**Week 1**

**Background: Microscopic observation of anemones**

Coral reefs depend on symbiosis with certain species of photosynthetic algae, which are nutritional endosymbionts (i.e., they live inside the cells of their hosts and provide their hosts with nutrients). In this class, we use the anemone *Exaptaisa pallida* as a model system for coral-algal symbiosis because it associates with the same types of algae, can be bleached like coral, and grows quickly in laboratory conditions. In class we will observe anemones in symbiosis with algae as well as aposymbioticanemones (i.e., anemones that have been bleached and do not contain algae within their cells).

**Background: Pipetting exercise**

The study of genetics depends heavily on the use of molecular biology techniques. In particular, genetics often requires that we isolate and manipulate DNA from our model organisms. Because the cellular environment is aqueous, many molecular biology techniques ultimately revolve around the manipulation and combination of small volumes of liquids that contain our molecules of interest (e.g., DNA). In this class we will practice pipetting because good pipetting technique is critical to the success of our experiments throughout this semester.

**Background: Mutagenesis of *Breviolum minutum***

*Breviolum minutum* is a species of unicellular dinoflagellate algae and nutritional endosymbiont of corals, anemones like *Exaiptasia pallida* and other cnidarians. They play an active role in establishing and maintaining a symbiotic partnership with their cnidarian hosts, and because of this their genetics are also interesting to scientists studying coral bleaching. In this class we are attempting a **forward genetic screen** in which we cause random mutations with UV exposure and screen for a phenotype (here, resistance to 5-fluoroorotic acid or 5-FOA). This screening is accomplished with the use of selective media (plates containing 5-FOA) which should prove lethal to wildtype algae but is not lethal to *Ura3* null mutants.

**Protocol for pipetting exercise:**

Warm-up exercise:

What are the ranges of the pipettes at your table?

1)

2)

3)

4)

Select the correct pipette for desired volume:

1) 1.25μl 5) 9.8μl

2)165μl 6) 2.00μl

3) 200μl 7) 12.8μl

4) 20μl 8) 465μl

**Exercises 1 & 2**

Each student should receive four 1.5 ml tubes. Before pipetting label your tubes A, B, C, D.

**Exercise 1:** **Adding & removing large volumes**

1.Add 370μl Blue reagent to tube A.

2.Add 225μl of Red reagent to tube C.

3.Add 495μl of Yellow Reagent to tube B.

4.Add 160μl of Blue reagent to tube D.

5.Remove 155μl from tube A and dispose.

6.Remove 190μl from tube B and add it to tube D (do not mix).

**Exercise 2. Adding vol. into vol. & common mixing techniques** (Note before and after mixing).

1.Add 285μl of Red reagent to tube A and mix by pipetting up and down.

2.Add 195μl of Blue reagent to B and mix using inversion.

3.Add 275μl of Yellow reagent to tube C and mix using flick/tap method.

4.Add 150μl of Red reagent to tube D and mix by vortexing.

**Exercises 3 & 4**

Each student should receive four 200ul tubes & label them E, F, G, H.

**Exercise 3: Adding small volumes**

1) Add 20μl of Blue reagent to tube E.

2) Add 11μl of Red reagent to tube F.

3) Add 2.5μl of Yellow reagent to tube G.

4) Add 1μl of Blue reagent to tube H.

**Exercise 4: removing & mixing small volumes**

1.Remove 10μl from tube E and dispose of it.

2.Remove 1μl from tube F and dispose of it.

3.Add 7.5μl of Red reagent to tube G and mix by pipetting up and down.

4.Add 9μl of Yellow reagent to tube H and mix by pipetting up and down.

**Exercise 5. Making a master mix.** (1-1.5 ml tube per person)

Often to save on time and to reduce variation between reactions, it is helpful to make a master mix when doing multiple reactions. Master mixes are made by making reaction mixes that are larger in total volume but contain the same ratios of reactants as 1x. It is often customary to make master mixes larger than what is desired to account for pipetting error.

Example: Desired=5x, Make = 5.5x

Desired =20x, make=22x

1x Reaction Desired=9x Make=\_\_x reaction

10μl Yellow reactant. \_\_Yellow Reactant. \_\_Yellow Reactant 9μl Blue reactant \_\_Blue Reactant \_\_Blue Reactant

1μl Red reactant \_\_Red Reactant \_\_Red Reactant

20μl Total \_\_Total \_\_Total

**Protocol for *Breviolum* mutagenesis

Note to scientists in training:**

As a rule, read all protocols from beginning to end before starting. Ensure that you have access to all required materials and equipment. Make sure that you understand both how to perform each step and why you’re performing the step. Understanding why you’re doing something is important for avoiding mistakes AND critical for troubleshooting. If there is any confusion, resolve it before beginning.

