Microbial Ecology

Released on: Saturday, Jun 3rd 1995. Copyright Bill Watterson and Universal Press Syndicate.

EcoPlates are multiwell test plates that allow rapid determination of the metabolic capabilities of a bacterial population without tedious and time-consuming reagent preparation. After inoculation with a suspension of bacteria washed from a soil or plant sample, the incubated plate returns a unique set of positive (purple) and negative (clear) reactions that allows you to assess whether two samples contain similar or dissimilar bacterial populations. *

Community-Level Physiological Profiling (CLPP)[1]

Each species of bacteria has a specific set of carbon compounds that can and can not be used for energy. In fact, this set of compounds is usually unique at the species level with a limited amount of variability (some mutants of particular species will lose the ability to use a carbon source, but rarely will a mutant gain the ability to use a brand new carbon source that is not used by other wild-type members of that species). Likewise, each community of bacteria (all the bacterial species that live in one particular area) also have a specific set of other carbon sources that can and can not be used for energy. Describing that pattern of carbon-usage is called **community-level physiological profiling (CLPP)**.

Learning to Score An EcoPlate

Figure 1 shows the layout of carbon substrates on our EcoPlates. Each substrate (carbon source) has been given a unique number. Referring to Table 2, you can see which substrate (carbon source) is found in each of the wells. For example, substrate #10 is i-Erythritol. Like all the substrates here, i-Erythritol is found three times on the EcoPlate. It is found in the following wells: C2, C6, and C10.

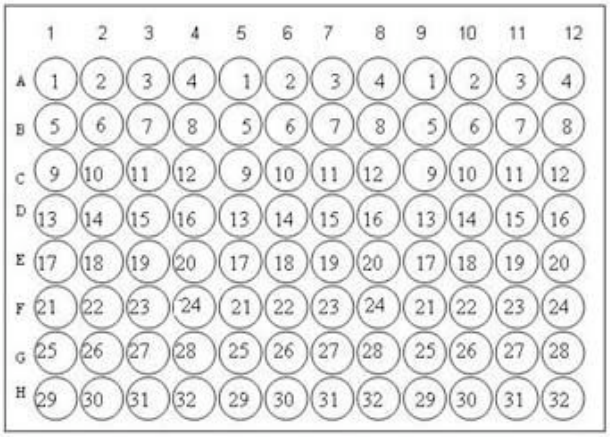


Figure 1. EcoPlate. A single substrate and a dye is present in each well. The substrate is a unique carbon source that can be used as energy by the bacteria. The numbers inside the circles each correspond to a particular substrate. Please refer to Table 2 to determine what substrate corresponds to what number.

[1] H. Edenborn. National Energy Technology Laboratory. U.S. Department of Energy, Pittsburgh, PA 15236

Before you begin

Before you begin, please practice scoring the EcoPlates. Turn in the completed worksheet.

There are three main calculations that you should be able to do *and* to understand:

% Functional Diversity = 100 * number of positive (purple/pink) carbon source wells / Total number of carbon source wells (31)

This value varies from 0 to 100% with 0 being low diversity and 100% being highly diverse.

You will need to report the inconsistency found within each sample.

% Variation of Results within Sample = 100* i/31

i = the number of carbon sources in which the three replicates were not ALL positive or ALL negative. On Table 2, i = sum of everywhere there is a 1 or 2 (but not 3 or 0)

% Similarity (S_{SM}) = 100* $\frac{a + d}{a + b + c + d}$

*Please note that water is not a carbon source.

a = Number of carbon sources used by both sample A and sample B

b = Number of carbon sources used by Sample B but not by Sample A

c = Number of carbon sources used by Sample A but not by Sample B

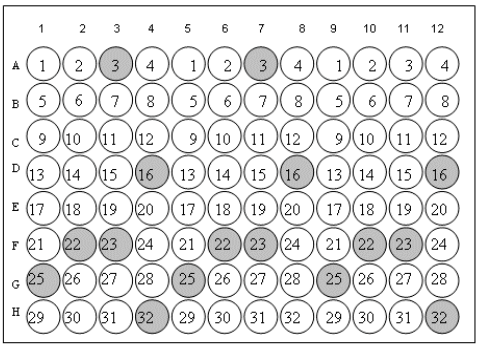
d = Number of carbon sources not used by bacteria in either sample

If the two samples give identical "fingerprints" on the EcoPlates, S_{sm} will be 100. If the two samples give exactly opposite "fingerprints", the value will be 0.

