**Effect of H2O2 on LOH, December 30, 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

**Day 1:**

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

**Day 2:**

1. Check OD600. Restage to OD=0.6 in fresh YPD in new glass tubes. Final volume should be 4-6ml. Grow in 30C shaker for 2 hours, measure OD600nm values. The OD600 values for all strains should be around 0.8-0.9.

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

3. Pour off YPD and add equal volume of ddH2O

4. Spin cells down and wash ddH2O two more times

5. Immerse plastic tubes in waterbath sonicator and sonicate for 4 minutes at the default setting to ensure uniform segregation of cells

6. Make 2X H2O2 solutions of 0.15%, 0.1%, 0.075%, 0.05%, 0.025%, 0.01%, 0.005, 0%.

7. 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.

8. Wrap tubes in parafilm (because H2O2 may cause tubes to pop during incubation).

Incubate at 30C shaker for 3 hours at 30C. (*This step can be skipped for small volumes*. )

9. 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).

10. Terminate the H2O2 treatment reaction by adding 960ul water (50x dilution) and chill on ice.

**11. Sonicate all the tubes again in waterbath for 2 mintues. (Previous protocal gives higher frequency of half blacks than full blacks, which raises the possibility of many cells stuck together).**

12. Because we aim to put 150 colonies on 100mm MLA plates (or 500 colonies on 150mm plates), additional dilutions are probably needed again. Usually, we can add 150ul liquid on small plates and 250ul liquid on large plates. Leave at 30C for overnight or RT for the weekend.

Because there are fewer cells at higher concentration of H2O2, so more cells should be added to MLA plates for treatment with higher concentration of H2O2. This can be done by using less dilution. Proper dilution can be estimated from previous experiments.

**Day 3: Counting colonies**

1. Cells grow slower on MLA plates. Count colonies by color-section patterns: White, Blacks, HalfBlacks, QuarterBlacks, QuarterQuarterBlacks, ThreeQuarterBlacks, Others. Ignore any section patterns that is less than 1/8.

2. Input the counting results on spreadsheet in GoogleDoc and share with lab members.

**Notes:**

1) It is possible that strains show various shades of gray or brown colors. So “white” and “black” are relative terms in each strain.

2) It seems colors darkens over time in some strains (especically in M14). So, plates should be counted and images should be taken as soon as they are ready.