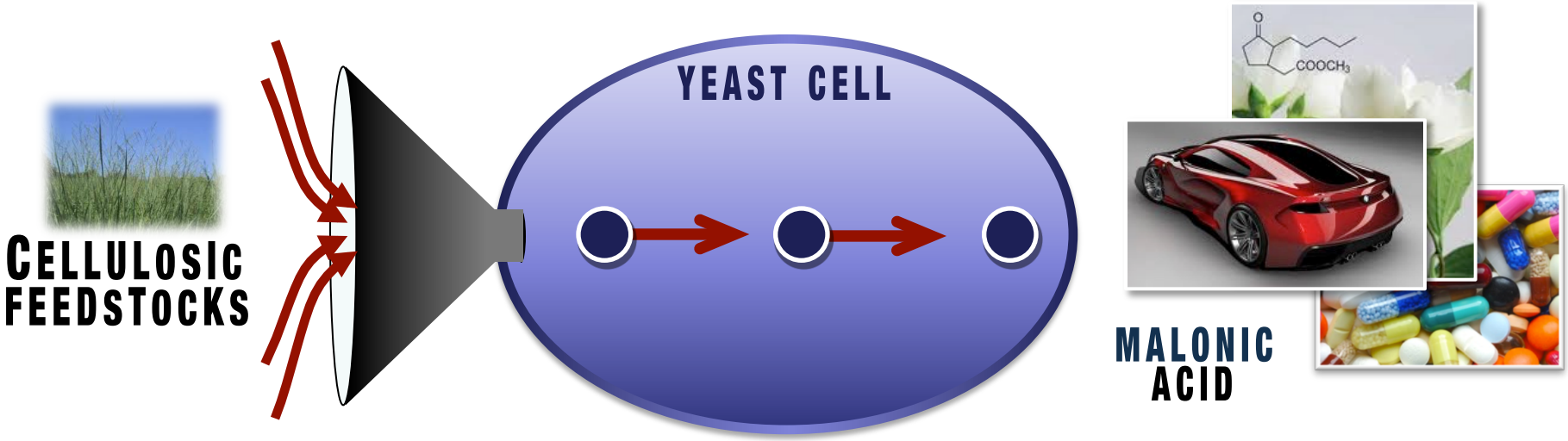
LYGOS

Design & Optimization of a Biochemical Production Platform with Biosensor-Guided Synthetic Evolution



March 23, 2015
DOE BETO Conversion Review

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Lygos, Inc.

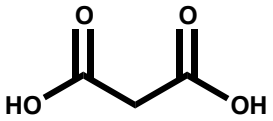
Malonic Acid Is An Ideal Biological Product

CELLULOSIC SUGARS

MALONYL-COA → FATTY ACIDS

LYGOS Enzymes

MALONIC ACID



- **Malonyl-CoA lies on the carbon superhighway in biology**
  - Pathway is compatible with all available, low-cost feedstocks
  - Malonyl-CoA is basis of fatty acid production (90%+ yields reported)
- **Malonic acid is an ideal molecule to produce biologically**

  Theoretical Yield (lb-product / lb-sugar): 1.73

  2/3 $C_6H_{12}O_6$ + 2 CO_2 ➔ 2 $C_3H_4O_4$

Goal Statement

Develop an integrated approach to biochemical pathway optimization for production of malonic acid & demonstrate path toward commercially-relevant fermentation metrics.

Relevance to US & BETO mission:

- Reduce dependence on foreign oil
  - 130 million pounds produced in foreign countries
- Increase production of fuels & chemicals from lignocellulosic feedstocks
- Decrease greenhouse gas (GHG) emissions
  - 100 million pounds of CO_2 sequestered
  - Eliminate 34 million pounds of sodium cyanide use
- Identified as Top 30 bioproduct by DOE

Quad Chart Overview

Timeline

- Start: Aug 31, 2013
- End: January 1, 2016
- Project is 47% complete by spend (as of 12/31/15)

Budget, $2.06MM

	FY 13 Costs	FY 14 Costs	Planned FY 15
DOE Funded	$310,481	$561,458	$996,468
Lygos Cost Share	$34,498	$62,387	$99,648

Barriers

- Bt-J: Cost effective production of bioproducts
- Productivity, yield
- Reduce cycle time for biocatalyst development
  - Design tools
  - Construction tools
- Bt-B: biomass variability

