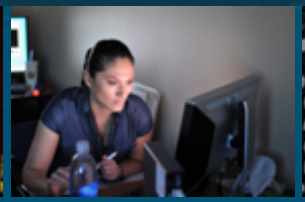
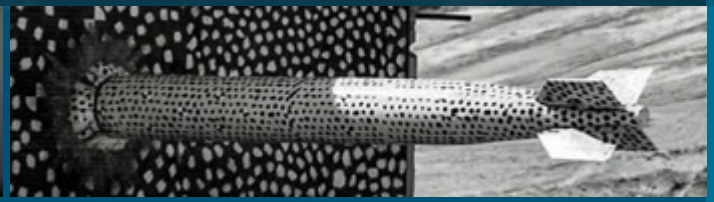
UT Austin | Sandia Technical Exchange





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Synthetic Biology

Anne Ruffing (aruffin@sandia.gov)

Synthetic Biology for Biofuel and Biochemical Production

CRISPRi modification of *Synechococcus* sp. PCC 7002 for enhanced biomass production

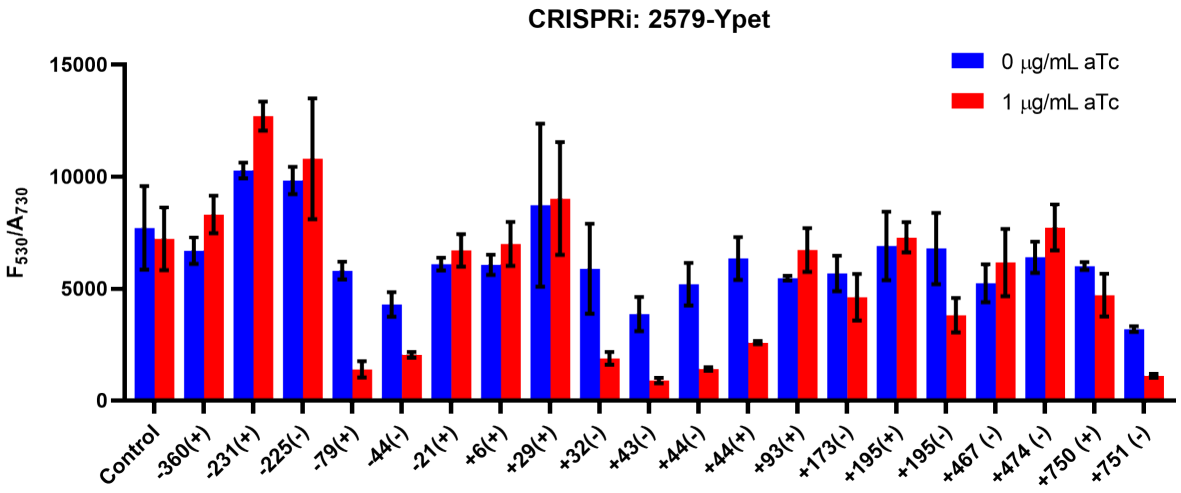
- CRISPRi design rules for cyanobacteria
- Shuttle vector for gRNA expression in 7002
- Improved operon prediction using RNA-seq data and machine learning algorithms
- Metabolic Flux Analysis (Collaborator: John Morgan, Purdue University)

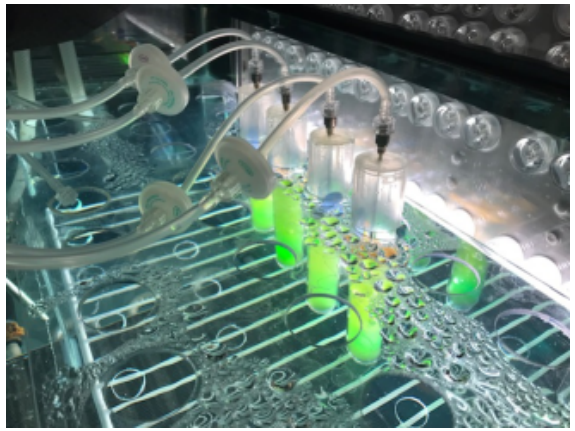
Engineering *Microchloropsis gaditana* to reduce dark loss for increased biomass production