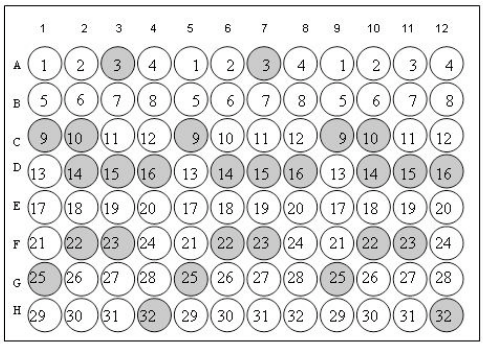
Please score EcoPlates 1, 2, & 3 by filling in table 1 on page 6.

The shaded wells are the ones that turned pink or purple.

Sample EcoPlate #1



Sample EcoPlate #2



Sample EcoPlate #3

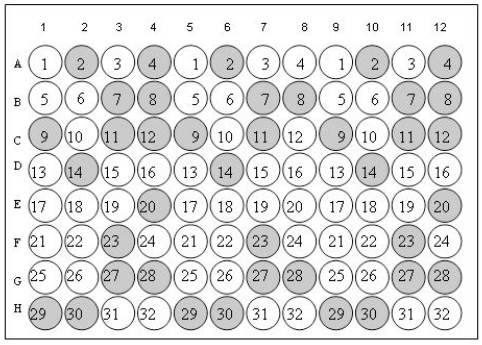


Table 1. Sample Plate #1 is already scored for you as an example. You need to score sample plates 2 and 3. Place a zero in the cell table if none of the three replicates turned pink or purple. Place a 1 in the table if ONE of the three replicates turned pink or purple. Place a 2 in the table if TWO of the three replicates turn pink or purple, and place a 3 in if all three replicates are positive (pink or purple).

Substrate Name	Substrate #	Sample Plate #1	Sample Plate #2	Sample Plate #3
Water	1	0		
β-Methyl-D-Glucoside	2	0		
D-Galactonic Acid γ-Lactone	3	2		
L-Arginine	4	0		
Pyruvic Acid Methyl Ester	5	0		
D-Xylose	6	0		
D-Galacturonic Acid	7	0		
L-Asparagine	8	0		
Tween 40	9	0		
i-Erythritol	10	0		
2-Hydroxy Benzoic Acid	11	0		
L-Phenylalanine	12	0		
Tween 80	13	0		
D-Mannitol	14	0		
4-Hydroxy Benzoic Acid	15	0		
L-Serine	16	3		
α-Cyclodextrin	17	0		
N-Acetyl-D-Glucosamine	18	0		
γ-Hydroxybutyric Acid	19	0		
L-Threonine	20	0		
Glycogen	21	0		
D-Glucosaminic Acid	22	3		
Itaconic Acid	23	3		
Glycyl-L Glutamic Acid	24	0		
D-Cellobiose	25	3		
Glucose-1-Phosphate	26	0		
α-Ketobutyric Acid	27	0		
Phenylethylamine	28	0		
α-D-Lactose	29	0		
D,L-α-Glycerol Phosphate	30	0		
D-Malic Acid	31	0		
Putrescine	32	2		
Total # of substrates used		**6**		
% Functional Diversity		**19.35%**		
% Variation of Results within Sample		**6.45%**		

Worksheet. To be completed before you begin any work.

1. How many *different* carbon substrates are there on the EcoPlate? _____
2. What is the name of the carbon substrate designated number 15? _____
3. Using the letter to designate the row and numbers to designate column, list the wells containing substrate #15. (For example, i-Erythritol is found in C2, C6, and C10.)_____
4. What is the control substrate in this plate? _____Should the control wells turn pink? _____
5. Using the letter to designate the row and numbers to designate column, list the wells containing the control. _____

Table 2. Please fill in the information in this table based on your calculations

Sample Plate	Functional Diversity	% Variation of Results
2		
3		

Table 3. Please fill in the information on this table based on your calculations.

