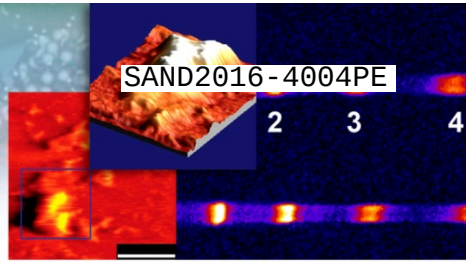
SANDIA BIOSCIENCE

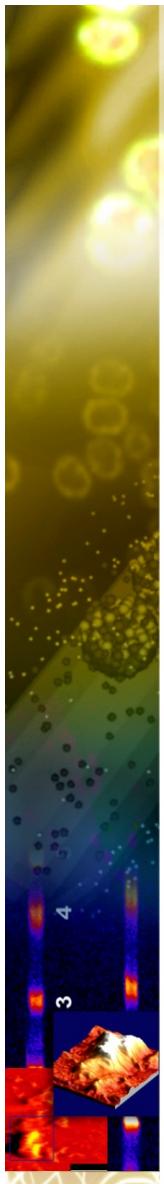


Bioinformatics and Computational Biology at a National Laboratory

Corey Hudson
MUII Conference
April 5, 2016

Mission of biology at a National Lab

- The mission of the national labs:
  - **Biodefense**: Tools to predict and detect biological attacks
  - **Bioenergy**: As a Department of Energy facility, national labs are heavily involved in the US government energy mission - including bioenergy
    - **Historically, this meant developing developing next generation biofuels**
    - **More recently, biological production of high value petroleum derivable chemicals, e.g., *adipic acid* which is petro-derived and produces huge amounts of N_2O.**



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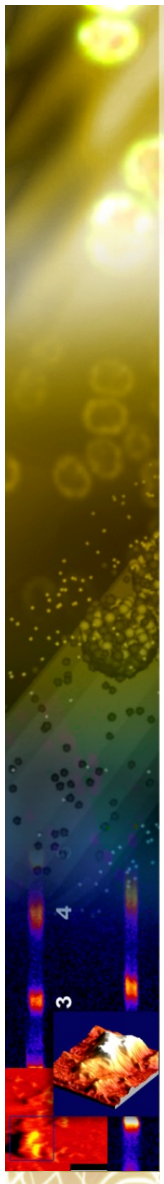
My postdoctoral work at Sandia

- Started **2012** as a **postdoctoral researcher**
- Projects:
  - Characterize genome and mobilome of highly antibiotic resistant bacteria: *Klebsiella pneumoniae* BAA-2146

    Responsible for nosocomial infections in India - known as the New Delhi *Klebsiella*. First US isolate.
  - Predict lignolytic enzymes in Sevilleta Arid Lands in New Mexico microbiome.
  - Build database of high quality mobile genomic islands in bacteria.



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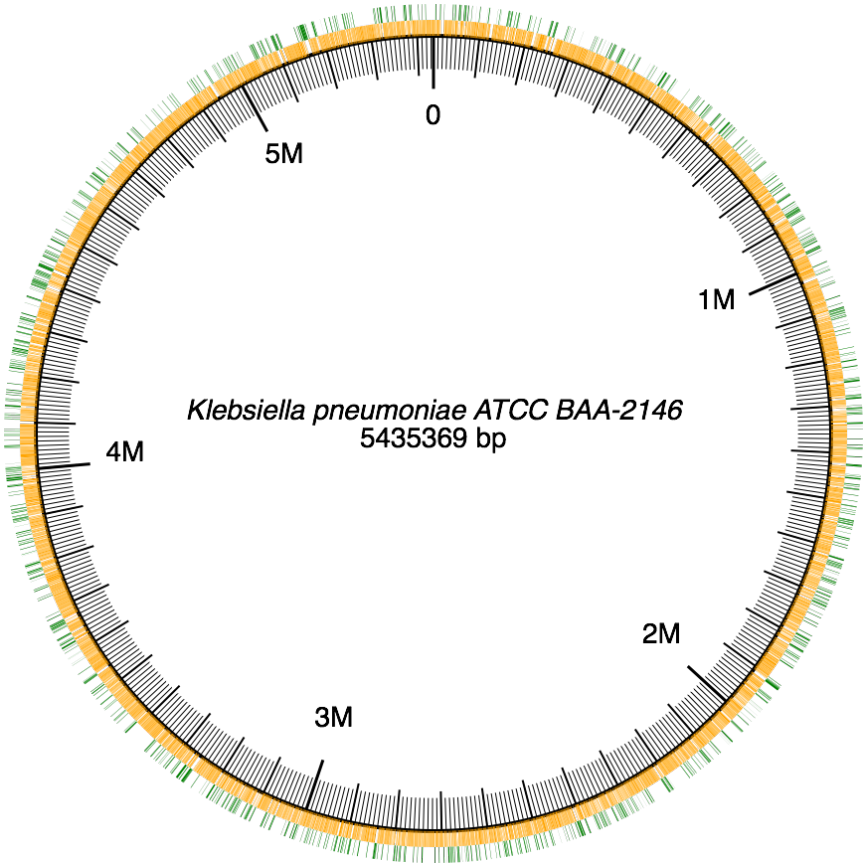
Identifying resistance elements in multidrug resistant genomes

- *Klebsiella pneumoniae* BAA-2146
- Strain sequenced from 2010 isolate
- Carries NDM-1 plasmid
- Resistance tested for 34 antimicrobial and antimicrobial/inhibitor combinations tested



Hudson, Bent, Meagher, Williams. (2014) Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding *Klebsiella pneumoniae* Strain. *PLoS One* 9(6): e99209

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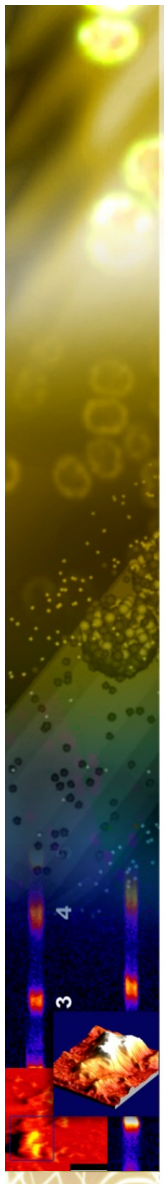
Mobile genomic elements as a source of pathogenicity and resistance

- Plasmids (inter-bacterial)
  - Primary source of resistance in *Klebsiella pneumoniae*
  - Can also include independently mobile resistance integrons
- Genomic islands (inter-bacterial)
  - Common source of pathogenicity in bacteria
- Chromosomal integrons (intra-bacterial)
  - Common source of resistance in bacteria
- Insertion sequences (intra-bacterial)
  - Often capable of affecting virulence through the activation and repression of neighboring genes



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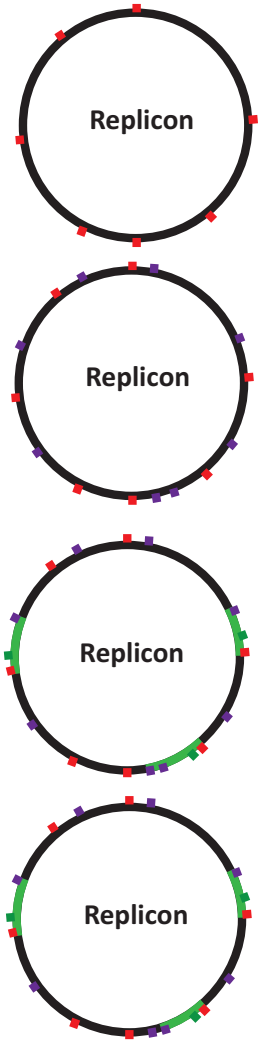
Features of genomic islands

- Genomic islands are large insertions into the genome by means of an integrase.
- These genomic regions can carry large numbers of genes.
  - Allows them to rework bacterial metabolism (e.g., iron uptake)
  - Many carry bacterial toxins
  - Secretion systems and effector proteins
  - Capsule synthesis proteins