We will be mutagenizing *Breviolum minutum* strain SSB01 using ultraviolet light. Each table will be administering a different dose of UV to randomly induce mutagenesis in a large population of cells. In the following weeks, we will select for mutants which possess the phenotype of interest. This protocol has been carefully designed to eliminate any UV exposure to experimenters

Each table/group is assigned a UV exposure time: 0 min (negative control), 15s, 30s, 1min, 2min, 4min.

1. Each table will receive a 6-well plate. Each student will label their own well accordingly, with the following information:

a. Table #

b. Section #

1. Experimenter name

d. Duration of exposure to UV

e.g. T6, S07, Josh, 32 min

1. Locate the T75 culture flask containing a dense culture of SSB01 on your table and swirl to homogenously suspend cells. Density is approximately 5 x 105 cells/ml.
2. Each student must transfer 1ml of SSB01 culture suspension into one well of the plate (each table will use four wells total). If the culture has settled, swirl before pipetting. Gently swirl plate to distribute culture across bottom of well.
3. When all tables are ready, students can begin carefully putting their plates into the UV chamber under the supervision of their TA. Each table should leave their plate in the chamber with the UV source on for the appropriate time before moving to the next step.
4. As soon as a sample’s exposure is complete, add 8 ml of the provided recovery medium. Recovery medium for this particular screen is Daigo’s IMK medium supplemented with 1g/L uracil.
5. Incubate at 25°C under a 12:12 light cycle, with 10-50µE/m2s1 PAR for 7 days.

**TAs: Ensure that the UV source is OFF before leaving.**

**For your lab notebooks:**

As a rule, when you are provided with a protocol, you do **not** need to copy the entire protocol into your lab notebook. It is perfectly fine to write something along the lines of “we performed the mutagenesis according to the protocol we were given in class.” However, it **is** important to include any mistakes or deviations from the protocol if any occur, as well as all data and everything listed in the lab manual for the week. You should also include the date and title of that week’s lab at the beginning of every entry. Lastly, you should not use a notebook that you are using for another course as your lab notebook, as your TA may need to keep it for a week or more when grading it. For this week, you should include the following information and answer the following questions:

1. Include photographs in your notebook or draw pictures of the symbiotic and aposymbiotic anemones you’ve observed. Note the differences in pigmentation. Explain what accounts for the differences in appearance you observe.
2. Write in your notebook the answers to the warmup exercises and exercise 5. For exercises 2-4, write the final volumes that each tube should have had. Did your tubes have those volumes when you were finished with the exercise?
3. For this experiment, each table will perform one treatment, and the experiment as a whole will be completed by the class. Because of this, it’s important that you write down all data. Record in your notebooks the exposure times that each table used. Which exposure time did your table use? Write your predictions of which tables (if any) will generate mutant algae, and which will generate the most.

**Week 2**

**Background: plating of *Breviolum minutum***

Previously, we mutagenized cultures of the symbiotic algae *B. minutum* (strain SSB01) with ultraviolet radiation. Now, we hope to screen these cultures of randomly mutagenized cells for a specific phenotype: resistance to the compound 5-fluorooritic acid (5-FOA). 5-FOA is broken down by action of the protein Ura3 into the toxin 5-fluorouracil (5-FU). *Ura3* knockout mutants will not metabolize 5-FOA into 5-FU and as such will be immune to its deleterious effects. We’re plating our mutagenized *B. minutum* cells onto media containing 5-FOA to screen for *Ura3-* (i.e., knockout) mutants.

**Protocol for plating of *Breviolum minutum***

One week ago, you irradiated your *Breviolum* with ultraviolet light to cause DNA damage and induce random mutations in the cells. We’re performing a mutant screen for tolerance to 5-FOA. This week, you will plate these mutagenized cells on agar plates containing 5-FOA at a concentration of 10ug/ml which is lethal to wild type cells. Your plates also contain 1g/l uracil added to the medium. SSB01 cells grow slowly, so it will take several weeks for any mutant colonies to become visible. Because of this long incubation time, cultures are susceptible to mold and bacterial growth, so **it is critically important that you work with good sterile technique**.

