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Appendix 4: Spot Assay of wild type and deletion strains on rich and stress response media

A spot assay is used to compare growth of wild type and deletion yeast strains. A spot assay on rich medium will compare growth rates in the most ideal conditions and may indicate a change in growth rate between the two strains. A spot assay can also be done on a specific selected medium, such as high salt or an alternate carbon source, to investigate the function of a gene.

Materials:

- Yeast suspension at OD600 0.1 for WT yeast
- Yeast suspension at OD600 0.1 for *ORF* deletion yeast
- 1 YPD plate
- One of the following or other selective plate of choice:
  - 1 YPD + 0.25 ng/mL Cycloheximide
  - 1 YPD + 1M NaCl
  - 1 YPD + copper (100 μM CuIISO4)
  - 1 YPD + 0.76 μg/mL hydroxyurea
- Sterile water
- Sterile microtiter plate
- 200 μl micro-pipette
- 20 μl micro-pipette
- Serial dilution grid template (included at the end of this appendix)
- Tape
- Sharpie pen
- Parafilm and scissors
- 30^{o}C incubator

Procedure

1. Label your petri plates with your name or initials, date, and media type. Be sure to label the bottom of the plate - the part of the plate that contains the agar.

2. Each person will do their own experiment on WT and one ORFan deletion strain. You will create a 10-fold serial dilution in the wells of a microtiter plate, as pictured below (figure 1). The top three rows are the WT yeast serial dilution. The next three rows will be the deletion strain.

   You will only use half of your microtiter plate; if you mess up and want to have a second try you have plenty of room!

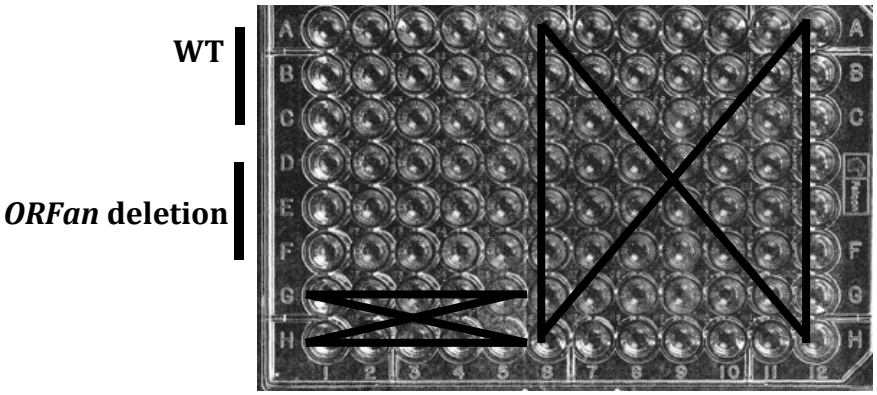
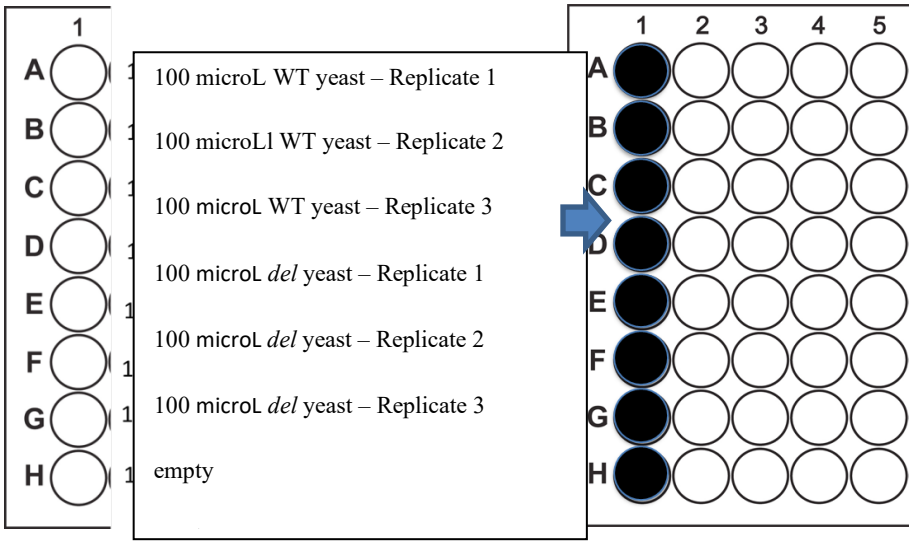


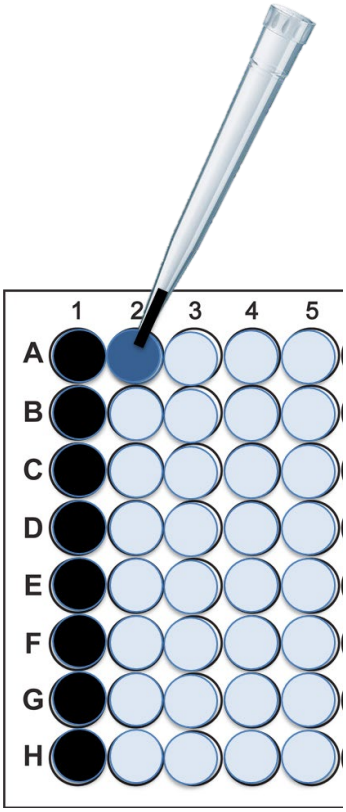
Figure 1: WT and deletion layout in a microtiter plate.

