Project 3: Pond Crash Forensics

5/20/2013
Algae Peer Review

Todd W. Lane Ph.D.
Sandia National Laboratories

Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Goal Statement

- The goal of this project was twofold:
  - to develop tools and methods that will be used to identify the <u>biological</u> agents of pond crashes through the forensic analysis crash samples.
  - To develop technology for the rapid, pond side, early detection of these pond crash agents

- The creation of tools for the diagnosis and detection of biological agents of pond crashes will be critical to informing the development of inexpensive screening and monitoring tools for early crash detection, as well as engineering and biological countermeasures that will enhance pond stability and increase long-term productivity.

- This will decrease the loss of production time to crashes and therefore decrease the cost of the final product.

Quad Chart Overview

Timeline

- Project start date: 10/01/2010
- Project end date: 9/31/2012
- Percent complete: 100%

Barriers

- Barriers addressed
  - FT-A. Feedstock availability and cost
  - Im-F. Cost of production

Budget

Total project funding: $800K
- DOE share: $800
- Contractor share: $0

Funding received in FY12: $400
Funding for FY13: $0
ARRA Funding: $0

Partners

Interactions/ collaborations

Texas Agrilife (TAMU)
PNNL, University of Arizona
NAABB
NASA

Project management

Project Lead: Todd Lane
Sandia National Labs, Livermore

The difference between short term & sustained productivity is the problem

Short term areal production
of 30-50 g/m^2/day
–Commonly claimed

Annualized areal production rates of
13.2 g/m^2/day: ANL, NREL, PNNL 2012

Sub optimal growth conditions lead to lower annualized production.:

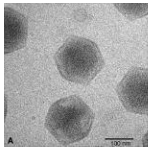
- Irradiance, temp, salinity etc.
- Pond/PBR collapses caused in part by biological agents (ultimate suboptimal condition)

Real time data on predator/pathogen load enables proactive pond management: We intend to create tools to enable such management

Presence of the biological agent can be necessary but not sufficient to crash

Agent *Algae* *Environment* *Collapse*

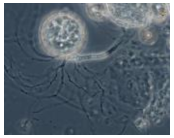
Viruses



Bacteria

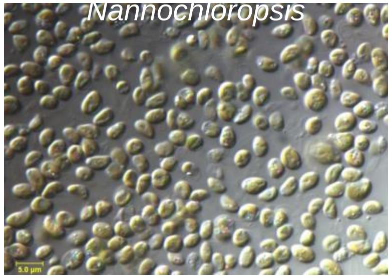
Predators

Fungi

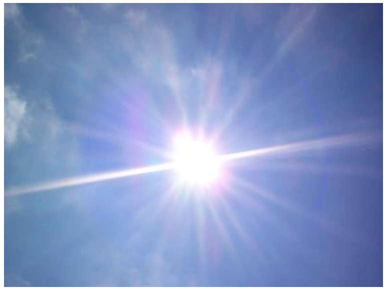


Patterson & Laderman, 2001.

\+

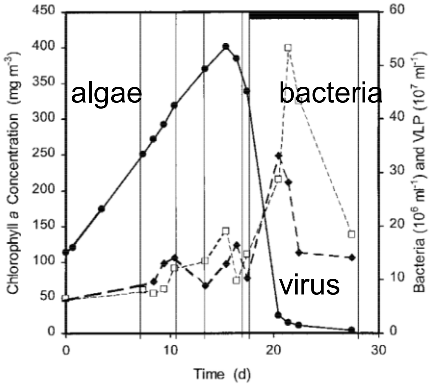


\+



Environment (Temp, salinity, pH, CO2, nutrients)

=



Herman Gons et al., Antonie van Leeuwenhoek, 81: 319-326, 2002.

"Perhaps the most worrisome component of the large-scale algal cultivation enterprise is the fact that algal predators and pathogens are both pervasive and little understood."

- DOE Draft Algal Biofuels Technology Roadmap (2009)

Project Overview

- Annualized areal production is <u>in part</u> limited by pond crashes caused by biological agents. These crashes increase algal production costs and are an economic barrier to the commercialization of algal biofuels
- Goals:
  - Develop diagnostic tools and methods to identify the root causes of pond instabilities through the forensic analysis of samples taken from raceways and PBRs post-crash.
  - Identify and demonstrate potential technologies for rapid, inexpensive pond side diagnostics
  - Develop spectroscopic indicators for early stages of algal infection.
- Leverage:
  - Internal Sandia $12M investment in Biodefense technology which <u>enables ultra high throughput sequencing to rapidly and cheaply identify an etiologigical agent without the need for isolation.</u>
  - Internal and NIH investment in fieldable diagnostics: SpinDX
  - Sandia's hyperspectral imaging capabilities.

1-Approach

- Leveraged Sandia investments in biodefense and clinical diagnostics
- Developed methods to enrich for nucleic acids that are likely to derive from the etiological agent of the crash.
- Utilized second generation sequencing to identify agents.
- Created quantitative assays that facilitate the detection of agents at low concentration.
- Utilize advance spectroscopic methods to detect early hallmarks of algal stress.
- Technical metrics of progress
  - Speed of analysis: 48 hours for identification, ~30 min for detection
  - Sensitivity of detection: <1 organism
  - Cost of analysis
- Unique aspects:
  - The use of nucleic acid target enrichment followed by ultra high throughput sequencing as a strategy for agent identification
  - Fieldable diagnostics for pond side detection

2-Technical accomplishments.

