JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Overview of DNA synthesis Platform and applications

Sam Deutsch
DNA Synthesis Science

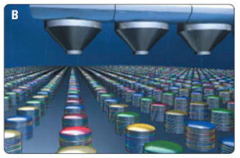
JGI 'Build' Pipeline

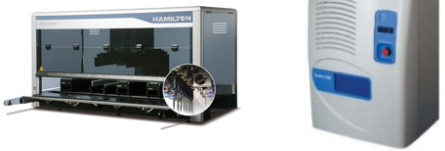
Project Management
Calls
Review
ELSI
Initiation

phytozome
UniProt
img
Data mining

GENE DESIGN
j5
BioStudio
Design Software

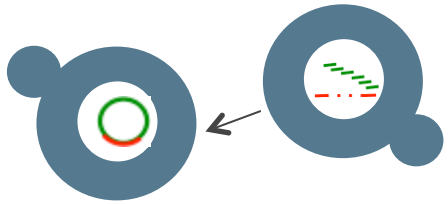
Gen9
IDT INTEGRATED DNA TECHNOLOGIES
1-Kb blocks



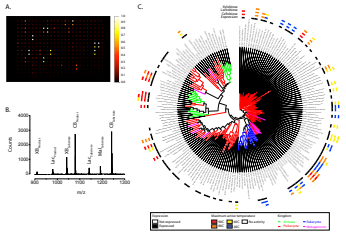


Liquid handling systems

Cloning and picking

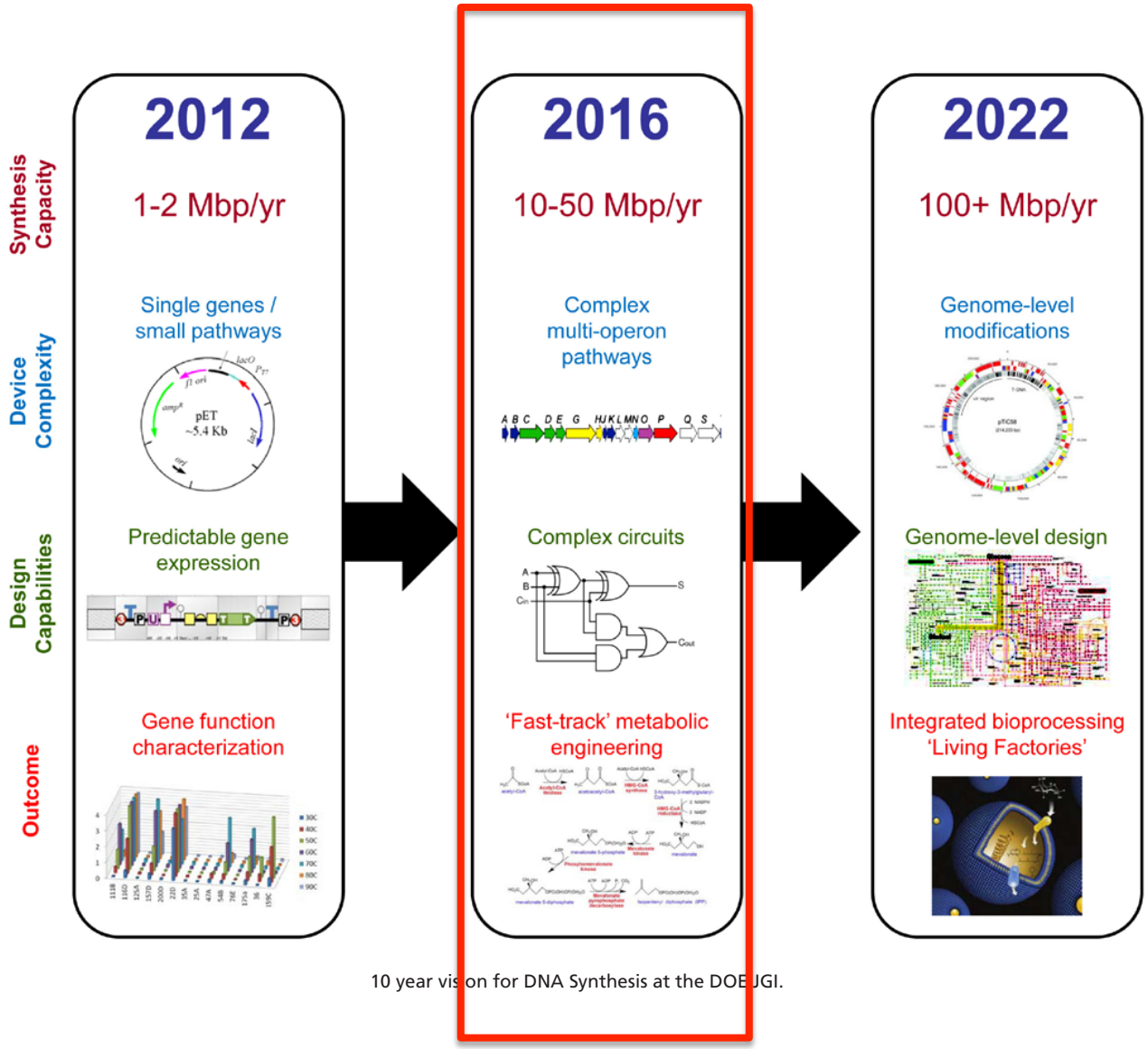


Multi-fragment assembly



Functional Characterization

10-year vision for evolving capabilities



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

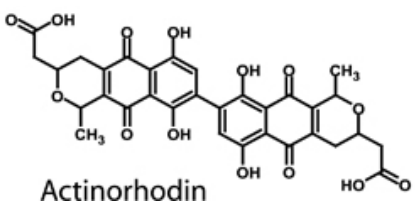
- Data integration and learning

Analyzing strain characterization data to improve design

Highlight projects

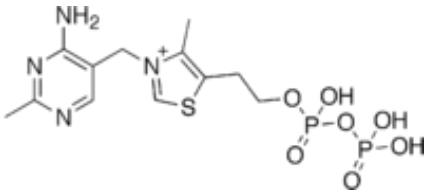
Actinorhodin

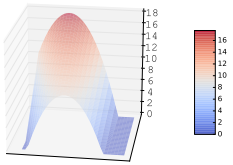
Engineering complex biosynthetic pathways