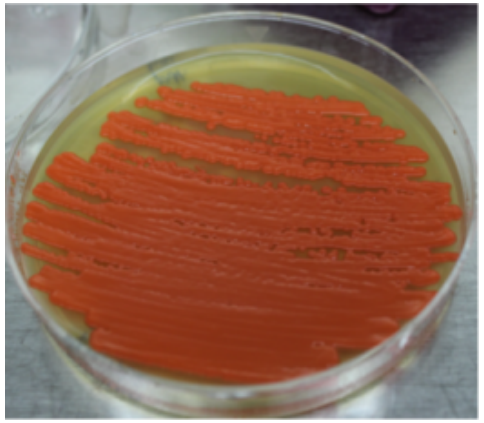
- CRISPR/Cas9 editing for gene knockout
- CRISPRi (dCas12a) for gene knockdown

Development of RNAi and CRISPRi tools for gene knockdown in *Rhodosporidium toruloides* (Agile Biofoundry)

- Improve terpene production







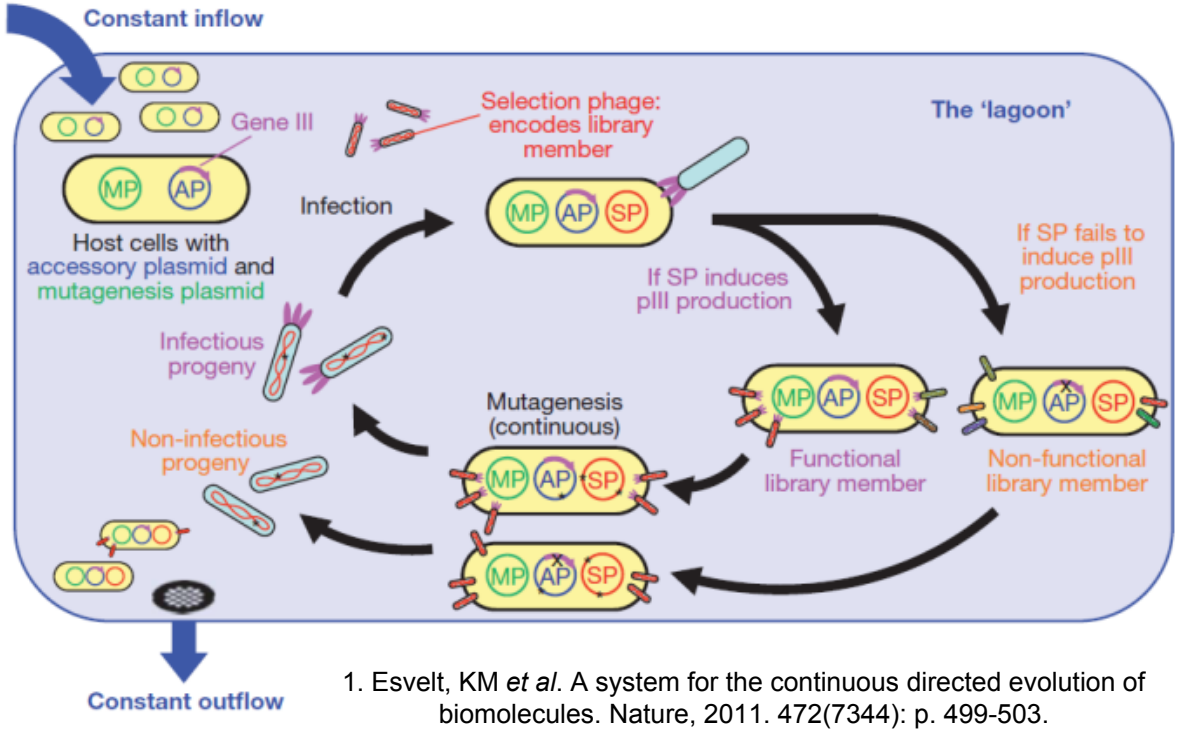
Synthetic Biology for Biosensor Applications

Engineering noble gas biosensors using phage-assisted continuous evolution (PACE)

