Knowledge Foundation's 5th Annual International Conference

SAMPLE PREP 2012

Sample Preparation for Virus, Toxin & Pathogen Detection & Identification

May 3-4, 2012
San Diego, CA USA

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InnovaPrep LLC

James M. Birch, PhD,
Monterey Bay Aquarium Research Institute

Steven S. Branda, PhD,
Sandia National Laboratories

Mark W. Eshoo, PhD,
ibis Biosciences, Inc., a subsidiary of Abbott Molecular

Stephane Evoy, PhD,
University of Alberta, Canada

Dennis W. Harris, DPhil,
IntegenX, Inc.

Michael J. Heller, PhD,
University of California San Diego

Bruce Irvine,
Claremont BioSolutions LLC

Raj Krishnan, PhD,
Biological Dynamics, Inc.

John P. Langmore, PhD,
Rubicon Genomics, Inc.

Raymond P. Mariella, PhD,
Lawrence Livermore National Laboratory

Andre Marziali, PhD,
Boreal Genomics Inc.; University of British Columbia, Canada

Richard A. Montagna, PhD,
Rheonix, Inc.

Rolf Müller, PhD,
Biomatrica, Inc.

Michael Pollack, PhD,
Advanced Liquid Logic, Inc.

Eric R. Rhodes, PhD,
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Alexis Sauer-Budge, PhD,
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Stephanie Thatcher,
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CONFERENCE AGENDA

Thursday, May 3, 2012

8:00 *Registration, Poster Setup, Coffee and Pastries -* ***Exhibit Hall Open***

8:50 **Organizer's Welcome and Opening Remarks**

PLENARY SESSION

9:00 **KEYNOTE ADDRESS**

Sample Preparation "The Achilles Heel for Pathogen Detection and Early Disease Diagnostics"

Michael J. Heller, PhD, Professor, Depts of Bioengineering and Nanoengineering, University of California San Diego

With regard to detecting and identifying low levels of pathogens and disease related biomarkers in complex samples, the following colloquialism says it best "Finding the needle in the haystack is not the same problem as detecting a needle". Yet today many researchers continue to put most of their time, money and intellectual efforts into coming up with new ultrasensitive detection technologies, biosensors, nanosensors, etc for detecting single molecules. While most acknowledge the importance of sample preparation in developing the total analytical/diagnostic system for pathogens and biomarkers, many do not understand just how challenging this problem really is. These challenges can range from dealing with large volumes of complex clinical/biological/environmental samples, to the loss of the organisms/biomarkers after an extended sample preparation process has been carried out. While sample preparation may be the Achilles Heel of pathogen detection, a number of new technologies are now starting to address this issue much more seriously.

9:45 **KEYNOTE ADDRESS**

Advances in Sample Preparation using Microfluidic Technologies

Raymond P. Mariella, PhD, Senior Scientist, Lawrence Livermore National Laboratory

Abstract is not available at time of printing. Please visit www.KnowledgeFoundation.com for the latest Program updates.

10:30 *Networking Refreshment Break, Exhibit/Poster Viewing*

11:00 **Ultra-Rapid Sample to Sequence**

Mark W. Eshoo, PhD, Director of New Technology Development, ibis Biosciences, Inc., a subsidiary of Abbott Molecular

Abstract is not available at time of printing. Please visit www.KnowledgeFoundation.com for the latest Program updates.

11:30 **Automated Molecular Biology Platform Enabling Rapid and Efficient Sequence Analysis of Pathogens in Clinical Specimens**

Steven S. Branda, PhD, Senior Member of Technical Staff, Biotechnology and Bioengineering Department, Sandia National Laboratories*

Effective response to an infectious disease outbreak critically depends upon rapid and accurate identification and characterization of the causative pathogen. Probe-based methods are problematic due to need for *a priori* knowledge of pathogen properties such as nucleic acid (NA) sequences. Unbiased Second Generation Sequencing (SGS) of NA extracted from clinical specimens has enabled discovery of novel pathogens, but this brute-force approach is inefficient and frequently ineffectual, primarily because the signal-to-background (pathogen-to-host NA) ratio in clinical specimens is often very small. We are developing a microfluidics-based automated molecular biology platform that enables selective depletion of host NA in extracts from clinical specimens, and preparation of the remaining (pathogen-enriched) NA for SGS analysis. The platform is designed to interface directly with personalized SGS systems (e.g., Illumina's MiSeq), to take advantage of their short turnaround times; the trade-off in bandwidth (reads/run) is acceptable because sequencing is focused on informative (pathogen-derived) NA. This new technology platform, together with its dedicated bioinformatics pipeline, comprises a Rapid Threat Organism Recognition (RapTOR) system for highly efficient sequence analysis of pathogens in clinical specimens. RapTOR's implementation will greatly accelerate identification and characterization of novel pathogens, and thereby support rational and effective response to infectious disease outbreaks. **In collaboration with: K.Patel, J.S.Schoeniger, S.A.Langevin, V.VanderNoot, H.Kim, Z.Bent, K.P.Williams, O.D.Solberg, P.Lane, D.Curtis, A.Sinha, M.Misra, N.Thaitrong, B.Carson, J.B.Ricken, E.La Bauve, R.F.Renzi, M.Bartsch, N.D.Pattengale, R.Meagher, E.May, A.J.Powell, T.W.Lane*

12:00 **Sample Preparation Using Digital Microfluidics**

Michael Pollack, PhD, CTO, Advanced Liquid Logic, Inc.