Thiamine

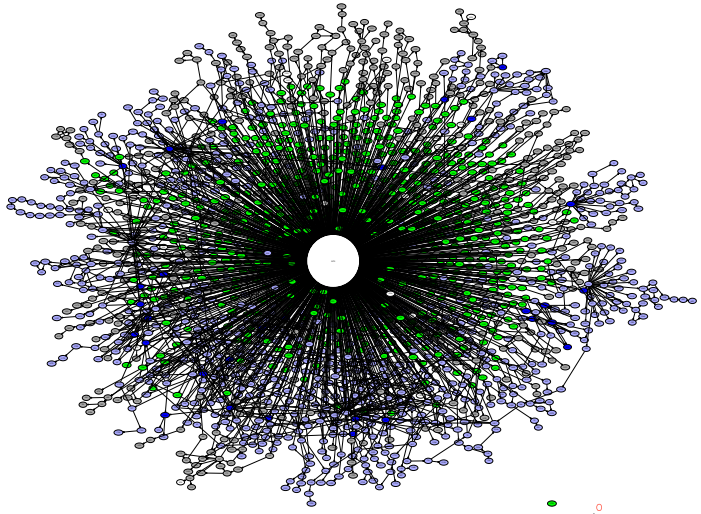
Pathway optimization and data driven learning

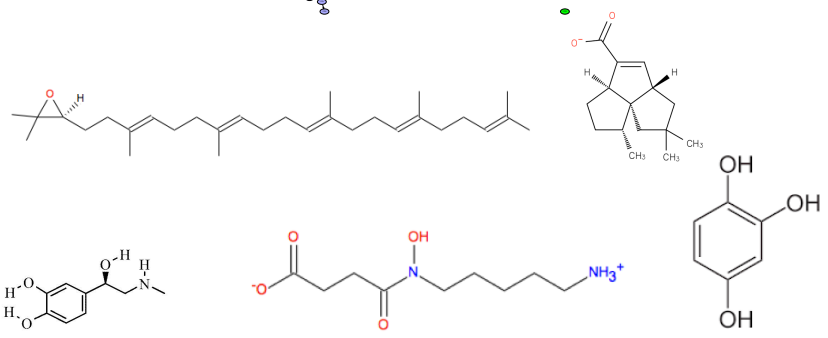




Targets of opportunity

Enabling biomanufacturing

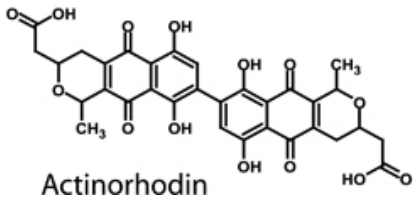




Design, OMICS

Complex Biosynthetic cluster: Actinorhodin

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



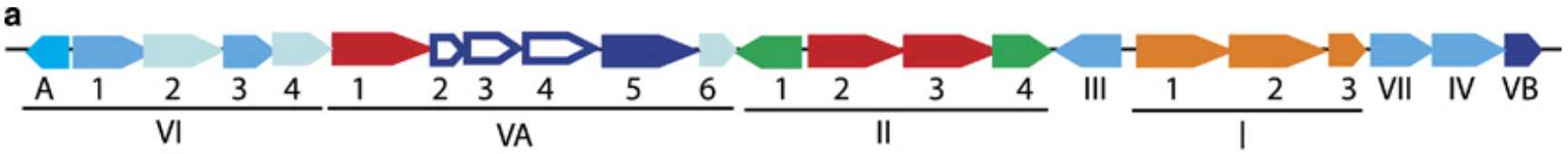
Why Actinorhodin?

- 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

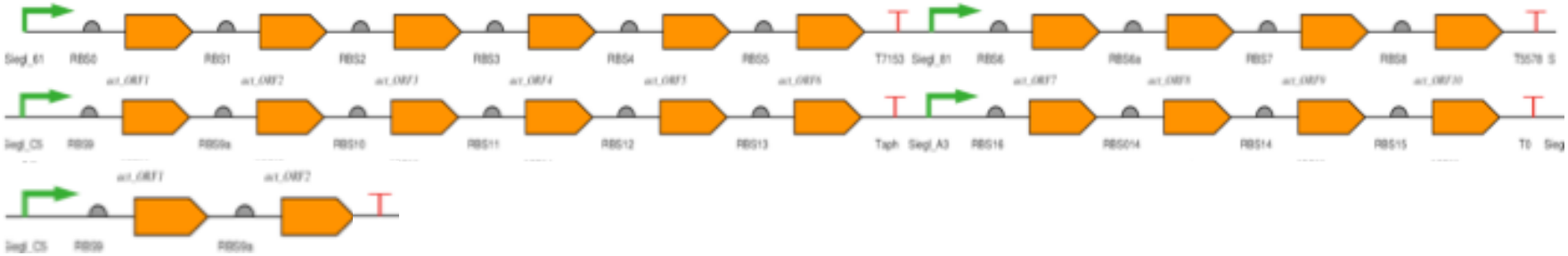
Actinorhodin strain characterization

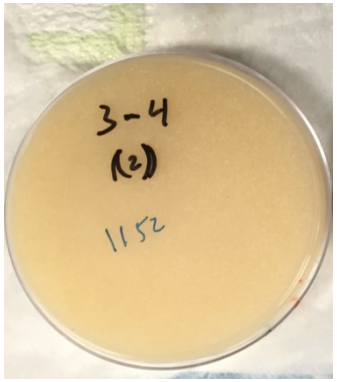
Native pathway:

