Note to scientists in training:

As a general 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.

PCR clean-up protocol with ExoSAP-IT™

TA will complete Step 1.

1. Make a 1:10 dilution of ExoSAP-IT™ reagent. (2 µl of the 1:10 working solution is required for each reaction.) For reactions sufficient for 24 students + 6 extra reactions:
    a. Pipette 54 µl of molecular biology grade water into a microcentrifuge tube. Label this tube **ExoSAP 1/10**.
    b. Remove stock of ExoSAP-IT™ reagent from –20 °C freezer and place it on ice. Do not defrost the ExoSAP-IT™ stock – it is in a glycerol solution and does not freeze at -20 °C.
    c. Briefly spin down the ExoSAP-IT™ stock in a microfuge (~3 sec) and return it to ice.
    d. Pipette 6 µl ExoSAP-IT™ stock into the tube you labelled **ExoSAP 1/10**. Close tube, briefly flick to mix, and briefly spin down in microfuge.
        i. Immediately return the ExoSAP-IT™ stock (NOT your dilution) to the -20 °C freezer.

ii. Keep your dilution (the tube labelled **ExoSAP 1/10)** on ice. It cannot be frozen. This dilution can only be used today; any leftovers should be discarded.

2. Students will collect their PCR products from the TA.

3. Each student must label a new PCR tube. They need to be able to uniquely identify their tubes.

4. Briefly spin down PCR products to collect at bottom of tube. Transfer 5 µl of the PCR product into the labeled PCR tube.

> *If your electrophoresis experiment failed or you have no more PCR products due to pipetting mistakes, ask your group member who had a successful experiment to give you 5 µl of his/her PCR product.* ***Note that you will have to use an appropriate primer later on.***

5. Pipette 2 µl from **ExoSAP 1/10** into the labelled PCR tube containing 5 µl of your PCR product.

6. Close the PCR tube, flick to mix, and spin down in a balanced microfuge (Guided by the TA).

7. Using the thermal cycler, incubate at 37 °C for 30 min to degrade remaining primers and nucleotides, and then for at 80 °C for 15 minutes to inactivate ExoSAP-IT™ reagent. This should be automatically accomplished using a program called EXOSAP; **check the program before running it.**

7. Keep on ice while you prepare for the next step.

Cycle sequencing with BigDye Terminator Kit

Notes before beginning:

Before setting up your cycle sequencing section, you need to dilute the primers you're using for sequencing. First, be sure that you're using the appropriate primer for your PCR product (OR your borrowed PCR product). For instance, if the PCR product you are sequencing is SCAR7, you should be using the SCAR7 FORWARD primer. (If you're borrowing your labmate's SCAR7 PCR product, you should be using the SCAR7 REVERSE primer.) **Attention to detail is critical** - note that if you have a SCAR**5** PCR product but use a SCAR**7** primer for sequencing, your cycle sequencing reaction will fail.

We want each sequencing reaction to contain 0.935 picomoles (pmol) of one (and only one) primer. A typical concentration used for primer working stocks is 10 uM; you are being provided with primers of 10 uM. This is equivalent to 10 pmol/µl. This presents a problem: if you were to use this directly, that's far too small of a volume to accurately pipette. The solution to this is to dilute your 10 uM stock to 0.4675 uM. You can then add 2 µl of this 0.4675 uM dilution to your reaction; this equates to 0.935 pmol, and 2 µl is a volume that you can pipette with reasonable accuracy.

1. In order to make this dilution, first pipette 95.33 µl of molecular biology grade water into a microcentrifuge tube.
2. Then acquire your appropriate primer stock for the PCR product you are sequencing.
3. Label the tube to which you have added 95.33 µl of water with the name of your primer (e.g. 23S-FORWARD) and "0.4675 uM." Add 4.67 µl of the 10 uM stock primer to tube you just labelled. Close tube and flick to mix. Spin down tube briefly to collect contents at bottom.

Setting Up the Cycle Sequencing Reaction Mix

- Uniquely label a PCR tube.
    - It need not appear on the tube, but each student must capture the following information in **their lab notebook**:
        - Unique genet ID (e.g. ARB-42)

- Amplicon (e.g. 23S, SCAR3, SCAR5, or SCAR7)
- Primer (JUST THE ONE!) used for sequencing (e.g. 23S FORWARD, SCAR5 REVERSE, SCAR5 FORWARD, etc.)

  Students: this must be recorded in your lab notebooks in order for any data to be useful. You are responsible for this information and will be asked for it in coming weeks.

- Add the following to the labelled PCR tube:

  3 µl water (molecular biology grade)

  2 µl 5x buffer

  2 µl ExoSAP-IT treated PCR product

  2 µl primer (0.4675 uM)

  Double check that you're using the appropriate primer for the PCR product you are using!

