Plating *Symbiodinium* on 5-FOA Medium

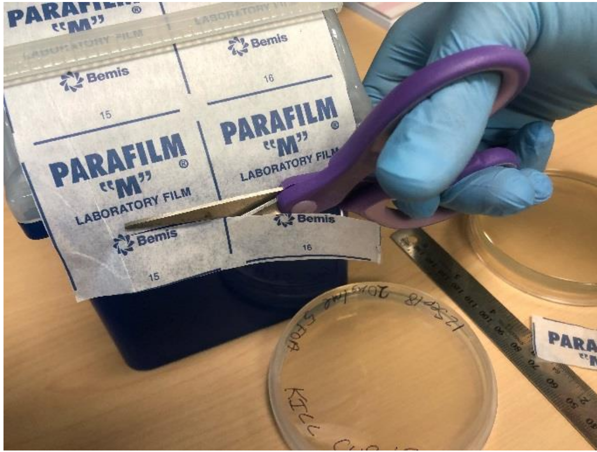
One week ago, you irradiated your *Symbiodinium* with ultraviolet light. The intent was to cause DNA damage and induce random mutations in the cells. We're performing a mutant screen for one particular phenotype – tolerance to 5-FOA. As discussed in your lab lecture, we believe that most mutants with the 5-FOA tolerant phenotype should be loss-of-function mutants for *URA3*. *URA3* loss-of-function mutants will not be able to synthesize their own uracil and will require an exogenous source of the nucleobase. This is why the recovery medium you used last week included 1 g/l uracil. This week, you will plate these mutagenized cells on agar plates containing 5-FOA, at a concentration of 10 ug/ml, a concentration which is lethal to wild type cells. Your plates also contain 1 g/l uracil added to the medium. SSB01 cells grow slowly; it will take several weeks for any mutant colonies to become visible.

Maintain aseptic condition as best as you can. Most importantly, avoid talking while plating.

1. Retrieve your UV-irradiated *Sybiodinium* from the incubator.
2. Obtain a 2 ml tube and label with your name.
3. Transfer 2 ml of the *Symbiodinium* liquid culture (from your well in your table's 6-well plate) into a 2 ml tube labelled with your name.
4. Centrifuge at 10,000g for 1 min.
5. Be mindful of where the *Symbiodinium* pellet is and be sure to not disturb it. IF YOU DISTURB THE PELLET, repeat the centrifugation. Using a pipette, remove 1.5 ml from the upper portion of the liquid (the supernatant) and dispose in the waste cup provided

on your table. If you accidentally pipette off or disturb *Symbiodinium*, pipette the cells back into your tube and repeat centrifugation to collect the cells again.

6. Add 1.5 ml of the *Symbiodinium* culture to fill up the tube to 2ml.
7. Repeat step 4, 5 and 6 until you have no liquid culture remaining and all cells are collected in a pellet in 0.5 ml volume in your 2.0 ml tube. Resuspend your cells (in approx. 0.5 ml volume; use the graduation on the tube to confirm.) The suspension should be dark brown in color; this is because the *Symbiodinium* is now densely concentrated
8. Obtain a media plate from your TA and label appropriately. The label must include your name, your section, your table, and treatment (0, 2, 4, 8, 16, or 32 min).
9. Pipette 0.5 ml of the concentrated *Symbiodinium* culture onto the center of the plate.
10. Use the sterile plastic spreader to spread the cell suspension evenly.
11. Cover plate and do not move for ~10 minutes to allow the liquid to absorb.
12. Cut a 1 cm wide strip of Parafilm. Carefully wrap your plate with Parafilm to prevent evaporation and contamination. Note that if you do not wrap your plate properly, your media will dry, killing any mutants.



13. Turn your plate upside down so that condensation does not drip onto the plate and interfere with the *Symbiodinium* being cultured.
14. Incubate at 25°C under 12:12 light cycle (~10-40 μE m^{-2} s^{-1} PAR).
15. Inspect in 4-6 weeks.