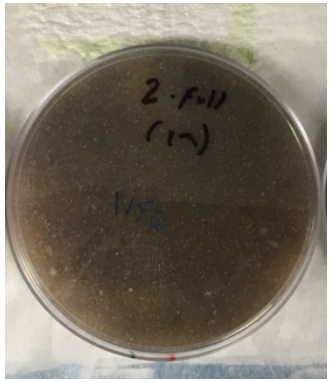


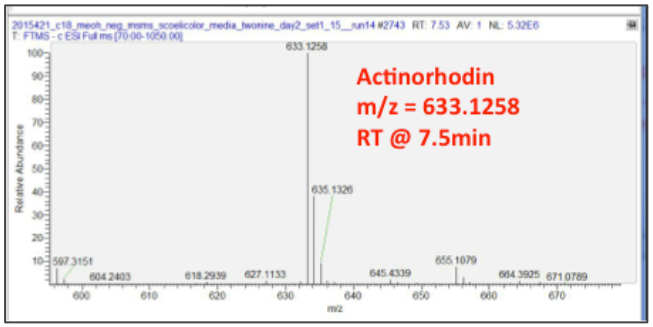
Highly regulated expression; unknown control system

Refactored Design:









S. coelicolor Δ*act strain* (control)

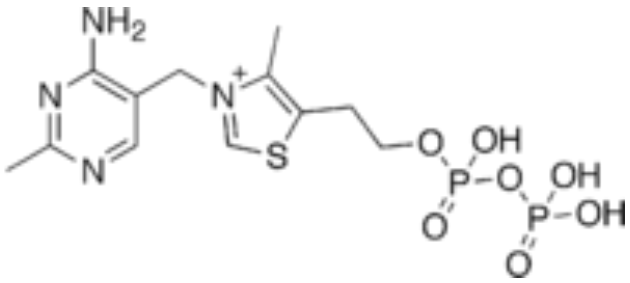
Refactored Actinorhodin strain

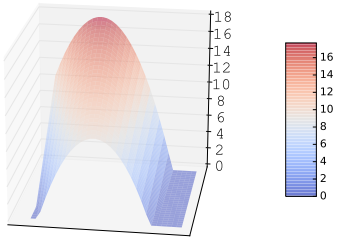
Metabolomics (LC-MS)

Highlight projects (II)

Thiamine

Pathway optimization and data driven learning



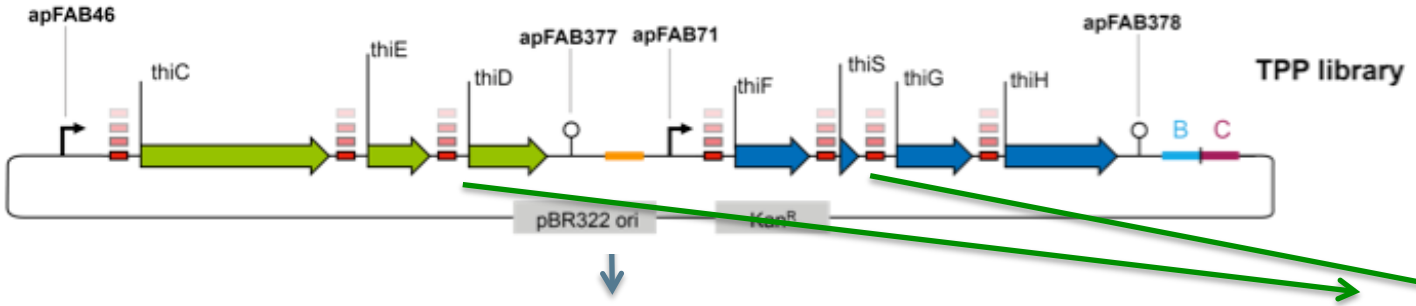


Deutsch, Biosensor grant. Collab with M . Sommer (CFB)

