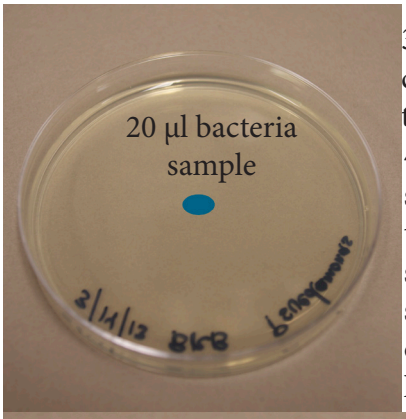
ASSET: How to Make a Spread Plate

CAUTION: When working with any form of bacteria, even nonpathogenic bacteria like *Pseudomonas fluorescens*, take extreme caution. Use sterile technique and seal all containers that bacteria are stored in. Make sure you always clean your work area before and after handling bacterial cultures. Wash your hands when finished.

Spread plates are used to distribute bacterial cells evenly across the surface of an agar plate. Bacterial cells suspended in a small drop of liquid are distributed using a sterile spreader. If the concentration of bacteria is low, individual colonies will result from the spreading process. If the concentration of bacteria is too high, a confluent bacterial "lawn" will result and individual colonies will not be distinguishable.

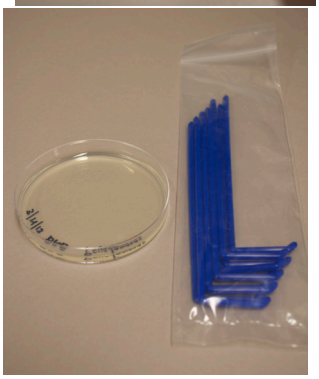
1. Using a marker, label the **BOTTOM** of your spread plate with the date, your initials and the species of bacteria you are plating. **ALWAYS KEEP THE LID ON** your agar plate until you are ready to complete your spread. This minimizes the risk of contamination.

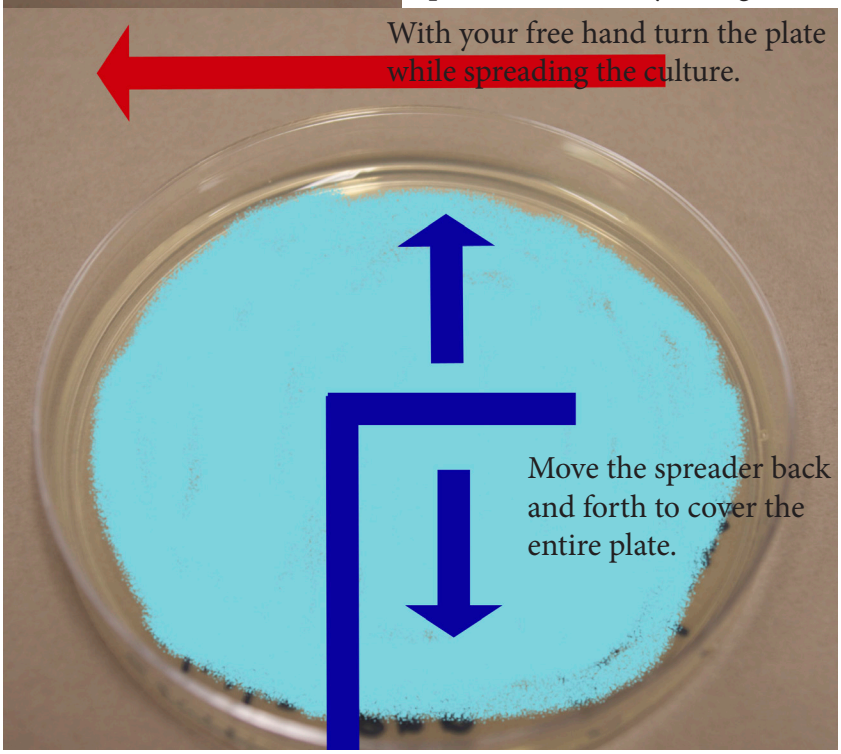
2. When spreading a plate, it is important to use sterile spreaders. Your spreaders may be individually wrapped or in a bag. They should be opened just prior to use. If spreaders are individually wrapped, open at the handle end. If the spreaders are packed in bulk, one person should distribute the spreaders when students are ready to use them. The L-shaped end should **NOT** come in contact with anything but the liquid sample and the agar.



3. Using a micropipette, place 20 µl of a dilute bacterial culture onto the center of the agar plate without touching the agar.

4. Using your sterile spreader, gently rub across the agar surface while rotating the plate with the other hand. See the diagram below for an example. **DO NOT** allow the spreader to dig into the agar. It is important that you spread the sample across the whole agar plate so it makes counting colonies easier. Continue spreading until the liquid is absorbed by the agar.





5. Most bacterial samples will not be colored, but in the example shown the light blue represents the extent of the plate surface that should be spread with the bacterial culture. You will not be able to see the bacteria you spread on the plate until after the bacteria have grown.

6. After completing the spread, decontaminate the spreader with alcohol or bleach, and dispose of the spreader in the waste beaker. Put the cover on the plate and seal it around the edges with either Parafilm or tape. Place the cultured plate upside down. Condensation may form during incubation. If it drips onto the agar, all of your colonies will run together. If it stays in the lid, no harm will be done. Check the plate for bacterial growth 2-3 days later, if you are incubating at room temperature. Colonies will form faster if the plates are incubated at 37 C.

7. Once your colonies have grown, spend some time analyzing your results. **DO NOT** open the lid to count your colonies. Look closely at the size, shape and texture of your colonies. Individual colony differences in size, shape, texture, or outline, as compared to the characteristics of the ancestral colonies, are indicative of genetic changes in the bacteria giving rise to that colony. When you are done, decontaminate the plate with bleach and dispose of your sealed plate in an appropriate waste container.

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