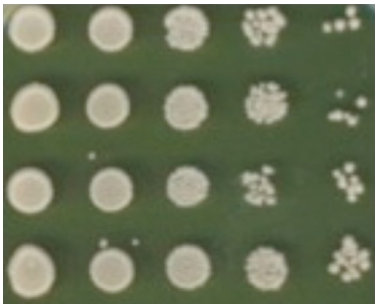
Yeast spot plates

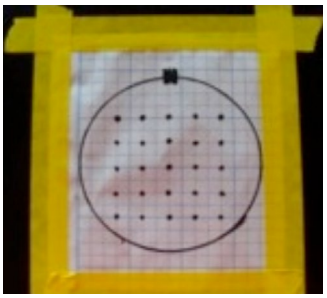
Scientists use spot plates both to calculate the number of cells in cultures and to obtain information about the growth properties of strains on different media. The figure below shows an example of a typical spot plate. Each row represents a dilution series from a different yeast culture. The same volume of diluted culture is used for each spot. The dilution series is planned so that the most dilute spots contains a small number of individual colonies that can be distinguished from one another, typically less than ten.



Spot plate.
Each row on the plate represents a series of 1:10 dilutions of a liquid culture of *S. cerevisiae*. Five µL of each dilution was spotted on the plate. The plate was incubated for two days at 30˚C. Individual colonies are apparent at the highest dilution of each extract.

Most commonly, investigators make a series of 1:10 dilutions in **sterile** water and then spot a few microliters of each dilution in a row. In this experiment, 5 µL aliquots were spotted from the serial dilutions. Note that it is possible to count individual colonies in the most dilute samples. This in turn enables you to calculate the number of viable cells in the original culture. In the top row, you can distinguish 4 colonies in the sample that has been diluted 100,000-fold. The original culture would have contained 400,000 cells in 5 µL, which corresponds to 80 million cells per mL (8×10^7 cells/mL).

Preparing the spot plate
See the diagram on the back of this page.



1. Alignment grids are useful for preparing good-looking spot plates! Obtain an alignment grid (right) and mark the target positions for culture dilutions. Place an orientation mark at one point along the circumference.

2. Label the plate with your initials and date with small letters around the BOTTOM rim of the dish. Put a hash mark on the edge of the plate to serve as an alignment marker.

3. Prepare a series of five 1:10 dilutions from each culture using sterile distilled water. (Diagrams in your lab notebook are often helpful in designing dilution series.) To prepare a serial dilution, first pipette 90 µL sterile water into five microcentrifuge tubes. Next, use a P20 to transfer 10 µL from the original culture into the first tube. ***Vortex the cell suspension***, and then transfer 10 µL from this tube to the second tube in the series, and so on. *Use the same pipette tip for the entire dilution series.* Eject the tip into the appropriate waste container.

4. *Beginning with the last dilution in the series*, spot 5 µL spots in a row. Vortex each dilution before spotting it, because cells may have settled. Again, you will be able to use a single pipette tip for a dilution series, since you started with the most dilute sample.

5. Repeat step 3 for each culture that you are analyzing. Be careful to note in your lab notebook which culture has been spotted into each row on the plate!

6. Leave the plate right side up for ~30 minutes, to allow time for the yeast to settle and attach to the medium.

7. When the cells have settled, invert the plates and incubate them at 30°C. Plates are inverted to prevent water droplets that form on the inner surface of the lid from falling on the colonies. Plates can also be kept at room temperature, but cells will grow more slowly. Do NOT incubate the cells above 30°C, which stresses the yeast.