Currently plant derived → Bacterial fermentation

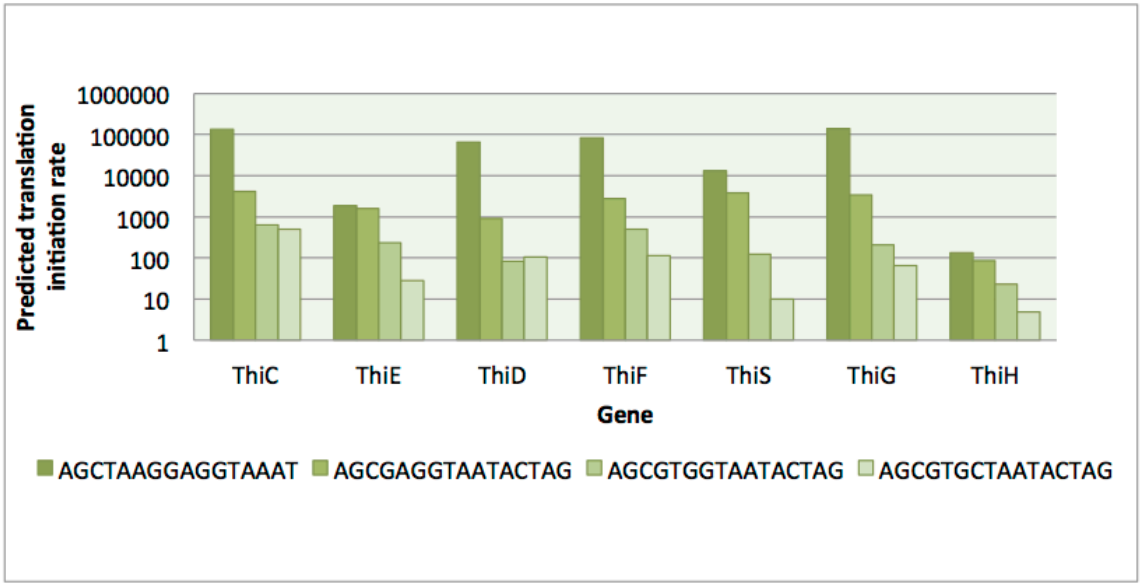
Refactoring thiamine pathway

- **Thiamine biosynthesis combinatorial libraries**



~16000 variants total

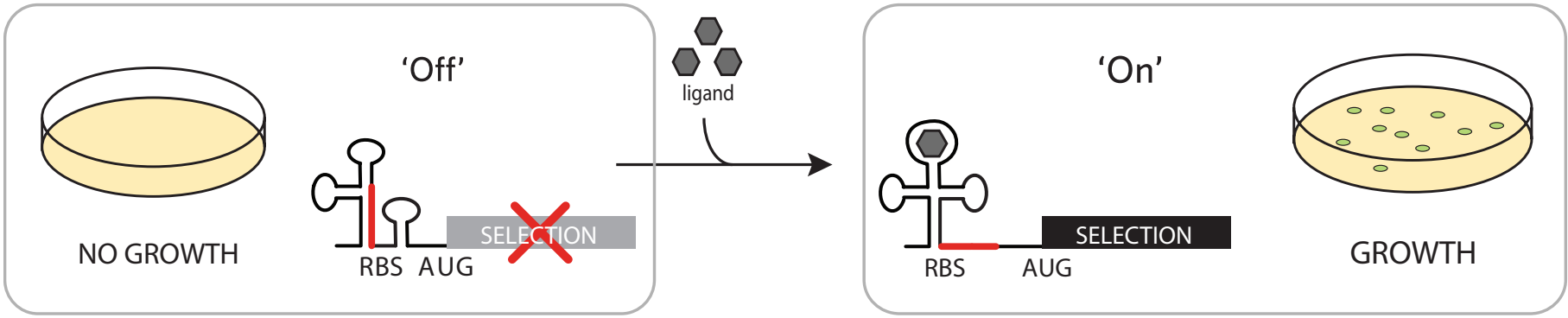
Regulatory variants



Predicted by RBS calculator to have strong expression effects

How to screen large numbers of variants ?

Screening: Thiamine biosensor system

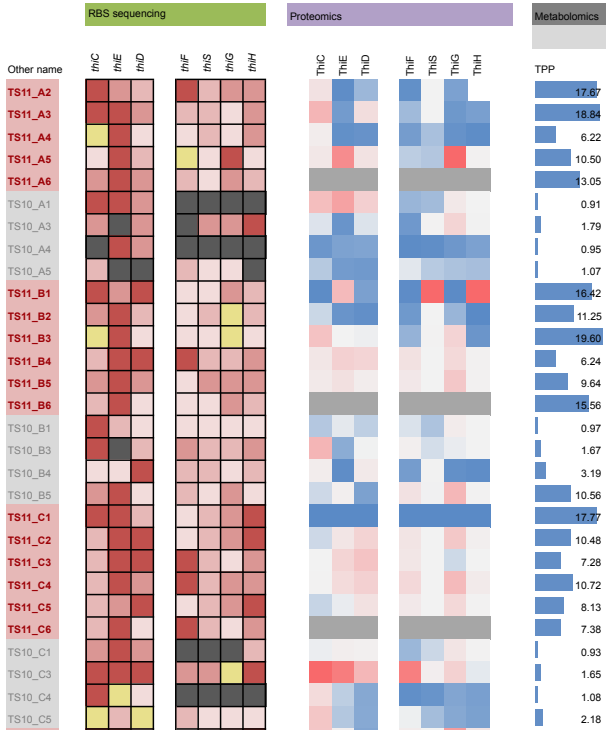


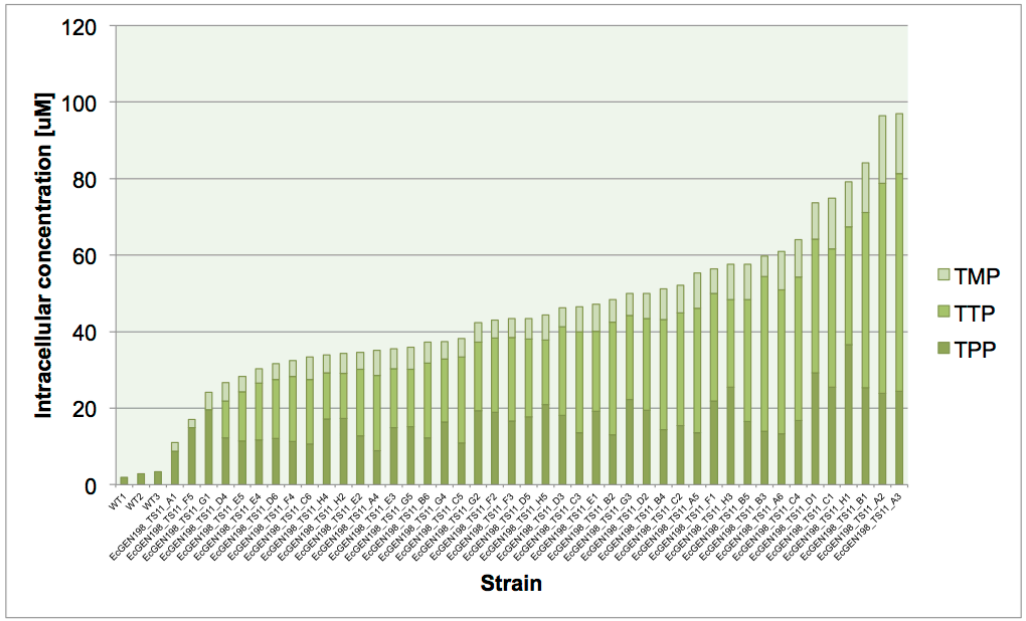
- At 50uM chloramphenicol only cells that produce > 3uM internal thiamine (~5X Wild type) will survive.