- Genetically identify unknown etiological agents of pond crashes without the need for agent isolation.
  - Developed collaborations and obtained samples from groups running pilot scale open ponds
  - Tested sample preparation and analysis trained on laboratory and field samples
  - Developed host and background subtraction reagents
  - $10-$30 per sample sequencing cost
- Developed inexpensive yet rapid and sensitive pond side diagnosis system
  - Demonstrated the bench top technique for
  - 30 minutes sample to answer
  - Single organism detection for many predators.
  - Estimated ~Dollar per sample/ ~$1000 instrument

Euk SSU rRNA variable regions

- **Variable Region 4: ~ 400 bp amplicon (*S. cerevesiae*)**
  - **Significant length heterogeneity**
  - **Highest degree of variability**
  - **Generally thought to be sufficient to identify eukaryotes to the genus level**
  - **50-75 % coverage with 150bp PE (no overlap)**
  - **MiSeq 250 bp PE (Beta) 35 hours 10-14M reads**

- **Variable Region 9: ~168 bp amplicon (*S. cerevesiae*)**
  - **High variability**
  - **Used for genus level ID**
  - **MiSeq100 bp PE 14-19 hours 10-14M reads (PE)**

- Often used in combination

- Other molecular bar coding regions
  - Internal transcribed spacer (ITS): various length, allows for species level ID, applied in fungal phylogeny
  - MtCO

Bioinformatics pipeline

178 million raw paired-end reads (150PE)

Qfilter

155 million quality-filtered & trimmed paired-end reads

Bowtie2

145 million paired-end alignments to Silva rRNA database

sam2tax

Silva results (all hits within 1% of best) mapped to NCBI taxonomy

lca

Last common ancestor based taxonomic summary for each hit:
132 million resolve at Genus level, 10 million at Species level