Islander: An algorithm for discovering high-quality islands

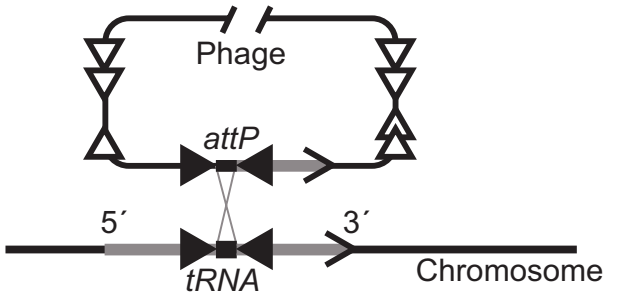


Place tRNA/tmRNA on replicon

BLAST tRNA/tmRNA against replicon to find fragments

Identify integrases occurring between tRNA/tmRNA and their associated fragments

Deduplicate overlapping regions



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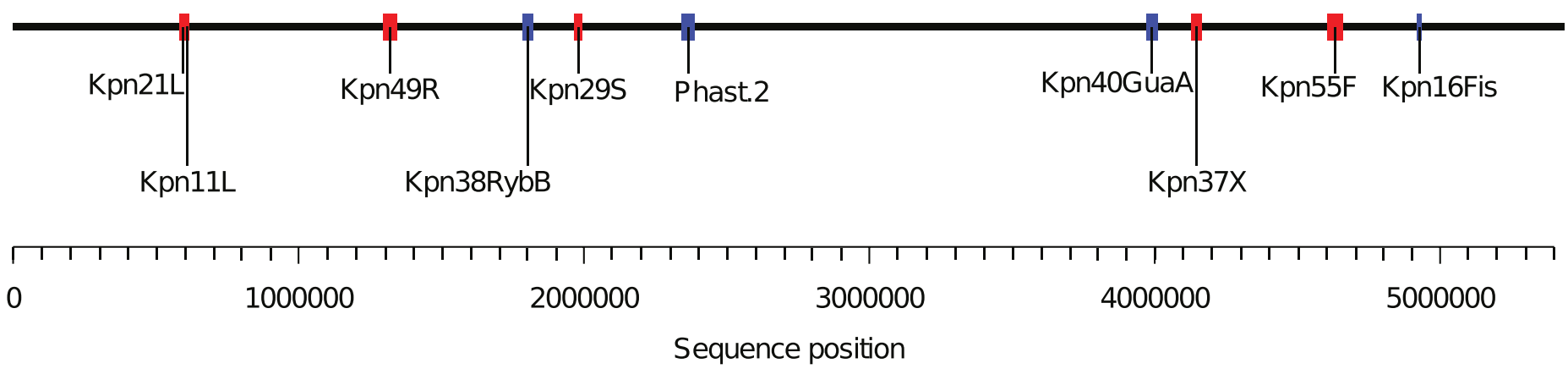
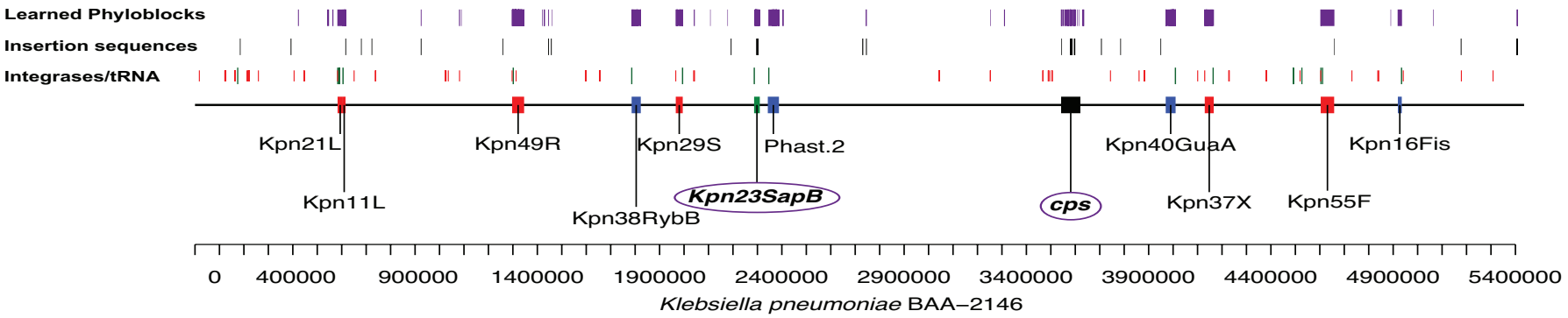
Annotating genomic islands in *Klebsiella pneumoniae* BAA-2146



- Annotated 6 genomic islands using this algorithm (Islander)
- Included 4 additional islands using PHAge Search Tool (PHAST)
- Amounts to 342 kb out of 5429 kb

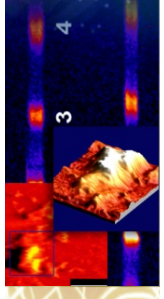
Using comparative genomics to ID islands and and insertion sequences



- Points to four insertion sequences
  - 2 IS*Ecp1*
    - 1 carrying β-lactamase CTX-M-15 that was recently transferred to the chromosome (a first in any complete genome) from one of its plasmids and in plasmids, predicted to confer resistance to β-lactam
  - 2 IS*Kpn18*
    - 1 disrupting RamR, a negative regulator of RamA, predicted to confer tigecycline resistance

Switching gears: Breaking down lignin

- Lignin, an irregular, complex aromatic molecule in higher plants and some algae, is the second most abundant biopolymer on the planet, making up between 10% and 25% of plant biomass.
- Limits the ability to break-down plant materials into biofuels.
- Question: Can high-lignin turnover environments be prospected to identify new classes of lignin breakdown enzymes?



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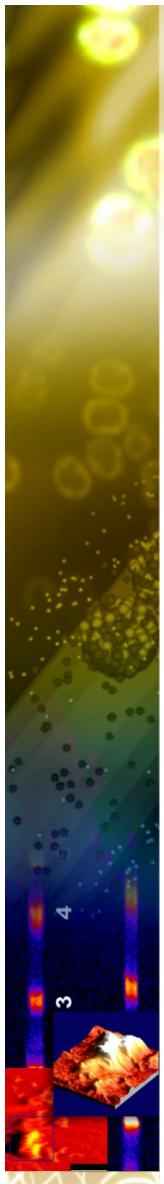
Hudson, et al (2015) Lignin-Modifying Processes in the Rhizosphere of Aridland Grasses. *Environmental Microbiology* 17(12)

Tools for engineering microbial lignolytic breakdown

- Most microbiome annotators work at the broadest of functional categories (e.g., *secondary metabolism* or *virulence, disease* and *defense*)
  - Not useful discovering novel genes in a metagenome
  - Inefficiently annotate entire metagenome
- Need for tools that are targeted
  - Include expert knowledge of gene feature
  - Target annotation
  - Use rule-in / rule-out gene families with careful thresholding to remove spurious reads



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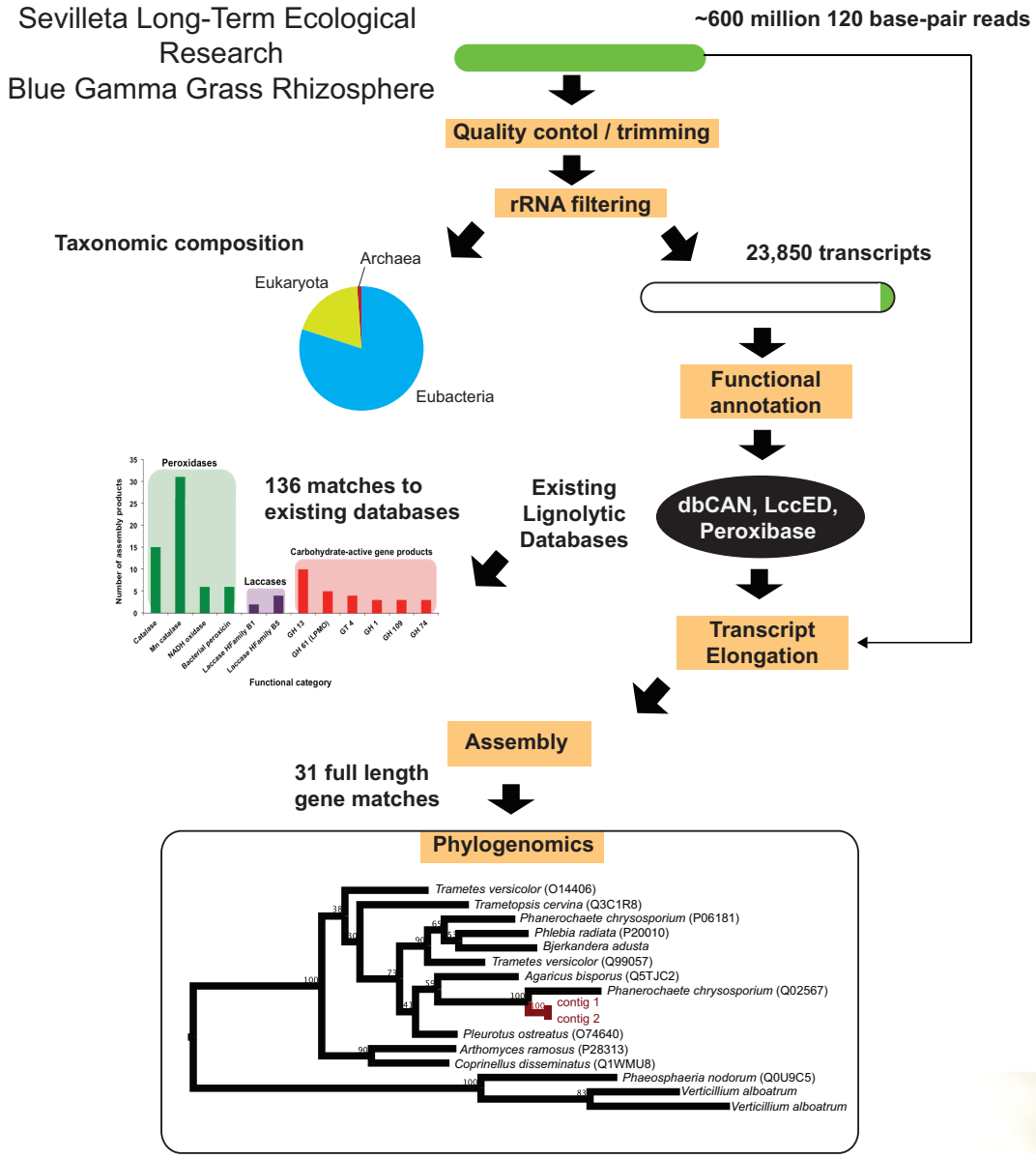
Pipeline for Lignin-active Enzymes



