**BACTERIAL PRODUCTION ASSAY**

Pre-protocol steps:

* Make working stock of C14-leucine (40 uM)
* Aliquot the amount of 100% TCA needed for each timepoint into separate tubes
* Make 5% TCA solution
* Make 80% ethanol
* All stocks/solutions should be diluted with MQ H2O

On the boat:

* Collect 10 mL of lake water in a 15 mL Falcon tube
* Fill incubation thermos with lake water
* Put Falcon tube in well-sealed plastic bag and place in thermos
* Start the protocol within 30 min of sampling

In the lab:

1. Add 1.5 mL of lake water to 6 microcentrifuge tubes. Label ONLY the lids.
2. Add 80 uL of 100% TCA to the two negative control tubes
3. Add 10 uL of C14-leucine working stock to all six tubes. Start with the experimental samples and end with the controls
4. Close lids tightly and invert all tubes 5 times to mix. Record the time on the metadata sheet.
5. Put all tubes in a plastic bag with as much air removed as possible
6. Place bag of tubes in thermos of lake water and incubate for 55 min.
7. After 55 min, remove the tubes from the thermos and arrange on bench. Exactly 5 min after removal from the thermos, begin the next step. Record the time on the metadata sheet.
8. Add 80 uL of 100% TCA to the four experimental tubes. Invert 5 times to mix.
9. Leave samples in the fridge for 1 hour.
10. After 1 hour, place samples at -20 degrees.

\* Repeat steps 1 – 10 for each sampling timepoint. Samples can be stored at -20 for a few days\*

1. Thaw samples on benchtop.
2. Centrifuge samples at max speed for 15 min. Label the lid of each tube with the orientation of the tube in the centrifuge, as you may not be able to see a pellet after centrifugation.
3. Pour supernatant into C14 liquid waste container
4. Tap each tube on a Kimwipe to remove any remaining liquid. The Kimwipe is now solid radioactive waste.
5. Add 1 mL of 5% cold TCA to each tube. Do not shake, resuspend, pipette on, or otherwise disturb the pellet (which you may not be able to see).
6. Centrifuge samples at max speed for 5 min in the same orientation as previously. Repeat steps 13 and 14.
7. Add 1 mL of cold 80% ethanol to each tube. Do not disturb the pellet.
8. Repeat step 16.
9. Get out a new tube and add 10 uL of the C14-leucine working stock. This is a positive control.
10. Add 1 mL of scintillation cocktail to each tube.
11. Vortex all samples for approx. 3 seconds.
12. Place tubes in scintillation vials and take to the liquid scintillation counter. Label the top of the vials with the name of the sample inside.
13. Count samples on the C14 protocol
14. Report the activity of each vial on the metadata sheet. Average the activity of the experimental samples and subtract the average activity of the two control samples for each timepoint. Report this value and the standard deviation of both the experimental samples and the control samples on the metadata sheet.

Supplies need (per timepoint):

* 6 microcentrifuge tubes (2 mL, screw cap)
* 15 mL Falcon tube
* Thermos
* 2 Ziploc bags
* 400 uL 100% TCA
* 60 uL of C14-leucine working stock (+ 10 uL for positive control for – 1 needed per lake)
* Centrifuge
* Kimwipes
* Vortexer
* 6 mL cold 5% TCA
* 6 mL cold 80% ethanol
* 6 mL scintillation cocktail
* 6 glass scintillation vials
* Liquid scintillation counter