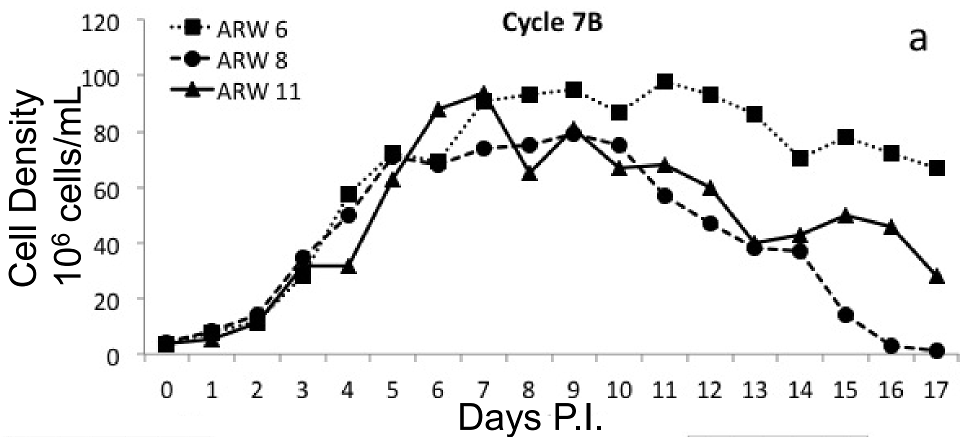
In-house tools

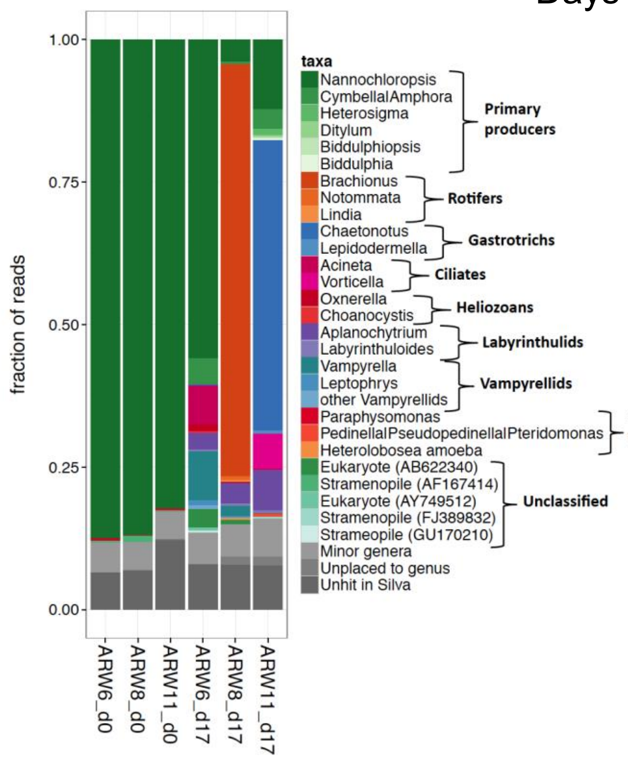
Off-the-shelf tools

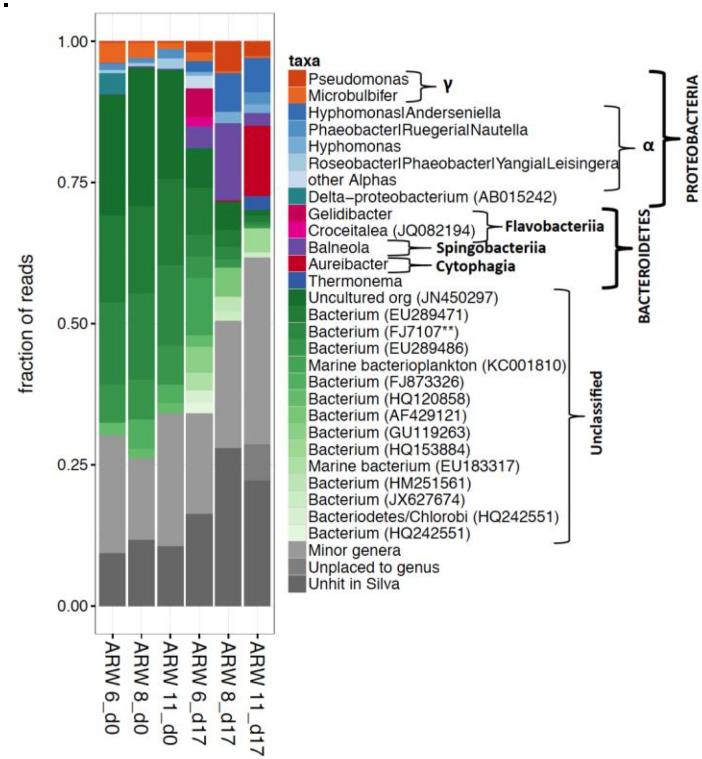
silva comprehensive ribosomal RNA databases