Thiamine library characterization

Full OMICS characterization of 50 strains from library that survived selection

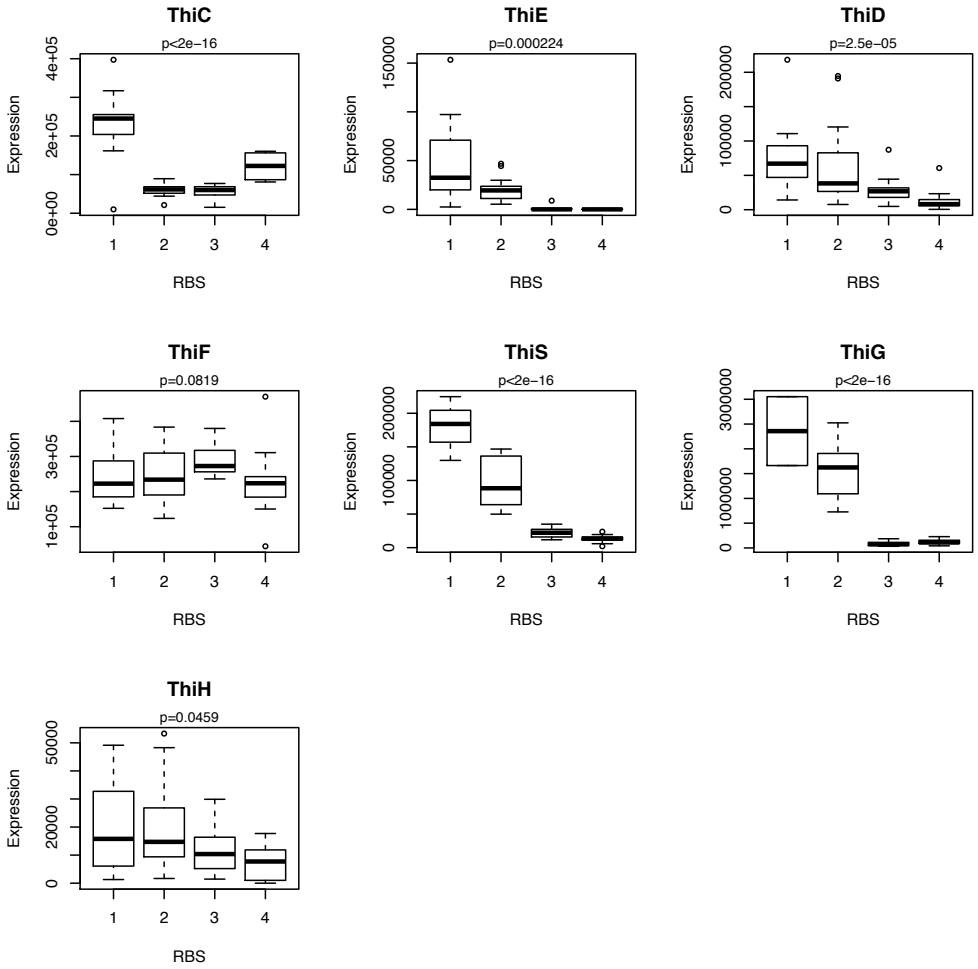
Sequencing Proteomics Metabolomics



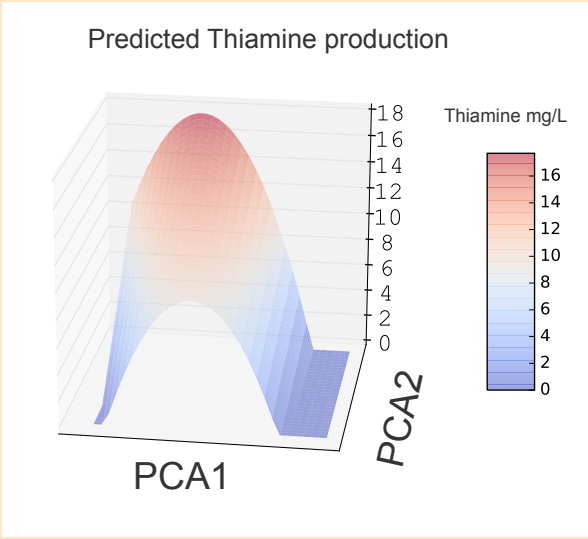


- Top strains produced 50X wild type levels !

How are RBSs associated to Thiamine production ?