Partners

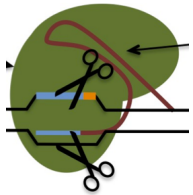
- Cellulosic sugars
- Fermentation Scaling
  - DOE-funded ABPDU

Project Overview & Technical Approach

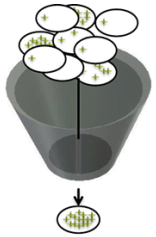
Overarching Goal: Develop an integrated approach to biochemical pathway optimization for production of malonic acid

- **Objective #1: Genome-scale metabolic node perturbation**
  - ***In silico*** **biocatalyst design & management software**
  - **Multiplexed biocatalyst engineering, diversity creation, & targeting tools**
- **Objective #2: Deploy biosensor screening method to enable faster identification of improved biocatalysts**
  - **Biocatalyst evaluation via biosensor screen**
- **Objective #3: Use statistical approach to guide biocatalyst design**
- **Objective #4: Translate benchtop fermentation metrics to pilot scale**

in silico **design**



biocatalyst construction tools



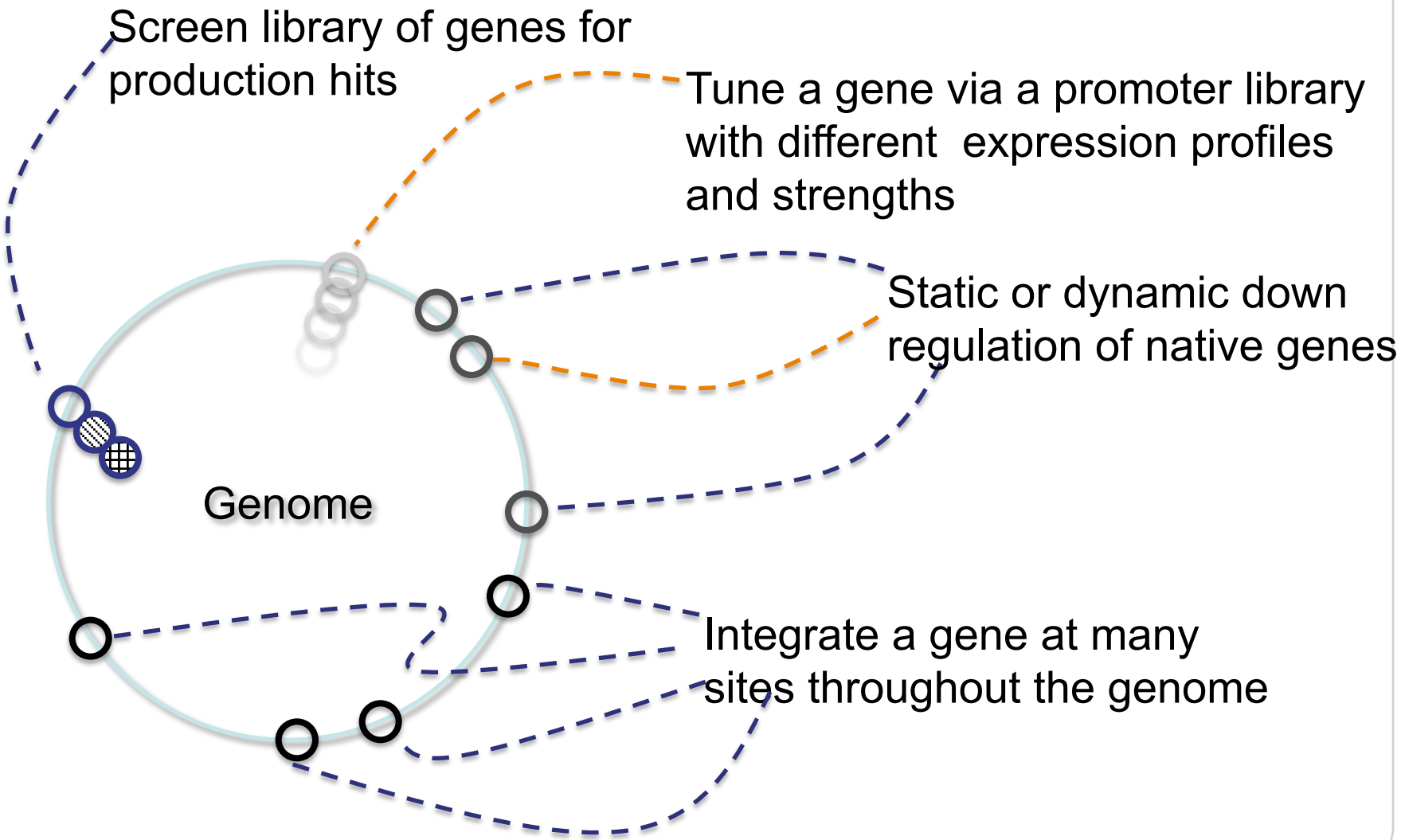
