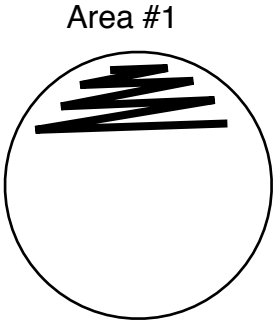
Pure cultures from single colonies

A common method for establishing the purity of bacterial cultures is to isolate single colonies. A colony that is physically isolated or separate from other growth is usually derived from a single bacterial cell. This was first observed by Robert Koch who noticed that when he left a cut potato open to the air, isolated colonies would develop on it. He found that each contained a pure culture, Koch figured out that each colony was initiated by a single cell that landed on the potato. This led to the development of solid culture media placed in petri dishes, which is now the most common method for culturing bacteria.

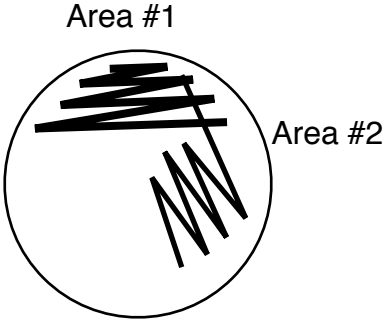
One way to isolate single colonies is the three-way streak method. The principle underlying this method is that when the bacteria are sufficiently diluted (this happens when they are spread out by streaking across the agar plate), individual cells will be deposited on the surface of the agar. After the single cells divide, a colony will be visible. A colony contains a pure culture because it is derived from a single cell.

The three-way streak method involves the following steps:

1. Flame sterilize and cool a wire loop. Transfer the cells to the fresh plate by touching a colony with the loop then gently spreading the material by moving the loop back and forth across about 20% of the surface of the agar. This is Area #1.

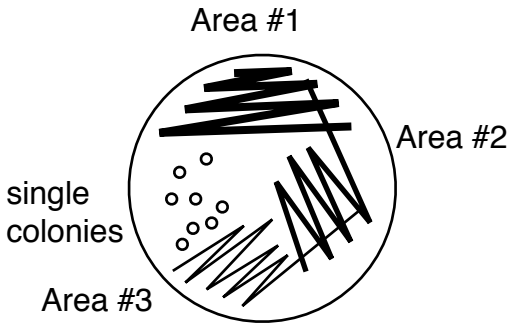


2. Flame sterilize and cool the loop again. Drag the sterile loop across the end of the spread area. Spread this material across a clean area of the plate, which will be Area #2. Do not reenter Area #1.



