Overview of DNA synthesis
Platform and applications

Sam Deutsch
DNA Synthesis Science
The DOE JGI's primary role in synthetic DNA projects will be to support users in the computational design of desired target constructs, in the creation of these large and complex DNA molecules and in their introduction into suitable host cells. In contrast, in-depth functional characterization of the resulting transformed host organisms will primarily rely on expertise and assays established in the respective users' laboratories. Nevertheless, in order to be able to support users in the ability to generate synthetic systems required to address energy and environmental challenges, the DOE JGI will also develop experimental paradigms in which functional readouts can be closely linked to synthetic sequence.

IN SILICO TO IN VIVO: PRODUCING AND INTRODUCING THE DNA

Currently the most expensive and time-consuming step in large-scale synthetic DNA projects is the assembly of small oligonucleotides into larger fragments. The DOE JGI will be at the forefront of implementing new technologies into the DNA synthesis pipeline. These goals will be supported by the DOE JGI's longstanding experience in developing cutting-edge molecular processes, wet-lab automation and process optimization.

The DOE JGI DNA Synthesis Pipeline will also benefit from the DOE JGI's state-of-the-art sequencing and computational analysis capabilities, which are central to this activity.

10 year vision for DNA Synthesis at the DOE JGI.
Focus Areas

• Design

Major bottleneck at present. Our goal is to enable design of 1000s of constructs whilst maximizing success rate

• Pathway refactoring/engineering

  • Rapid pathway build
  • Combinatorial libraries
  • Refactoring of large gene clusters

• Data integration and learning

  Analyzing strain characterization data to improve design
Highlight projects

Actinorhodin

Engineering complex biosynthetic pathways

Thiamine

Pathway optimization and data driven learning

Targets of opportunity

Enabling biomanufacturing

Design, OMICS
Actinorhodin: polyketide antibiotic produced by *Streptomyces coelicolor*, requiring 22 genes for biosynthesis. Cluster ~ 25 kb, %GC >70

Why Actinorhodin?

- Representative of many biosynthetic clusters (size, complexity, GC content)
- Pathway is well characterized (but never before refactored)
- Connects with JGI interests in microbe-microbe and plant-microbe interactions

Collaboration between JGI, JBEI and Radiant Genomics: Biomanufacturing grant
Actinorhodin strain characterization

Native pathway:

Refactored Design:

Highly regulated expression; unknown control system

S. coelicolor Δact strain (control)

Refactored Actinorhodin strain

Metabolomics (LC-MS)

Actinorhodin
m/z = 633.1258
RT @ 7.5min
Highlight projects (II)

Thiamine

Pathway optimization and data driven learning

Deutsch, Biosensor grant. Collab with M. Sommer (CFB)

Currently plant derived  Bacterial fermentation
Refactoring thiamine pathway

- Thiamine biosynthesis combinatorial libraries

Regulatory variants

~16000 variants total

Predicted by RBS calculator to have strong expression effects

How to screen large numbers of variants?
Screening: Thiamine biosensor system

- At 50uM chloramphenicol only cells that produce > 3uM internal thiamine (~5X Wild type) will survive.
Thiamine library characterization

Full OMICS characterization of 50 strains from library that survived selection

Sequencing  Proteomics  Metabolomics

Top strains produced 50X wild type levels!
How are RBSs associated to Thiamine production?

OMICS based model

PPP_int ~ ThiH_exp * ThiG_exp + ThiE_exp + ThiC_exp
p<2.2e-16, Rsq=0.75
Highlight projects (III)

Targets of opportunity

Enabling biomanufacturing

Collaboration with JBEI, KBASE: Agile biomanufacturing

DOE INTEREST IN SUSTAINABLE BIOPRODUCTS
Enabling biomanufacturing

Chassis Organism

\[ E. \textit{coli} \]

Biochemical Reactions

Retrosynthetic Design Space (RDS)
Targets of Opportunity (Hubs)

1 step from *E. coli*

Selected Targets (30)

Selected + 1 step (193)

Selected + 2 step (380)

Selected + n steps (~1000)
Targets of Opportunity pilot

- Completed the Design and Build of ~100 pathways
- Characterization of strains is in progress

![Chemical structures](images)

FPP → PtlA (S. avermilitis) → PtlL + NADPH + O₂ (S. arenae) → 1-deoxypentalenate

BioDevisor/SPL

Protocol / Assembly Strategy
Strain Characterization

Single gene synthesis  Assembly  Cloning  Sequence QC

Control  DP strain

Deoxypentalenate

[M-H]: 233.15
Capabilities served to user community

- CSP 1405 – Justin Siegel (Davies): Metagenomic based operons for increased alkane production
- CSP 1880 – Kristala Prather (MIT): Combinatorial assembly and screening of heterologous glucaric acid pathways in yeast
- CSP 1882 – Brandon Chen (Genomatica): Engineering efficient methanol utilization for renewable chemicals
- BRC 2073 – Cameron Currie (UW): Combinatorial pathways to increase cellulolytic capacity in Streptomyces sp.
- CSP 1755 - Tobias Erb (ETH): Refactoring novel carbon fixation pathways
- CSP 1585 - Mark Blenner (Clemson): Refactoring lignin degradation and utilization pathways
- CSP 1878 - Mike Smanski (UM): Refactoring natural product clusters from disease suppressive soils
- Internal Science: Refactoring phenazine pathways for fungal pathogen biocontrol
Conclusions

Described Synthesis platform capabilities in the context of:

- Design and synthesis of large biosynthetic clusters
- Combinatorial libraries for pathway optimization
- Large scale design for chemical biomanufacturing

All projects involved strong Design and OMICS components and DBTL iterations

The capabilities that were showcased are being served to user community as part of our strategic plan to provide integrated capabilities which go beyond DNA synthesis
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