

Inoculation Materials:

- Individually wrapped 1mL sterile transfer pipettes
- 5mL (or larger) sterile glass sample vials
- Enough 3M Petrifilm plates for all water samples
- 3M Petrifilm spreader
- A sharp-tipped permanent marker

Inoculating and incubating Petrifilms is easy, but a little practice helps. Please read over these instructions before you begin. You may want to practice an inoculation and your sample handling process in a “dry run” before you use up your Petrifilms.

1. Obtain sterile pipettes with volume measurements on the sides as well as small (~5mL), sterile sample vials for samples.
2. Use sterile pipettes to take samples from different locations and store in the containers. Ideally, you should collect the samples and perform the inoculation and begin incubation within one hour, or as soon as possible, but not more than 24 hours. Store the samples in a room temperature environment away from sunlight.
3. You may find it useful to label each vial/jar with a Sharpie. Carefully record the date, time, and place of sample collection and name of the person sampling to associate with the mark on the vial. Because that is too much to write on a vial or a Petrifilm, you will want to associate each sample with a short, abbreviated codename like “A1” or “TUR-3”.
4. When you are ready for inoculation, place the 3M petrifilm plate on a level surface. Use a permanent marker to label the corner of the petrifilm with the codename of the sample that it will be used for.
5. Shake the sample gently to make sure the bacteria are not at the top or bottom of the sample. With a sterile pipette, draw 1mL of samples from the chosen sample vial.
6. Lift the clear top film and with the pipette perpendicular to the inoculation area slowly dispense 1mL of samples onto the center of the bottom film.
7. Roll the top film down onto the sample to prevent trapping air bubbles.
8. Place the 3M Petrifilm spreader with the flat side down on the center of the 3M Petrifilm plate. Press gently on the center of the 3M Petrifilm spreader to distribute the sample evenly. Do not slide the 3M Petrifilm Spreader across the film.
9. Remove the 3M Petrifilm spreader and leave the 3M Petrifilm plate undisturbed for at least one minute to permit the gel to form.

10. Your samples are ready to be placed in the Armadillo for incubation. Be sure to handle the Petrifilms carefully when transferring to the Armadillo.
11. You are allowed to stack as many 20 petrifilms together, but not more than that. The Armadillo allows you to incubate 40 petrifilms with a small cardboard stand for a second stack of 20.
12. When the 48 hours is up, IMMEDIATELY take the petrifilms out and photograph them clearly, including the sample label in the photograph. Petrifilms change their appearance slowly after incubation, so it is important to take photos immediately.
13. Once you safely have photos, we recommend you dispose of the Petrifilms as they are a slight biohazard.