3. Flame sterilize and cool the loop again. Drag the sterile loop across the end of Area

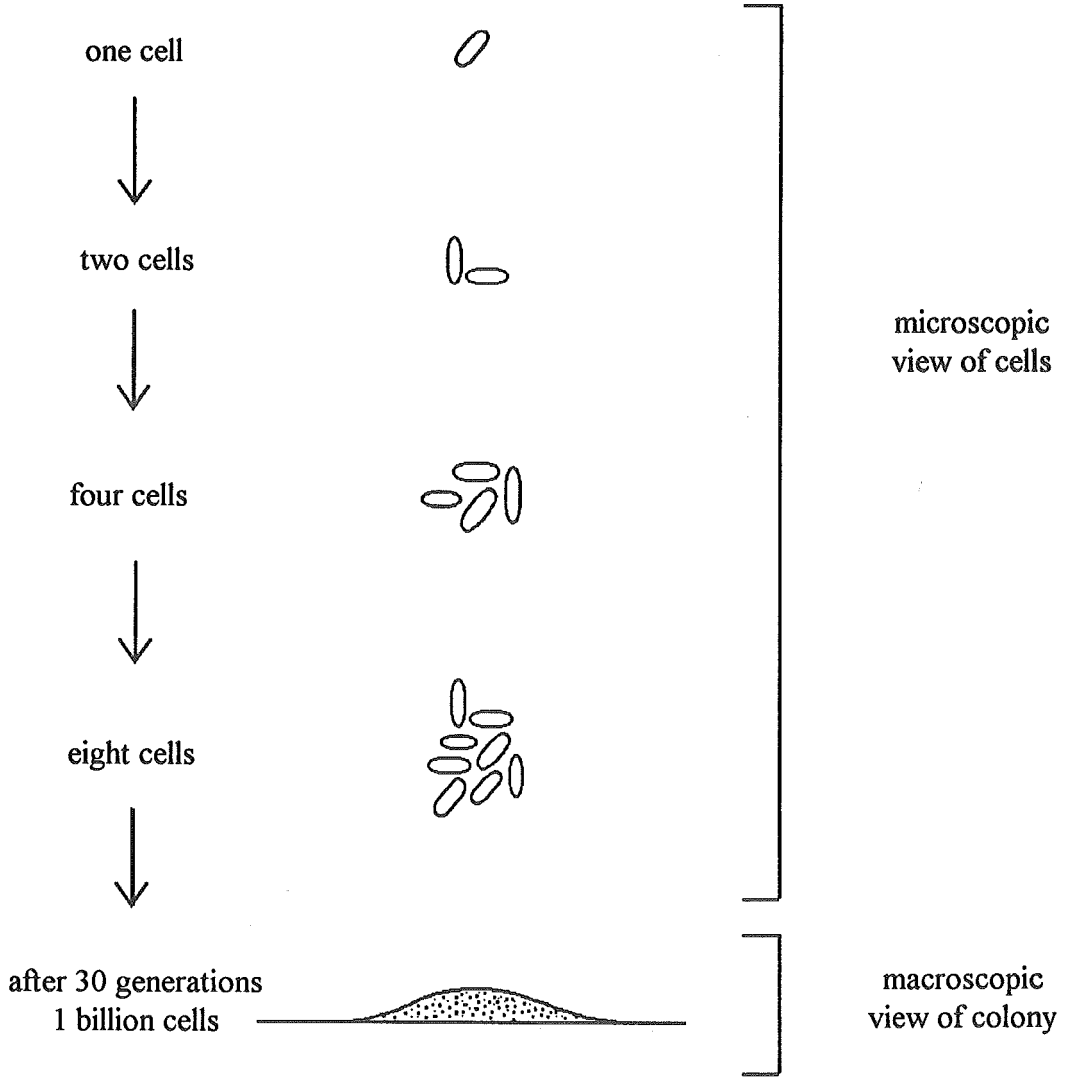
#2. Spread this material across a clean area of the plate, which will be Area #3. Do not reenter Area #2. You should have single colonies at the end of the Area #3 streak.



✳ Make sure you cool the loop! If you hear your loop sizzle when you touch a colony, you have successfully fried the colony and killed the bacteria -- not what you want. You can cool the loop by waiting patiently and counting to 10 slowly. You can also touch the loop to a clean part of the fresh plate to make sure it is cool - no sizzling sound.

Growth of a bacterial colony

When a bacterial cell is deposited on a solid surface and provided with nutrients, it can form a colony. A colony is a group of cells that are derived from a single cell, and thus, cells in a colony are usually genetically identical to each other and to the original cell that gave rise to the colony. Many bacteria grow very rapidly so that a single cell on a rich nutrient medium can form a colony visible to the human eye in a few hours or days. For example, the common gut bacterium, *Escherichia coli*, can divide every 20 minutes when it is grown under optimal conditions. A single cell of *E. coli* will divide forming two cells, the two cells divide forming four cells, the four cells divide forming eight cells, and on and on until they run out of nutrients. In just 10 hours, or 30 generations, one cell can grow to be one billion cells! Other bacteria grow far more slowly. The pathogen that causes tuberculosis, *Mycobacterium tuberculosis*, divides every 24 hours. It takes 5 weeks for *M. tuberculosis* to form a colony with one billion cells!



Adapted from Handelsman J., Houser B., Kreiger H. *1997*. Biology Brought to Life. Times Mirror Higher Education Group, Dubuque, Iowa.