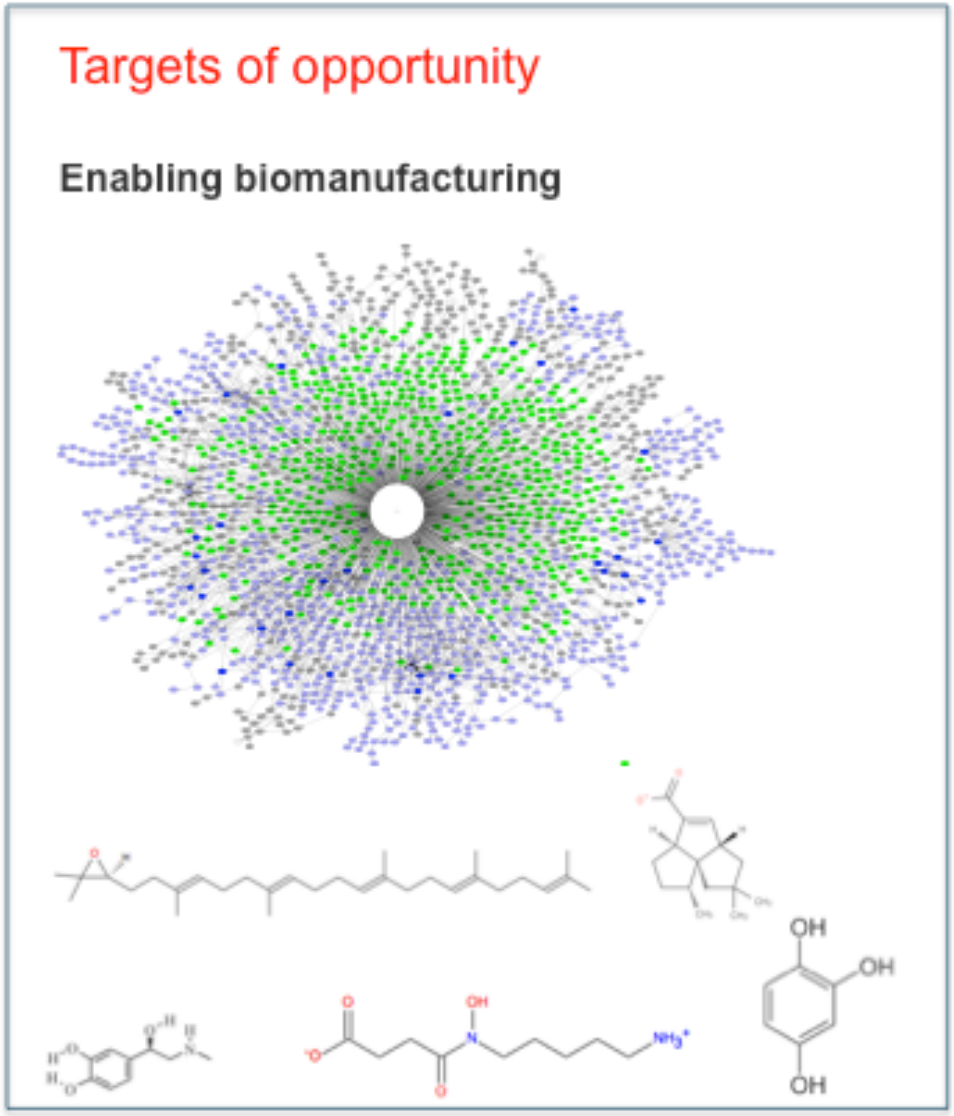


OMICS based model




```
TPP_int ~ ThiH_exp * ThiG_exp + ThiE_exp
+ ThiC_exp
p<2.2e-16, Rsq=0.75
```

Highlight projects (III)



