Streaking Microbial Cultures on Agar Plates

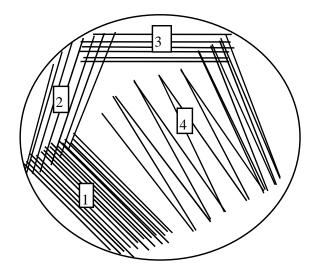
Agar streak plates are an essential tool in microbiology. They allow bacteria and fungi to grow on a semi-solid surface to produce discrete colonies. These colonies can be used to help identify the organism, purify the strain free of contaminants, and produce a pure genetic clone.

In order to obtain well-isolated discrete colonies, the quadrant streak technique should be used. This allows sequential dilution of the original microbial material (broth culture or colonies on a plate or slant) over the entire surface of a fresh plate. As the original sample is diluted by streaking it over successive quadrants, the number of organisms decreases. Usually by the third or fourth quadrant only a few organisms are transferred on the inoculating loop and these produce a few isolated colonies.

The quadrant streak technique is described below. Study the diagram and read the "Tips" below the diagram before you begin the streak plate.

- 1. Flame the inoculating loop until it is red hot and then allow it to cool.
- 2. Remove a small amount of bacterial growth (either a loopful from a broth culture or a single colony from a plate or slant) with the sterile inoculating loop.
- 3. Immediately streak the inoculating loop very gently over a quarter of the plate using a back and forth motion (see area 1 in the figure below).
- 4. Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2).
- 5. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3).
- 6. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate (area 4).

Quadrant Streak



Inoculation of a streak plate

- 1. Area of initial inoculation and first streaks yields heavy growth.
- Area of second streaks from area
 yields less dense growth
- 3. Area of third streaks from area 2 yields weak growth
- 4. Area of fourth streaks from area 3 yields single colonies.

Tips

- Use the entire surface of the plate, not just the middle of the plate.
- Streak only one loopful of a broth culture.
- Remove only one colony or a barely visible amount of cells from a plate or slant.

- Flame the loop after you streak each quadrant.
- Streak lightly so that you do not gouge the agar.
- Cool the loop after flaming by gently touching the edge of the agar where you have not streaked.
- Examine the plate carefully after colonies have grown. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate. You will have to determine which colony is the strain of interest.

Related articles:

<u>Tips for Pouring and Storing Agar Plates</u>
<u>Sterilizing Laboratory Materials for the Classroom</u>
<u>Nutrient Broth, Plates and Slants</u>

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