**New layout**

**Introduction**

**Materials and Methods**

* **Algae culture** 
  + Strain
  + Preculture and cold exposure
* **RNA extraction**
* **RNA sequencing**
* **Gene annotation? (Nico)**
* **Identification of genes of interest (cold responsive and cell wall related) (Nico)**
* **Calculation of gene expression in B2-2 (Nico)**
* **Detection of DEGs? (Nico)**
* **TEM imaging**

**Results**

1. **Effect of temperature on cell growth** 
   * Reference to Lorenza’s paper where she shows comparison to UTEX strain
2. **Overall transcriptome changes with different amounts of cold exposure– effect of cold on the overall genetic regulation of the cell metabolism** 
   * PCA diagram showing differences between timepoints
   * Investigate the gene expression associated with more/ less cold exposure
     1. GO enrichment between different time points
     2. KEGG
   * Venn diagram (comparison between timepoints?)
   * Industrially relevant changes= increase in lipids/carbohydrates? Any other obvious changes in pathways?
3. **Cold responsive genes**

* Establish that 8 gene clusters are present; 8 predominant expression patterns. Explain what happens in these clusters (up or down regulation and which pathways do they correspond to?)
* Diagram shows the gene activation is **time point sensitive** for cold exposure
* Cold shock proteins?
* Non-responsive cold responsive genes? Interesting if the algae has somehow evolved to keep the genes in a normal functional state (doesn’t alter their regulation by turning them on or off with cold… or maybe they need temperatures lower than 5C)/ or they are incorrectly labelled)
* **Recovery happens after 24 hours for most cold responsive genes?** Ask Nico

1. **The cell wall: a physical protective barrier against the cold?**

* TEM images and measurements show an increase in cell wall thickness when exposed to cold stress
* Identification of cell wall genes and how they change temporally (table?)
* Enzymatic pathway with enzymes that might be upregulated to explain the increase in thickness?
* Cell wall genes : Log 2-fold change between 0 hours & 4 hours / 0 hours and 120 hours? Volcano plot?
* Cell wall synthesis pathway? (like the lignin one I got)

**OR Results section**

1. **Effect of temperature on cell growth**
2. **Cold Responsive Genes**
   1. PCA Diagram showing differences between timepoints
   2. Venn diagram
   3. KEGG + GO pathways
   4. Soft Clusters
   5. Recovery after 24 hours? Is there data for this?
   6. Non-responsive cold responsive genes
3. **Effect of Cold Exposure and of the Regulation of Cold Responsive Genes on the cell metabolism**
   1. Industrially Relevant changes (more lipids/carbs?)
   2. Other interesting changes – ex: cold shock proteins?
4. **Cell Wall TEM Measurements**
   1. Identification of cell wall genes that are altered
   2. How do they change temporally?
   3. Enzymatic changes in cell wall pathway – putative cell wall related genes
   4. TEM images and measurements