high throughput screening

scale up

Project Approach - Management

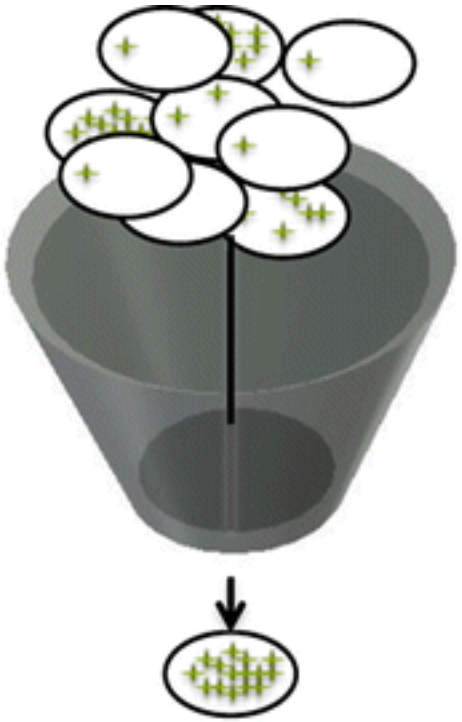
- Lygos is responsible for biocatalyst design, screening, and fermentation process development
- Leveraging expertise of partners
  - Lignocellulosic feedstocks – commercial providers
  - Fermentations scale up – eg., ABPDU
- Materials & facility scheduling are planned in advance
- Go/no-go decision points support objectives
  - DNA construction capacity
  - Screening capacity
  - Fermentation metrics (yield, productivity, scale)

Task A: Software tools for designing & constructing biocatalysts



Task B: Biosensor-based screening

Genetic diversity is created which results in individual biocatalysts that produce different levels of malonic acid - but we don't know which ones.



Large, diverse population

↓

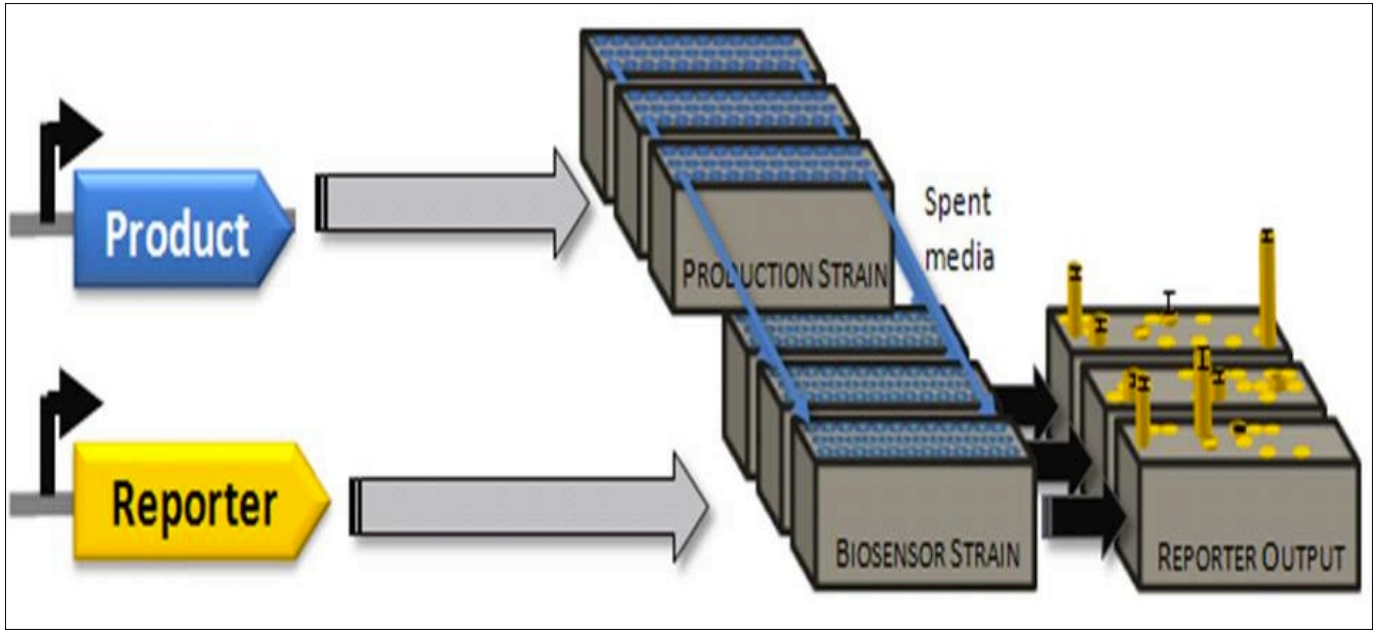
Identify improved production strain(s)