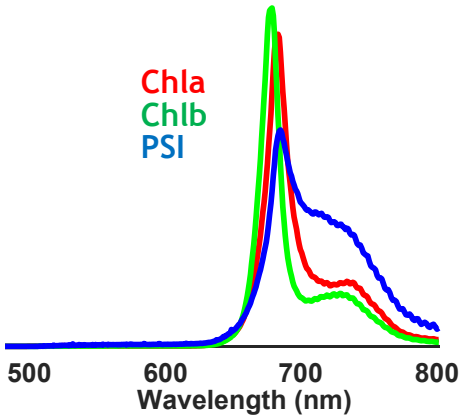
- Understanding mechanisms of noble gas interactions in *E. coli*
- PACE for rapid protein evolution (Collaborator: Ahmed Badran, Broad Institute)

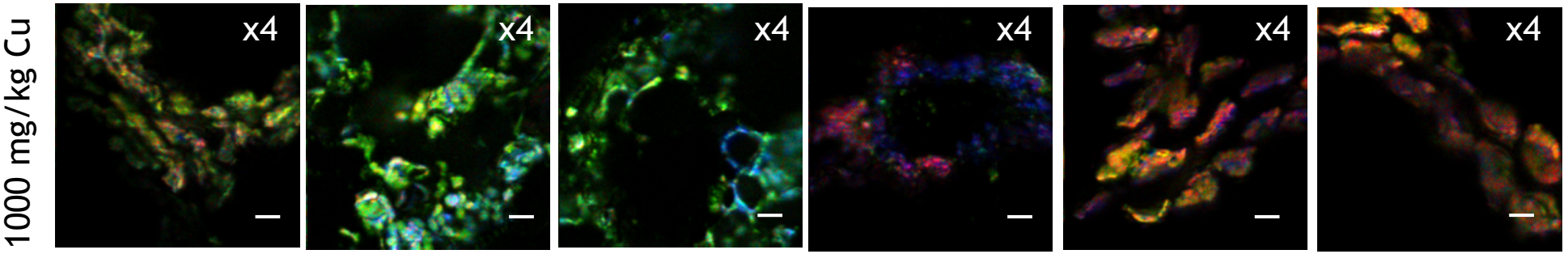
Using bioindicators to detect source emissions

- Hyperspectral imaging and multivariate curve resolution analysis to detect spectra in tall fescue
- Hyperspectral confocal fluorescence microscopy



1. Esvelt, KM *et al*. A system for the continuous directed evolution of biomolecules. Nature, 2011. 472(7344): p. 499-503.

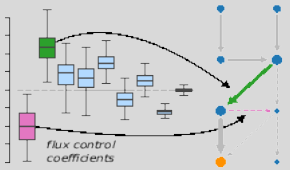




Sandia Biomanufacturing- the Agile BioFoundry

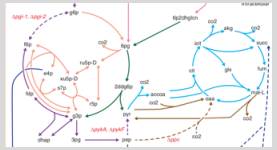
John Gladden (jmgladd@sandia.gov)

