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Supplemental Materials

for

Mass Spectrometry as a Tool to Enhance "-omics" Education

Michael J. Wolyniak[1]*, Nathan S. Reyna[2], Ruth Plymale[2], Welkin H. Pope[3], and Daniel E. Westholm[4]

[1]Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943, [2]Department of Biology, Ouachita Baptist University, Arkadelphia, AR 71998, [3]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, [4]Department of Biology, The College of St. Scholastica, Duluth, MN 55811

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*Corresponding author. Mailing address: Department of Biology, Hampden-Sydney College, Box 183, Hampden-Sydney, VA 23943. Phone: 434-223-6175. E-mail: mwolyniak@hsc.edu.
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Appendix 1: Experimental protocol for mass spectrometry of *M. smegmatis* cell pellet infected by mycobacteriophage

1) Inoculate several cultures of *M. smegmatis* (e.g. dilute 50/100/200/400/800 µl smeg culture into 50 ml sterile growth media-the goal is to have one culture at an OD of 0.4 the next day).
2) Incubate at overnight at 37^{o}C with shaking.
3) Measure OD600 of cultures (set OD= to 3.5x10^{7})
4) Take 5 ml of culture with OD=0.4 and spin down in 15 ml conical tube @ 500$_{x}$g for 5 min.
5) Remove 4 ml of supernatant and resuspend pellet in remaining broth.
6) Infect concentrated culture with phage at an MOI=10. Mix gently.
7) Incubate for at 37^{o}C for 15 min.
8) Bring volume of conical tube back to 5 ml with pre-warmed broth culture media.
9) Transfer contents to sterile 50 ml flask containing several cut pipette tips
10) Incubate flasks at 37^{o}C with shaking for 3-3.5 hours.
11) Transfer 1.5 ml from flask to two sterile microcentrifuge tubes.
12) Pellet the phage-infected cells at 14,000$_{x}$g for 5 min.
13) Remove supernatant. Resuspend each pellet in 750 µl of phage buffer. Combine resuspended pellets into one tube.
14) Spin combined pellets at 14,000$_{x}$g for 8 min.
15) Remove supernatant.
16) Store infected cell pellet at -80^{o}C.

Sample Multiplicity of Infection (MOI) calculation:

The *M. smegmatis* culture OD600 spec reading gave a concentration of 2.19E^{7} cells/ml. Your phage lysate is at 6E^{9} pfu/ml. You want to infect 1 ml of bacterial cells (2.19E^{7} cells) with an MOI of 10 (10 phage particles per cell in infection).

Calculation:
(2.19E^{7} cells)x(10 phages/cell)x(1ml/6E^{9}pfu)=0.1825 ml=182.5µl phage lysate for infection