RapTOR

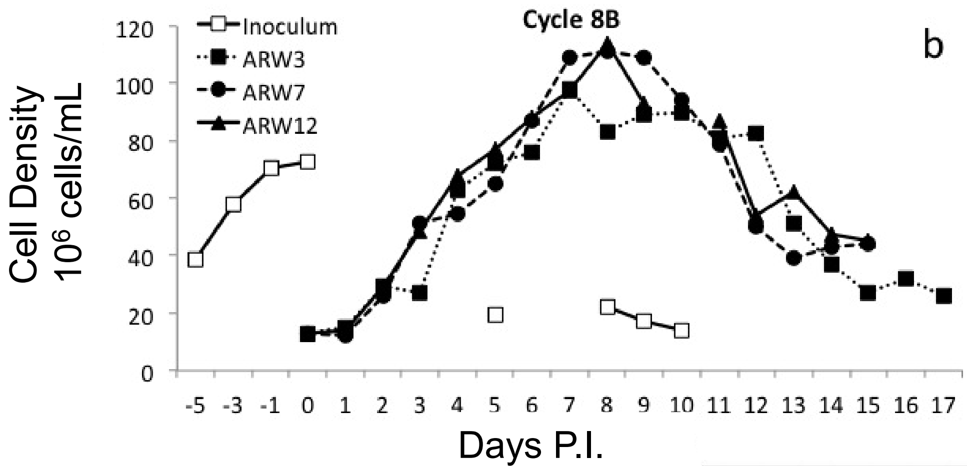
Forensic Analysis of Pond Crashes

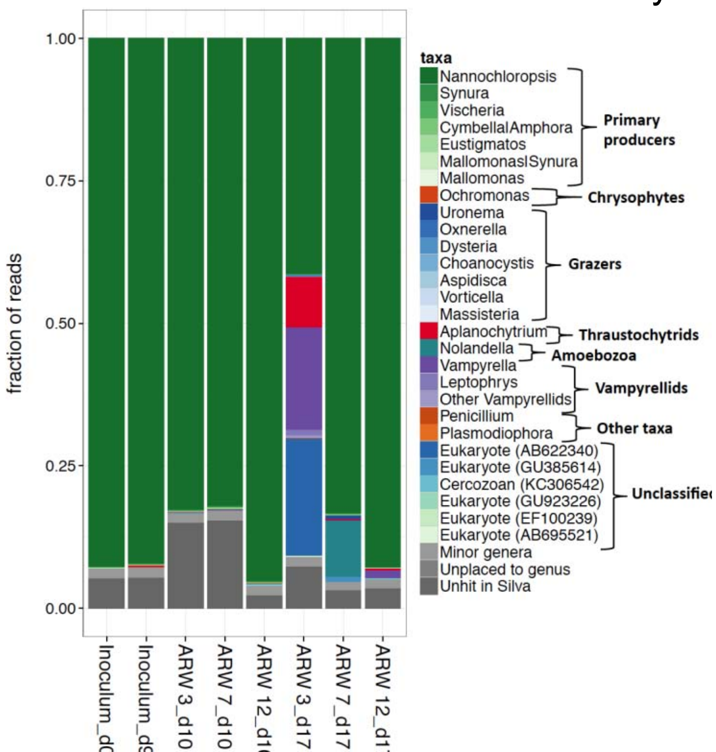


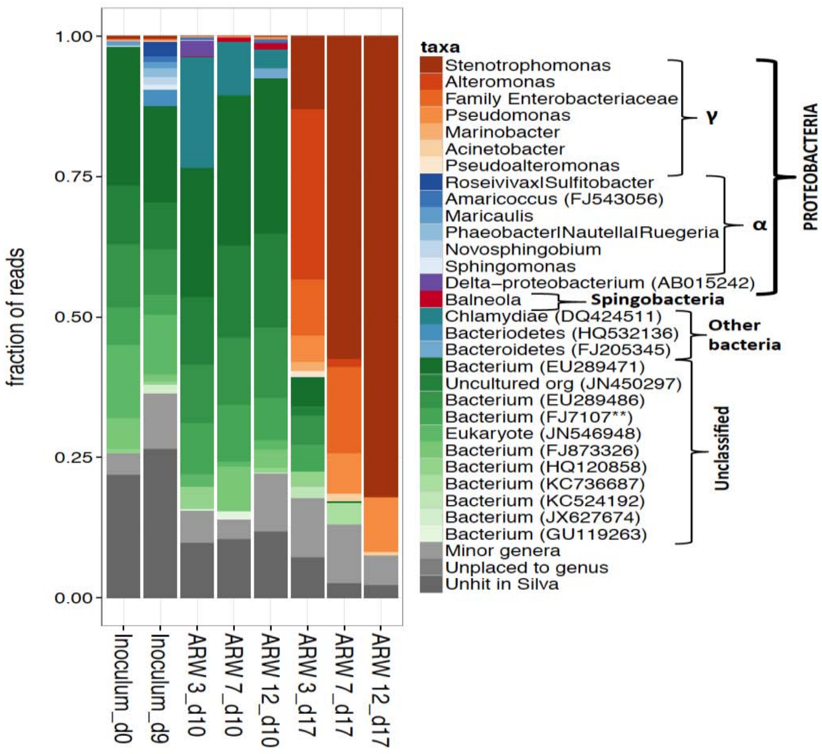




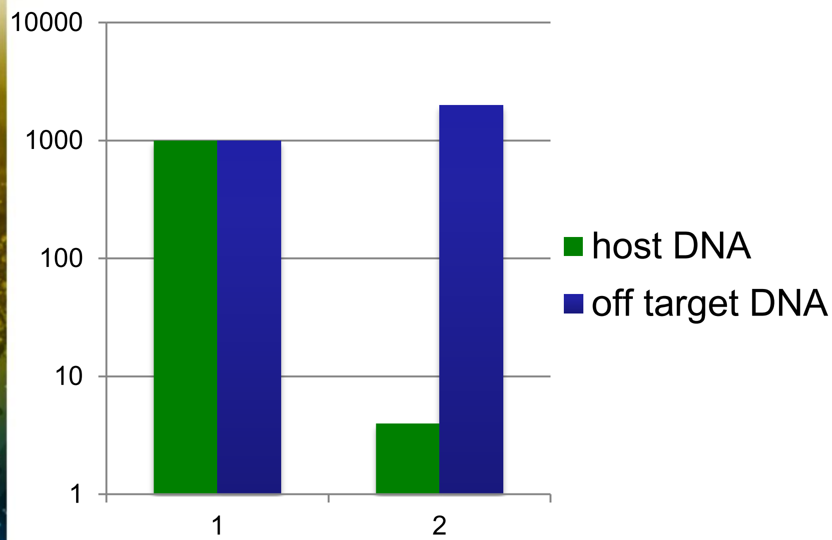
Evaluation of pond intervention

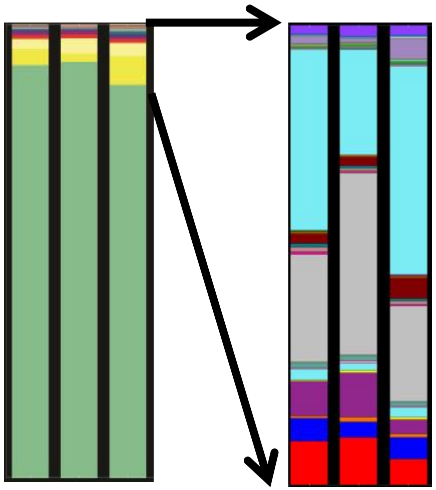


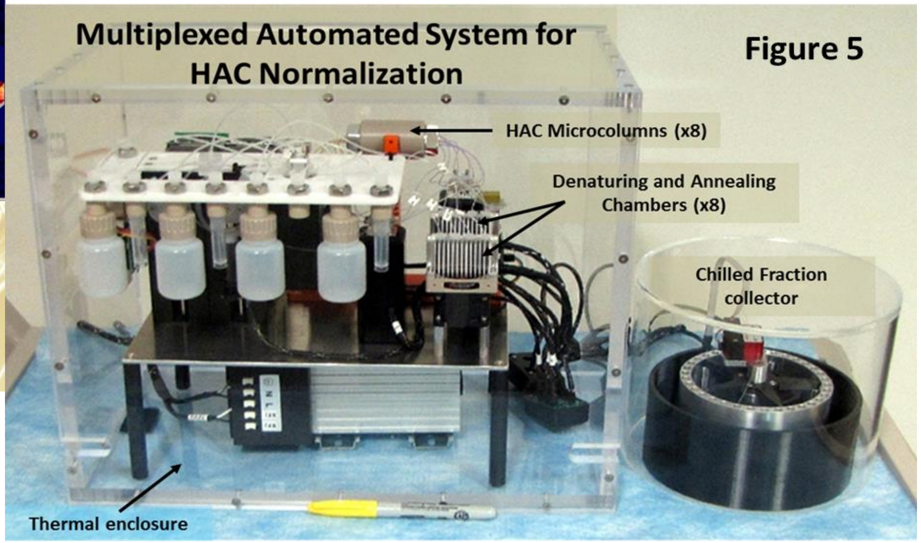