ABF DBTL Infrastructure

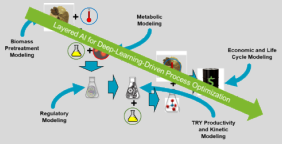


Bayesian Inference: Metabolic Kinetics

Kinetic Learning



Metabolic Modeling



Deep Learning

art

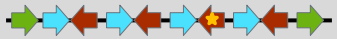
Design

DiVA

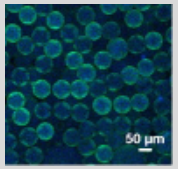
Sequence Validation

Learn

Build



EASy



Biosensors

LabKey

Transcriptomics, Metabolomics, Proteomics, & Lipidomics

Test

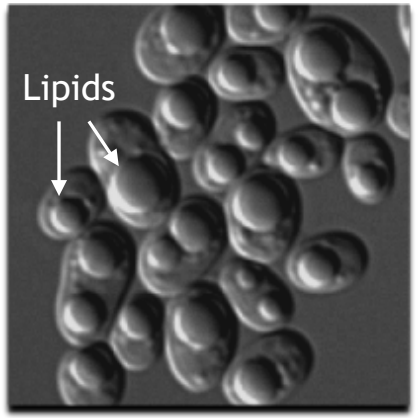
EDD

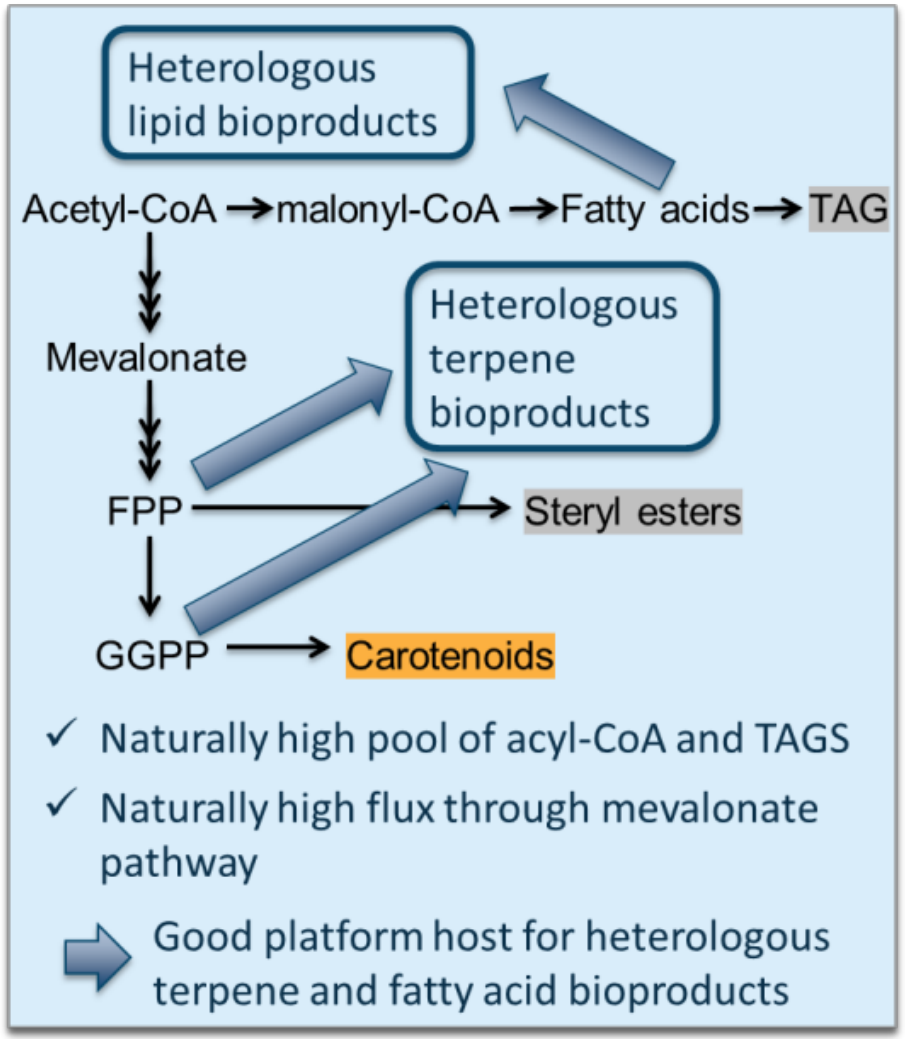
Data Quality Assessment

ABF Host- Rhodosporidium toruloides

Rhodosporidium toruloides

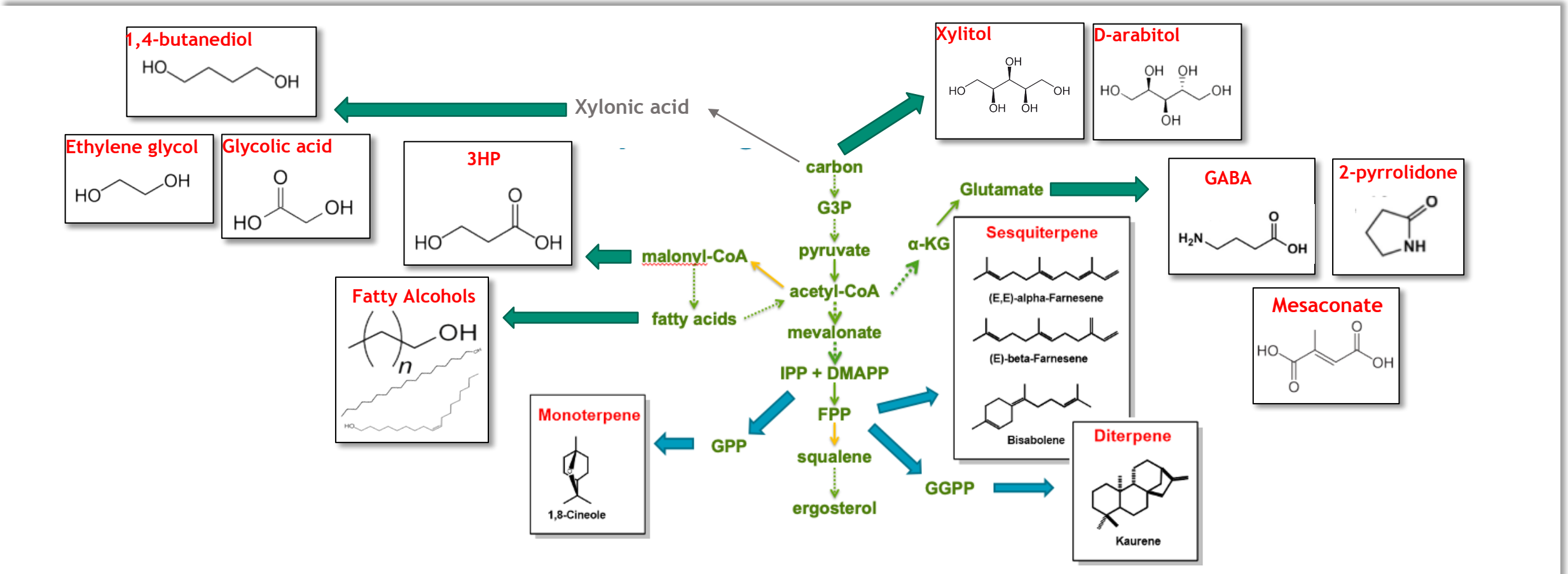
- Utilized lignocellulos
- Fast growing
- Oleaginous, carotenogenic
- Metabolically versatile
- Genetically tractable





Current Targets in *R. toruloides*

- Pyrophosphates: Terpenes- biofuels and bioproducts (adhesives, insect repellents, polymers, fragrances, food additives)
- Malonyl-CoA: Fatty Alcohols- Detergents, lubricants, plastics and cosmetics. $5.2 billion in 2011 globally. Grow at 4% CAGR in next decade. 3HP transfer target- acrylate polymers, biodegradable polymers
- Xylose: sugar alcohols and xylonic acid- top value-added chemicals from biomass to make polymers, plasticizers, concrete dispersal agents, adhesives, etc.
- Glutamate: mesaconate, GABA, and pyrrolidone- polymers, solvents, hydrogels, dyes, and flame-retardant materials



Potential Partnerships

Research interests:

- Developing non-model microbial biomanufacturing hosts
- Expertise in fungal and bacterial systems

Potential areas for partnership:

- Characterization on non-model organisms (growth, physiology, genomics, metabolic models, etc.)
- Synthetic biology tool development
- Automation- development of high-throughput engineering methods
- Metabolic engineering for production of biofuels and bioproducts

Biotechnology & Bioengineering

Ramdane Harouaka (rharoua@sandia.gov)