Biosensor screens can offer the most cost effective and direct measurement.

Dietrich JA et al. ACS Synthetic Biology 2013

Task B: Biosensor-based screening

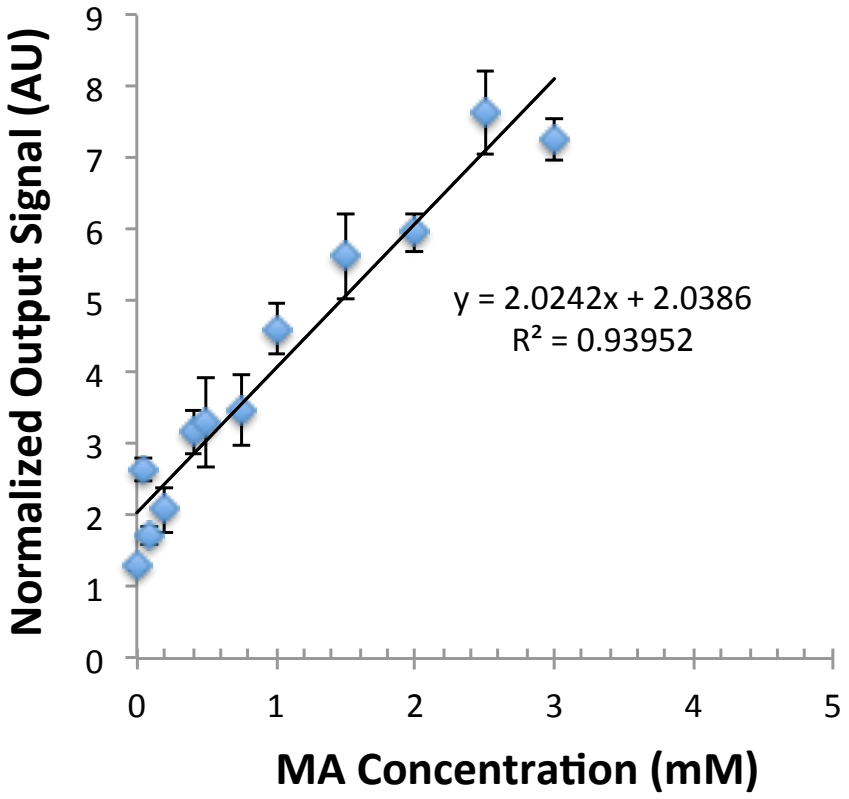
Malonic acid concentration is detected via biosensor strain



Step 1: Malonic acid (MA) producing strains are grown
Step 2: Spent media is transferred to a standalone biosensor strain culture
Step 3: Biosensor strain grows proportionally to MA concentration

