



Aug 21, 2020

Streaking and Isolating Bacteria on an LB Agar Plate - CHEM 584

Forked from [Streaking and Isolating Bacteria on an LB Agar Plate](#)

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In Development

dx.doi.org/10.17504/protocols.io.bj5mkq46



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ABSTRACT

This protocol describes how to streak and isolate (single colony) bacteria on an LB agar plate. To see the full abstract and additional resources, please visit <https://www.addgene.org/protocols/streak-plate/>.

Sample Data



Figure 1: Streaked out plate with single colony (red circle).

EXTERNAL LINK

<https://www.addgene.org/protocols/streak-plate/>

DOI

dx.doi.org/10.17504/protocols.io.bj5mkq46

PROTOCOL CITATION

Addgene The Nonprofit Plasmid Repository, Ken Christensen 2020. Streaking and Isolating Bacteria on an LB Agar Plate - CHEM 584. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bj5mkq46>



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CREATED

Aug 21, 2020

LAST MODIFIED

Aug 21, 2020

PROTOCOL INTEGER ID

40845

GUIDELINES

Streaking diagram and guide for spreading bacteria



Diagram: streaking motions on a plate for achieving single colonies

Streak #1. Using a sterile loop, pipette, or toothpick, touch the bacteria growing within the punctured area of the stab culture or the top of the glycerol stock and *gently* spread the bacteria over a section of the plate in a zig-zag motion, as shown in the diagram above.

Streak #2. Using a fresh, sterile toothpick or freshly sterilized loop, *gently* drag through streak #1 and spread the bacteria in a zig-zag motion over a second section of the plate.

Streak #3. Using a third sterile pipette tip, toothpick, or sterilized loop, *gently* drag through streak #2 and spread the bacteria over the last section of the plate.

MATERIALS TEXT

Reagents

- Sterile toothpicks or wire loop
- Bunsen burner (or other small flame source)
- Incubator
- Marker

Reagents

- LB agar plate (with appropriate antibiotic)
- Bacterial stab

Day 1

- 1 Obtain an LB agar plate with appropriate antibiotic.

- 2 Label the bottom of the plate with the plasmid name and the date. It is also a good idea to add the antibiotic resistance and your initials. Labeling within a laboratory setting is important for organization, and it is recommended that you keep a standard labeling system for all your objects/solutions.
- 3 Sterilize your lab bench by spraying it down with an appropriate disinfectant and wiping it down with a paper towel. Maintain sterility by working near a flame or bunsen burner.
- 4 Obtain the appropriate bacterial stab, overgrown plate, or glycerol stock.
- 5 Using a sterile loop, pipette tip or toothpick, touch the bacteria growing within the punctured area of the stab culture or the top of the glycerol stock.



Pro-Tip

If you use a wire loop you can sterilize it by passing it through a flame, just be sure to allow enough time for the loop to cool before touching it to the bacteria.

- 6 *Gently* spread the bacteria over a section of the plate, as shown in the diagram (see Guidelines & Description), to create streak #1.



Pro-Tips

- Hold your toothpick at an angle, the way you would hold a pencil so that you can make a broad stroke. Only touch the surface of the plate, do NOT dig into the agar.
- Another very popular technique is to draw in discontinuous lines. Start by streaking a vertical line of bacteria along one edge of the plate. Then streak horizontal lines in another section of the plate, and then diagonal lines in another section of the plate. Make sure that the first line (and only the first) in each new section crosses at least one line of the previous section so that it will contain some bacteria.

- 7 Using a fresh, sterile toothpick, or freshly sterilized loop, *gently* drag through streak #1 and spread the bacteria over a second section of the plate, to create streak #2.
- 8 Using a third sterile pipette tip, toothpick, or sterilized loop, *gently* drag through streak #2 and spread the bacteria over the last section of the plate, to create streak #3.
- 9 Incubate plate with newly plated bacteria overnight (🕒 12:00:00 - 🕒 18:00:00) at 🌡 37 °C .



Pro-Tip

Some plasmids or bacteria need to be grown at 🌡 30 °C instead of 🌡 37 °C . This is often true for large

unstable plasmids, which sometimes recombine at 37°C . Be sure to check this before incubating your plate.

Day 2

- 10 In the morning, single colonies should be visible. A single colony should look like a white dot growing on the solid medium. This dot is composed of millions of genetically identical bacteria that arose from a single bacterium. If the bacterial growth is too dense and you do not see single colonies, re-streak onto a new agar plate to obtain single colonies.
- 11 Once you have single colonies, you can proceed to recovering plasmid DNA or use the individual colonies for other experiments.