**Sample collection for B12/DMB experiments with *B. bigelowii* and isolated UCYN-A**

Goal: Collect samples from *B. bigelowii* cultures suitable for metabolomic analysis of B12 compounds, and other metabolites. *B. bigelowii* cultures will be grown in 3 conditions: +B12 (0.4 nM B12), -B12 (0 added B12) and -B12 +DMB (0 added B12, 100 nM DMB). Each condition will include triplicate (biological) cultures. Samples will include whole culture filtered on to 1 µm Omnipore 25mm PTFE filters, and isolated UCYN-A nitroplasts filtered onto the same type of filter. Given biovolumes of ~150-200 µm3 for *B. bigelowii* and 10-20 µm3 for UCYN-A, we will aim for 5-6 million *B. bigelowii* cells per sample and 50-100 million UCYN-A to achieve ~1 µL biovolume.

The UCYN-A isolation procedure includes cell lysis via 0.15% Triton-X detergent, density gradient centrifugation using Percoll, then washing and resuspension of UCYN-A in an isolation buffer which can be removed via filtration.

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| **Isolation buffer** |  |
| 0.5 M | sorbitol |
| 6 mM | Na2EDTA |
| 5 mM | MgCl2 |
| 10 mM | KCl |
| 1 mM | MnCl2 |
| 50 mM | HEPES |

Samples will be collected at day and night time points from triplicate cultures in exponential growth phase, resulting in 36 initial samples:

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| **Culture condition** | **Sample type (3 triplicates each)** | **Time** |
| 0.4 nM B12 | whole cells on filter | Day |
| 0.4 nM B12 | isolated UCYN-A on filter | Day |
| 0.4 nM B12 | whole cells on filter | Night |
| 0.4 nM B12 | isolated UCYN-A on filter | Night |
| 0 added B12 | whole cells on filter | Day |
| 0 added B12 | isolated UCYN-A on filter | Day |
| 0 added B12 | whole cells on filter | Night |
| 0 added B12 | isolated UCYN-A on filter | Night |
| 0 added B12 100 nM DMB | whole cells on filter | Day |
| 0 added B12 100 nM DMB | isolated UCYN-A on filter | Day |
| 0 added B12 100 nM DMB | whole cells on filter | Night |
| 0 added B12 100 nM DMB | isolated UCYN-A on filter | Night |

Blanks required:

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|  | **Blanks** | **description** |
| A | filter blank (+B12, whole cells) | Omnipore PTFE filter with new media passed through |
| B | filter blank (-B12, whole cells) | Omnipore PTFE filter with new media passed through |
| C | filter blank (-B12+DMB, whole cells) | Omnipore PTFE filter with new media passed through |
| D | filter blank (UCYN-A) | Omnipore PTFE filter with clean isolation buffer passed through |
| E | media blank (+B12) | 40 mL new media in Falcon tube |
| F | media blank (-B12) | 40 mL new media in Falcon tube |
| G | media blank (-B12, +DMB) | 40 mL new media in Falcon tube |