### Chromatin landscaping in algae reveals novel regulation pathway for biofuels production

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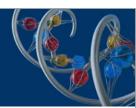
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#### Summary

The diminishing reserve of fossil fuels calls for the development of biofuels. Biofuels are produced from renewable resources, including photosynthetic organisms, generating clean energy. Microalgae is one of the potential feedstock for biofuels production. It grows easily even in waste water, and poses no competition to agricultural crops for arable land. However, little is known about the algae lipid biosynthetic regulatory mechanisms. Most studies relied on the homology to other plant model organisms, in particular Arabidopsis or through low coverage expression analysis to identify key enzymes. This limits the discovery of new components in the biosynthetic pathways, particularly the genetic regulators and effort to maximize the production efficiency of algal biofuels. Here we report an unprecedented and de novo approach to dissect the algal lipid pathways through disclosing the temporal regulations of chromatin states during lipid biosynthesis. We have generated genome wide chromatin maps in chlamydomonas genome using ChIP-seq targeting 7 histone modifications and RNA polymerase II in a time-series manner throughout conditions activating lipid biosynthesis. To our surprise, the combinatory profiles of histone codes uncovered new regulatory mechanism in gene expression in algae. Coupled with matched RNA-seq data, chromatin changes revealed potential novel regulators and candidate genes involved in the activation of lipid accumulations. Genetic perturbation on these candidate regulators further demonstrated the potential to manipulate the regulatory cascade for lipid synthesis efficiency. Exploring epigenetic landscape in microalgae shown here provides powerful tools needed in improving biofuel production and new technology platform for renewable energy generation, global carbon management, and environmental survey.

#### Chlamydomonas as model for algal biofuel feedstock

Microalgae potentially produces the highest oil yield with the smallest land area required.

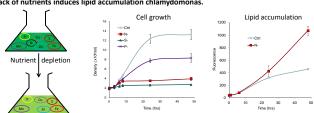
Unique Advantages of Algae as Biofuel Feedstock

- · High area productivity
- · Non-seasonal, fast growing
- · Minimum competition with agriculture
- · Renewable energy
- Recycles stationary emissions of CO<sub>2</sub>

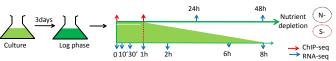
#### Chlamydomonas reinhardtii as a reference model

- Unicellular green algae (Chlorophyta), found all over the world, in soil, fresh water, oceans
- The most widely used laboratory species is Chlamydomonas reinhardtii
- Cells can grow on a simple medium of inorganic salts, using photosynthesis to provide energy
- Reference genome available

#### Lack of nutrients induces lipid accumulation chlamydomonas.

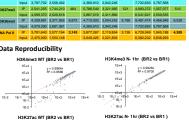


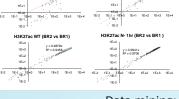
Experimental Design



#### Chromatin Landscape

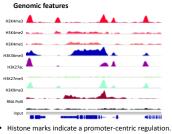
# ChIP-seq Data summary



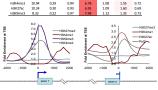


0.4% 0.0% 2.3% 0.0%

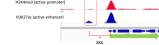
0.91 0.33 0.52 1.07 1.72 0.39 1.43 4.78



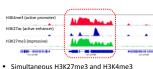




## H3K27me3 marks heterochromatin



H3K27ac-specific marks indicates potential distal regulatory elements/enhancers.



marking indicates potential bivalent domain.

#### Transcriptome

#### **Data summary**

Total 9 timepoints x 2 conditions, Total reads of >50m per lib

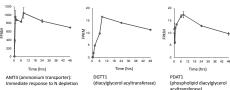
N: Nitrogen, S: Sulfur, P: Phosphorus

Genome ver5			
	Reference	Cufflink	Modified/filtered
Genes	18,773	17,730	16,467
Transcripts	19,529	33,929	32,395
Unique Transcriptome size (Mb)	94.6	97.8	96.1
Mean transcript length	5411	5922	6203



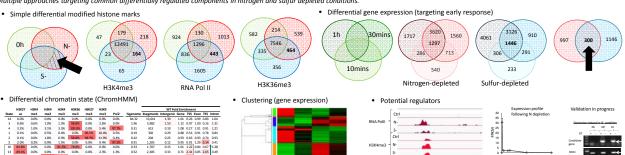
- Extension (6100) Novel genes (184)
- Antisense genes (1727) Merged (685)
- · Novel exon (544)

#### Control genes expression profile



#### Data mining: Searching for common players in lipid biosynthesis pathway

Multiple approaches targeting common differentially regulated components in nitrogen and sulfur depleted conditions.



#### Discussion

Here, we report a pilot attempt to understand the regulation of algae lipid biosynthetic pathways by genome wide chromatin landscaping. Deep sequencing of transcriptome also provide supporting data to further dissect the regulatory pathways.

Computational analysis have enabled us to narrow into a smaller group of highly potential regulators. In-depth analysis is still on going to map out a potential interaction or inter-regulatory pathways. Effort to further validate these findings in the biological system is currently underway.

This approach shall provide a more feasible way of dissecting regulatory elements in algae and potentially overcome the limitation of the laborious and lowthroughput mutagenesis