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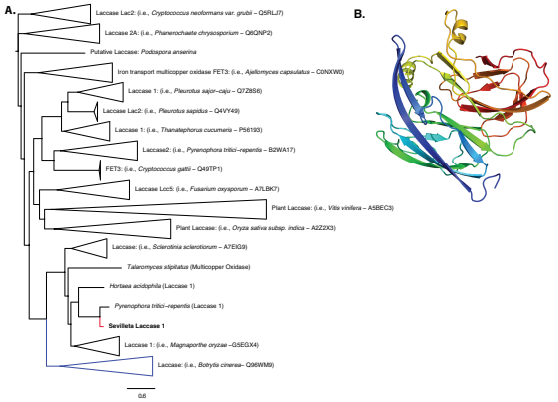
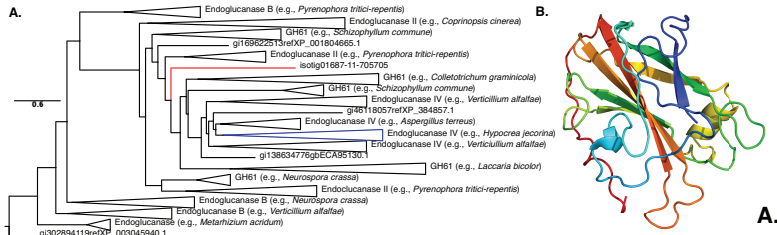
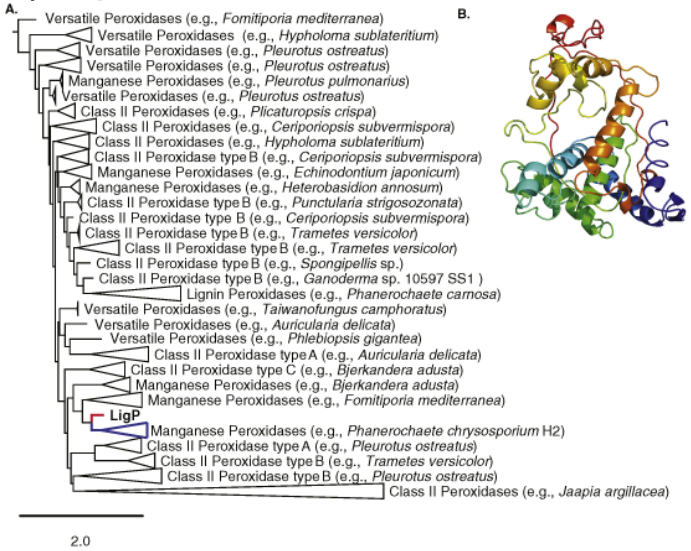
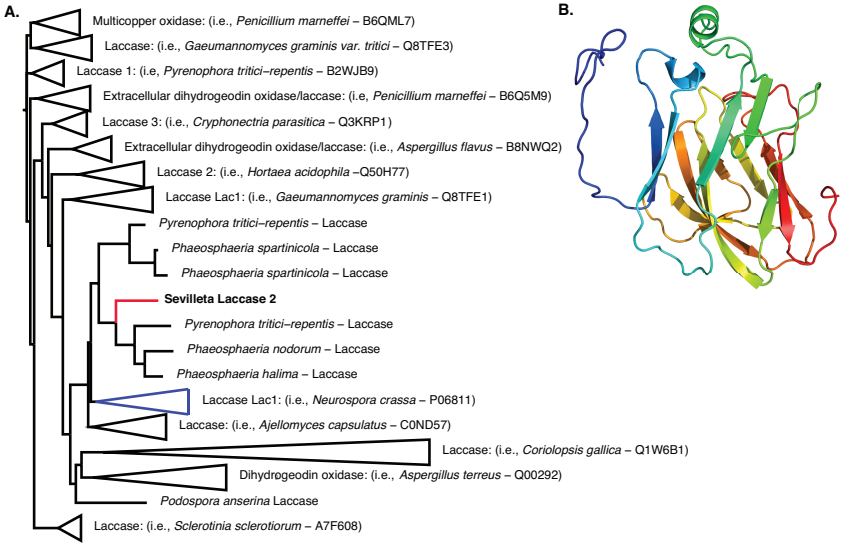
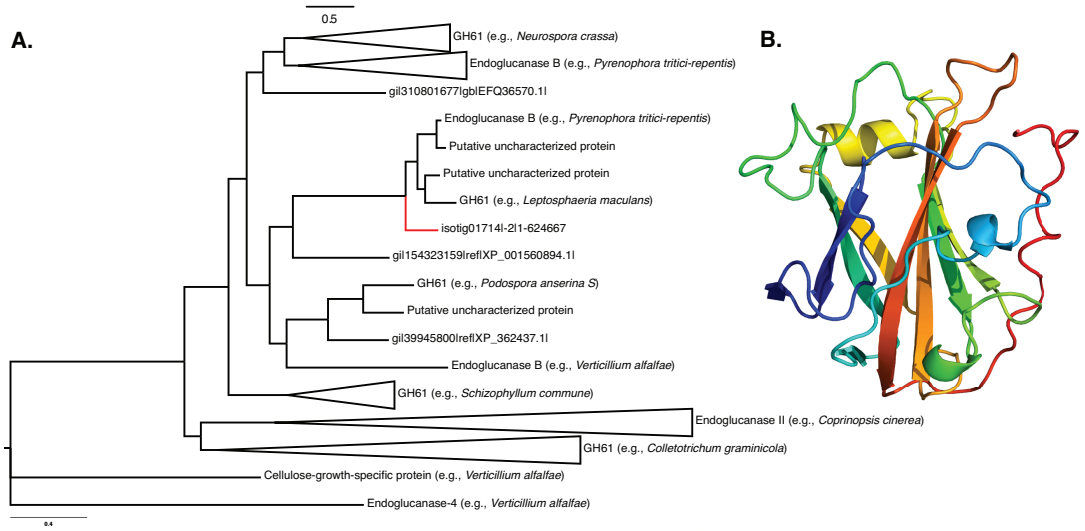
Five new families of enzymes











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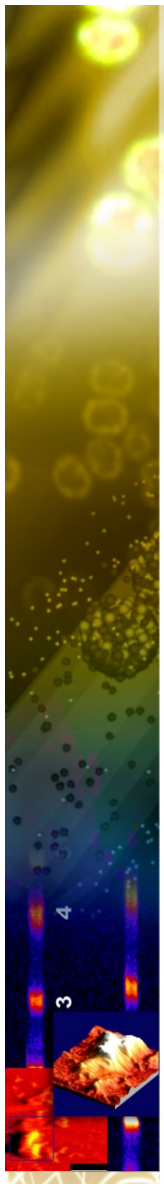
Update on project, since publication

- Enzymes are currently being synthesized to allow *in situ* testing of the their efficacy in lignin breakdown.
- Early days for this work, and involves considerable work in optimizing production and activity.
- *Remains to be seen how much lignin breakdown will stay a focus of the DOE.*



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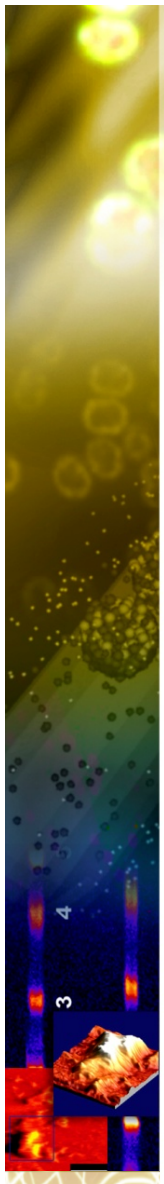
My staff work at Sandia

- Started **2014** as a **Senior Member of the Technical Staff**
- Projects:
  - Experimental identification of mobile genetic elements
  - Lab coordinator for DTRA Cooperative Biological Engagement Program at Lakka Beach in Freetown, Sierra Leone
  - Triage of biologically produced chemicals of high value as drop-in fuel blendstocks
  - Identification of engineered biological organisms
  - User-in-the-loop active machine learning in cybersecurity (not part of today's talk)



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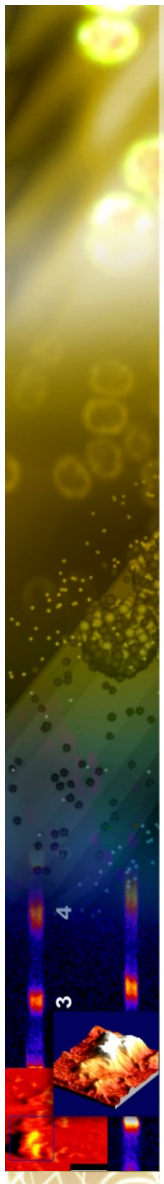
Experimental characterization of mobilome

- Bacteria release mobile elements
  - Under stress
  - In stationary phase
- Research question: **Can we supplement and improve bioinformatic tools for mobile element detection using experimental means**?



