**General Extraction of M. maripaludis metabolites**

1. Harvest cells (~1 million) – 1 ml of OD660 0.6 cells (~1 billion)
2. (Cold) Pellet at 13,000 rpm for 1 min and discard the supernatant
3. Re-suspend the pellet in PBS (5-10 mL) for washing the cells and mix well – Instead of PBS, use modified cold \*H2 assay buffer (1 ml wash volume).
4. (Cold) Pellet at 13,000 rpm for 1min and discard the supernatant
5. Resuspend the pellet using 0.1 mL of wash buffer, then immediately add 1 mL 80:20 methanol:water (-75oC) on dry ice (~-75oC) to quench metabolism – cells will lyse here
6. Vortex to mix, 30 min incubation at -75oC
7. Spin the mixture at 5000 rpm for 10 min at 0-4oC
8. Remove the soluble extract into an Eppendorf vial (1.5 mL, locked) and place it on dry ice
9. Resuspend the pellet in 500 uL of 80:20 methanol:water (0-4oC) and vortex to mix
10. Sonicate cell suspension in an ice bath for a few seconds
11. Centrifuge at 5000 rpm for 10 min at 0-4oC and combine the resulting extract with 250 ul of the initial extract (the pellet can be measured for total protein). This will result in two Eppendorf vials for each sample, each with 750 uL of extract.
12. Completely dry samples using the Speedvac at 30 oC

\*Modifiled H2 assay buffer: 50 mM MOPS , 400 mM NaCl, 20 mM KCl, 20 mM Mg2Cl, 1 mM CaCl

Questions and notes:

We are interested in severely limiting the duration of spin and wash steps to minimize metabolic changes. Thus we have modified the procedure as shown above.

Since we only use minimal growth media (Salts, vitamins, trace minerals and 10 mM acetate), would a 1 ml wash be sufficient? We also modified the wash buffer as our marine cells lyse easily in buffer with lower osmotic pressure like PBS.

We typically correlate OD660 with cell dry weight and so should be able to normalize our calculations that way.

After drying with speed vac could we store it at -80 deg C before sending it to you? Or should we store it in desiccant at room temperature?