3. Uniformly suspend the yeast suspension by flicking the side of the tube with your finger to create a vortex. Add 100 microliters of the yeast suspension to the appropriate wells in the **first** column of the microtiter plate.



4. Add 90 microliters of sterile water to columns 2 through 5 in each row.

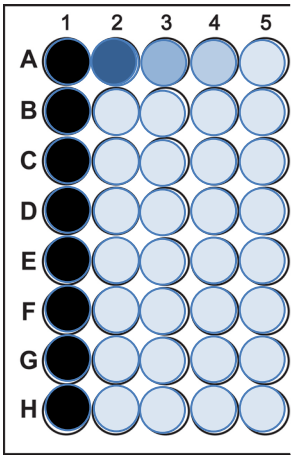
5. Starting with the first row (row A, column 1) carry out a 10-fold dilution by moving 10 µl of the cell suspension in row A column 1 to 90 µl sterile water in the adjacent well (row A column 2) of the sterile microtiter plate.



Carefully and slowly pipet the liquid up and down to mix the cells in the wells before you transfer the cells. Yeast are actually quite large for a microorganism, and will settle to the bottom of the well. Pipetting up and down slowly will suspend the yeast.

BE CAREFUL! Pipetting quickly will splash the yeast suspension between wells and confuse your results. Once you have transferred the 10 microliters to the adjacent well, pipet up and down again to mix the cells thoroughly using the same pipette tip. With the same tip, transfer 10 µl of this yeast suspension to the next well to get a 1:100 suspension of yeast. *Hint: as you pipette up your 10 µl, take a moment to look at the tip with the liquid in it. Does it look ok? Does it look like 10µl? If not, squirt it back into the well and try again. Sometimes air bubbles will get trapped in the tip, throwing off the volume. If this happens, it is ok to get a new tip.*

6. Do this again until you have carried out a 10-fold serial dilution across the top row of the microtiter plate through column 5. Repeat this for WT in rows B and C. Use your deletion strain for serial dilutions in rows D, E and F. Use a fresh pipet tip for each row. If you look carefully, you should be able to see that the yeast suspensions in the microtiter plates are becoming less opaque as you dilute the cells.



When complete, you will have 5 rows of 10-fold serial diluted yeast. The first column contains undiluted yeast (dilution factor of 1), the second row will have 10X fewer cells in it, because you added 10 microliters of yeast to 90 microliters of water resulting in a suspension of yeast that has 10X fewer cells per amount of volume in the well. 10 microliters of cells/100 total microliters of liquid = 1/10 the concentration, or an actual dilution of 0.1. These values are important because you can use them to determine the number of colony forming units (CFU) present in your original yeast suspension.

Transferring the serial dilutions to the surface of the petri plates:

7. Once your dilutions are complete, label your petri plates with the date and your initials. If the plate type is not already indicated, label that as well.

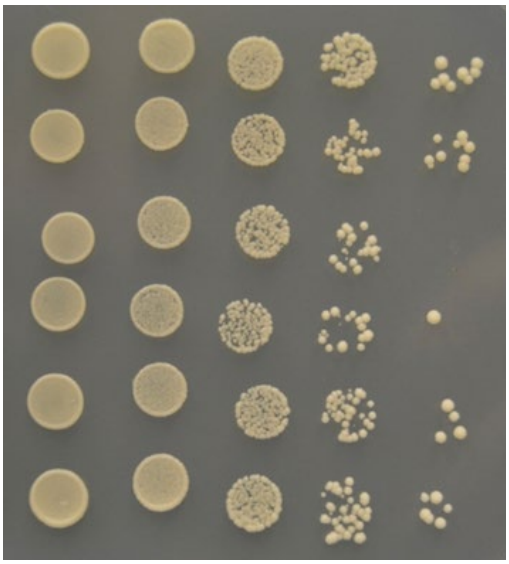
8. Using a micropipette, transfer 2 µL of each cell suspension to the surface of the petri plate. Do this for the entire grid – all of the replicates of the entire dilution series that you made.
   Hint: Use the template provided as a guide for your spots. Print out the template and position the plates over the circles so that you can see where to place your 2 µL spots. Placing tape between the petri plate and the template will ensure that the plate will not move as you transfer samples. Be careful not to jostle your plates as you work so that the spots of liquid won't run into each other. Also, use the reflection of light off the surface of the petri plate to better see where you have placed your spots.

   As you pipette up your 2 µL, take a moment to look at the tip with the liquid in it. Does it look ok? Does it look like 2 µL? If not, squirt it back into the well and try again. Sometimes air bubbles will get trapped in the tip, throwing off the volume. If this happens, it is ok to get a new tip.

   Let the spots of culture dry on the plate, invert the plates, and place them in the 30°C incubator.

   Capture an image of your plate when colonies have formed in the spots with the most diluted yeast.

Once the colonies have formed on the plates, you will see fewer colonies forming as you move from column 1 to column 5. This is because the concentration of yeast has decreased by 10 fold in each subsequent column, so fewer cells were placed on the surface of the plate. Each cell forms a colony. When the colonies are crowded, the spot of yeast will be confluent, so that no individual colonies will be observed (see column 1). As you move to the columns to the right, you eventually dilute the yeast enough so that you can see individual colonies form. These are your data – you will count the number of colonies and note which column had "countable" colonies. You can use the colony counts and dilution factor to calculate the concentration of cells in your original yeast suspension. *To the right is an example of what the growth on your plate might look like.*



[Photo credit: Steve Johnston]

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