Microfluidics & Microfabricated Platforms

High-throughput screening

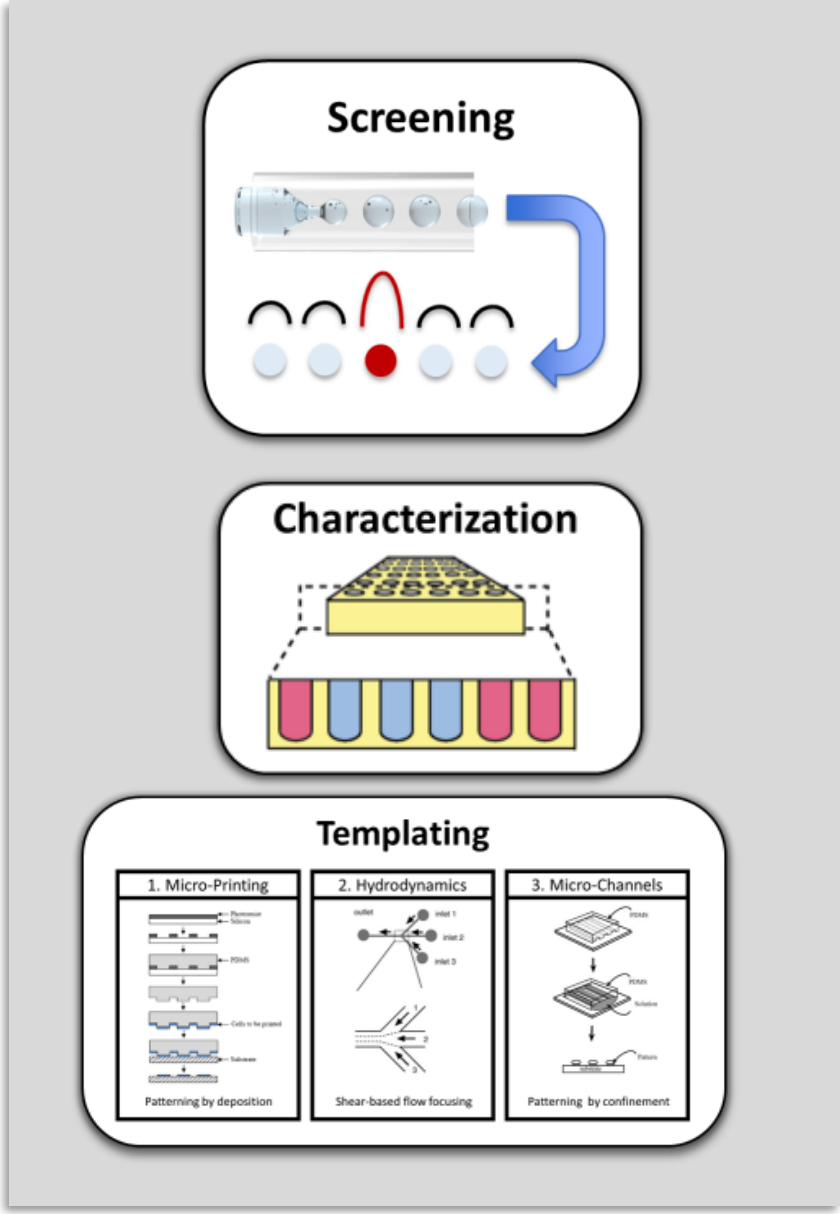
- Droplet assays for clonal identification/selection
- Optical, chemical, functional, proteomic, etc

Single-cell Characterization

- Multi-species Combined / Dual RNA-Seq
  - Massively multiplexed probe libraries
  - Semirandom amplification
- Microwell platforms for combined Imaging + Transcriptomics

Microfluidic Templating

- On-chip biosynthesis and packaging
- Fabrication of ordered structures



Virus Engineering

Todd W. Lane | DMTS (twlane@sandia.gov)

Virus Engineering for Advanced Algal Biofuels & Bioproducts

Target 1: Polinton Like Viruses

- Polintons are virus-like self-synthesizing transposons wide spread in the genomes of diverse eukaryotes
  - All encode a protein-primed DNA polyermase and retrovirus-like integrase
  - Most encode:
    - an ATPase and maturation protease homolgous to enzymes of NCLDV
    - homologs of major and minor capsid proteins
- PLVs share characteristics and some core genes with both Polintons and virophages

- *Tetraselmis striata* virus (originally *Tetraselmis viridis* virus)
- 31 kb genome dsDNA
- 60 nm Icosahedral virion
- 33 ORFs

- "Restore" the polymerase and integrase functions of the Polinton either by complementation in the host or engineering of the virus
- Remove either or both of the major and minor capsid proteins.
- Create a production host for viral packaging
- and a insertion competent host.