Automated, mutiplexed and manual systems for capture-based suppression







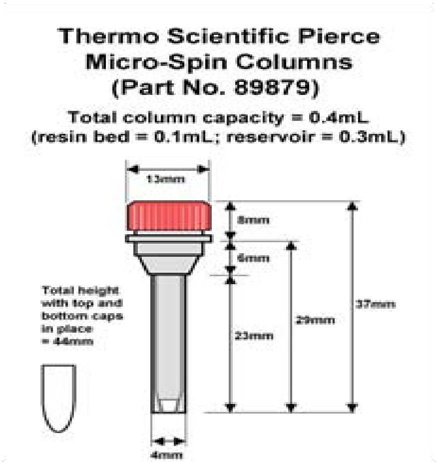
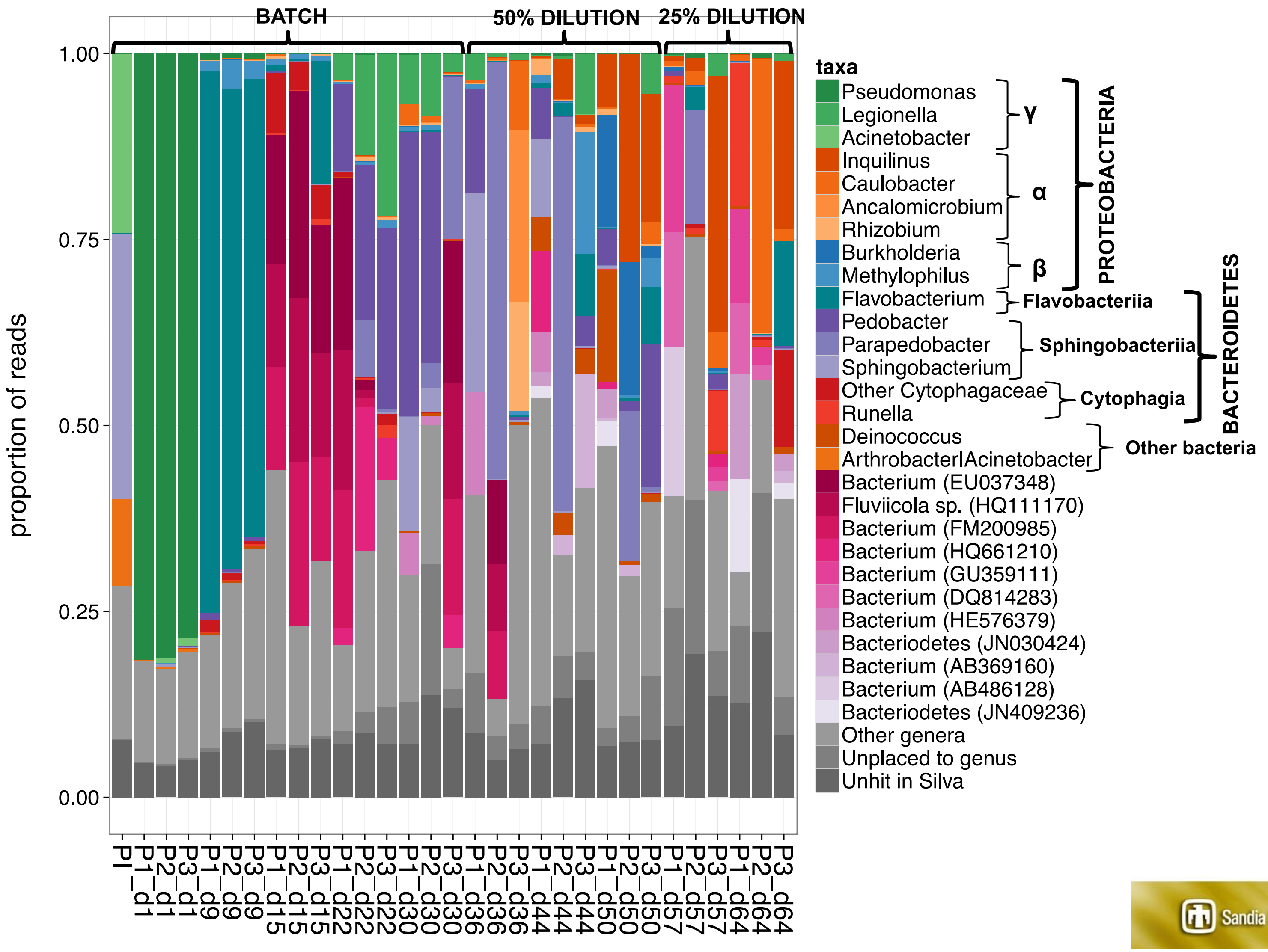


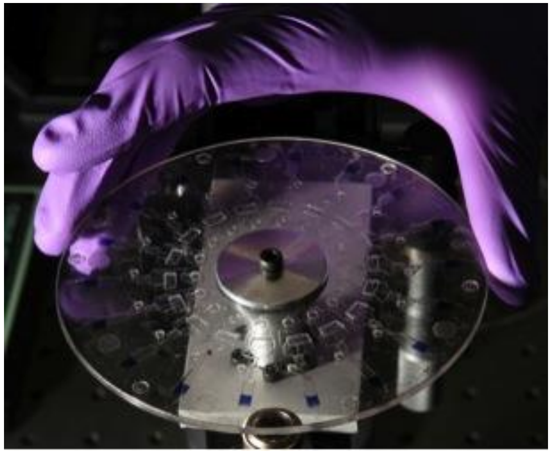
Figure 10: Dimensions of the commercially available spin columns

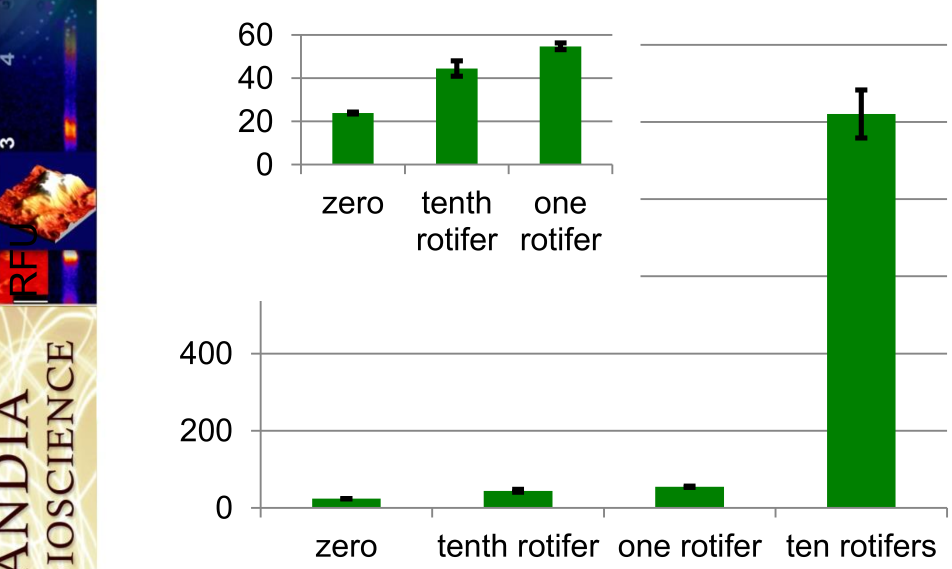
Long Term Microbiome Analysis Indicates Community Divergence