1. Retrieve your cells from the incubator.
2. Obtain a 2ml tube and label it with your name.
3. Transfer 2ml of the liquid culture (from your well in your table’s 6-well plate) into a 2ml tube labelled with your name.
4. Centrifuge at 10,000g for 1 min.
5. Be mindful of where the pellet is and be sure to not disturb it. **If you disturb the pellet, repeat the centrifugation**. Using a pipette, remove 1.5ml supernatant and dispose of it in the waste cup provided on your table.
6. Add 1.5 ml of the culture to fill up the tube back up 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 0.5ml volume in your 2.0ml tube. Resuspend your cells in ~0.5ml volume. The suspension should be dark brown in color because the cells are 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 0.5ml of the concentrated culture on to 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 a few minutes to allow the liquid toabsorb.
12. Cut a 1cm 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 and your cells will die.**
13. Turn your plate upside down so that condensation does not drip onto the plate and interfere with the cells.
14. Incubate at 25oC under 12:12 light cycle (~10-40μE m-2s-1PAR).
15. Inspect in 4-6 weeks.

**For your lab notebooks**

It is not important to copy the entire protocol down into your lab notebook, unless you feel compelled to do so. Instead, you may summarize what you’ve done in class today. More importantly, state clearly *why* we did this protocol—what do we hope to achieve? If necessary, write down any mistakes or deviations from the protocol, what you expect the outcome of those deviations will be, and what you’ve done to keep the protocol on track. Finally and most importantly, what do you think will be the outcome of this experiment? Write down in detail what you predict the results will be across the whole experiment (remember, each table is one treatment, so the experiment is all of the groups together). Why do you think this will be the case?

**Week 3**

**Background: anemone stress challenge**

Coral bleaching is the breakdown of symbiosis between the coral animal and its endosymbiotic algae. The loss of the pigmented algae results in the white coral appearing to have “bleached.” Other cnidarians including the anemone *Exaiptasia pallida* and the jellyfish *Cassiopea xamachana* also engage in symbiosis with photosynthetic dinoflagellates and can be used as models to study coral bleaching. This week, we will begin the second major experiment in this course: the anemone stress challenge. We will be quantifying a phenotype (tolerance to thermal stress or menthol exposure) and then spend the rest of the semester seeing if variations in this phenotype correspond to differences in genotype. Little is known about the mechanisms underlying coral bleaching, so understanding genes involved in this process is of great value to the scientists who study coral bleaching and hope to prevent the loss of coral reefs to climate change.

Every table will have one **genet** of anemones (a clonal colony propagated asexually from a single individual). Each anemone in a genet is called a **ramet**. Students will place one ramet in each of four different treatments: 25oC seawater, 32oC seawater, 34oC seawater, and 25oC seawater with 0.19mM menthol. Menthol is known to bleach anemones, although the mechanism is unknown. These treatments then represent, respectively, a normal temperature they would experience in nature and a **negative control** treatment, mild heat stress, more extreme heat stress, and a **positive control** treatment. Each anemone will be left in the appropriate temperature for one week.

 Before going into the incubator, students will measure the color of each anemone using the coral health chart. This is an indirect measure of symbiosis, because more algae in an anemone’s cells means more pigment and a darker color. They will then be measured at the end of one week, and loss of pigment (and therefore algae) can be discerned as the difference between color before and after the stress test.

 **Tolerance** to thermal stress of menthol can here be defined as the difference in color of an anemone before and after a stress treatment compared to the difference seen in a control anemone in the same genet. A genet in which a thermally stressed anemones go from very dark to completely bleached is less tolerant to thermal stress than a genet in which thermally stressed anemones go from very dark to only partially bleached (assuming their respective controls behave consistently).

**Protocol: anemone stress challenge**

Each table will receive one genet (clonal population) of at least 4anemones.

