EMS Mutagenesis of Yeast

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1. Grow a 50 ml O/N YPD yeast culture at 30 degrees C.

2. Determine cell density and transfer an amount of culture corresponding to 1 x 108 cells to several 15 ml conical tubes. Spin down cells and wash with 5 ml sterile water. Spin down cells and wash with 5 ml 0.1M sodium phosphate (NaH2PO4) buffer, pH 7.0. Spin down cells and resuspend in 1.7 ml buffer.

3. Transfer cells to glass culture tubes. In hood, add 50 ul EMS (ethyl methanesulfonate) (also have one control tube without EMS). Incubate on roller at 30 degrees for varying amounts of time, between 20 min. and one hour.

4. At each time point, add 8 ml sterile 5% sodium thiosulfate (autoclaved). This will stop the mutagenesis by inactivating the EMS. Each cell suspension should contain 1 x 107 cells/ml. Save an aliquot of cells at this point to use in figuring out the cell survival.

* All glassware, plasticware, and solutions that comes in contact with EMS should be rinsed
with 5% sodium thiosulfate to inactivate the EMS.

5. Spin down cells in 15 ml conical tubes and resuspend in 9 ml sterile water. Figure out actual cell titer after wash by plating out varying dilutions onto YPD plates at 25 degrees C. Store cells at 4 degrees C.

6. To determine the cell survival, use an aliquot of cells from step 4, dilute, and plate onto YPD plates to give about 500 cells/plate. Incubate at 30 degrees C. Count the number of colonies and compare each EMS time point to the nonmutagenized cells. Use the time point that gives a cell survival of 60-70%.

7. Use stored cells to plate out varying numbers of cells onto YPD plates. Also plate out non mutagenized cells as a control. (Optional: briefly sonicate cells, ~10 sec, to make sure that there are no clumps of cells present before plating). If searching for suppressors of a ts strain, incubate one plate at 25 degrees C and one plate 37 degrees C, look for colonies that grow better on the 37 degrees C plates. If

searching for ts strains, incubate all plates at 25 degrees C until colonies come up, replica-plate to two new YPD plates, incubate one plate at 25 degrees C and one plate at 37 degrees C, look for ts colonies on the 37 degrees C plates.