EMS MUTAGENESIS FOR *ARABIDOPSIS*

1. Weigh out 10,000 seeds (approx. 200 mg) and place in 50 ml Falcon tube.

2. Wash seeds in 10 ml of 0.1% Tween20 (10 μl Tween20 in 10 ml dH_2O) for 15 mins.

3. Remove Tween20 and add 15 ml dH_2O followed by 15 – 45 μl EMS (0.1% - 0.3%) depending on concentration chosen. (30 μl EMS in 15 ml dH_2O = 0.2% solution). Replace cap and seal with parafilm.

4. Shake overnight in tube rotator placed within a tray in the fume hood.

5. Next morning let seeds settle and pipette off the EMS into 5M NaOH (leave this a further night and then dispose of as NaOH).

6. Add as much dH_2O to the seeds as Falcon tube can hold. Mix. Remove water into 5M NaOH. Repeat rinse 8 times. Let seeds soak in the last rinse of 10 ml dH_2O for 1 – 2 hours to let EMS diffuse out of seeds.

7. Pour seeds and water into 90 ml of 0.1% agar (0.1 g agar in 90 ml dH_2O, autoclave to dissolve, then cool) to make final volume of 100 ml.

8. Sow seeds at rate of 0.5 ml per pot (i.e. need 200 pots) using 1ml pipetteman, cover with clingfilm and vernalise. This averages 50 seeds per pot of which 1200 seeds from each pot (pool) will have to be screened. It's a good idea to fertilise pots so that plants grow healthily. Also, sow some unmutagenised seed alongside to monitor kill rate.

EMS = Ethyl Methane Sulfonate, Sigma (M0880). Must be used fresh. EMS is a volatile carcinogen and must be stored in the Poison cabinet – handle with double gloves, wear goggles and carry out all experiments in the fume hood. Destroy by exposure to 5M NaOH and dispose in same way as NaOH after 24 hours.