value as possible by continuing to add more bacteria with the swab to the tube.
2. Work carefully and keep the tube capped. If necessary, use another swab if you think it has touched another surface.

11 Once you've reached the target cell density at **95%T**, cap the tube and proceed to the next Activity.

Activity 3-Biolog plates preparation

12



Pour the cell suspension into the multichannel pipette reservoir.

13 Load 8 tips onto each gasket of the multichannel pipet and fill the tips by drawing up 100 µL of cell suspension from the reservoir.

14 Use the multichannel to add 100 µL of diluted bacterial culture to each of the 96 wells of the GEN III MicroPlateTM.

Note

Use the technique practiced in Activity 1.

Safety information

If the tips touch the wells, replace your tips before delivering inoculation fluid to the next column. Work carefully to avoid contamination!

15 Eject the pipettor tips if any one tip touches the wells of the plate as this can cross contamination wells with chemicals. Reload tips as needed.

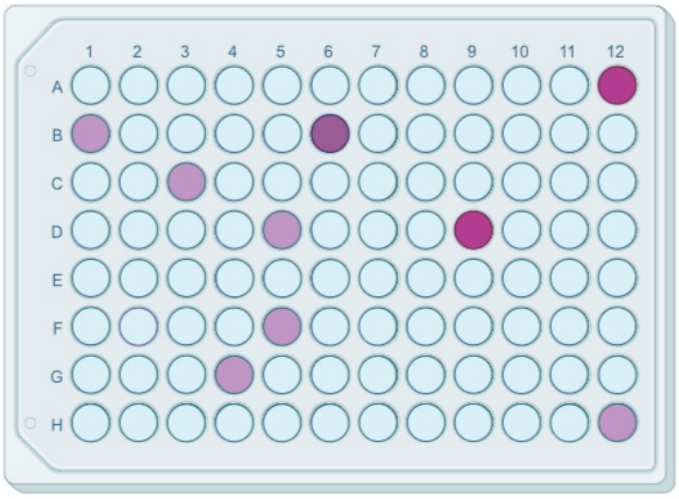
16 Check that all wells have liquid

17 Cover the GEN3 microplate with its lid and eject the pipette tips.

18 Incubate at 28 °C for . 72:00:00 - 120:00:00 hours

Reference image:

Biolog GEN3 Bacterial Growth



Incubate the plate at the desired temperature for 3 to 5 days.

Note

Critical Thinking Questions for Preparing Biolog Growth Plates

1.What are some advantages of using a multichannel pipette?

2.What are some disadvantages of using a multichannel pipette?

3.During the inoculation, describe how you ensure that you isolate a "pure culture." Why is a "pure culture" needed for this assay?

4.What is a turbidimeter and why would measuring turbidity be important for this assay?