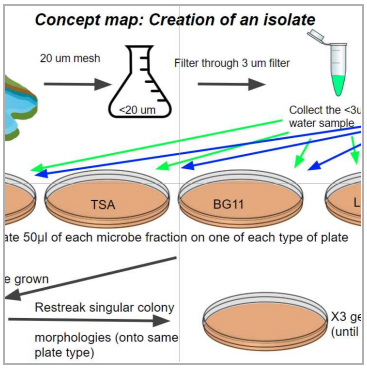

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We use this protocol and it's working

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Water Sampling and Plating Generations (Antimicrobial Group)

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ABSTRACT

Protocol developed by the Antimicrobial Group class project. The goal was to isolate microbes from freshwater lakes that produce antimicrobial compounds in order to study antibiotic production in nature and environmental reservoirs of antibiotic resistance.

Protocol for class project in **Microbes in the Wild: Advanced Environmental Microbiology Lab (EEB 447)** course at University of Michigan Biological Station and on campus.

1 Collect whole water at six sites using a Van Dorn water collector for deep (~122.5-123.7 m) samples and 2 L containers for surface samples (~15 cm below the surface).

2 Set site names and classifications

Lake	Location Type	Location Name	Latitude	Longitude	Depth
Lake Michigan	Pristine	Off Shore A) Shallow B) Deep	45.28287	-85.25048	A) 15cm B) 123.7M
Lake Michigan	Pristine	Near Shore A) Shallow B) Deep	45.21355	-85.16976	A) 15cm B) 122.5M
Lake Michigan	Disturbed	Clad 2	45.2778	-85.135415	15cm
Douglas Lake	Disturbed	Septic	45.555901	-84.665300	15cm

Table 1: Sampling site specifics. We took samples from both Lake Michigan and Douglas Lake (inland). We took note of whether the site was anthropologically disturbed or pristine, gave it a specific location title, noted latitude and longitude of the site, and noted the depth at which the sample was taken.

Sites samples and description

3. Prefilter whole water using 20µm mesh.
4. Set up peristaltic pump with 3 µm filter
5. Filter approximately 500mL of sample water.

6. Collect the filter in an eppendorf with 1mL of PBS as the particle-associated large fragment

3 Collect the <3µm filtered water as the free-living (FL) smaller fraction.

4 For the particle-associated (PA) fraction, vortex tube, then partially remove filter with an edge under the closed cap

5 Centrifuge at 1500 RPM for 1 minute

6 Fully remove filter and resuspend pellet

7 Pipette 10µL of both PA and FL onto separate plates of 4 agar types (TSA, LB, BG11, and LAKE) and spread.

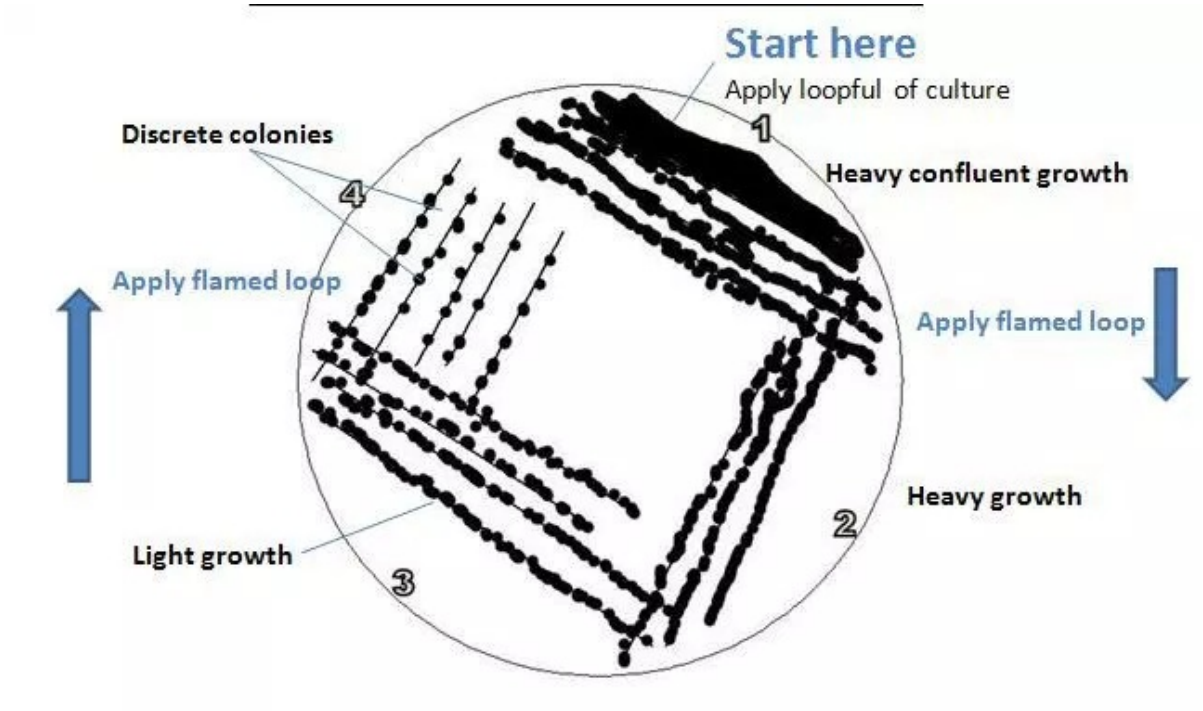
This is the parent generation.

8 PROPAGATE NEXT GENERATIONS:

8.1 From the lawns initially plated, by sterile loop pick up a single colony.

8.2 Select a plate of the same media.

8.3 Steak for isolation using a three phase streak pattern as shown below, sterilizing the loop in between each quadrant.



4. Label these plates "generation 1"
5. Continue until generation 3 is obtained.
6. Use generation 3 for testing.