DOE INTEREST IN SUSTAINABLE BIOPRODUCTS

Collaboration with JBEI, KBASE: Agile biomanufacturing

Enabling biomanufacturing

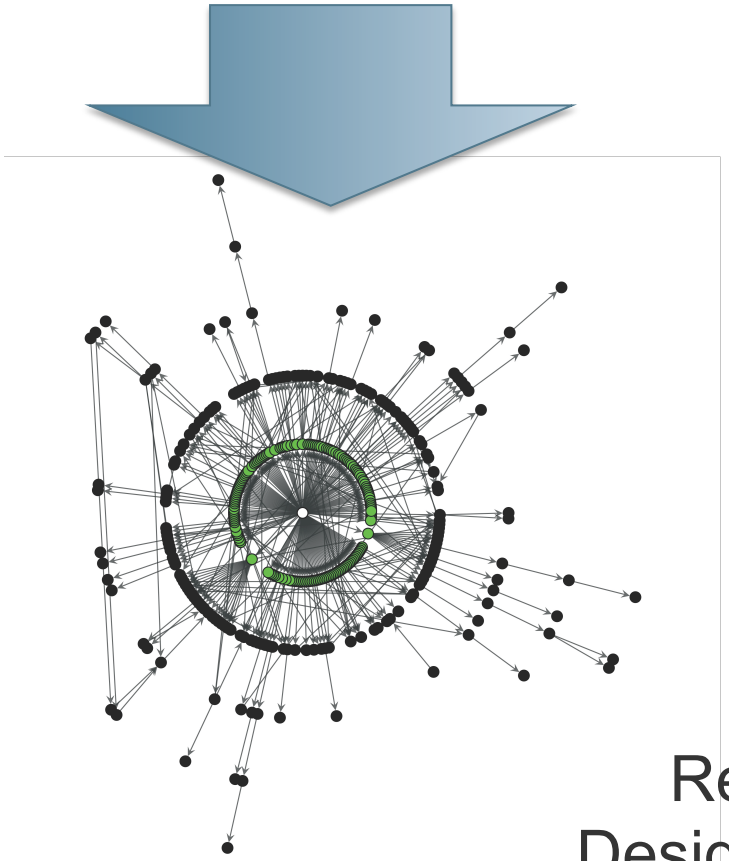
Chassis Organism

E. coli

+

Biochemical Reactions

MetaCyc

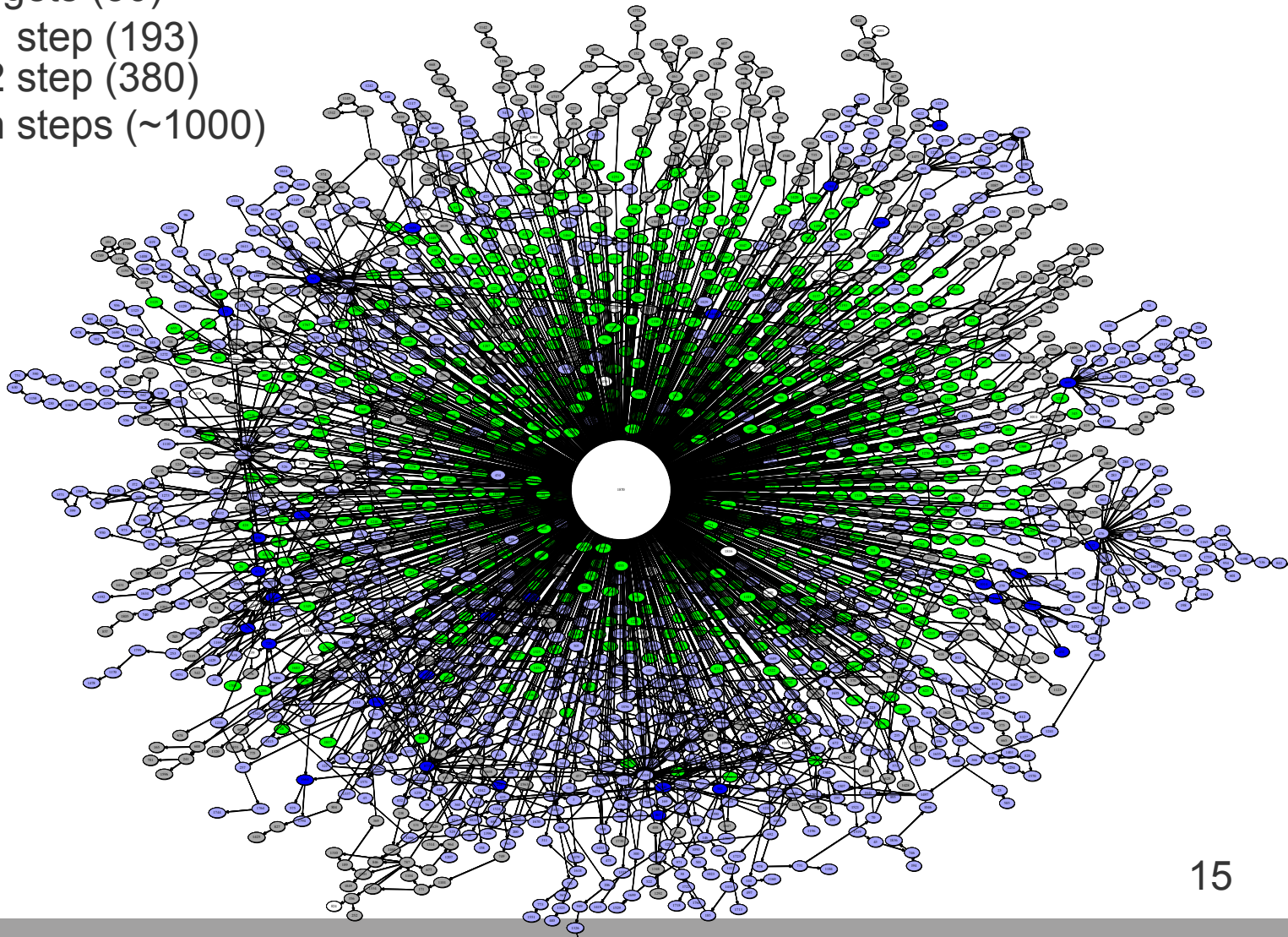


Retrosynthetic Design Space (RDS)

Targets of Opportunity (Hubs)

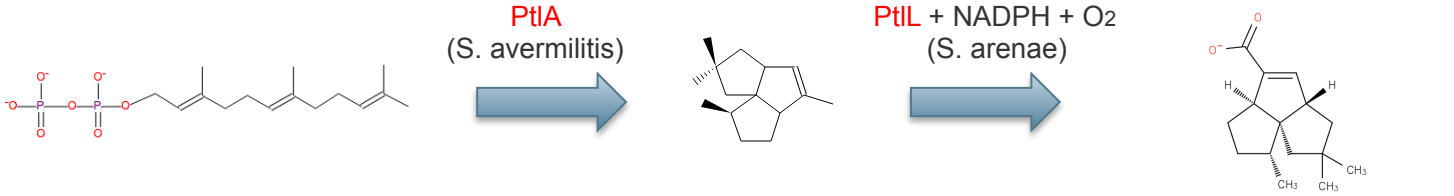
- 1 step from *E. coli*
- Selected Targets (30)
- Selected + 1 step (193)
  Selected + 2 step (380)
  Selected + n steps (~1000)