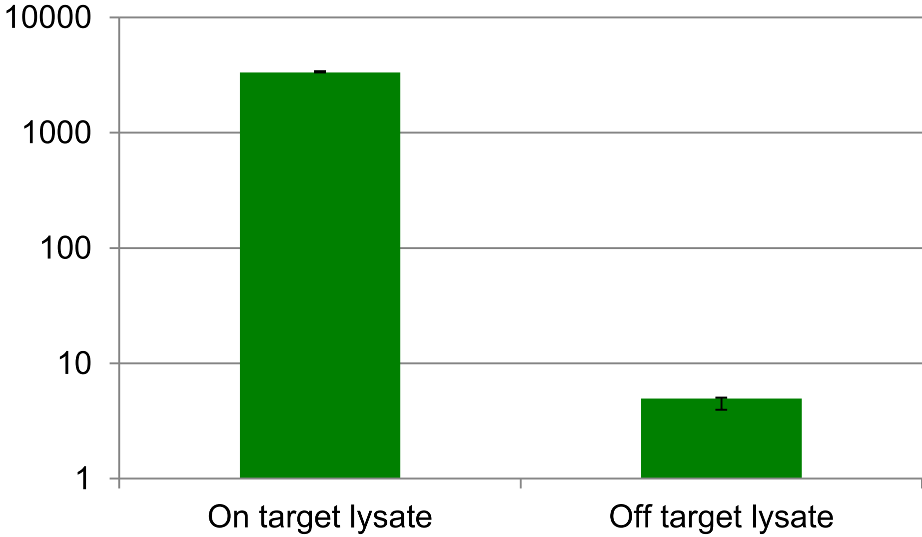


The goal of all this sequencing? Direct from the sequencer to assay.

- Target probes for predators pathogens and parasites in the pond
- SpinDX system of centrifugal fluidics and detection
  - Originally designed for clinical or environmental agent detection in “low resource” environments.
  - Rapid prototyping
  - Optimization
  - Validation
  - Genus level probes target agent and nearest neighbors

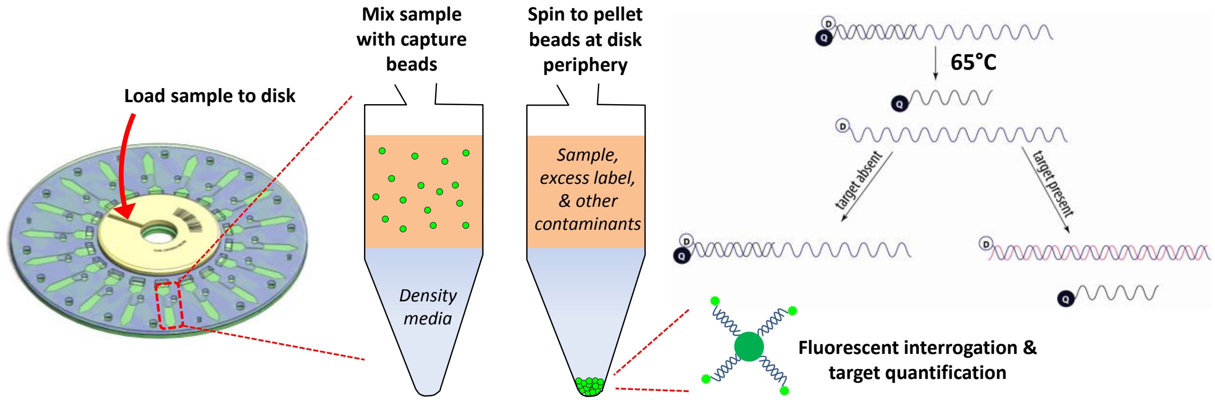


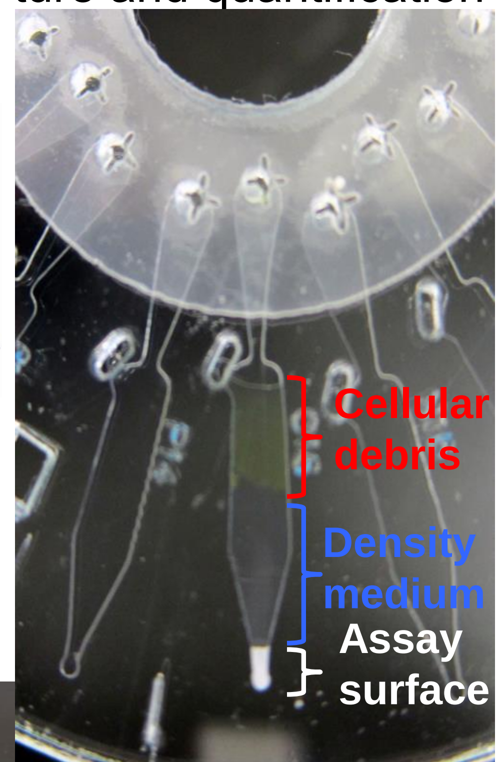




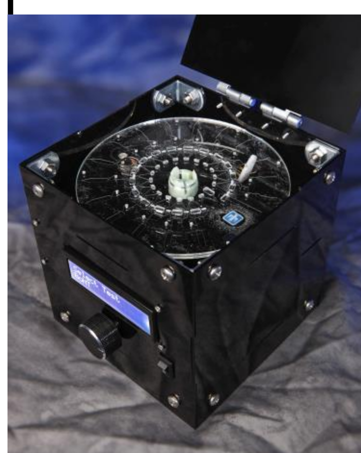
SpinDx^{TM} has the necessary characteristics for a field assay for pond management