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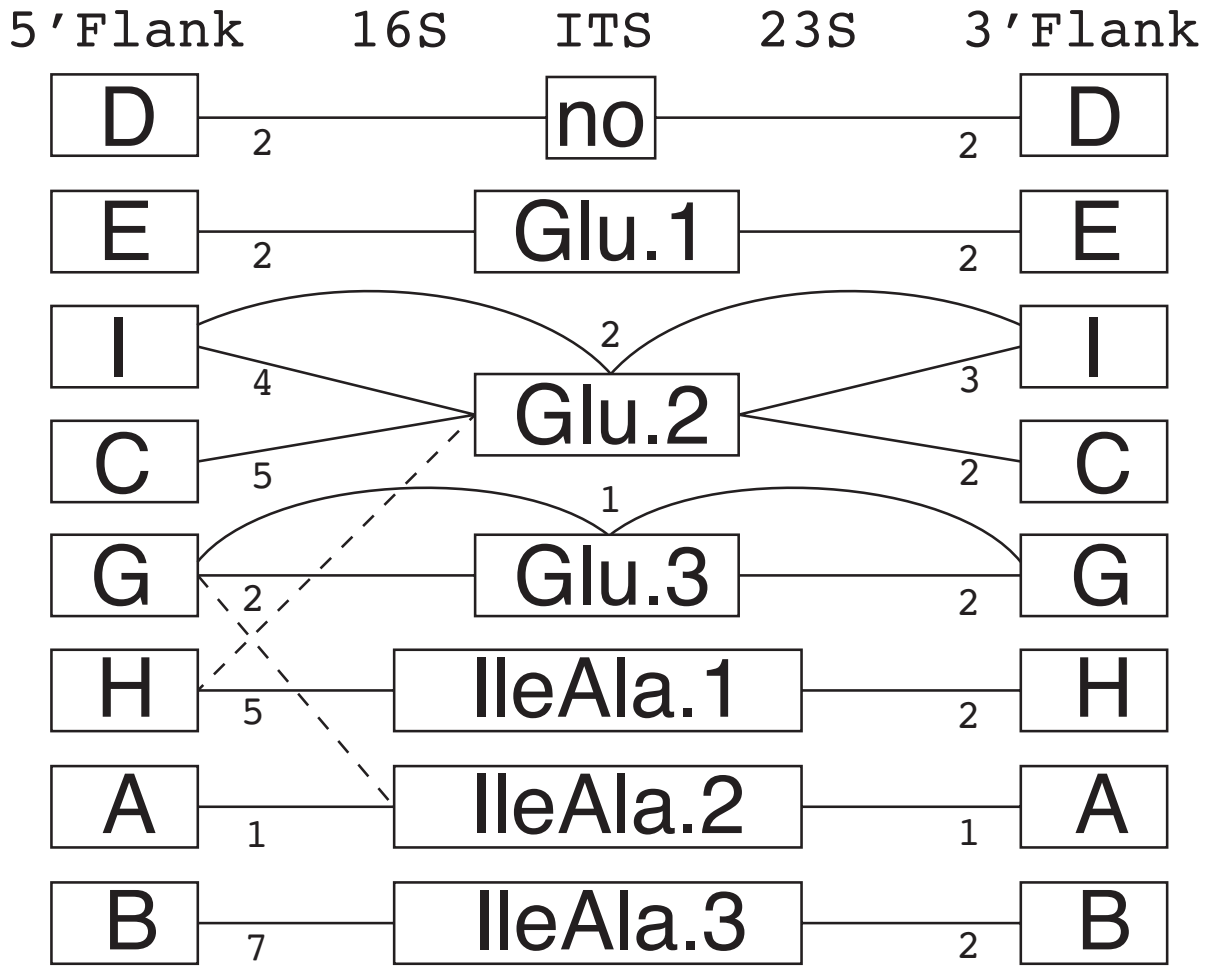
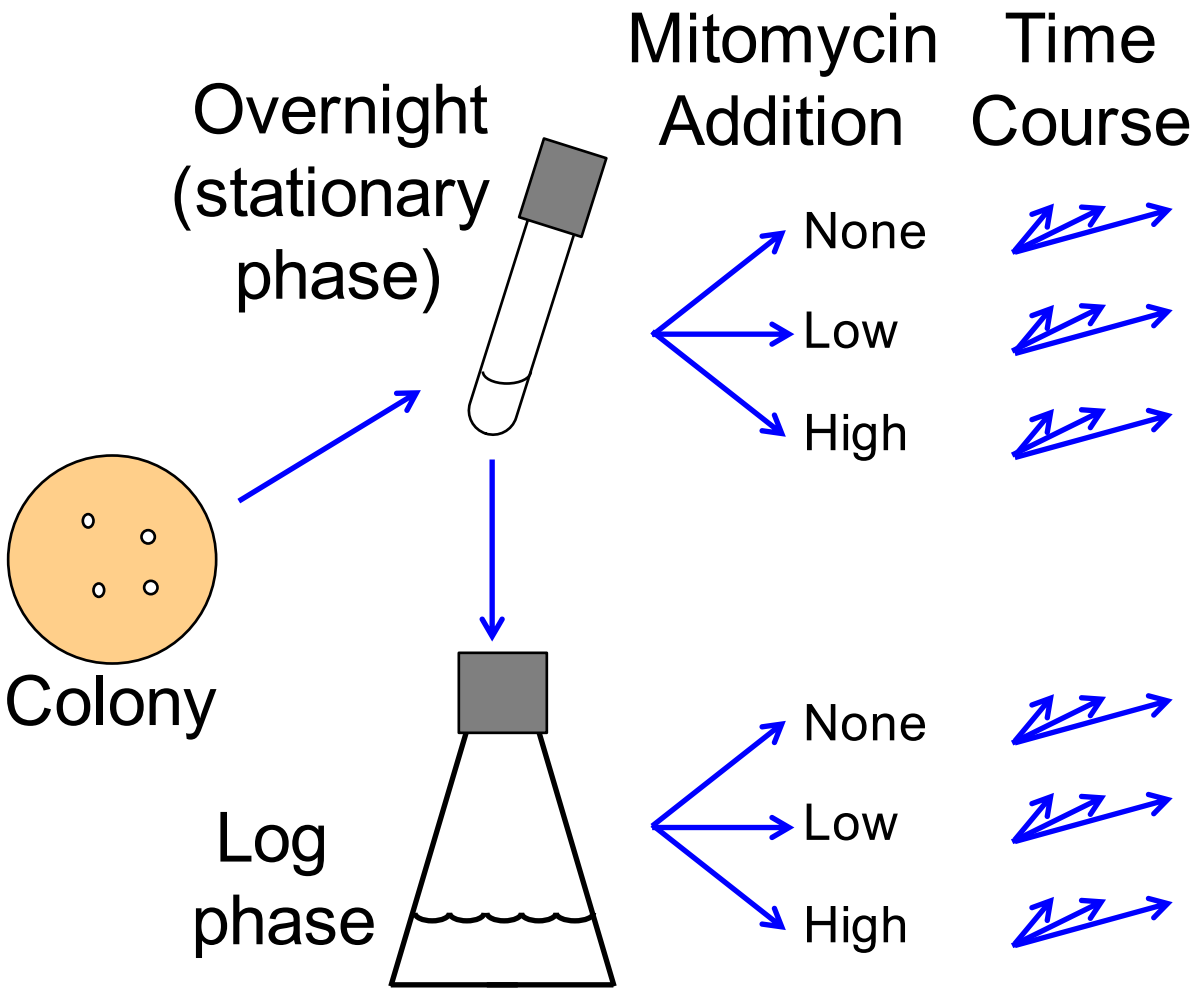
Discovery while assembling genome: PacBio identification of recombinant subpopulation



