Coccolithus braarudii HET and HOL protein mass spectrometry

1. Coccolithophore culturing, harvesting, and protein extraction

Haploid and diploid cultures of *C. braarudii* were cultured at 15 °C and harvested during mid-exponential growth. Proteins were extracted using RIPA buffer and liquid nitrogen grinding.

1. Coccolithophore protein mass spectrometry (MS)

TMT labelling, Liquid Chromatography Mass Spectrometry (LC/MS) and subsequent raw data analysis were carried out by the Proteomics Facility of Bristol University, UK.

The raw data files were processed and quantified using Proteome Discoverer software v2.1 (Thermo Scientific) and searched against the latest release version 2021\_2 of the *C. braarudii* predicted protein database (25204 proteins) from Uniprot ([www.uniprot.org](http://www.uniprot.org)), and a common contaminates database using the SEQUEST HT algorithm.

The reverse database search option was enabled, and all data was filtered to satisfy false discovery rate (FDR) of 5%, which is classed as FDR confidence = Medium. Data were normalized to the total peptide amount in each sample by Proteome Discoverer to equalize peptide abundances across all TMT channels and correcting for differences in sample loading. Normalized proteins were subsequently quality controlled.

Differential abundance between life phases was assessed using LIMMA (Ritchie *et al.*, 2015), with a significance level of < 0.05.

1. Notes on the data spreadsheet

Accession: Uniprot Accession Number for *Coccolithus braarudii*

logFC, AveExpr, t, P.Value, adj.P.Val, B: For information on analysing RNAseq or protein MS data, see package LIMMA: <https://kasperdanielhansen.github.io/genbioconductor/html/limma.html>

# Unique Peptides: Number of unique peptide for each protein

Protein FDR Confidence: False discovery rate (FDR), 5 % = Medium, 1 % = High

Blast2GO: Protein annotation

Description: Protein description from Uniprot

Best BLAST hit & e value: NCBI Blast hit with highest e-value