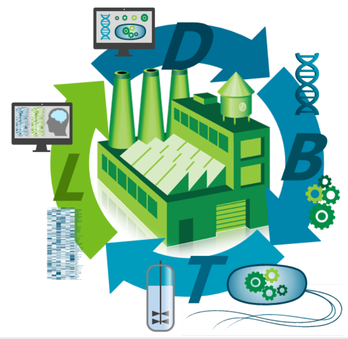
Agile BioFoundry

Agile Genetics for Biomanufacturing

CPS# 33729e, WBS#2.5.3.705

Ramesh K. Jha



Project Type

- *DFO with Agile BioFoundry*
- ***DFO Goal**: Accelerate innovation and adoption of new biomanufacturing approaches and foster the growth of the US bioeconomy*

Project Timeline

- *Project Start Date: 10/1/2018*
- *Project End Date: 09/30/2020*
- *Percent Completed: 12.5%*

Funding

		FY19	FY20
DOE Funding to Labs	Total	250,000	250,000
	LANL	140,000	140,000
	NREL	110,000	110,000
Academic Cost Share		107,143	107,143

Management Approach

- *Collaborative effort between LANL, NREL and UGA*
- *Conference call every two weeks, additional ad hoc discussions via email*

Team

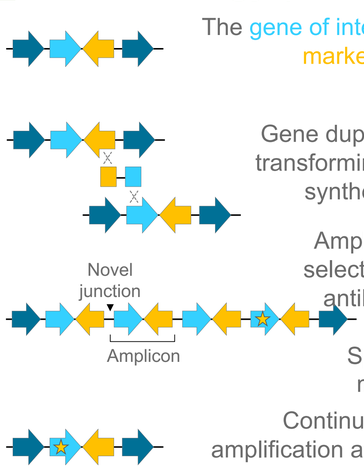
Name	Role
Ramesh Jha, Ryan Bermel	Biosensor, protein design Molecular biology, strain screening
Taraka Dale	Mentorship, management
Isabel Pardo Chris Johnson Gregg Beckham	EASy, microbial engineering Metabolic engineering Mentorship, management
Stacy Bedore Alyssa Baugh Ellen Neidle	*Acinetobacter baylyi* ADP1 expertise and consultation

Project Background

ADP1 as a platform organism

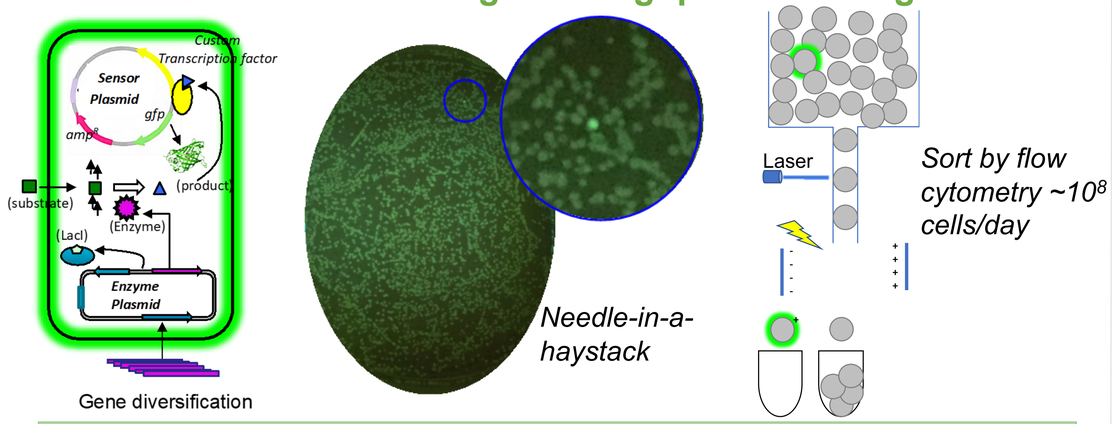
- Naturally competent
- Unique genetic system: exceptionally high frequency of natural transformation and recombination
- Easy to alter the chromosome
- Readily uptakes plasmids and linear DNA from the media
- Whole genome resequencing allows facile identification of chromosomal mutations
- Grows quickly (aerobically)
- Metabolic capabilities that differ from other model organisms
- New synthetic biology tools being developed