Protocol

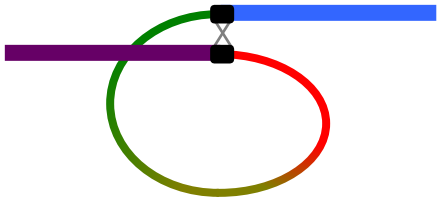


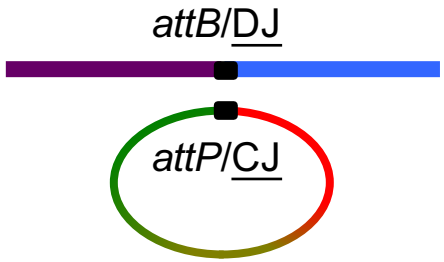
Per sample:

- Treat with or without DNase overnight
- Nextera
- SE Illumina NextSeq
- Juxtaposer software

Detecting Excision and Circularization

Mobility event



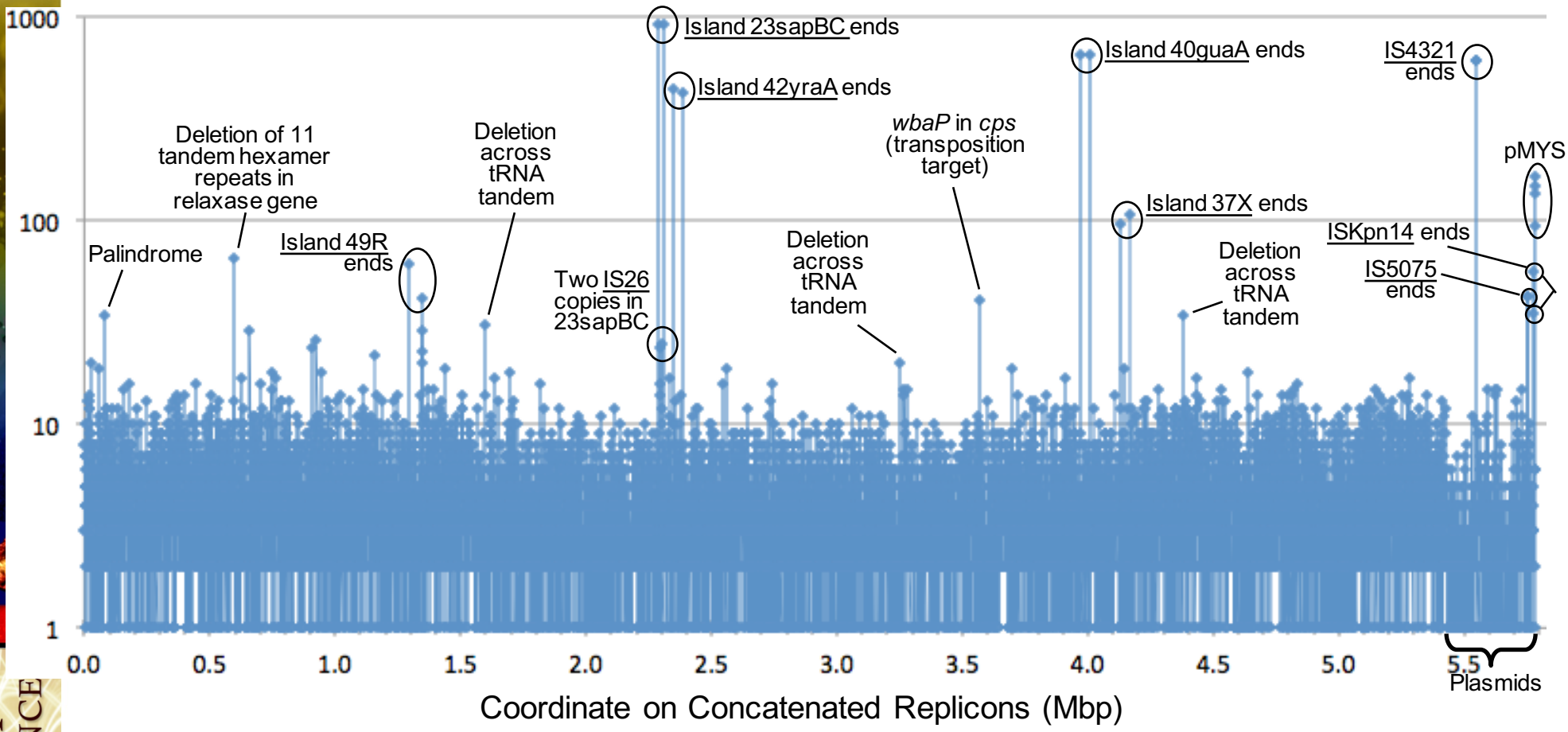


NGS reads

Standard reads

Recombinant reads

Juxtapositions in the genome



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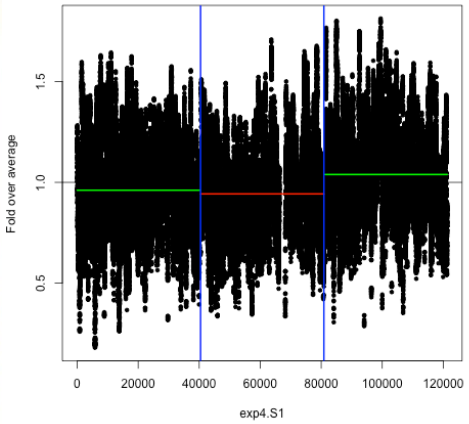
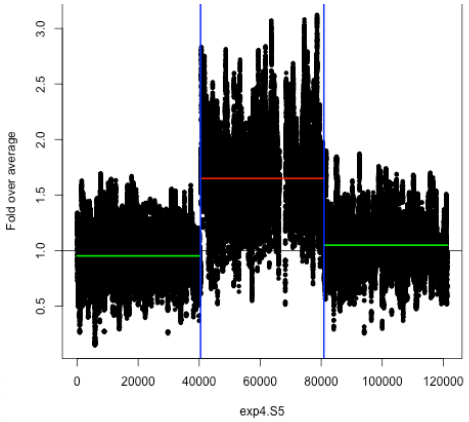
Mitomycin and Exonuclease Treatment

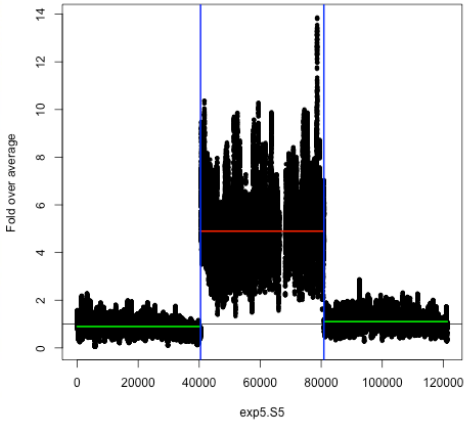
Island Kpn40guaA



No MMC
(no Exo)

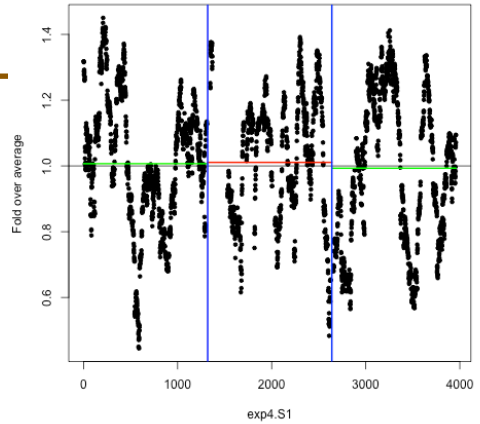


High
Mitomycin 2h
(no Exo)



High MMC 2h,
Exo-treated

Insertion seq. IS4321



Log Phase
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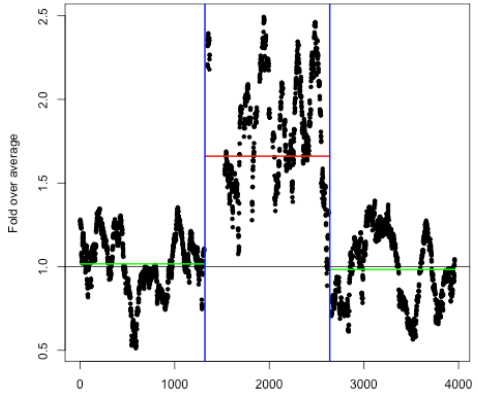
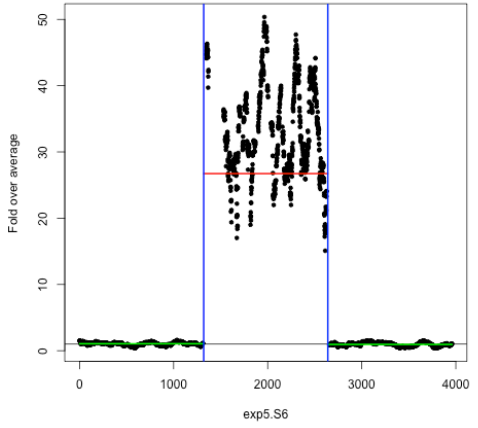