Task B: Biosensor-based screening

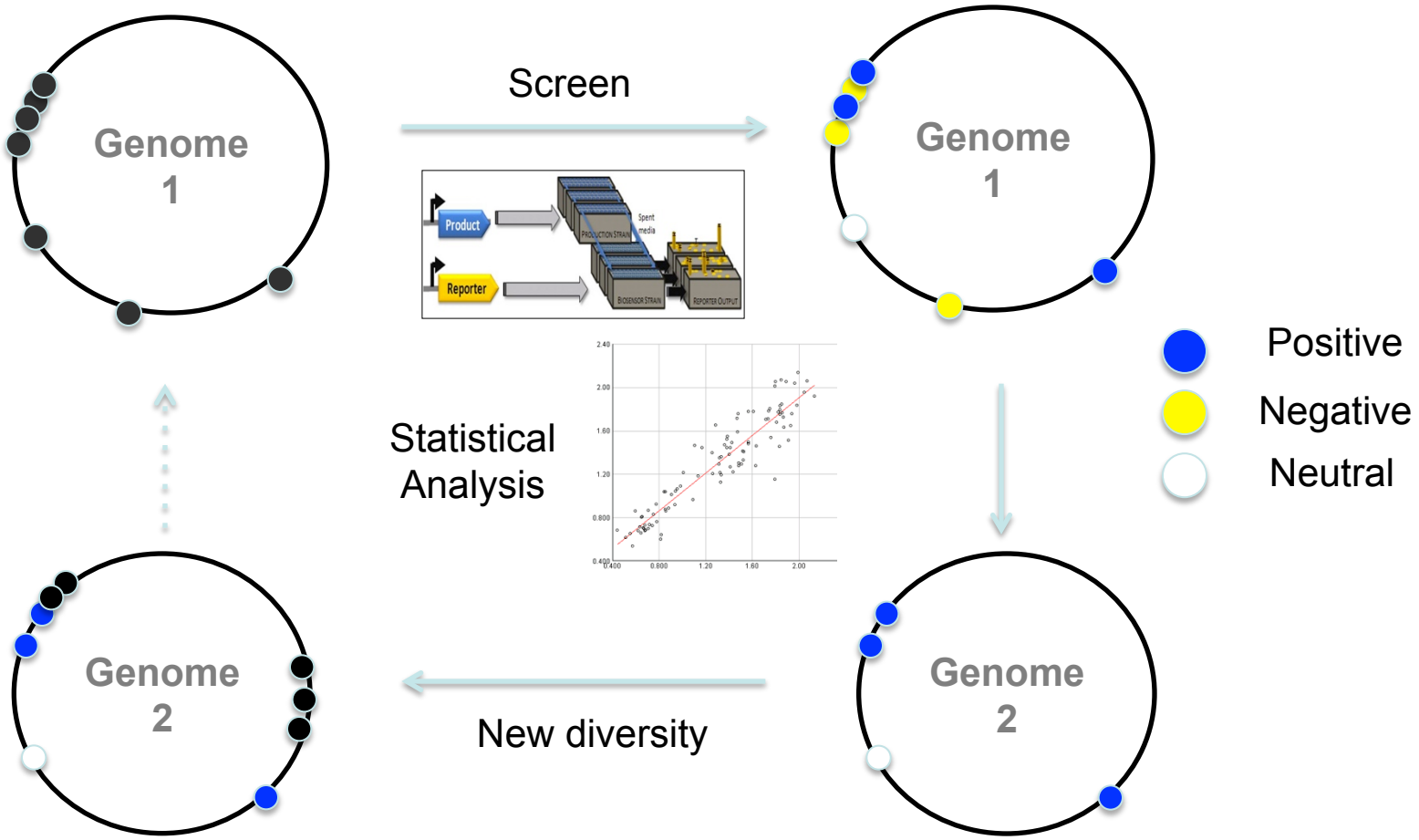
Output of the biosensor screen demonstrates a linear range of malonic acid detection



Improved biocatalysts are identified

Task C: Metabolic hotspot ranking

Metabolic hotspots are ID'd & ranked via statistical analysis in order to more efficiently identify subsequent engineering targets



