

Instructor's Notes – Preparing and Pouring Media Plates

The following are the lists of equipment, supplies and preparation instructions for pouring the media plates that your students will need in the initial plating of bean beetle microbiomes. Pouring your own nutrient agar, eosin methylene blue, and phenylethyl alcohol agar plates is easy and cost effective. The quantities listed are those needed for a class of 24 with students working in 6 groups of 4 students. We are assuming that each group will evaluate the microbial community from two beetles - one from each of two experimental treatments. The quantities per group would need to be increased if the number of treatments were greater than two.

Equipment

- Autoclave
- Water bath
- Glass flasks (500mL or 1L)
- Autoclave gloves
- Electronic balance
- spatula

Supplies

- Aluminum foil to cover flask mouth
- Weighing dishes
- Sterile 100mm plastic petri dishes (80 plates assuming that a minimum of 20 plates are poured at a time)
- Disposable gloves
- Nutrient agar (NA) – (Difco, #211665)
- Eosin Methylene Blue (EMB) – selective for gram-negative bacteria – (Fluka, #62087)
- Phenylethyl alcohol agar (PEA) – selective for gram-positive bacteria – (Criterion, #C6611)
- Stab agar
 - nutrient broth
 - NaCl
 - agar
 - cysteineCl
 - thymine
 - 2mL vials with Teflon cap and bar code label

Preparation of Media

Although a single class of 24 will use only 36 plates of nutrient agar and 12 plates each EMB and PEA, it is best to prepare extra plates as insurance against contamination that may occur. Plates that are not needed can easily be held in a sealed bag in the refrigerator.

The general rule of thumb for pouring plates is a yield of 20 plates from 250mL of media and 75 plates from 1L of media. When you pour plates you only just need to cover the bottom, so pour enough to cover one-half to two-thirds of the plate and then gently swirl the plate to get full coverage.

Prepare media by using a flask that is double the volume of the amount of media you plan to make, if you plan to make 250mL of media, use a 500mL flask. You will be autoclaving your media, so nothing needs to be sterile at this point. Let's assume that you are making a 250mL volume of media. Measure 250mL of distilled or deionized water into a 500mL flask. Then weigh the appropriate mass of powdered media (see below), add to the flask containing the water. Swirl the flask to get everything mixed. Cover the flask mouth with aluminum foil and autoclave on liquid cycle.

Powdered Media Needed per Volume of Liquid Media

Nutrient agar (NA) – (Difco, #211665): 23g/L, 5.75g/250mL

Eosin Methylene Blue (EMB)– (Fluka, #62087): 36g/L, 9g/250mL

Phenylethyl alcohol agar (PEA) – (Criterion, #C6611): 36g/L, 9g/250mL

When the autoclave cycle is completed, carefully move the hot flask of media to the bench where you plan to pour plates or to a water bath set at 55°C. Gently swirl the flask to ensure the media is fully mixed. The hot media cannot be pouring into plates until it cools enough for you to hold the flask comfortably (without an insulated hot glove). The water bath would permit the flask to cool but still keep the media liquid hot. Arrange the empty sterile plastic plates, lid up, on a work bench where you can easily pull them to the bench edge. Placing an absorbent bench pad on both the bench edge and the floor where you will be pouring is helpful for catching spills. Once the flask cools enough to handle, pour media in each plate, just opening the lid enough to pour into it. With the lid on the plate, swirl to spread the hot media, then move the plate aside to solidify. With a little practice, you should be able to pour stacks of 5 plates at a time, pouring into each plate, then swirling the stack. Wearing a disposable glove on the hand holding the flask will permit you to start pouring cooled but still hot media with minimal discomfort.

Once the media has solidified, turn the plates upside down to prevent condensation from dripping back onto the media. Plates may be re-bagged in the plastic sleeves that the plates came in and stored upside down in the refrigerator, but they should be fine at room temperature for a week without being re-bagged. Plates stored in the refrigerator should be brought to room temperature before use in class.

Plates needed for a class of 24 working in 6 groups of 4 students in each group:

Each group needs:

NA: 6 plates, 2 at full concentration of microbiome, 2 at 1/10 dilution, and 2 negative controls

EMB: 2 plate at full concentration

PEA: 2 plate at full concentration

Total needed for class:

NA: 36 plates

EMB: 12 plates

PEA: 12 plates

Colony Archiving Vials for Stab Cultures

We will provide 2mL vials (with bar code labels) containing sterile stab agar (see below) that are ready for each student to inoculate with the same bacteria that they picked for PCR and Sanger Sequencing. The quantity of stab culture vials needed is 24 for a class of 24.

These 2mL vials for stab cultures were prepared by making stab agar and filling sterile vials 2/3 full.

Stab agar

- 10 g nutrient broth
- 5 g NaCl
- 6 g agar
- 10 mg cysteine Cl
- 10 mg thymine
- H₂O to 1 L

Autoclave and then pour into sterile 2mL vials 2/3 full and cap with sterile Teflon caps.