	Percent Similarity		
	Sample Plate 1	**Sample Plate 2**	**Sample Plate 3**
Sample Plate 1			
Sample Plate 2			
Sample Plate 3			

6. Please use Table 3 to answer this question. These three plates came from the following sources:
   Bacterial wash from the unwashed left hand of Joe Smith
   Bacterial wash from the unwashed right hand of Joe Smith
   Bacterial wash from the unwashed left hand of Jane Doe.

   Based on Table 3, propose a hypothesis for which plate contains the bacterial community of Jane Doe's hand. Please explain your reasoning

Design your own Experiment

Spatial comparison: "Is the bacterial community found here the same as the one found there?"

How Inoculate EcoPlates

All work must be done aseptically.

1. Collect your sample. It is essential that you do not contaminate your sample with bacteria from your hands or other places. Use sterile collections test tubes with lids are provided, and if at all possible, you should collect your samples directly into these sterile tubes without any utensils. To avoid contamination by unwanted bacteria here are a few tips:

   Do not touch the soil, creek sediment, or plant that you plan to sample with your hands or fingers.
   Do not leave your sterile tubes uncapped and open for airborne bacteria to enter.
   Do not choose samples from soil or plants that you think someone has touched.
   Do not breathe or sneeze on your samples or into your tubes.
   Continue to use aseptic techniques when diluting your sample in step #2.

   If you will need to take your samples with forceps or other tools in order to get the sample into the sterile tubes, please talk to your instructor about a suitable disinfection procedure for your tools. Several grams per sample should be more than enough for the next step.

2. After you have taken your samples to the lab, you will need to dilute them in sterile phosphate buffered saline (PBS). Mix your sample in sterile PBS in a set dilution (wt/vol) so that you dislodge the bacteria into the solution (Figure 2). To get good results from your EcoPlate, you don't want too many or too few bacteria, just the right number. You will have to guess to a certain extent at your dilution, depending on what is being sampled. A good starting point ist to dilute the sample until it is barely turbid (barely cloudy). This is often a 10^{-3} *weight/weight* dilution for soil (1 g of soil/1000 g final solution (1 ml of PBS = 1 g). For plant matter, a dilution of 10^{-2} (1 g of plant matter/100 g final solution) is probably better.

   For each sample, put the sterile tube or the cap to the sterile tube on the scale and tare it (or use clean weigh boats or weigh paper will work as well). Then add sample to the tube (or to the cap) using a sterilized utensil so that you have less than 4 g of soil. Fill your tube with 9× as much PBS. Since each ml is approximately 1g, you have just made 1/10 or 10^{-1} dilution. Vortex this original dilution at high speed (or shake thoroughly) for 1 minute. Using 3 ml of the 10^{-1} dilution and 27 ml of PBS to create 30 ml of a 10^{-2} solution in a new sterile tube. To obtain the final dilution, take 3 ml of the 10^{-2} solution and add 27 ml of pure PBS. You will ultimately need 100 µl of solution per well and *at least* 10 ml of diluted sample per plate.

   If you find that your solution is too cloudy after mixing it, you will want to dilute the sample further with the buffer. Keep track of how diluted your final sample is and include this information in your laboratory report.