Task C: Metabolic hotspot ranking

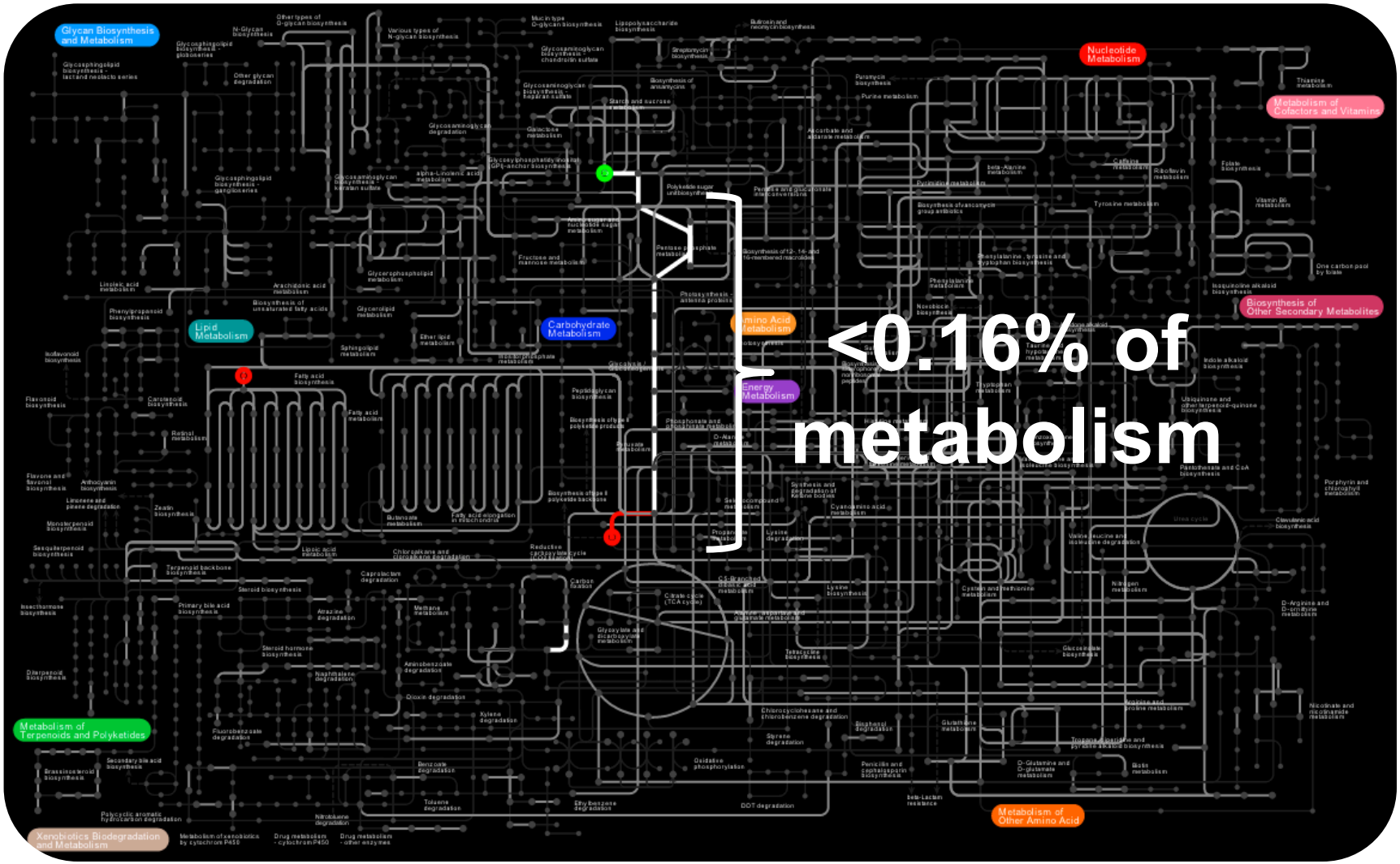
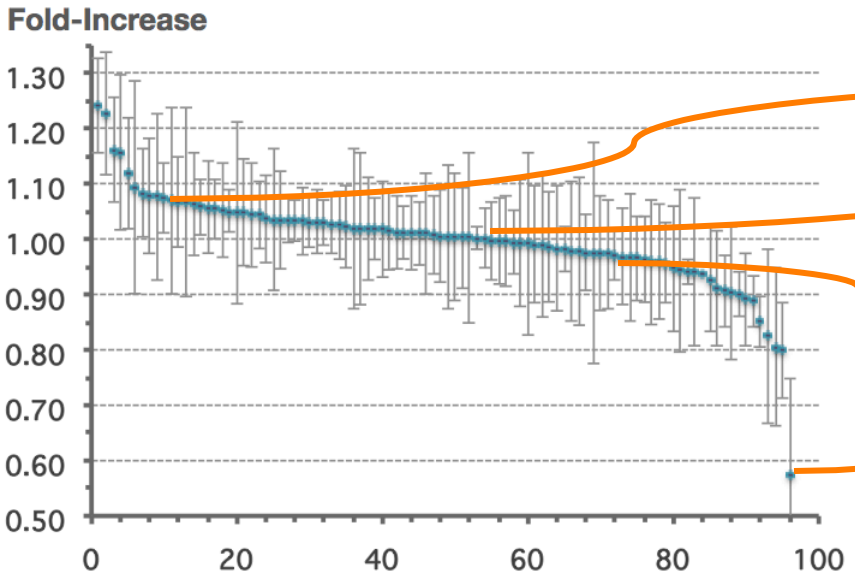
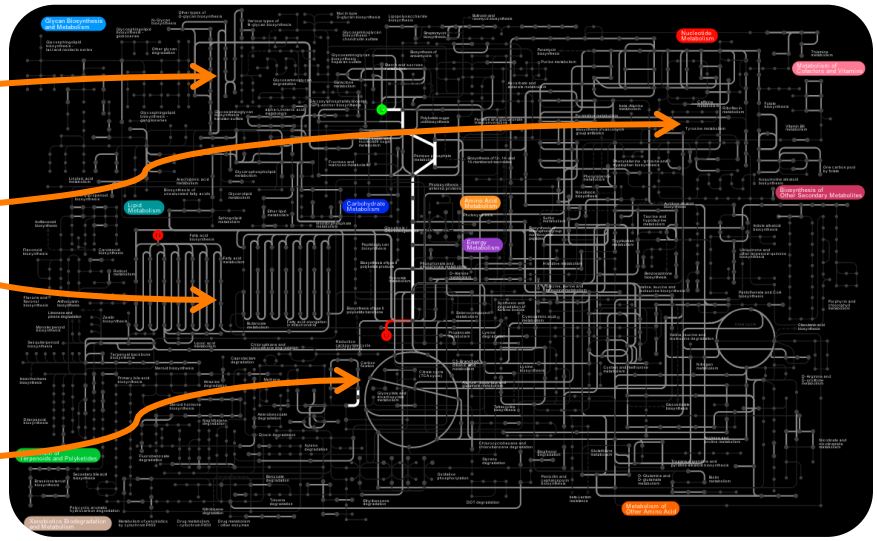