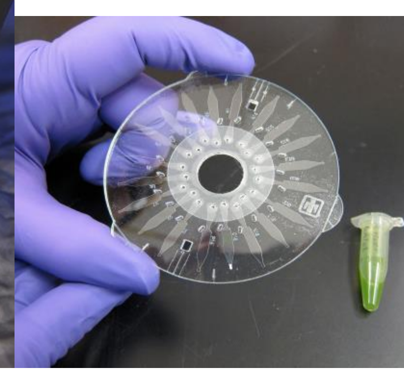
ture and quantification



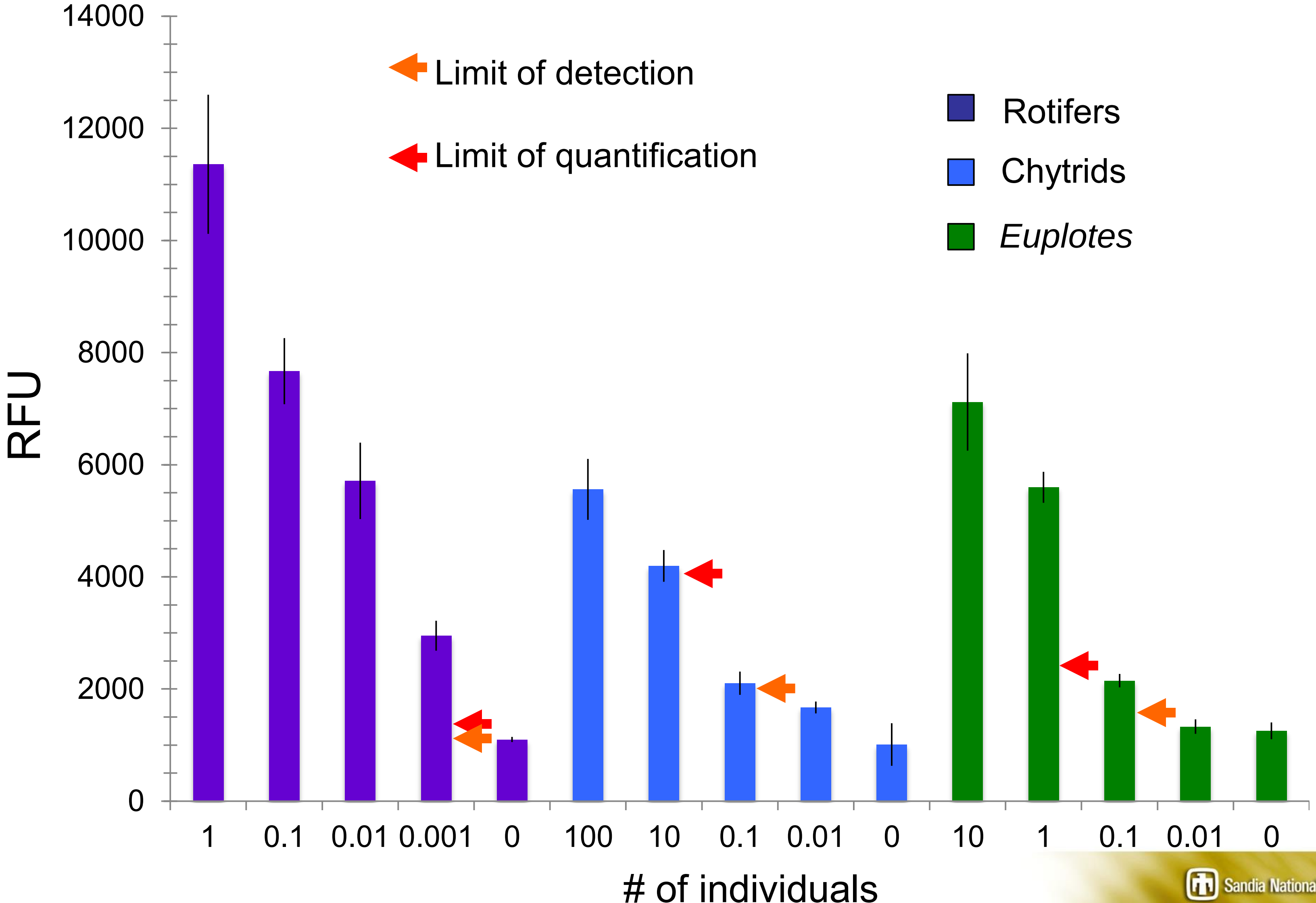


- Assay time: approx 30 min
- 36 channels per disc
- Potential for multiplexed assays in each channel
- Low reagent costs
- Low material costs
- Low instrument cost ($1000)
- Fieldable





SpinDX detection/quantification of pest species



3 - Relevance

- ***BETO Multi-Year Program Plan (2012):*** ***"***The feedstock supply and logistics performance goal is to increase the projected productivity of large-scale algae cultivation …
  - Current annualized production is limited in part by losses due to crashes and etiological agents of these crashes are "pervasive and poorly understood"
- The objectives of the project have contributed to meeting these goals by
  - Creating the ability to identify etiological agents
  - Produce sensitive and rapid assays that allow early detection
- Application of project results to the algal industry
  - Early detection will enable the "salvage" of biomass prior to crash
  - Will facilitate rapid pond remediation and return to production
  - Will enable the development and deployment of countermeasures.

