Yeast Growth Assay (“Spot tests”)

1. Prepare yeast cultures overnight to saturation at 30°C.

2. Create a detailed key in notebook detailing (i) strains being tested, (ii) the order they will be spotted onto the plate, (iii) the overnight culture media and temperature used, (iv) and the plate types being used for spotting, and (v) how many days incubated and at what temperature.

3. A sterile 96-well plate should be prepared. 12 strains can fit onto one 96-well plate. For more than 12 strains being spotted, use another plate, etc. However, only a maximum of 8 strains x 6 lanes (48 wells) can be spotted onto a single petri dish.

4. Fill a sterile petri dish with sterile distilled water. Use the multi-channel pipettor to transfer 160 uL of water to all wells (same tips can be used).

5. In the far left lane for each strain, pipette approximately 30-50 uL of the overnight culture for a rough starting optical density (O.D.) of 1.0. Transfer the culture media after vortexing the culture to resuspend prior to transferring. Gently pipette up and down into the first well for each strain.

6. Continue for all strains being tested.

7. Use the multichannel to transfer 40 uL from the first lane of 8 strains into the second lane and pipette up and down carefully (no bubbles!) 10-15 times. Then immediately transfer another 40 uL from the second lane into the third lane and pipette up and down. Repeat until 6 lanes have been filled including the first lane.

8. Sterilize the 48-pin “pronger.” Dip the pronger into a small glass petri dish filled with 95% ethanol and flame. Repeat 2x times. The ethanol bath should only have a small amount of liquid in it and be placed far away from the open flame. Use caution and common sense. Finally, use a bath of 70% ethanol to sterilize the pronger and allow to cool.

9. Use the pronger to carefully dip into the 96-well plate (48 pins, so top “half” of 8x6 grid onto a single agar plate) and transfer the liquid with yeast to the agar plate. Press gently and ensure liquid transfer.

10. Dip the pronger into a petri dish of water to shake off any media and water and yeast. Then repeat the sterilization process in Step 8. As a control, after sterilization (once yeast are transferred), spot the empty pronger onto a YPD plate and ensure no yeast grow after 2 days!

11. Repeat for additional 96-well plates and strains if needed.

12. Alternatively, but more expensive, a multichannel pipettor can be used to transfer individual lanes of diluted yeast to plates; the pronger is more precise and cost effective.