**April 5, 2011. This protocol has been used for M5\*, M8\***

**Rapamycin + H2O2 on LOH in 101S\* strain. Feb 17, 2011**

Grow 101S\* strain overnight in 5ml YPD

Next day, Check OD (should be around 1.6-1.7).

Add 0.75ml overnight culture to 5ml YPD, and the grow for 2hour to reach OD600= 0.8 ~ 0.9.

Spin down 1ml the cells in eppendorf tubes, wash with sterile water, spin down again

Immerse tubes in waterbath sonicator and sonicate for **5** minutes to ensure uniform segregation of cells. (water bath sonicator use only 1 power setting).

For each rapamycin dilution x H2O2 dilution (12 total here), acquire a 1.5 ml tube, and add 4ul cells, 15ul water

To make rapamycin 100x working solutions:

Sigma stock of Rapamycin = 2.5mg/mL in 200ul DMSO

Make 100x work solutions in DMSO.

Take 1ul 2.5mg/mL Rapamycin + 24 ul DMSO -> 0.1mg/mL.

Then two fold dilution for other solution (5ul + 5ul).

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| --- | --- | --- | --- | --- |
| 20x solutions | 0.1mg/mL | 0.04mg/mL  (4 ul 0.1mg/ml + 6 ul DMSO) | 0.02mg/mL  (4ul 0.04mg/ml + 4ul DMSO) | 0% control (DMSO only) |
| yeast mix in water | 19ul | 19ul | 19ul | 19ul |
| 20x Rap soln | 1ul | 1ul | 1ul | 1ul |
| Final Rapamycin | 5ng/mL | 2ng/mL | 1ng/mL | 0ng/mL |

Place tubes horizontally in 30C shaker for **2** hours

Add 20ul of H2O2 solution of 0%, 0.1%, 0.2%, 0.3% (This is 10X dilution of cells, but 2x dilution of H2O2 stocks. reach final concentration of 0%, 0.05%, 0.1% and 0.15% of H2O2 ). Vortex to distribute cells. (*E. Jackson wrote and record the 2X H2O2 stock concentration on the plate. So final concentration during analysis should be half of those numbers.*)

Place tubes horizontally in 30C shaker for **3** hours.

Add 960ul H2O to terminate reaction

**Sonicate all the tubes again in waterbath for 2 minutes to prevent cell clusters.**

For plating we use 150mm plates. Put 250ul on each large MLA plate

For 0% H202 use 100x dilution (990 ul H20+ 10ul yeast soln), For 0.2% and 0.4% use 10x dilution (900ul H2O+100ul yeast soln)