4 - Critical Success Factors

- Increase interaction with industrial partners
  - We are reaching out to and have been approached by a number of private sector entities interested in both the identification and detection elements of Pond Crash Forensics.
- Increase the TRL of spinDX system and deploy prototype
  - We are seeking funding from diverse sources for the development of a deployable prototype system.
  - We have identified partners for beta testing of system
  - We continue to leverage improvements in both sequencing and detection technologies from investment in biodefense and clinical diagnostics

Summary

- Relevance of objectives
  - Development of technology to for early detection of pond crash agents, enabling the development and utilization of countermeasures, reducing costs.
- Approach
  - Second generation sequencing for identification
  - Pond side SpinDX for detection
- Technical accomplishments
  - Developed and demonstrated methods for agent identification
  - Developed and demonstrated rapid, inexpensive, pond side diagnostics
- Future work
  - Applied for funding to complete development/deployment of pond side diagnostics
  - Molecular Identification of algal predators and pathogens for ATP^3
- Success factors and challenges
  - Increased partnership and interaction with industry
- Technology transfer
  - Companies interested in testing and evaluation of pond side diagnostic system

Acknowledgments

DOE EERE Office of Biomass Programs

Sandia National Labs

- **Laura Carney**
- **Pamela Lane**
- **Owen Solberg**
- **Chung-Yan Koh**
- Lara Jensen
- Aaron Collins
- Deanna Curtis
- Jeri Timlin
- Kelly Williams

NAABB

- Josh Wilkenfeld
- Tzachi Samocha
- Braden Crowe
- Jonathon VanWagenen
- Michael Huesemann

NASA

- Jonathon Trent
- Sigrid Reinsch

RapTOR

OMEGA PROJECT 2010 – 2012

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

ARC • SFPUC • URS

Pacific Northwest NATIONAL LABORATORY

EUREKAGENOMICS

AgriLIFE RESEARCH Texas A&M System

SANDIA BIOSCIENCE

Additional Slides

Publications and Presentations

Publications

1. Carney, L.T., Wilkenfield, J.S., Lane, P.D., Solberg, O.D., Fuqua, Z.B., Cornelius, N.G., Gillespie, S., Williams, K.P., Samocha, T., **Lane, T.W.** 2013. Pond Crash Forensics: Molecular diagnosis of pond crashes in replicate raceway mass cultures of the alga *Nannochloropsis salina*. *Submitted for publication*
2. Carney, L.T., Reinsch, S.S., Lane, P. D., Solberg, O.D., Jansen, L.S., Williams, K.P., Trent, J. D. and **Lane, T.W.** 2013 Microbiome analysis of a microalgal mass culture growing in municipal wastewater in a prototype OMEGA photobioreactor. *Algal Research* *Submitted for publication*

Presentations

1. Lane, T.W., "Pond crash forensics: Identification and early detection of pond crash agents" Algal Biomass Summit, Denver CO, September 24-27, 2012
2. Kessler M.K., Collins A.M., Jones H.D.T., Carney L.T., Lane T.W., Timlin J.A., "Tracking Early Infection Events of the Chlorella Virus PBCV-1 with Hyperspectral Confocal Microscopy", presented at Microscopy & Microanalysis, in Phoenix, AZ, July 30 - Aug 2, 2012.
3. Carney L.T, Koh C.-Y. Lane P.D. Sommer G.J, Lane T. W. Forensics of Algal Production Pond Crashes ASLO Aquatic Sciences Meeting, Lake Biwa, Otsu, Shiga, Japan July 8-13 2012
4. Lane T.W., "Pond crash forensics: Identification and early detection of pond crash agents", Algal Biofuels, Biomass and Bioproducts, San Diego, CA , June 10-13, 2012
5. Carney L.T, Koh C.-Y. Lane P.D. Sommer G.J, Lane T. W. Pond Crash Forensics: Rapid detection of predators and pathogens in open pond systems. Algal Biofuels, Biomass and Bioproducts, San Diego, CA , June 10-13, 2012
6. Lane T.W., "Automated Sequencing Library Preparation and Suppression for Rapid Pathogen Characterization", Sequencing Finishing & Analysis in the Future 2012, Santa Fe, NM, June 5-7, 2012
7. Lane T.W., "Pond Crash Forensics", Algal Biomass Summit, Minneapolis, MN, October 25-27, 2011
8. Lane, T.W., "Pond Crash Forensics", 4th Congress of the International Society for Applied Phycology, Halifax, Canada, June 19-24, 2011
9. Lane, T.W., "Pond Crash Forensics", Algae Platform Peer Review, Annapolis, MD, April 8, 2011

Definitions

- Metagenome: all the genetic material present in an environmental sample, consisting of the genomes of many individual organisms.
- Suppression: A general term for the elimination of host and background nucleic acid sequences
- cDNA: complementary DNA (cDNA) is DNA synthesized from a mature mRNA template
- gDNA: Genomic DNA
- PCR: Polymerase chain reaction, at technique to amplify a single or few copies of a piece of DNA across several orders of magnitude
- SGS: Second generation sequencing