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Soil Plating Protocol

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ABSTRACT

Protocol for serial dilution of soil samples and plating.

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Protocol status: Working
We use this protocol and it's working

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Sample Preparation

1 Collect Soil Sample

1.1 Collect a small amount of soil into a Ziploc bag. Your soil sample will contain some bits of roots, leaves, and rocks, but try to get a sample that is mostly soil

2 Weigh 1 g of PBS

2.1 PBS PBS PBS Take your PBS sample to the balance

2.2 Place an empty weigh boat or piece of weighing paper on the plate of the balance

2.3 Press the "zero" button on the balance (this subtracts the weight of the weigh boat/weighing paper so that the balance now reads 0 g . This means the mass you see when you add your soil is just the mass of your soil, not your soil plus the weigh boat/weighing paper)

2.4 Slowly transfer a bit of your soil onto the weigh boat/weighing paper until you reach 1 g (if you transfer too much, you can pour some back into your sample bag)

3 Dilute PBS in PBS

3.1 Transfer the 1 g of soil you weighed into the empty 50 ml conical tube you are given

3.2 Using a serological pipet, add PBS to the conical tube containing your 1 g of soil until you reach the line labeled 10 mL
- pipet slowly - you do not want to accidentally go past the 10 ml line
- you are aiming for the bottom of the meniscus (the downward curve made by the liquid in the tube) to touch the 10ml line

3.3 Label this tube with your initials and the number 1

Serial Dilution

4 Label your tubes

4.1 You will be given five additional, smaller tubes, each containing 9 mL of PBS. Label these tubes with your initials and the numbers 2-6

5 Mix your soil and PBS mixture

5.1 Use the vortexer to mix your soil and PBS well (the soil will not completely dissolve, but try to get it as well dispersed as you can

6 Create your serial dilution

6.1 Once your mixture in tube 1 is well dispersed, transfer 1 mL of this mixture into tube 2

6.2 Cap tube 2, and use the vortexer to mix it

6.3 Once the mixture is tube 2 is well dispersed, transfer 1 mL of this mixture into tube 3

6.4 Cape tube 3, and use the vortexer to mix it

6.5 Once the mixture in tube 3 is well dispersed, transfer 1 mL of this mixture into tube 4

6.6 Cap tube 4, and use the vortexer to mix it

6.7 Once the mixture in tube 4 is well dispersed, transfer 1 mL of this mixture into tube 5

6.8 Cape tube 5, and use the vortexer to mix it

6.9 Once the mixture in tube 5 is well dispersed, transfer 1 mL of this mixture into tube 6

Plating your serial dilutions

7 Label your plates

7.1 You will be given three types of plates: R2A, PDA, and TSA. Each of these plates contains

agar, along with nutrients for the microbes. The three different types of plates contain different nutrients, and this means that different types of microbes might grow on each type of plate

7.2 With your plates closed, turn them over so that the bottom (the side containing the agar) is facing up

7.3 Label each plate with your initials, the type of plate, and if you are given more than one of any of the types of plates, a number (for example, if you have two TSA plates, label them TSA 1 and TSA 2)
- It is a good idea to write small and label your plate near the edge so that your writing does not make it difficult to see the colonies later

8 Plate your microbes

8.1 Turn your first plate back right-side up, with the side containing the agar on the bottom

8.2 You will be plating dilution tubes 3-6.
- You will plate 4 different plates for each agar type, with each plate being a different dilution.

8.3 Check the dilution tube you will use first to make sure the soil is still dispersed. It is a good idea to give it another vortex

8.4 Remove the lid from your plate, and quickly pipet 100 µL of your dilution onto the plate using the p200 pipet. Then place the lid back on the plate, and cap your dilution tube
- Be sure not to leave the plate open any longer than absolutely necessary to avoid contamination
- When pipetting, hold the pipet so the tip is just above the surface of the agar, but not touching or poking the agar

8.5 Open the plate again, and use a disposable spreader to gently spread the liquid around the plate

- Do not press down, just lightly glide the spreader back and forth across the agar. You do not wat to break the agar
- Try to reach all areas of the plate - you want to spread the liquid out as much as possible
- Continue spreading until you no longer see liquid on the surface of the agar

8.6 Close the plate

8.7 Repeat these steps for each plate

9 Seal and incubate your plates

9.1 Carefully stretch Parafilm around the edges of each plate so that it is sealed shut

9.2 Turn the plates upside down - with the side containing the agar facing up - and stack them together

9.3 Place your plates in the 30 °C incubator

- The plates need to grow at this temperature for several days. When they are finished growing, we will send you photos, and we will store the plates in the refrigerator so that you can do more experiments with them later.