**General Extraction of M. maripaludis metabolites**

1. Harvest 1 mL of OD660 0.6 cells (~10 million cells)
2. (Cold) Pellet at 5,000 rpm for 1 min and discard the supernatant
3. Re-suspend the pellet in H2 assay buffer (1 ml wash volume) for washing the cells and mix well
4. (Cold) Pellet at 5,000 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. Combine these two samples into one of the tubes.
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

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.

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