S. cerevisiae MMS Mutagenesis

NOTE 1: (i) MMS = methanesulfonicacid methyl ester; methylating agent
(ii) induces : GC -> AT
GC -> TA
AT -> TA
AT -> GC
GC -> CG

NOTE 2: Protocol from Dr. Christine Moore-Rodriguez

Protocol used: Generally to further mutagenize a mutant strain and look for synthetic or bypass phenotypes. You're trying to balance the number of *de novo* mutations induced in each cell: too many and you'll never be able to track down which one is interacting with your study mutation. A balance between the induced mutation rate and killing curve will help you guesstimate the appropriate exposure time.

Protocol:

1. Grow 3mL culture of yeast strain in YPD overnight (to saturation).

2. Collect cells by centrifugation (microfuge 6K, 5min, RT°). Wash in 3mls sterile ddH_2O.

3. Resuspend cells in 3ml 50mM KP buffer pH 7.0. Take a 1mL aliquot from each sample and place in 1mL of sterile water for a 1:1000 "nonmutagenized" (NM) cell control.

KP buffer pH7.0 (0.1M stock; dilute to 1L)
61.5 mL of 1M K_2HPO_4
38.5 mL of 1M KH_2PO_4

4. Add MMS to 0.5% final in the KP resuspension @ RT° for 30 minutes (see **Note 3**) with gentle shaking. (0.5% = 15µL per 3mL reaction)

5. Stop mutagenesis by adding an equal volume of **freshly made** 10% Sodium Thiosulfate (in ddH_2O) to inactivate the mutagen.

6. Spin down cells (microfuge: flash spin up to 10K) and wash 2x with 1ml ddH_2O. Resuspend cells in 1 mL sterile water, plate on YPD and incubate appropriately:
(i) if mutation under study is temperature sensitive (*ts*) and you're trying to isolate bypass mutants, you would incubate plates at the non-permissive temperature.
(ii) plate at the appropriate density: ≤ 300 growing cells per 8cm plate is about as dense as you can comfortably work with)

7. Select spontaneous and induced revertants after 3 days incubation. May want to continue to score each day from day 3 to 6.
Controls: 100µL of 1:1000 dilution of cells at permissive Temp.
100µL of the NM control at Permissive Temp. Determine the % killing with these two controls.
May want to plate 100µl of the ts cells (undiluted and 1:100) at the non-permissive temperature.

NOTE 3: A killing curve was performed on strains YSB307 & YSB308 (9/12/95). The average of the data is as follows (**expressed**: % survivors / min exposure to 0.5% MMS)

	% Survivors
0'	100
30'	31.8
45'	19.7
60'	5.2