1. Before transferring anemones, score the color of each individual using the coral health chart provided. Take photos of your anemones with reference chart in background.
2. Label your Falcon tubes with genet ID (location), section number, table number, student name and treatment (e.g. ARB31/U02/T3/Josh/Menthol)
3. Each student will transfer ~40ml of seawater into 50ml calibrated Falcon tube. Note: for anemones in the menthol treatment, use 40ml of pre-made mentholated seawater instead.
4. Each student will transfer one anemone using a cut-off transfer pipette to a Falcon tube containing 40ml of seawater or mentholated seawater. Note: the anemones like to adhere to their cups, and sometimes work themselves into corners and are difficult to remove. A firm but delicate touch is best, and if you are afraid you are causing significant damage to your animal, don’t hesitate to call your TA over to help. Remember, although they are simple, they are animals and should be treated with respect and care!
5. Loosely cover the top of each tube in plastic wrap and place each tube in the appropriate incubator for seven days.

**Notes:** if a table has less than four students or a student is absent, each group should still put an anemone in each of the four treatments. Return any remaining anemones to your TA to preserve the lineage. If your cup contains exactly four anemones, your TA may ask you to take additional steps to maintain the genet next week.

**For your lab notebooks**

Write a brief overview of the whole experiment. Why are we doing what we’re doing, and what do we hope to achieve? Record the genet ID of your anemones, and write down the coral health chart score for each individual. How do you predict each of your four anemones will fare? Which treatments will see the most bleaching, and which will see the least? Do you expect to see variation in tolerance among different groups, and if so do you think that there may be a genetic basis for that variation? Why or why not?

**Week 4**

**Background: anemone stress challenge (cont.) and DNA extraction**

This week we take our anemones out of their respective incubators and examine the effect that the different treatments had on their pigmentation (which is a proxy for how much algae they have lost, i.e., how much bleaching has occurred) relative to the control group. These data points—along with our pre-treatment data points—will allow us to assess the tolerance of each genet to temperature and menthol stressors and to quantify that tolerance phenotype.

 In this experiment, we are interested in seeing if we can correlate those phenotypes to genetic differences. In order to determine the genotype of our anemones and their symbiotic algae, we must first extract DNA from them. This genomic DNA extraction will be used in subsequent labs for amplification of specific regions of DNA and finally sequencing of that DNA. DNA extractions can be performed without a kit, but such techniques may involve harmful chemicals like phenol and chloroform and may require more experience than we have time to provide you with on this one task. Kit extractions are far simpler but follow the same basic principles. Firstly, you must lyse the cells of the organism (or in our case, organisms, as we will be extracting DNA not only from our anemones but from the algae inside their cells). Once the cell and nuclear membranes are sufficiently disrupted, it is possible to access the DNA. At this point your solution will contain your DNA as well as many other types of water soluble molecules. In a traditional DNA extraction, some of these molecules will be removed by centrifugation and phase separation, and the phase containing the DNA is kept and further cleaned by pelleting the DNA in alcohol to remove more alcohol soluble molecules. In a kit extraction, purifying the DNA is achieved using a filter column with selective permeability; the first filter we will use in this extraction allows the DNA to flow through and collects certain large impurities, then the second column binds the DNA and allows us to wash alcohol soluble molecules out of the filter before the final elution step.

**Protocol: anemone stress challenge (cont.)**

Measure the color of your anemones using the coral health chart as you did last week. The genetics lab would like to maintain each genet, so if you do not have any anemones remaining in your cup from last week, it may be necessary to cut an anemone in half and return one half to your cup, saving the other half for DNA extraction. If necessary, cut the anemone **transversely** (i.e., cut it in half from the mouth to the foot). If a piece of an anemone contains part of the foot, it should be able to regenerate completely. Return one half to your cup and save the other for DNA extraction.

**Protocol: DNA extraction**

The full instruction manual for the DNA extraction kits we are using can be found here:

<https://files.zymoresearch.com/protocols/_d6016_quick-dna_tissue-insect_miniprep_kit.pdf>

When performing a DNA extraction from a kit, the specific names of tubes and buffers will vary from manufacturer to manufacturer, but the basic premise remains the same as described above. We will be making some minor deviations to the protocol as listed in the manual, so the instructions below have been modified for clarity and should be followed in class.