Overnight
(no Exo)



Overnight,
Exo-treated

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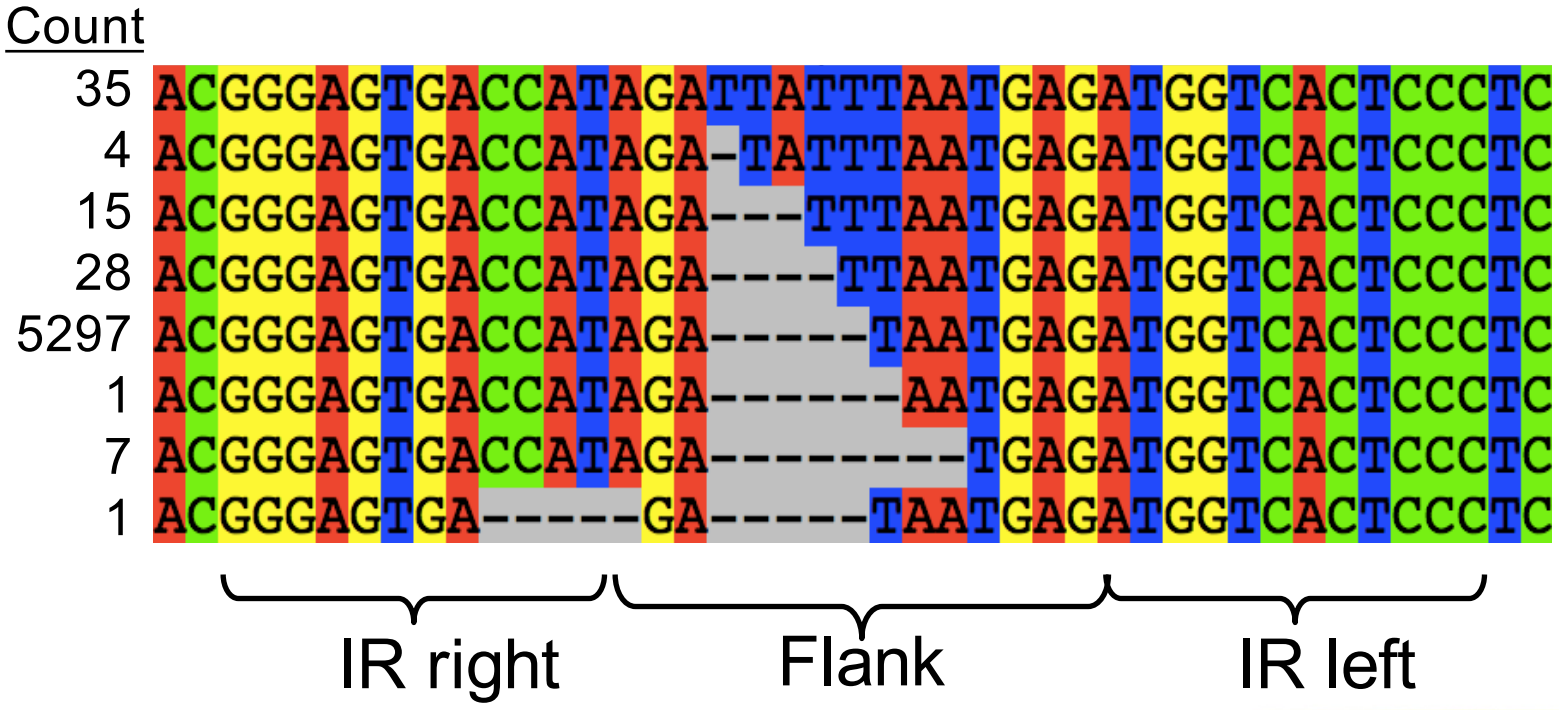
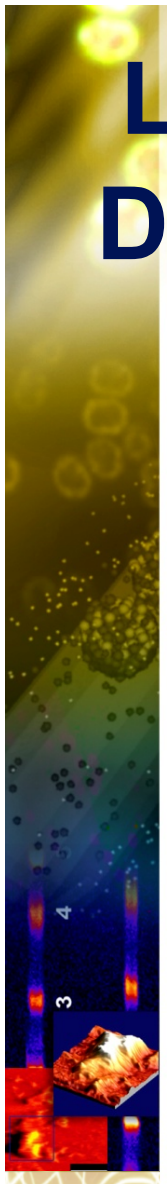
IS4321 and IS5075 precision

- Very similar, each at one copy at left end of Tn21
- Typically reach beyond their Inverted Repeats, i.e., they are site-specific
- Varying extent of flanking sequence captured in Circular Junctions



Lab Coordinator at Lakka Beach Ebola Diagnostic Lab: Freetown, Sierra Leone

- DTRA Cooperative Biological Engagement Program (CBEP) – Mobile Ebola Virus Disease (EVD) diagnostic laboratory
- Built and staffed by contractor MRI Global
- Sandia: Lab Coordinator and oversight role
  - Assist with daily operations
  - Interpret/verify test results and report out to local Ministry of Health and Sanitation (MoHS)
  - Lab liasion to MoHS, CDC, WHO, and NGOs
  - Attend (weekly) MoHS meetings at Former UN Special Court complex



