ESTIMATING BACTERIAL PRODUCTION FROM ^{14}C-LEUCINE INCORPORATION INTO PROTEIN

In this lab, we will estimate bacterial production in your Winogradsky column by measuring the incorporation of "hot" ^{14}C leucine into bacterial protein. To get a rate, we measure the amount of ^{14}C incorporated at 0, 15 and 30 minutes. We halt the uptake by killing the bacteria with 100% trichloroacetic acid (TCA). TCA also precipitates proteins. We collect the bacteria by centrifugation and remove free unassociated ^{14}C leucine by rinsing with 5% TCA and then 80% ethanol. A scintillation cocktail will then be added that converts beta emission from ^{14}C into light pulses by transferring energy given off by ^{14}C disintegrations to fluor molecules that emit light at 320-420 nm (more on this later). These bursts of light will be counted by a scintillation counter as CPM and converted to DPM by the machine.

EVERYONE MUST WHERE A LAB COAT, COVERED SHOES, GLOVES and EYE PROTECTION!

1. Label four 2 mL microcentrifuge tube ***caps*** "W", "0", "1", "2" which stand for "Working solution", Blank (0 min), 15 min incubation and 30 min incubation. Also place a small mark on the tube itself as well, which will allow us to place the tube in the centrifuge in the same orientation each time. Don't write on the side of the centrifuge tube (Why?)
2. Collect more than 5 mL of water from the top port of your Winogradsky column and place in a snap cap tube. We actually only need 4.5 mL.
3. Add 20 μL of the ^{14}C-leucine working solution (which is 12 μM Leu) to the bottom all four tubes. Keep all materials that come into contact with ^{14}C on the tray at your lab bench.
4. Firmly cap and set the "W" sample aside, as we will not use it until the last step.
5. Add to the "0" tube ONLY, 0.1 mL of 100% cold TCA (trichloroacetic acid).
    a. This is the killed control.
    b. Leave on lab bench until incubation of other samples is completed.
6. To the "0", "1" and "2" tubes, add 1.5 mL of your Winogradsky water. Cap firmly, then shake sample. Be careful to NOT splash the ^{14}C-leucine out of the tube when you add your sample.
    a. This will give a final leucine concentration of 160 nM.
    b. Do not touch the pipette tip to the liquid as it contains ^{14}C and possibly TCA. If you do, replace the pipette tip.
    c. NOTE THE TIME OF ADDITION!!
7. **The incubation period now begins**. In this case, just leave your samples on your tray in the rack. Incubations are typically done under conditions relevant to the samples.
8. After 15 min, kill sample "1" by adding 0.1 mL of 100 % cold TCA. Cap and shake.
9. After 30 min, kill sample "2" by adding 0.1 mL of 100 % cold TCA. Cap and shake.
10. Place your three samples in the centrifuge so that the mark on the tubes faces radially outward. NOTE the location of your samples in the rotor. **Make sure rotor is balanced with samples!!! Add water "blanks" (1.6 mL) if necessary to balance.**
11. Centrifuge samples on high for 15 min.

12. Remove tubes, and gently pour sample into the ^{14}C liquid waste container. A cell "pellet" may, or may NOT, be visible on the outer wall of the centrifuge tube. Regardless of visibility, we do not want to dislodge this "pellet", so do not touch any pipette tip in the expected location.
13. Gently tap the lip of the centrifuge tube on a folded Kimwipe placed on your workspace tray to removal all drops. Kimwipes need to be treated as solid ^{14}C waste, so keep on your tray, but out of your working area. Also, avoid tapping tube on any drop collected on Kimwipe, as we do not want to contaminate the outside of the tube with ^{14}C.
14. Add 1.0 mL of cold 5% TCA to each tube, **but to not shake** and avoid touching pipette tip on centrifuge tube or dispensing TCA onto the invisible pellet.
15. Place tubes back in centrifuge, again with mark facing outward and noting location of your samples in the rotor.
16. Spin on high for 5 min.
17. Again, pour out liquid into ^{14}C sample waste, and gently tap tube on NEW Kimwipe to remove drops.
18. Add 1 mL of cold 80% ethanol to each tube; again, do not shake and avoid disturbing the pellet.
19. Re-spin samples in centrifuge as before for 5 min.
20. Again, pour out liquid into sample waste, and gently tap tube on NEW Kimwipe to remove drops.
21. Place tubes in rack on your bench.
22. Add 1.0 mL of scintillation cocktail to each tube, INCLUDING sample "W". HOWEVER, be very careful not to splash ^{14}C leucine out from sample "W", and do not touch pipette tip to centrifuge walls.
23. Recap all samples and vortex for a few seconds.
24. Place centrifuge tubes in scintillation vials for counting. Place a mark on the top of each cent vial with your initials along with "W", "0", "1" or "2" as appropriate. Do not write on the side of the scintillation vials (why?).
25. The TA will take samples over for counting on the scintillation counter, which we will discuss at our next meeting.