1. Add each anemone to a bashing bead lysis tube. This is the tube containing small beads, which will help abrade the cells of the anemone, allowing you to access the DNA. Add 750μl of bashing bead buffer to the tube and cap tightly.
2. Vortex the tubes containing your anemones with buffer for ~1min or until the anemones are completely destroyed. It is important to that there are no visible pieces left after vortexing to maximize our DNA yield. **Note:** you may need to invert your tube several times to trap the anemone under the beads and prevent it from floating above the beads while you vortex.
3. Centrifuge the bashing bead tube in a microcentrifuge at ≥10,000 x g for 1 minute to pellet any large pieces of cellular debris.
4. The supernatant (i.e., the liquid above the beads) contains your DNA at this points, as well as many other water soluble molecules. Transfer up to 400μl supernatant to a III-F filter column (the one with the red ring) in a collection tube and centrifuge at 8,000 x g for 1 minute.
5. At this point, the filter has collected larger cellular debris, and your DNA and other molecules have flowed through the filter into the filtrate. Add 1,200μl of genomic lysis buffer to the filtrate in the collection tube from Step 4. Mix well by pipetting up and down.
6. Transfer 800μl of the mixture from Step 5 to a II-C filter column in a collection tube and centrifuge at 10,000 x g for 1 minute. **Note: DO NOT** discard the remaining 800μl of the mixture from Step 5! You will use it in the next step.
7. Your DNA is now bound to the filter with some other molecules. We can wash it further before causing it to unattach from the filter for collection. Discard the flow through from the collection tube and repeat Step 6. **Note:** when discarding flow through, you can pour the liquid onto a paper towel and dab the top of the collection tube dry instead of pouring the buffer down the sink. Throw your paper towels away in solid waste at the end of the class.
8. Now all of your DNA is on the filter. Add 200μl DNA pre-wash buffer to the column and centrifuge at 10,000 x g for 1 minute.
9. Add 500μl g-DNA wash buffer to the column and centrifuge at 10,000 x g for 1 minute.
10. Label a clean 1.5ml microcentrifuge tube with your section, table, genet ID and treatment and transfer the column to a clean 1.5ml microcentrifuge tube and add 100μl DNA elution buffer directly to the column matrix. It is important that you put the buffer directly on top of the filter, but do not poke the filter with your pipette. The buffer will cause the DNA to release from the column, as the electrostatic charge of DNA is pH dependent. Centrifuge at 10,000 x g for 1 minute to elute the DNA.

After elution, your DNA will be suspended in the buffer in your labelled microcentrifuge tubes. Return those tubes to your TA so that he or she can freeze them for next week’s protocol. When cleaning up, discard all tubes and filter columns that you have used during the extraction, but do NOT throw away any excess buffer, which may be used for later sections and should be disposed of properly by your TA.

**For your lab notebooks**

Record all coral health chart data from the anemone stress challenge. For easier comprehension, copy your observations from last week and your results from this week into a new table in this week’s entry. What did you observe? Are the bleaching results what you expected? Why or why not? How does the tolerance of your genet compare to other genets in the class?

For long protocols like today’s DNA extraction, it is not necessary to copy the entire protocol into your notebook (although you may, if you would like it for your own records). Instead, it is sufficient to write “We performed a DNA extraction as directed by the protocol in the lab manual,” or something along those lines. It is important, however, to write down a clear statement of purpose for any protocols performed in lab (“We performed a DNA extraction *because…*”) and any deviations from the protocol: if you made any errors or received alternate instructions from your TA, that should be noted. Often small errors in procedure can be rectified by other modifications to the protocol, and if you do make a mistake you should seek your TA’s advice before proceeding.

**Week 5**

**Background: PCR**

Polymerase chain reaction (or PCR) is a method used to amplify specific sequences of DNA found in low abundance in genomic DNA extractions to a workable quantity. Consider the extractions we did last week: we extracted whole genomic DNA from our anemones and the algae that live inside them. Your final samples should contain DNA representing the entire genome of both of these organisms, but those samples will not contain a particularly large amount of *any specific* region of the genome. If you’re interested in investigating the role of a specific gene or region of the genome in some process (in our case, coral bleaching) then you must first amplify that region of DNA so you have enough to study with downstream applications.

 PCR achieves this goal by approximately doubling the absolute quantity of a specific region of genomic DNA during a three-step cycle. This cycle is then repeated, often 30-35 times, resulting in a DNA sample in which the region of DNA you are interested in is greatly enriched. This is achieved using a reaction mixture containing machinery (an enzyme, primers) that accomplish this goal, as well as the building blocks of DNA, which are necessary for the synthesis of these new DNA strands. Firstly, your reaction mixture must contain a **polymerase**, most commonly Taq polymerase (“Taq” meaning it comes from the thermophilic prokaryote *Thermophilus aquaticus*). Additionally, it must contain single-stranded DNA **primers** which determine the region of DNA that you amplifying and are required for polymerase activity. Your reaction of course must contain DNA (here, from your extractions last week) to serve as a substrate for the polymerase reaction, and dinucleotide triphosphates or **dNTPs**, the building blocks of DNA.