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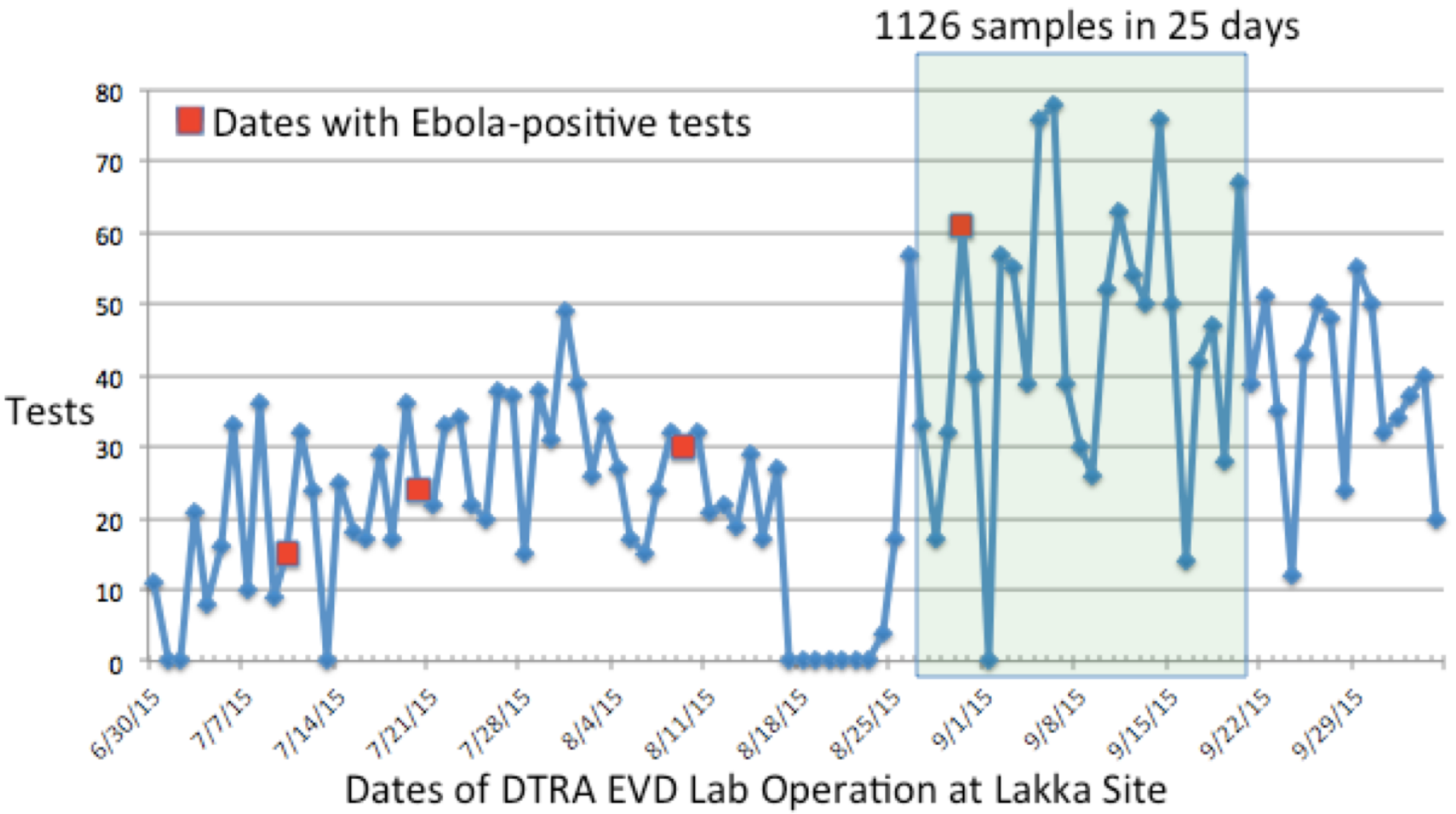
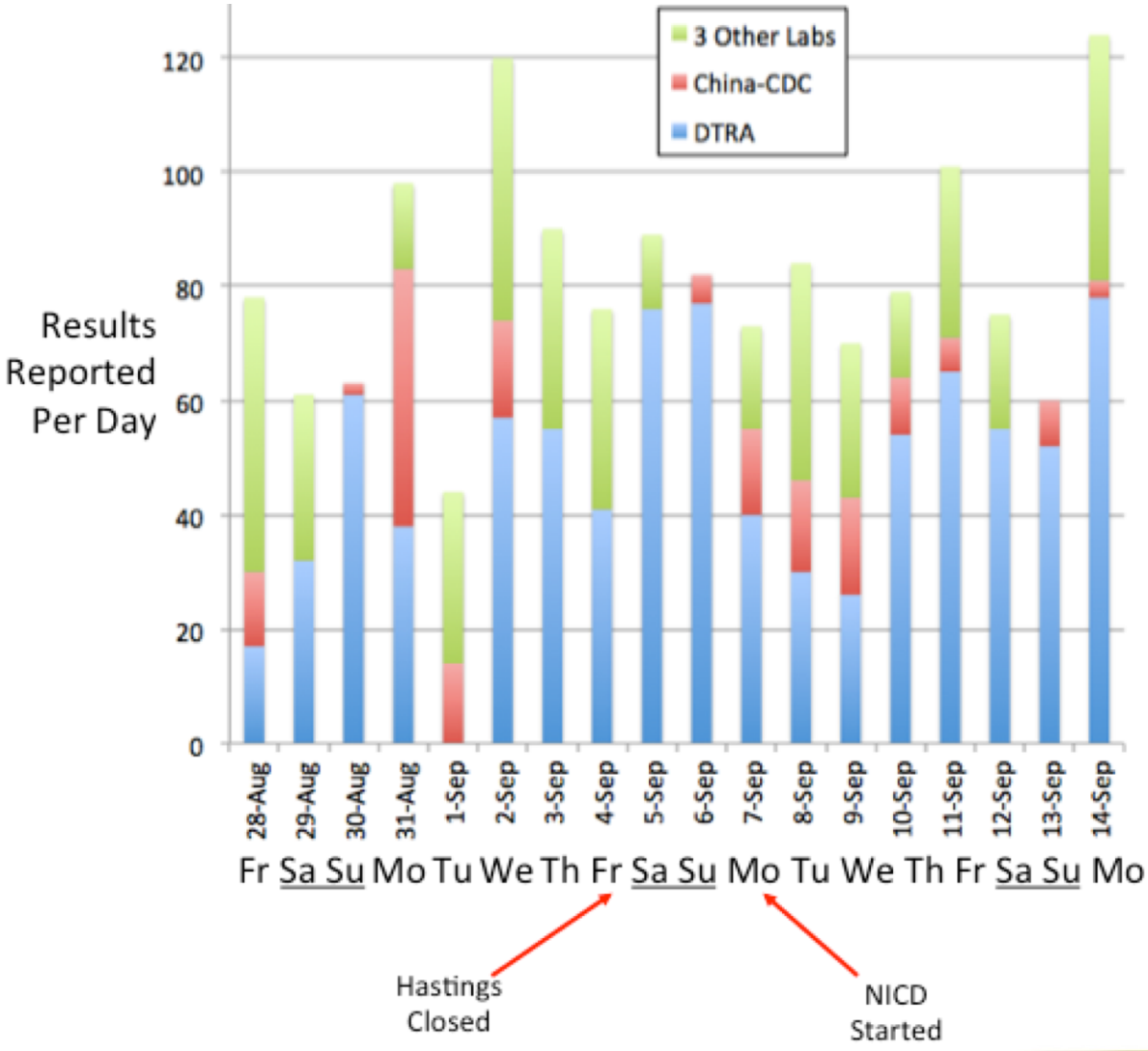
Sample processing



Relative volume of lab



Total Tests
DTRA: 854
Others: 613

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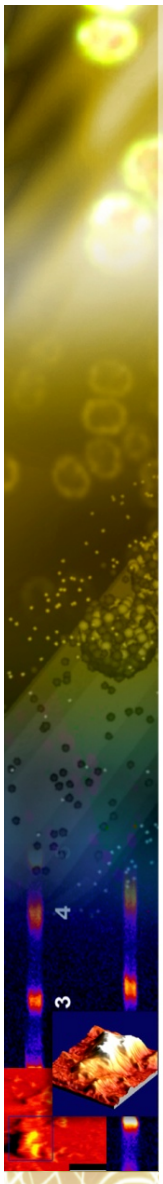
Screening chemicals for biofuel blendstocks

- Goal: Offset 30% of petroleum usage with Low Greenhouse Gas chemicals, without a dip in engine performance by 2025.
- Assumptions:
  - 1) Compounds with high Research Octane Numbers (RON: Translation-they burn well) will be able to substantially improve the quality of consumer grade fuel. (Caveats: A bunch of caveats, a whole bunch of caveats)
  - 2) We may not need to know the exact RON values.
  - 3) We have limited time and resources to critically evaluate all chemical properties of all potential chemicals.
  - 4) There are numerous chemicals.



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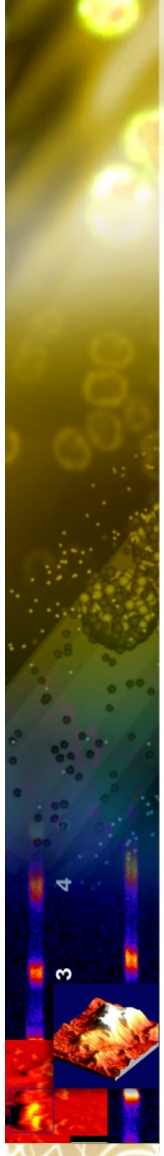
Enter Machine Learning

- Data collection of measured RON from 159 compounds
- Simple RON prediction using machine learning (i.e., Random Forest Classifier)
  - Model provides a very fast and course grained treatment for the assignment of chemicals into high/low RON class (e.g., > RON 85) and for triaging chemicals with no previous combustion measurements.
  - Mines data from public sources, including ChemSpider and PubChem.
  - ChemSpider properties primarily include chemical features (e.g., Boiling Point, Vapor Enthalpy, etc.)
  - PubChem includes 881 substructural fingerprint features: including hierarchical element counts, chemical ring systems, simple atom pairs, simple atom nearest neighbors, atomic neighborhoods, simple and complex smarts patterns.



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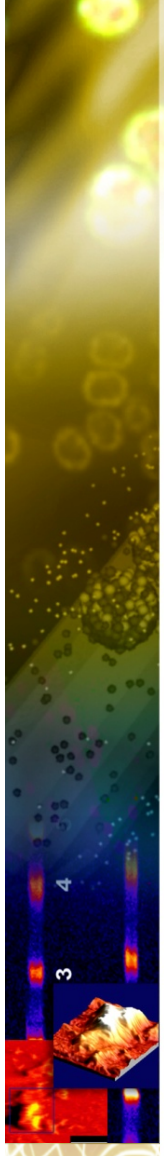
Quality of Predictor

Performance of classifier:

- 100 sub-sampled cross-validations (with 50% leave out)

Metric	Mean value	Std. dev
Accuracy	0.80	0.09
Precision	0.82	0.12
Sensitivity	0.80	0.16
Receiver Operator Characteristic (AUC)	0.88	0.07



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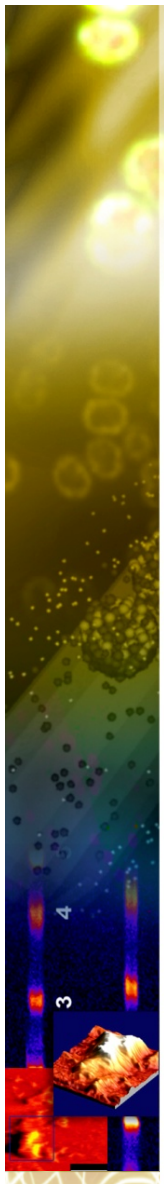
Chemoinformatics Data Mining

Features	Weight	Type
OH Rate Constant	0.0382	Physical
Surface Tension	0.0368	Physical
SMARTS Pattern: C-C-C-C-C-C	0.0349	Structural
KOC (pH 5.5)	0.0323	Physical
Vapor Pressure (mmHg at 25° C)	0.0313	Physical
SMARTS Pattern: C-C-C-C-C	0.0313	Structural
Octanol-Water Distribution Constant (pH 7.4)	0.0302	Physical



