**2012 June 25 Week, glucose effect on DHR, DHE signals by double-staining, by H Qin**

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

**2012 June 22, Friday.**

Grow BY4743 and M5 in 5 ml of media at 30C shaker.

The media is YP2%DS, YP1%DS, YP0.5%DS, YP0.1%DS, YP0.01%DS, YP0%DS. D means glucose, and S means sorbitol.

**2012 June 24, Sunday**

1:50 dilution of each culture to fresh 50 ml media in 125 ml flask, shake at 30C.

(I am not sure whether 50ml is too much in 125ml flask, so I need to try one on shaker first).

**2012 June 25, Monday**

**First, measure OD, spindown and sonicate.**

1. Take 1ml to measure OD at 600nm.
2. Then transfer this 1ml to eppendorf tube, spin down.
3. Pour off YPD and add 0.5 ml of PBS
4. **Point sonicate** at level 2 with quick push twice. (Make sure the probe is wipped clean with 70% EHOH).

**Second, DHE, DHR, and DHE-DHR labeling**

1. For each culture, acquire three 1.5 ml eppen tubes, and add 50ul cells per tube.
   1. We will do 3 labelings: DHE, DHR, DHE-DHR
   2. Make sure that unstained cells are left for controls.
2. DHE single-labeling,
   1. to the 75ul cells, add 1.0ul 5mM DHE and 125ul PBS (make master mix)
   2. 30 minutes incubation @ 30C nutator
   3. spindown,
   4. add 1ml PBS, proceed to measure in Calibur
3. **DHR single-labeling**
   1. to the 75ul cells, add 0.5ul 25mM DHR and 125ul PBS (make master mix)
   2. **overnigh incubation** @ 30C nutator
   3. next morning, spindown, and add 1ml PBS
   4. proceed to measure in Calibur
4. DHE-DHR double labeling
   1. First, do DHE labeling should proceed like step 6a, 6b, and 6c (not 6d).
   2. To the cell pellet, add 0.5ul 25mM DHR and 195ul PBS.
   3. Overnight incubation at 30C.
   4. Next morning, spindown, add 1ml PBS.
5. Measure both DHE and DHR in Calibur. Save instrument setting in the data folder.

**Note:**

1. Make sure the flow cytometer data are organized by their date take from the culturing flasks (not flow-cytomer measurement-date. This is absolutely critical).

Reference: Cossarizza 2011. Nat biotech.