 The three steps of the PCR cycle are **denaturation**, **annealing** and **elongation**. In denaturation, the reactions are heated to a temperature (usually >90oC) at which the hydrogen bonds holding opposite DNA strands will break, resulting in single-stranded DNA and allowing your primers to bind to their appropriate complements in your sample. During the annealing step, the primers will then bind to the newly single-stranded targets in your sample. This usually happens between 50oC and 60oC, although the exact temperature depends on the length of your primers and the ratio of As and Ts to Cs and Gs (which bind by triple rather than double hydrogen bonds and thus require more energy to break). Elongation is the step during which polymerase binds the DNA/primer complex and adds additional bases to the 3’ end of the primer, resulting in a new complementary strand attached to each of the single strands that were formed during the denaturation step, resulting in two complete double-stranded DNA molecules from the region of the genome that you specified for each one that existed before. The cycle is then repeated over and over to result in a potentially enormous quantity of your DNA of interest, or **amplicon**.

 Today in class we will be amplifying two different sorts of targets. From the anemone, we are amplifying 3 different population genetics markers, called **SCAR markers**. “SCAR” stands for **s**equence **c**haracterized **a**mplified **r**egion. Simply put, this means that these markers were randomly amplified by the researchers who developed them, sequenced (which we will discuss in two weeks) and the sequences of these regions were found to be sufficiently variable to characterize different populations of *Exaiptasia* from all around the world. The SCAR regions we are looking at in class today are also **ISSR** regions, or **i**nter-**s**imple **s**equence **r**epeats. A simple sequence repeat (or **SSR**) is a region of DNA containing a **v**ariable **n**umber of **t**andem base **r**epeats (thus it is also known as a **VNTR**). These occur throughout the genomes of organisms and differences in the number of these repeats can be used to infer population genetic information as well. ISSRs are simply the regions between these repeats. The researchers that developed the SCAR markers simply performed non-specific PCR off of SSRs, resulting in a large number of amplified ISSRs, some of which they sequenced and can now be called SCARs. The sequence of these SCAR markers will be used to infer relatedness of your anemone populations.

 To infer the origin of the algae in your samples, we will amplify the 23S ribosomal region. 23S sequences have been used to “barcode” organisms: simply, a method of identifying species using a short segment of variable DNA. Symbiont type, anemone type and symbiont/anemone interaction all make up components of how your genets will respond to the stress challenge, so it is important to get this information as well!

**Protocol: PCR**

The SCAR PCRs will be performed using the following primer pairs and otherwise identical mixtures: SCAR 3, SCAR 4 and SCAR 5. Your reactions for these samples should contain the following proportions:

1) 21ul SCAR master mix (premixed and aliquoted by your TA)

2) 3ul of the appropriate forward and reverse primer (again, premixed and aliquoted for you)

3) 1ul of genomic DNA from one of your DNA extraction samples to serve as a template

Because the SCAR markers are anemone sequences, it does not matter if the sample that you use for template comes from a bleached or a symbiotic anemone.

The 23S PCR mix is slightly different (note that different primers will often require very different reaction conditions based on their specific sequences!) and are as follows:

1. 23ul 23S master mix (**note:** this mix contains MgCl2 and different proportions of Taq mix and water and is **NOT** interchangeable with the SCAR master mix)
2. 1ul 23S F/R primer mix
3. 1ul genomic DNA (**note:**because the 23S template is an algal sequence, it **MUST** come from a sample that was obtained by a symbiotic anemone, as bleached anemones do not contain algae)

Clearly label your reaction tubes with your section and table number and so that you can tell which sample contains which reaction and give them to your TA, who will run the following cycle over the next few hours:

1) 94oC 1:30

2) 94 oC 1:00 (denaturation)

3) 55 oC 1:00 (annealing)

4) 72 oC 1:00 (elongation)

5) Go to 2 (34x)

6) 72 oC 5:00

7) 4 oC forever

**For your lab notebooks**

State clearly the purpose of performing this PCR today. Where does it fit in the overall context of the anemone stress challenge experiment? Record all of your reaction mixes, the reaction program, which samples you used for which reactions, and any deviations from the protocol as stated.