**Effect of H2O2 on LOH, November 29, 2010**

Goal: Study the dosage effect of H2O2 on LOH in various Met15+/- strains. Erin Jackson will use this protocol to test the effect of H2O2 on LOH in Met15+/- derivatives of some yeast natural isolates (YPS128\*, M13\*, M32\*, M5\*, M34\*, M2-8\*).

Procedure:

1. Grow each yeast strain overnight in 5ml of YPD in glass tubes at 30C to reach saturation of OD600.

2. Restage to OD=0.6 in fresh YPD in new glass tubes. Final volume should be 4-6ml. Grow in 30C shaker for ???? hours, measure OD600nm values. The OD600 values for all strains should be around 0.8-1.0.

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 1.5% 1.0%, 0.5%, 0%.

8. For each dilution, acquire a 1.5 ml tube, and add 4ul cells, 16ul water, and 20ul of H2O2 solution (This is 10X dilution). Vortex to distribute cells.

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

Incubate at 30C shaker for 3 hours at 30C

10. During the incubation period, do proper dilutions from the 1ml cell suspension and then use Bright-Line counting chamber to estimate the cell concentrations. (We aim to put 200 colonies on each 100mm plate).

11. Terminate the H2O2 treatment reaction by adding 960ul water (50x dilution) and chill on ice. Because we aim to put 150 colonies on 100mm plates (or 500 colonies on 150mm plates), additional dilutions are probably needed again. Usually, we can add 100ul liquid on small plates and 200ul liquid on large plates. Leave at 30C for overnight or RT for the weekend.