2012 July 23-25, strain M32, hydrogen peroxide effect on DHR, DHE signals by double-staining, by H Qin

Goal: Study the effect of H2O2 on DHR and DHE signals, using double-staining

Monday. July 23

Grow M32 in 5 ml YPD at 30C shaker

Tuesday July 24

First Restage the cells.

1. Dilute samples by 1:15. (Check OD600. Restage to OD=0.6 in fresh YPD in new glass tubes). Final volume should be 4-6ml.
2. Grow in 30C shaker for 2.0 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).
3. Transfer to 1.0x3 ml to 1.5ml eppendorf tubes and centrifuge at maximum speed for 5 minutes.
4. Pour off YPD and add equal volume of ddH2O
5. Spin cells down, merged the tubes from the same strains, wash ddH2O one more times, and then resuspend cells in 1.2ml water
6. **Point sonicate** at level 2 with quick push twice. (Make sure the probe is wipped clean with 70% EHOH).

Second, hydrogen peroxide treatment

7. Make **2X** H2O2 working stock solutions of 0.2%, 0.1%, 0.075%, 0.05%, 0.025%, 0%. (6 concentrations)
8. For each dilution, acquire a 1.5 ml tube, and add 150ul cells, and 150ul of H2O2 solution (This is 2x dilution of H2O2 working stocks). Vortex to distribute cells.
9. Wrap tubes in parafilm, because H2O2 may cause tubes to pop during incubation. (Skip these for large scale experiments with too many tubes).
10. Incubate on nutator for **1.5 hours** at 30C.
11. Terminate the H2O2 treatment reaction by adding 800ul water, spin down at max speed for 2 min.
12. Wash with 0.5ml PBS once to remove residual hydrogen peroxide. Gently remove the liquid using a pipette.
13. Resuspend the pellet in 300 ul PBS.

Third, DHR and DHE labeling

14. For DHR-DHE double labeling, to 75 ul cells, add 0.5ul 25mM DHR and 125ul PBS. Incubate at 30C for 1.5 hours. Then spindown cells, and add 200ul PBS + 1ul 5mM DHE. Inducate at 30C for 30 minutes. Spindown, add 1ml PBS.
    a. Note, unoxidized DHE is blue and may interfer with DHR in FL1.
15. For DHR labeling, to 50 ul cells, add 0.5ul 25mM DHR, and 150ul PBS. Incubate at 30C in dark, <u>one reaction for 1.5 hrs, and another for overnight.</u> Then spin down, and resuspend in 1ml PBS for flow cytometer. (Mater mix should be made).
16. For DHE labeling, to 50 ul cells, add 1ul 5mM DHE, 150ul PBS. Incubate at 30C in dark for 10 minutes. Then spin down, and respusend in 1ml PBS for flow cytometer.
17. Measure both DHE and DHR in Calibur. Save instrument setting in the data folder.

Wednesday July 25 (next day)

1. measure O/N DHR, and DHR-DHE double-stain

Note: All fcs file should be conpressed and then transfer to dropbox folder

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Invitrogen: The superoxide indicator **dihydroethdium**, also called hydroethidine, exhibits blue-fluorescence in the cytosol until oxidized, where it intercalates within the cell's DNA, staining its nucleus a bright fluorescent red. Dihydtroethidium is also available in conveniently packaged 5 mM solution stabilized in DMSO (D23107).

Reference: Cossarizza 2011. Nat biotech.