  1 µl BigDye™ Terminator

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  10 µl total volume for reaction.

  (If you were using more or less DNA template, you would need to adjust the amount of water used to maintain the same reaction volume, but everyone will be using identical volumes for this lab.)

  ***For cycle sequencing, you use a SINGLE primer, unlike a normal PCR. (Whereas amplification of normal PCR products is exponential, the accumulation of cycle sequencing products is linear.)**

- Close PCR tubes, flick to mix, briefly spin down, and keep on ice until the thermal cycler is ready to cycle your reaction.

Cycle Sequencing Program

- **For 35 cycles:**
    - 95˚C for 15 sec
    - 50˚C for 10 sec
    - 60˚C for 4 min
- **Then:** 4˚C Hold.
- **Note:** This program requires approximately 3 hours to complete.
- Store products at 4˚C (in the **FRIDGE**) until ready for capillary analysis in the DNA Core.

Symbiodinium Forward Genetics

Week 1: Mutagenesis

We will be mutagenizing *Symbiodinium 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. First, the UV source is situated to largely direct light onto samples. Any stray UV light is blocked by the glass window of the UV sash.

Each table/group is assigned a UV exposure time, i.e. 0 min, 2 min; 4 min; 8 min; 16 min; or 32 min.

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 #
    c. Experimenter name
    d. Duration of exposure to UV
    e. e.g. T6, S07, Josh, 32 min
2. Locate the T75 culture flask containing a dense culture of SSBO1 on your table and swirl to homogenously suspend cells. Density is approximately 5 x 10^5 cells / ml.
3. Each student must transfer 1 ml of SSBO1 culture suspension into the well plate. (If culture has settled, swirl before pipetting.) Gently swirl plate to distribute culture across bottom of well.
4. UV irradiation is to be performed as follows. **READ THIS COMPLETELY BEFORE PROCEEDING.**

a. Ensure that the UV source in the biosafety cabinet is OFF. Open sash.
b. Remove the cover from your 6-well plate. (The plate cover would block UV light.)
c. Carefully move your uncovered 6-well plate into position in the UV exposure station (254 nm UV source), located in the biosafety cabinet.
d. Close the sash.
e. Activate UV source by pressing the appropriate button on the biosafety cabinet. Set a timer on your phone for the appropriate amount of time.
    i. With the sash in place, look through the glass to confirm that blue light is being emitted by the UV source. **(ONLY DO THIS WITH THE SASH DOWN.)**
f. When time is up, turn off the UV source by pressing the appropriate button on the biosafety cabinet.
g. You can now open the sash and remove your sample. **(DO NOT OPERATE THE UV SOURCE WITH THE SASH UP.)**
h. Note that two plates can be placed in the UV irradiation source at one time. Since the treatment time is cumulative, this can be used to increase efficiency. We suggest the following sequence – be sure that you understand the objective (i.e. achieving a total exposure equal to the designated treatment) before beginning.
    i. Students exposing their **32 min** and **16 min** plates should perform begin their irradiation experiment first – both **16 min** and **32 min** plates should first be exposed for <u>16 minutes</u>.

ii. After 16 minutes of exposure time has elapsed, the students performing the **16 min** treatment remove their plate from the UV source. Students exposing their plate for **8 min** should now expose their plates for 8 minutes alongside the **32 min** plate.

iii. After 8 minutes of exposure time has elapsed, the students performing the **8 min** treatment remove their plate from the UV source. Students exposing their plate for **4 min** should now expose their plates for 4 minutes alongside the **32 min** plate.

iv. After 4 minutes of exposure time has elapsed, the students performing the **4 min** treatment remove their plate from the UV source. Students exposing their plate for **2 min** should now expose their plates for 2 minutes alongside the **32 min** plate.

v. After 2 minutes of exposure time has elapsed, the students performing the **2 min** treatment remove their plate from the UV source.

vi. If the above schedule has been adhered to, the **32 min** plate now has a cumulative exposure of 30 minutes (16+8+4+2=30). At this point, expose the **32 min** plate to an additional 2 minutes of exposure, for a final sum of 32 minutes.

5. As soon as a sample's exposure is complete, add 8 ml of the provided recovery medium. For the **32 min** exposure, add recovery medium only once the cumulative exposure of 32 minutes has been reached. (Recovery medium for this particular screen is Daigo's IMK medium supplemented with 1 g/l uracil.)

6. Incubate at 25 °C under a 12:12 light cycle, with 10-50 µE m^{-2} s^{-1} PAR for 7 days.

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

Before you leave the room, check that it is OFF, both on the switch in the biosafety cabinet and visually check that no light is being emitted. Otherwise, the next TA may not have a UV source to use.