E. coli RDS

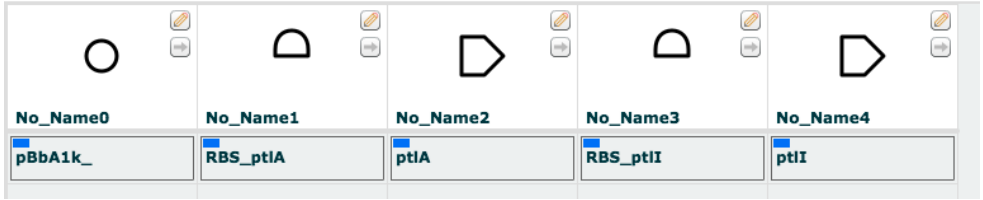


Targets of Opportunity pilot

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



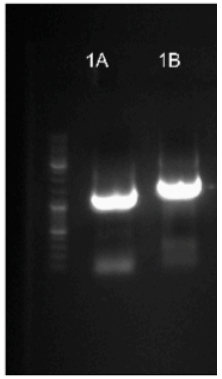
BioDevisor/SPL



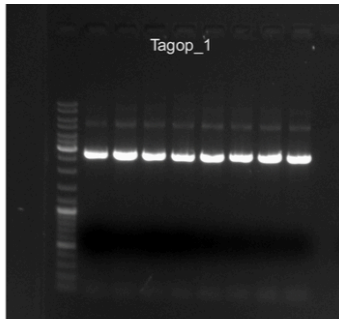
DIVA

Protocol / Assembly Strategy

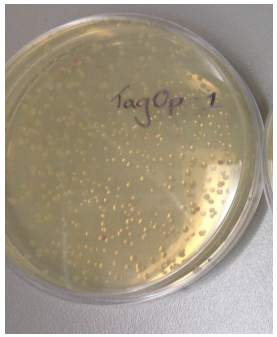
Strain Characterization



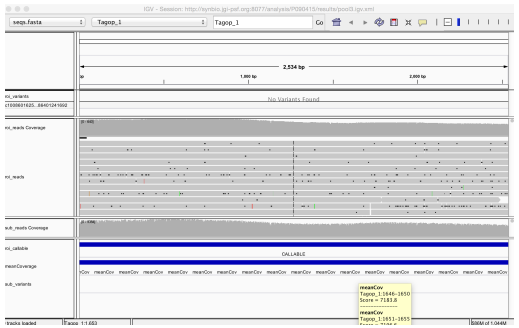
Single gene synthesis



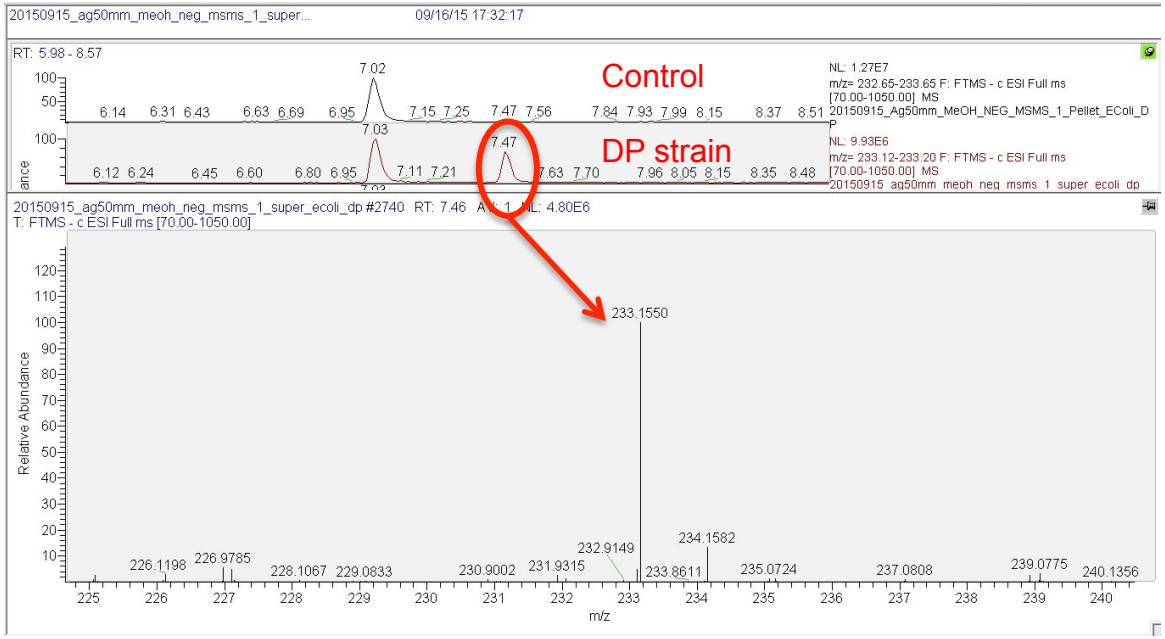
Assembly



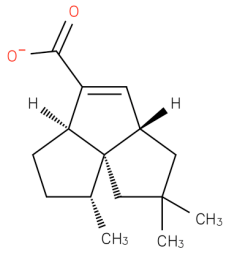
Cloning



Sequence QC



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

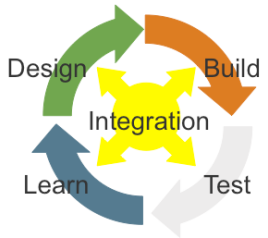
- Internal Science: Refactoring phenazine pathways for fungal pathogen biocontrol

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

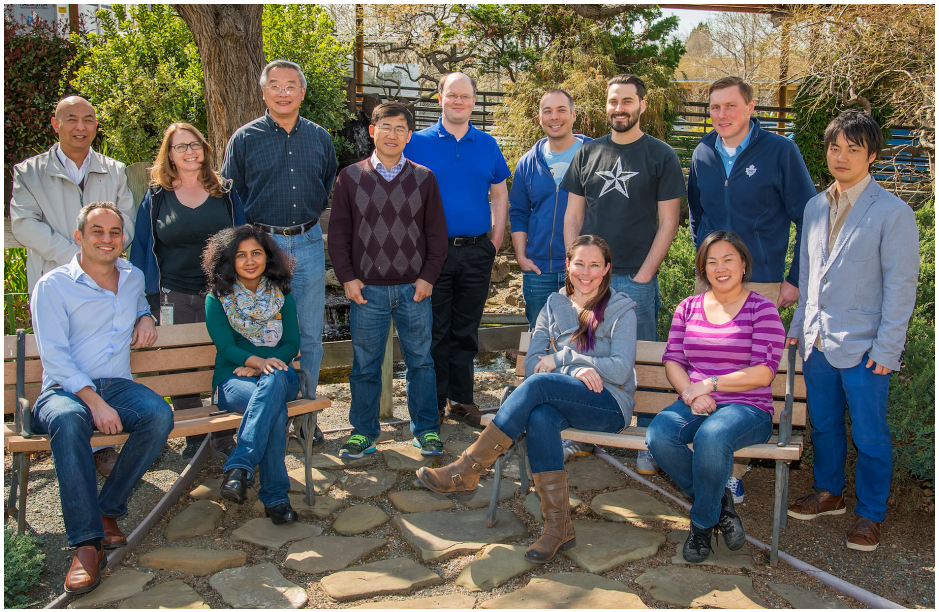
All projects involved strong Design and OMICS components and DBTL iterations



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

Acknowledgements

JGI DNA synthesis platform group



Biomanufacturing Group at LBNL

- Nathan Hillson
- Chris Petzold
- Paramvir Dehal
- Brian Olson

Northen Groups

- Katherine Louie
- Ben Bowen
- Trent Northen

Radiant Genomics

- Jeff Kim

Sommer Lab @ DTU

- Hans Genee
- Morten Sommer