Photo credit: Arkin et al. http://glamm.lbl.gov/

Task C: Metabolic hotspot ranking – transcription factors



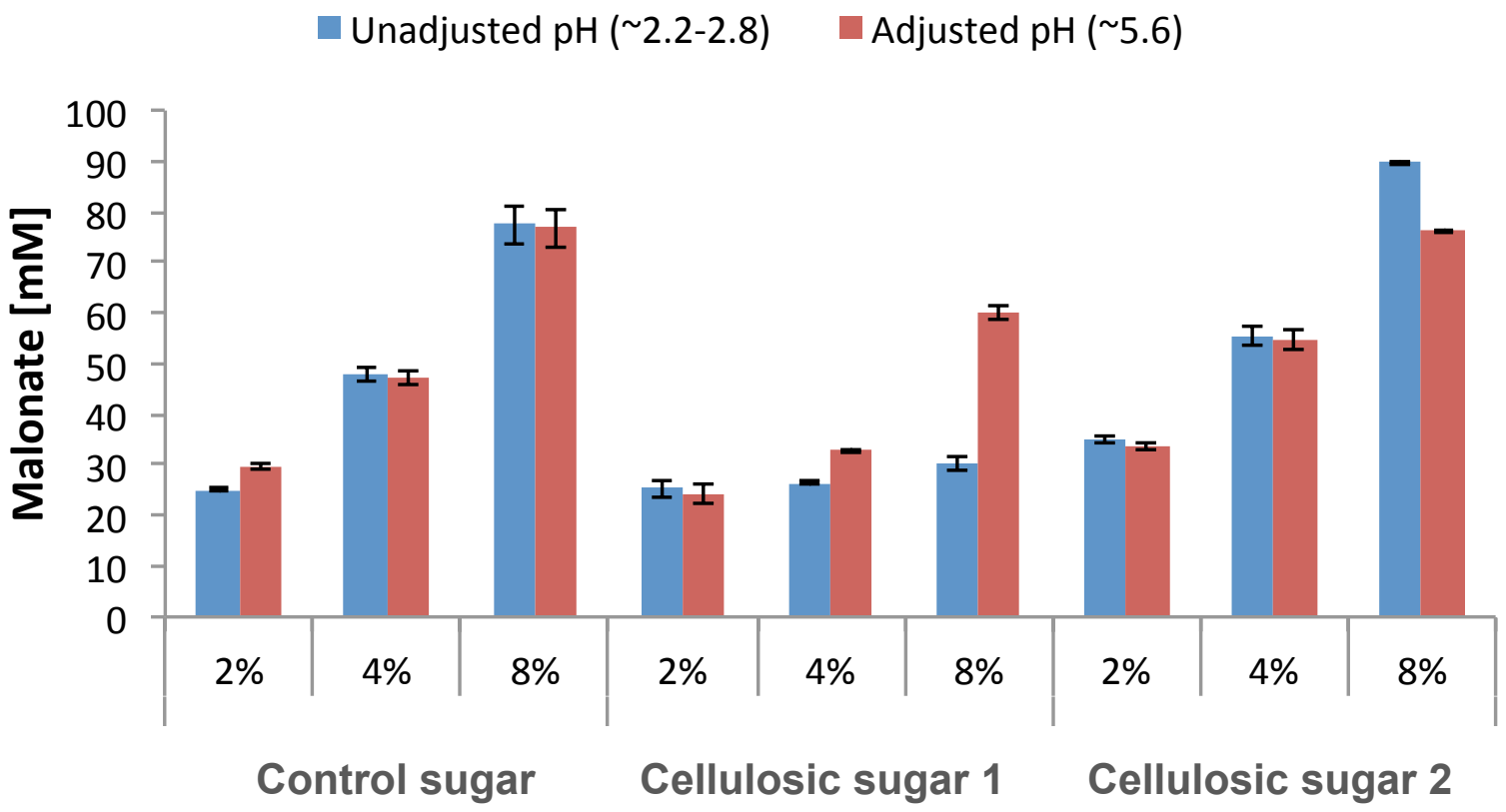


Metabolic Map

- New target identification
- Perturbation refinement
- Metabolic hotspot ID & rank

Guiding iterative metabolic engineering

Task C: Plate Performance Using Cellulosic Sugars

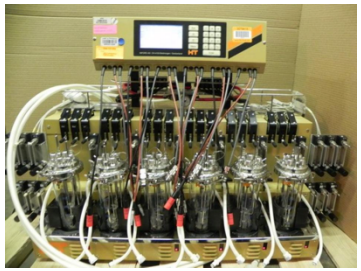


- Obtained samples from Commercial Suppliers
- Similar strain performance between glucose and cellulosic sugars
- Model organisms do not grow (data not shown)

Task D: Translate plate screening metrics to pilot scale

Addressing barrier to translating small scale experiments to relevant fermentation process

0.5 mL



500 mL

300 L

Successful initiation of process development & scaling to 300-L; Achieved 90% of metrics across scales

Relevance

The work in this project promotes:

- Tools for cost effectively building and validating biocatalysts
- Replacing petro products with lignocellulosic-derived products
- Technoeconomic analysis suggests that upon successful commercialization could result in production cost of $1.60/lb; down from project initiation of >$600K/lb
- The potential to reduce carbon dioxide emissions related to petroleum produced malonic acid
- Identification of pathway targets may aid in development of other bio-products & fuels
- Synthetic evolution process could be applied to other products

Future Work

- **Package & distribute automated design tools**
- **Further expansion of biocatalyst construction & screening capacity & workflow**
  - Key milestone (10-20 plates up to 100 plates)
- **Achieve plate and tank fermentation metrics**
  - Key milestone in plates (yield, titer, productivity)
  - Key milestone in 300-L (yield, titer, productivity)
- **Downstream purification process** (beyond scope of project)