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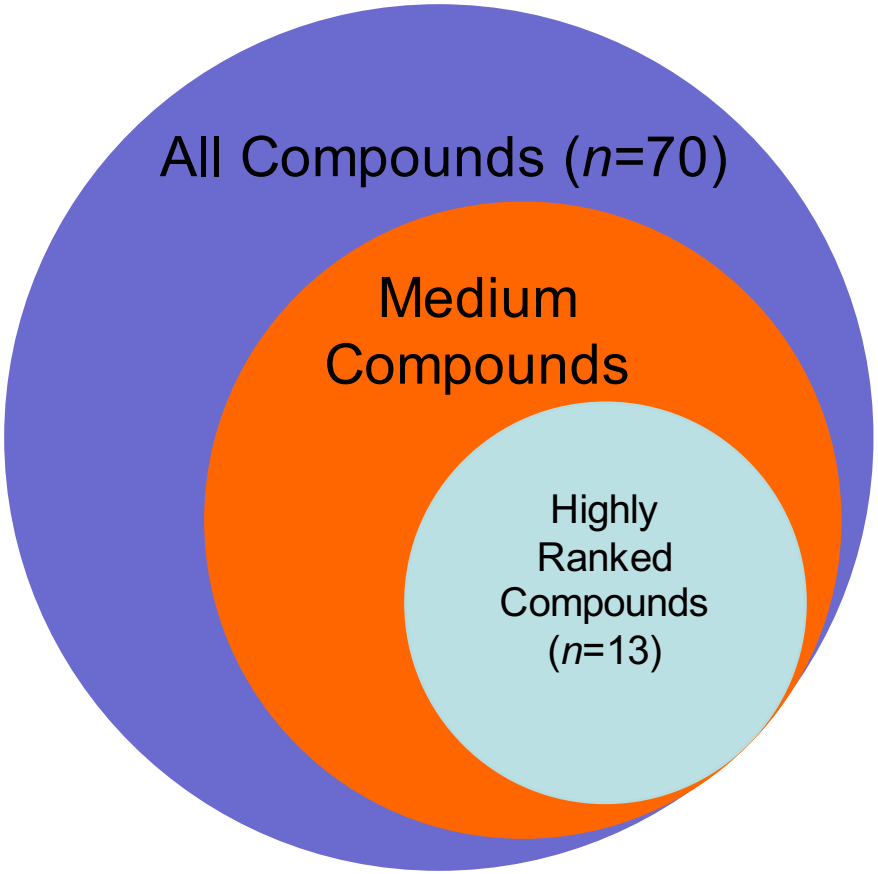
Initial Ranking for 70 Compounds of Interest



Compounds predicted to have RON > 85 in order of probability in class	
1. Isooctane	8. Isoprenol
2. Methylcyclopentane	9. Isobutanol
3. Ethanol	10. 3 methyl 1 butanol
4. Methyl butyrate	11. Butyl acetate
5. Ethyl isobutyrate	12. Toluene
6. Methyl 2-methylbutyrate	13. Isoamyl acetate
7. 2-methyl 2 butanol	

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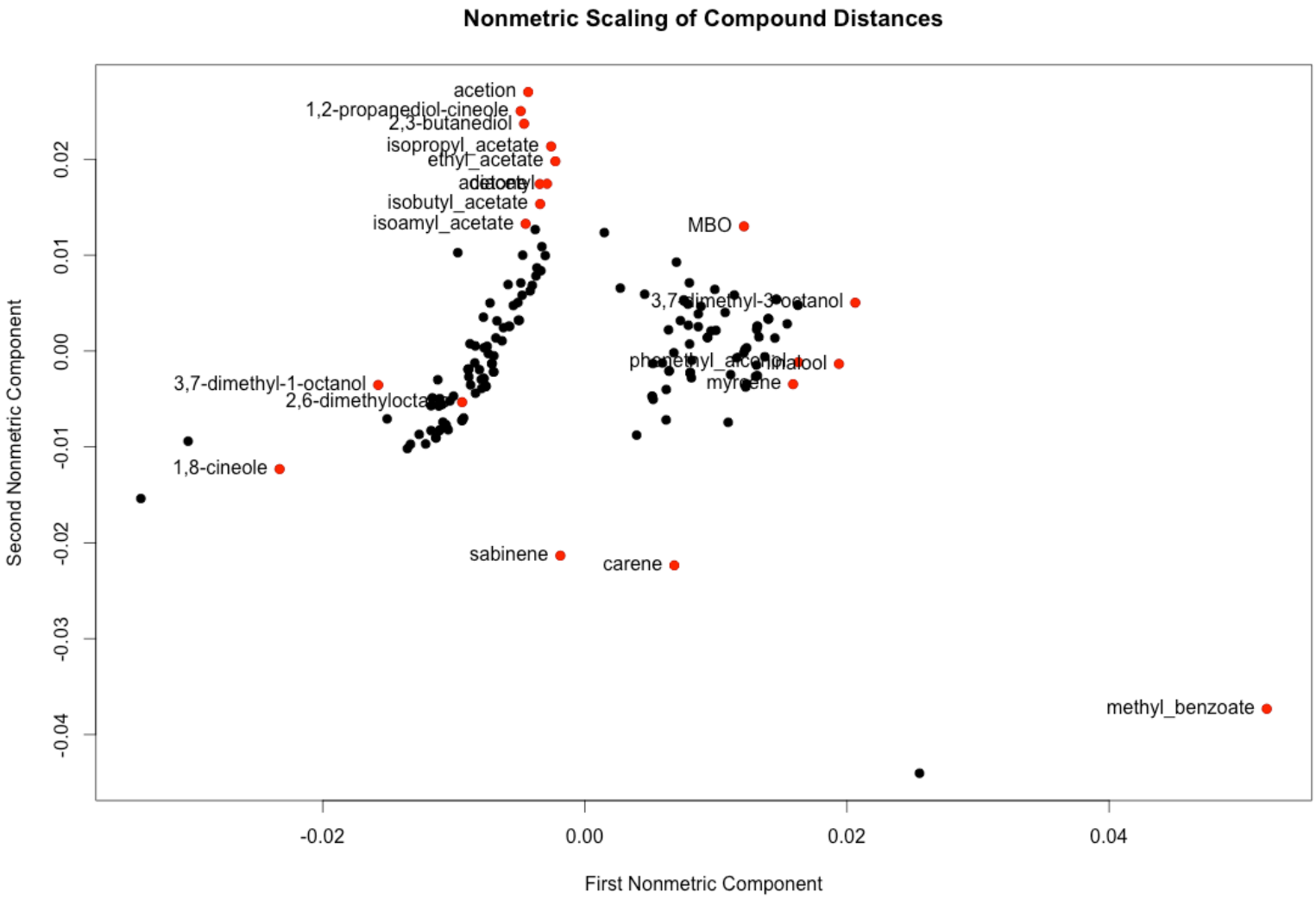
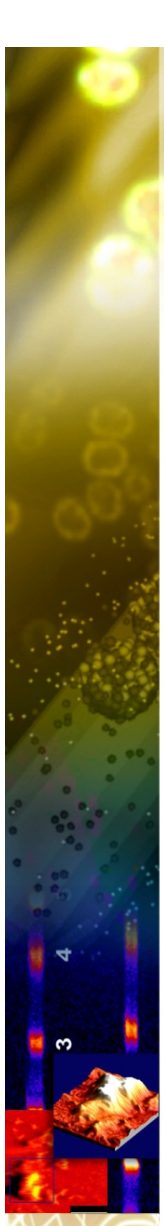
Testing 20 new compounds and the limits of the model



Wrap-Up

- Career Track in a National Lab
  - Postdoc
  - Senior Member of Technical Staff
- Biodefense: Basic and applied work in emerging infectious diseases
- Bioenergy: Work in the production of high-value chemicals
- Work on mission areas



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Collaborators

Kelly Williams	Gavin Conant	Leanne Whitmore
J. Chris Peers	Joe Schoeniger	Kunal Poorey
Seema Singh	Amy Powell	Debjit Ray
Zach Bent	Robert Meagher	Devin Petersohn
Britney Lau	Julian Wagner	

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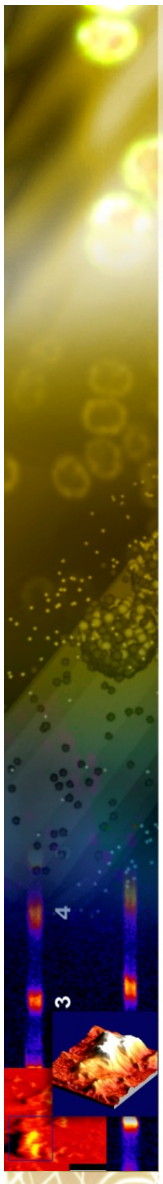

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