Evolution by Amplification and Synthetic biology (EASy)



- *Increased gene dosage allows evolution of genes that do not enable growth in single copy*
- *Combine with diversified DNA libraries*

Custom Biosensors for High Throughput Screening of Titers

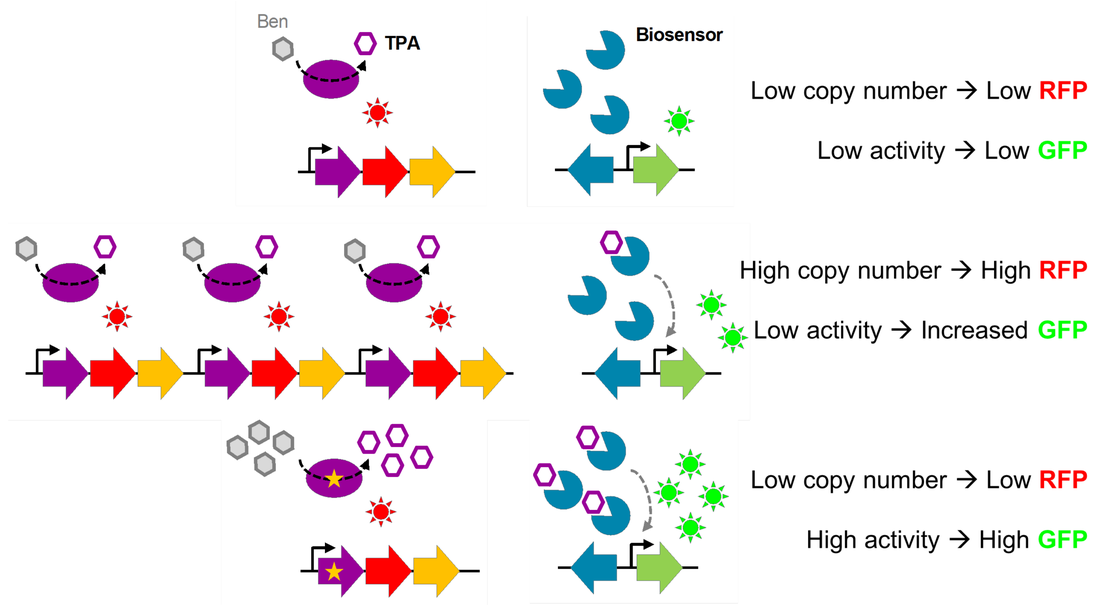


- *Link strain performance (metabolite titers) to detectable fluorescence signal*
- *Couple to flow cytometry exploiting the massive throughput for screening*

Overall Goal

Combine biosensor technology with ADP1 and EASy to rapidly create and screen for genomic variants with increased production of TPA

Technical Approach



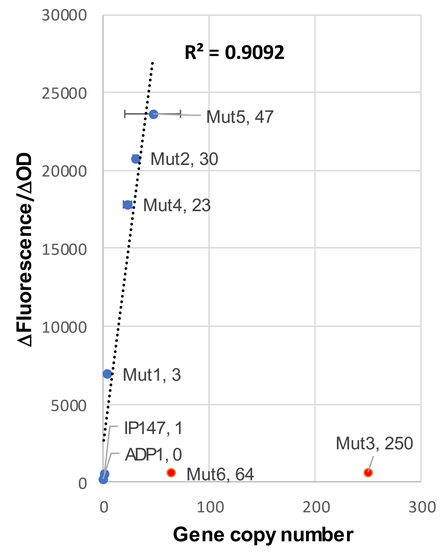
- *Gene copy number increases in high antibiotic concentration*
- *RFP as a quick indicator of gene dosage*
- *TPA biosensor as a direct indicator of metabolite production*