- **Benchtop Process Integration** (beyond scope of project)
  - Scale up integration risks
  - Feed & Process variation effects
  - Integration of biomass process
- **Derivative Product Development** (beyond scope of project)

Summary

- **Overview:** designing & constructing biocatalysts to consume cellulosic sugars to produce bioproducts (malonic acid)
- **Approach:** deploying synthetic biology techniques to accelerate the path toward commercialization (eg., design tools, screening tools, and validation).
- **Technical Accomplishments:**
  - Design software & tools; Biosensor screen
  - Continuously improved strains are being developed (Y,T,P)
  - Successful initiation of pilot scale fermentations
- **Relevance**
  - Bt-J Cost effective production of bioproducts ($1.60/lb)
- **Future Work**
  - Attain YTP milestones; expand workflow capacity

Acknowledgements

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- ABPDU
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Additional Slides

Responses to Previous Reviewers' Comments

- Project has not previously been reviewed

Publications, Patents, Presentations, Awards, and Commercialization

Patents:

- None published relating to this grant, but we are developing inventions previously described in PCT Pub. No. 2013/134424 in work supported by the grant.

Presentations:

- Steen EJ. *Synthetic biology for brewing.* Synbiobeta Lecture: Synthetic Biology for Computer Programmers. October 8, 2013, San Francisco, CA.
- Steen EJ. *An industrial perspective on synthetic biology.* Synbiobeta Lecture: Synthetic Biology for Computer Programmers. November 31, 2013 San Francisco, CA.

Commercialization:

- **Financing –** seeking capital for reaching next TRL
- **Offtake –** early discussions for use in a number of markets
- **Internal product development effort**