**Effect of H2O2 on LOH, Oct 14, 2010 by Hong Qin**

Goal: Study the dosage effect of H2O2 on LOH in various Met15+/- strains. Dominique Parker and Meighan Parker will use this protocol to test the effect of H2O2 on LOH in Met15+/- derivatives of yeast natural isolates.

Procedure:

1. Grow eatch yeast strain overnight in 5ml of YPD in glass tubes at 30C to reach saturation of OD

2. Take 1ml from the o/n culture and add to 4ml YPD in new glass tubes, grow at 30C for 1.5 hours, measure OD600nm values.

3. Transfer to 1.0 ml to 1.5ml eppendorf tubes and centrifuge at maximum speed for 5 minutes (RT is OK).

4. Pour off YPD and add equal volume of ddH2O

5. Spin cells down and wash ddH2O two more times

6. Immerse plastic tubes in waterbath sonicator and sonicate for 2 minutes to ensure uniform segregation of cells

7. Make 2X H2O2 solutions of 1.0%, 0.5%, 0.09%, 0%.

8. For each dilution, acquire a 1.5 ml tube, and add 20ul of cells and 20ul of H2O2 solution. Vortex to distribute cells.

9. Wrap tubes in parafilm (because H2O2 will cause tubes to pop during incubation).

Incubate horizontally for 3 hours at 30C

10. Use Bright-Line counting chamber to estimate the cell concentrations. (We aim to put 200 colonies on each 100mm plate).

11. Terminate the reaction by adding 960ul water (50x dilution). Determine proper dilution, plate 100ul (aim for 150 colonies per plate), leave at 30C or RT.