H2O2 growth restriction protocol 10/07/10

1. Grow yeast overnight in 10 ml of YPD (2x 10ml)

2. Take OD600 in the morning; restage to 1.0

3. Take 10ml of cells and centrifuge at 10-15 C/ 1000 rpm for 5 minutes (using 15 ml Falcon tubes)

4. Pour off YPD and add equal volume of ddH2O

5. Immerse plastic tubes in waterbath sonicator to ensure uniform segregation of cells

6. Spin cells down and wash ddH2O two times

7. Resuspend in original volume of ddH2O.

8. Make H2O2/water dilutions as follows:

1.0%: 30 microliters 30% H2O2 + 870 microliters water

0.8%: 24 microliters 30% H2O2 + 876 microliters water

0.6%: 20 microliters 30% H2O2 + 980 microliters water

0.4%: 12 microliters 30% H2O2 + 888 microliters water

0.2%: 180 microliters 1% H2O2 + 720 microliters water

0.1%: 90 microliters 1% H2O2 + 810 microliters water

0.06%: 100 microliters 0.6% H2O2 + 900 microliters water

0.03%: 50 microliters 0.6% H2O2 + 950 microliters water

9. For each dilution, acquire a 1.5 ml tube, and add 250 microliters of cells and 250 microliters of H2O2/ H2O dilution. For the 0% H2O2 tube, add 250 microliters of cells and 250 microliters of ddH2O.

10. Vortex to distribute cells.

11. Wrap tubes in parafilm (because H2O2 will cause tubes to open during incubation)

12. Incubate horizontally for 3 hours at 30C

13. Make 10x dilution of 0% H2O2 dilution , count cells in bright line chamber

14. Determine proper dilution (usually 104x), plate, leave at 30C