**Effect of H2O2 on LOH,for 101S\* strain**

Last updated, August 30, 2012 H Qin

Updated on May3 ,2012, by N Gupta

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(this also works for M5\*, M8\*, March 22, 2011)

Goal: Study the dosage effect of H2O2 on LOH in strain 101S\*. Erin Jackson will use this protocol to test the effect of H2O2 on LOH in Met15+/- derivatives of some yeast natural isolates. Based on Dec 14, 2010 results, there seems to be linear response of LOH. We need to verify this finding. We need >20 blacks colonies at each H2O2 treatment in order to get publication quality data.

**Day 1:**

1. Grow each yeast strain 101S\* overnight in 5ml of YPD in glass tubes at 30C to reach saturation of OD600.

2. Prepare 30 large MLA plates.

**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. (Do not dilute the culture even if the OD600 is beyond 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. Point sonicate at level 2 with quick push twice. (Make sure the probe is wipped clean with 70% EHOH).

6. Make **2X** H2O2 solutions of 0.3%, 0.2%, 0.15%, 0.1%, 0.075%, 0.05%, 0.025%, 0.01%, 0.005, 0% (ten concentrations).

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 of cells, but 2x dilution of H2O2 stocks). 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

9. During the incubation period, do proper dilutions from the 1ml cell suspension (**at step 5**) 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 for 4ul cell -> 1000ul final volume) 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. Label the MLA plate with strain, H2O2 fraction, and replicate number.

13. Because we aim to put 150 colonies on 100mm MLA plates (or 750 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.

Note: Erin Jackson usually do 100X more dilution at this step for low H2O2 levels, and 10X for high levels. This is likely 10x less than the theoretical calculation, which indicates that many cells do not grow on MLA plates.

**Leave at RT overnight or 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.

Based on Dec 14, 2010 data of 101S\*, viability drops to half at 0.09% H2O2 and to 5% at 0.25%. So, we probably need to put 10 times more cells for 0.1%, 0.15, 0.2%, and 0.3% H2O2 treatment.

**Three plates for each concentration are needed to estimate the standard deviations.**

**Day 3: Counting colonies**

1. DocIT using translight.

2. 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.

3. Input the counting results on spreadsheet in Dropbox 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.