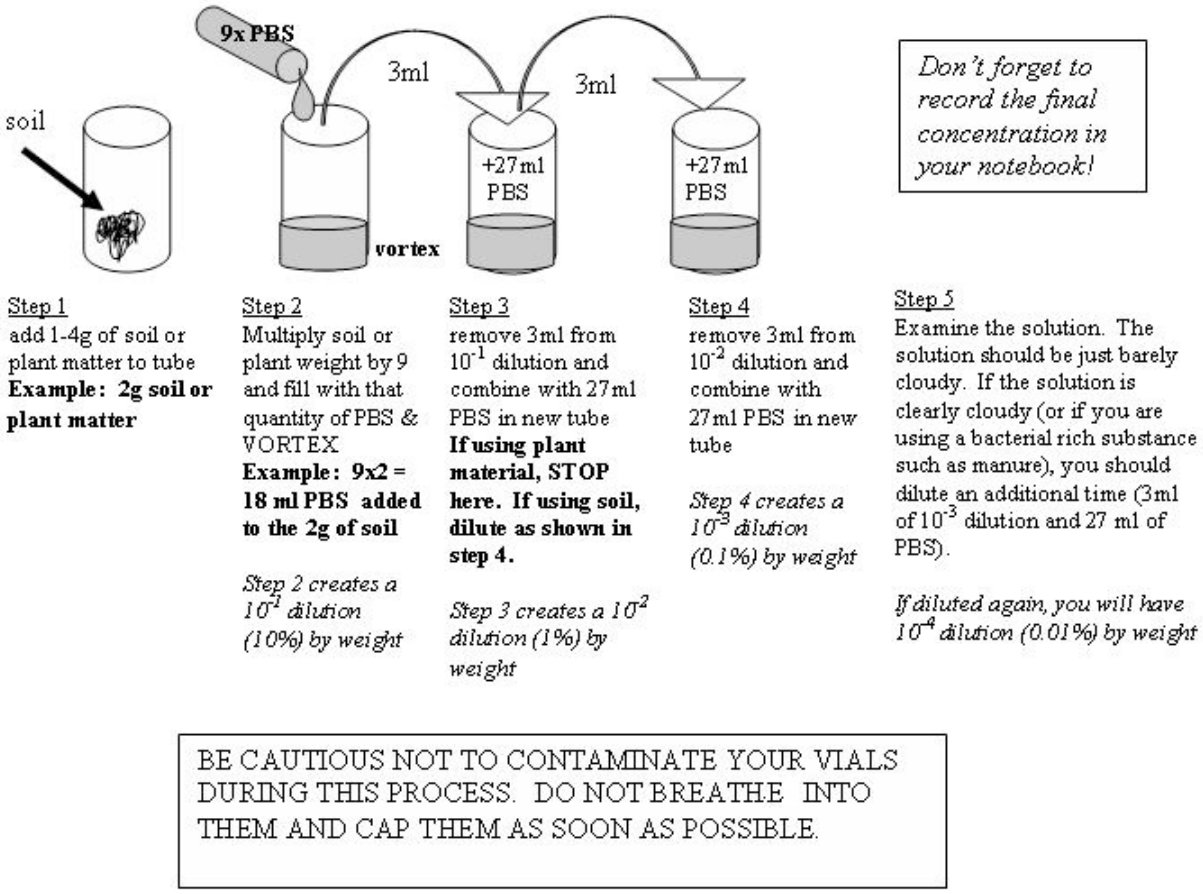


Figure 2. Summary of steps to create the appropriate concentration. The dilution series is created in order to achieve a final concentration of 1g of soil / 1000 g of final solution. The weight/weight dilution factors are commonly used in microbiology.

3. Aseptically inoculate the ecoplate by adding 100 µl of the diluted sample suspension to each well. *Keep the plates horizontal (flat), do not tip the plates*.

4. Place your plates in a plastic bag and incubate them at room temperature. *Keep the plates horizontal (flat), do not tip the plates*. A small piece of moistened paper towel inside the bag will help to maintain a constant high level of humidity and avoid evaporation from the wells. You will need to check your plate daily and fill out a data sheet showing which wells give a positive reaction (purple color) daily. It usually takes about 4 days for most of the positive wells to change color. *Keep the plates horizontal (flat), do not tip the plates*.

5. Data Collection. For experiments that are not examining a microbial community over time, you should inoculate all your EcoPlates in the same lab period and then examine them every day until the color begins to change. Record the "metabolic fingerprint" for each plate on the first day of color change and for three subsequent days. A sample data sheet is provided at the end of this handout that you can photocopy. You should mark down which wells were purple and which wells were not purple on the datasheet.

Data Summary Template