Potential Challenges

- *Biosensor may lack sensitivity and will be evolved for detection range*
- *Heterologous enzyme is a decarboxylase (catabolic TPA→Benzoate is a preferred reaction); the reverse reaction needs to be made preferable via evolution and engineering*
- *Achieving a high TPA titer and productivity from renewable substrates will be critical for Technical and Commercial viability*

Technical Progress

sfCherry amplification mutants



- *Protein based sensors that can bind to TPA and induce conformational changes, which can be exploited in some form of detectable signal*
  - *Aromatic molecule binders selected*
    - ➢ *Protein design to accommodate TPA molecule*
    - ➢ *Adaptation of the sensor to the ADP1 background*
- *sfGFP and sfCherry successfully expressed in ADP1*
- *Gene amplification of sfCherry and a limited correlation (up to a copy number of 50) between fluorescence and gene copy shown in ADP1*
- *TPA transporters established in ADP1 for initial optimization of biosensor*

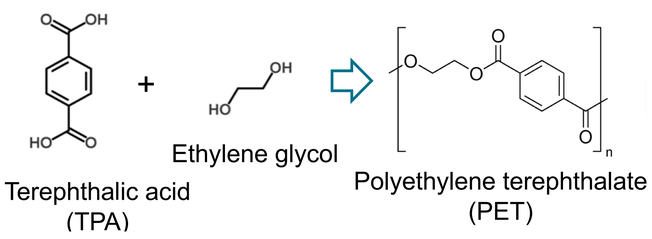
Future Plans

- *Establish TPA biosensor in ADP1*
- *Demonstrate a detection range between 100 µM and 5 mM for the TPA biosensor*
- *Investigate (de)carboxylases for aromatic molecules as potential scaffolds for design and evolution*
- *Show gene amplification for the carboxylase in ADP1*
- *Expedite the evolution process by rationally designed enzyme library*
- *Follow dynamics of gene dosage and TPA production over time*
- *Dual channel FACS for measuring the gene dosage (RFP) and the TPA titer (GFP)*
- *Achieve a TPA titer of >100 mg/L in ADP1 using Benzoate as a substrate*

Relevance and Impact

Terephthalic Acid (TPA): >$30 B Global Market

TPA consumption expected to be 65 million tons in 2018



- *New tools developed that are generalizable to different host strains and target molecules*
- *Platform chemicals and polymer precursor from renewables using engineered microbes*
- *Biomanufacturing will support biorefinery and boost bio-economy*

Supports 2020 BETO Goal:

*"**By 2020**, provide enabling capabilities in synthetic biology for industrially relevant, optimized chassis microorganisms and design-build-test-learn (DBTL) cycles for fuel and chemical production that reduce time to scale-up by at least 50% compared to the current average of ~10 years."*

References

(1) Elliott, K. T.; Neidle, E. L. *Acinetobacter baylyi* ADP1: Transforming the Choice of Model Organism. *IUBMB Life* **2011**, *63* (12), 1075–1080.
(2) Jha, R. K.; Kern, T. L.; Fox, D. T.; Strauss, C. E. M. Engineering an Acinetobacter Regulon for Biosensing and High-Throughput Enzyme Screening in *E. Coli* via Flow Cytometry. *Nucleic Acids Res.* **2014**, *42* (12), 8150–8160.
(3) Tumen-Velasquez, M.; Johnson, C. W.; Ahmed, A.; Dominick, G.; Fulk, E. M.; Khanna, P.; Lee, S. A.; Schmidt, A. L.; Linger, J. G.; Eiteman, M. A.; et al. Accelerating Pathway Evolution by Increasing the Gene Dosage of Chromosomal Segments. *Proc. Natl. Acad. Sci.* **2018**, 201803745.

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