SOP: General Sterilization Methods

When collecting tissue samples for DNA analysis, take steps to prevent cross-contamination due to DNA carrying over from one sample to the next, either by the utensils, your gloves, or the working surfaces (dishes, trays, etc.).

This is particularly important for molecular analyses examining symbionts, as the DNA of the symbionts is already in low quantity (compared to the DNA of the host) and the molecular assays are optimized to detect very low quantities of DNA.

There are two main methods to destroy undesired DNA from dissecting utensils:

(1) Bleach Wash:

a. Wipe any excess tissues off the utensil with a clean paper towel
b. Dip utensil in a diluted bleach solution (10-15%) for 5-10 seconds. Remove and shake off excess liquid.
c. Rinse under running freshwater or swirl in a water bath to remove residual bleach. Can use tap water, D.I. water is preferred if available.
d. With fresh paper towels, dry utensil as much as possible before proceeding. Once dried, no bleach remains, so ensuring the utensils are dry is one way to ensure that no bleach gets into your sample.
e. Once sterilized, do not place utensils onto a non-sterile surface or they will need to be re-sterilized.
f. Change out water bath every 30-50 samples.

NOTE: Bleach destroys DNA. Therefore, when using this sterilization method you want to take steps to prevent any bleach from coming into contact with, and potentially destroying, your samples.

Bleach can react negatively with other chemicals, including reagents used in DNA extraction (e.g., guanidine hydrochloride creating noxious chloride gas). Take precautions when using bleach in workspaces. Refer to SDS documents and read safety precautions carefully.

The water rinse step is meant to help remove residual bleach. If you are processing a large volume of samples, the water rinse in the second beaker will begin to accumulate bleach and essentially become a second diluted bleach rinse. Therefore, you will want to change/refresh the water in the second beaker at least 1x when processing a large number of samples (e.g., every 50 samples).

If there are a number of utensils, they can soak in the bleach solution and then all be rinsed and dried simultaneously. Do not let utensils sit in water for an extended time otherwise they risk damage from rusting.

2) Flame:

a. Only use dissecting instruments that are metal and can be exposed to high heat and flame. Plastic dissecting instruments will melt.

b. Wipe any excess tissues off the utensil with a clean paper towel.
c. Dip the working end of the utensil into a beaker of 95% ethanol
d. Ignite the working end and let the ethanol burn off (helps to place utensil in a Pyrex dish while the ethanol burns off). Be mindful of your hand placement in relation to the ethanol-soaked area before ignition.
e. Wait until the utensils are cool (~15 seconds) before proceeding to the next sample
f. Once sterilized, do not place utensils onto a non-sterile surface or they will need to be re-sterilized.

Negative Control:

If concerned about potential DNA contaminants in the surrounding sampling environment, collect a negative control sample.

a. Place an empty tube (e.g., 1.5mL microcentrifuge tube; same tube being used for samples) on the countertop where you are processing samples for genetics with the lid open.
b. When you are finished collecting samples, close the lid to the tube.
c. Label as "Atmospheric Control" with the collection date (mm/dd/yyyy). It will be processed in the same manner as the other genetic samples.
d. Repeat for each sampling event (assuming sampling events are occurring on different days).

Safety & Hazard Avoidance

a. Wear gloves when working with bleach and work in a well-ventilated area.

b. For flame sterilization:
- **Only work in designated spaces for hot work with adequate ventilation. Verify the Hot Work Permit for the space is current before starting.**
  - **S:\MarineDiseaseEcology\Safety Documents\Hot Work Designated Area Permits**
- **Participants must be knowledgeable of the location of the nearest fire extinguisher, manual pull station, phone, and exits.**
- **Check that the hot work equipment (burner, lighter) is in good condition before starting.**
- **Make sure to remove all combustible materials (when possible) before starting.**
- **Know the location of the closest fire extinguisher and exit.**
- **Ethanol is a flammable liquid used to sterilize metal utensils. As a precaution, use an extra Pyrex dish to cover the beaker of ethanol if it is accidently ignited by heated utensils.**