Metabolomic Technologies at JGI

JGI User Meeting
Trent Northen
Motivation: Microbial metabolism is central to global carbon cycling.
Motivation: Bioenergy and Bioproducts

- **Feedstock**
- **CO₂**
- **Biomass**
- **Pre-treatment**
- **Hemicellulose**
- **Cellulose**
- **Enzymes**
- **Sugar**
- **Microbes**
Motivation: understanding the diverse activities of microbial secondary metabolites

https://www.mpg.de/6656708/odour-activation-geosmin-fly
Need for rapid sequence-to-function technologies to complement sequencing

Huge need for functional data to complement sequencing

Current Opinion in Microbiology

Temperton, Current Opinion in Microbiology, 2012
Overview of metabolomics

Sequence

Extraction

HPLC

Function

Metabolite identification & Quantification

Mass Spectrometry
Metabolomics provides a functional complement genomics

Mass spectrometry based metabolomics provides a direct functional readout that can be linked to gene function
Vision: JGI effectively integrates metabolomics with sequencing/synthesis providing users new biological insights.
Experienced JGI Metabolomics Team

Trent Northen
Interest: metabolomics for microbial functional genomics and microbial community metabolism.
• PhD w/ 10 years metabolomics.
• Deeply connected to BER science missions through 8yrs involvement in BER science programs.
• TRNorthen@lbl.gov

Leslie Silva
Interest: microbial metabolomics
• PhD w/ 3 years metabolomics
• Key developer of JGI workflows.
• LPSilva@lbl.gov

Katherine Louie
Interest: microbial metabolomics
• PhD w/ 4 years of diverse metabolomics experimentation.
• Key developer of JGI workflows.
• KBLouie@lbl.gov

Ben Bowen
Research focus: metabolomics informatics
• PhD ~10 years mass spectrometry informatics
• BPBowen@lbl.gov
Initial products: analysis of primary and secondary metabolites from media and cells
• **Exometabolomic analysis of polar metabolites:**
  – Improve gene annotations
  – Examine nutrient exchange and resource competition
  – Provide critical insights into microbial foodwebs and carbon cycling ultimately to predict carbon cycling to metagenomic data

• **Secondary metabolite analysis**
  – Improve gene and pathway annotations
  – Improve our understanding of microbial communication
    • Plants
    • Microbes
Early Success Stories—Sequencing + secondary metabolite analysis

- Integrated metabolomics with transcriptomic analysis and mutagenesis to determine that psr1 is a key regulator of lipid metabolism in *Chlamydomonas reinhardtii* (Yee et al, *Nature Plant*, 2015)

- Integrated metabolomics with sequencing to verify accuracy of PacBio quantification of adenine methylation (Yee et al, *in review*)

- Integrated metabolomics with DNA synthesis to optimize secondary metabolite (violacein) production (Sam Deutsch - JGI, Nathan Hillson - JBEI)

- Integrated metabolomics with DNA synthesis to support refactoring actinorhodin biosynthetic pathway (Sam Deutsch)
Example: Metabolomics + DNA Synthesis Refactoring Actinorhodin pathway (21 genes)

Internal LBNL PI By Nathan Hillson, Col’s: Sam Deutch, Jeff Kim, Paramvir Dehal and Trent Northen

Challenge: Achieve predictable expression of a pathway by refactoring

- Representative of many biosynthetic clusters (size, complexity, GC content)
- Pathway well characterized (but never before refactored)

Native pathway – complex set of operons highly regulated under native conditions

Refactoring

Redesigned to predictably achieve phenotypic outcome under laboratory conditions

- Codon optimized genes: lower GC, remove repeats & secondary structure, preserve high translational potential
- Previously validated constitutive promoters and terminators
- Calculated optimal RBSs specific for each rCDS

Actinorhodin

Sam Deutsch (JGI)

Nathan Hillson (JBEI)
Pathway output is 100% of Wildtype; different timecourse

Actinorhodin production

<table>
<thead>
<tr>
<th>Strain</th>
<th>Day in culture</th>
<th>Supernatant</th>
<th>Pellet</th>
</tr>
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<tbody>
<tr>
<td>Del</td>
<td>2 3 6</td>
<td></td>
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<td>Ref</td>
<td>2 3 6</td>
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<td>WT</td>
<td>2 3 6</td>
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Peak area (au)
Accumulation of intermediates gives insight into pathway dynamics

Accumulation of intermediates or shunt products indicative of activity in different parts of pathway and between strains, etc
Metabolomics Bioinformatics to explore the biochemical space: MIDAS and Pactolus, chemical networks for integration with IMG

- Linking genomics with metabolomics
  - Compare and query *in silico* spectra with measured and theoretical spectra to algorithmically determine chemical identities.

**Annotated Spectra**

- Propagate chemical annotations within and across datasets

**In silico fragmentations and chemical identities**

- Integrate and match experimental and simulation spectra

**Raw spectra with unknown peaks**

- Propagate chemical annotations within and across datasets

**Raw spectra with:**
  - Probabilistic chemical IDs
  - *In silico* chemical IDs
Consider including metabolomics as part of new CSPs

- **Metabolomics should be in all cases tightly linked with sequencing and/or DNA synthesis**
- Typical metabolomics experiments are around 50-200 samples for polar metabolite analysis and 50-500 samples for secondary metabolite analysis.
- Larger requests will be considered on a case by case basis.


For questions about metabolomics please contact Trent Northen: **TRNorthen@lbl.gov**