Digital microfluidics, characterized by precise and direct manipulation of liquid droplets using electrowetting, is being applied in a variety of applications in research and diagnostics markets. The programmable flexibility of digital microfluidics permits a wide range of protocols to be implemented on a single platform or even on a single cartridge while providing substantial benefits in terms of reagent savings, throughput, ease of use and performance. Advanced Liquid Logic has implemented a broad range of sample preparation capabilities using digital microfluidics. Research applications include automation of highly complex workflows such as DNA fragment library preparation for next-generation sequencing. On the clinical side, digital microfluidics enables sample preparation from raw specimens for integrated sample-to-answer analysis. The basic concepts and capabilities underlying digital microfluidic-based sample preparation will be presented as well examples of applications which integrate sample preparation with downstream analysis.

12:30 *Luncheon Sponsored by the Knowledge Foundation Membership Program -* ***Exhibit Hall Open***

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2:00 **DNA Target Enrichment and Background Depletion for Detection of Rare Sequences**

Andre Marziali, PhD, President and CSO, Boreal Genomics Inc.; Director, Engineering Physics, University of British Columbia, Canada

An electrophoretic sample preparation method that is capable of both enriching a sample for multiple rare sequence targets from a large wild-type background with single nucleotide resolution, as well as depleting specific abundant sequences from a sample to allow sequencing of unknown rare mutants. As a front end to 2nd generation DNA sequencing technology, this method will allow much more accurate and sensitive determination of rare sequences in a sample. The method, based on the commercialized SCODA non-linear electrophoresis technology, takes advantage of the non-linear response to electric fields felt by nucleic acid fragments migrating through a gel matrix that is covalently decorated with a complementary oligo sequence and held at the duplex melting temperature. We have recently demonstrated that the method can be run in rejection mode, to remove specific sequences from a sample, allowing accurate sequencing of rare mutants that would otherwise be swamped by wild-type or background sequence. We will present recent data and performance metrics in both sequence enrichment and depletion applications.

2:30 **Isolation of Dilute Bacteria from Blood for Rapid Diagnostics**

Alexis Sauer-Budge, PhD, Senior Research Scientist, Fraunhofer Center for Manufacturing Innovation, Fraunhofer USA

Traditionally, bacterial pathogens in the blood have been identified using culture-based methods that can take several days to obtain results. This can lead to physicians making treatment decisions based on an incomplete diagnosis contributing to patient morbidity. To decrease diagnosis time, we are developing a novel technology that will be able to identify the type of bacteria, as well as any antibiotic-resistance information, in less than one hour.

3:00 **Bacteriophage Tail Spike Proteins for the Specific Capture of Pathogenic Bacteria**

Stephane Evoy, PhD, Associate Professor, Dept of Electrical and Computer Engineering and National Institute for Nanotechnology, University of Alberta, Canada*

The use of beads to capture bacterial pathogens has received increased attention for sample preconcentration and preparation. The high level of specificity of phages offers a potent alternative to antibodies for targeting purposes. Over the last seven years, we have developed bacterial capture platforms leveraging bacteriophage. These activities were recently expanded to the use of the actual tail spike proteins (TSP) responsible for their specificity. The smaller size of the TSP indeed provides more uniform coverage to the capturing surface. We have thus demonstrated the use TSPs for the specific capture of *Salmonella, Shigella* and *Campylobacter*. We have also developed beads functionalized with these TSPs, and used them as part of a sample preparation procedure that distinctively improves the detection threshold of PCR in complex matrices. Target pathogens include *Ecoli, Salmonella, Shigella, Campylobacter,* and *Mycobacterium* tuberculosis. **In collaboration with: A.Singha, D.Arutyunovb, C.M.Szymanskib.*

3:30 *Registration, Poster Setup, Coffee and Pastries -* ***Exhibit Hall Open***

4:00 **Next Generation 'Nanosome' Isolation from Blood and Other Solutions for Rapid Pathogen Detection Using an AC Electro-Kinetic Method**

Rajaram Krishnan, PhD, Chief Executive Officer, Biological Dynamics, Inc.*

The rapid isolation and detection of pathogens directly from blood and other complex solutions is a significant challenge and is often tedious, time-consuming and complex using a combination of chemical and mechanical steps. Recent design and construction of a microelectrode array by Biological Dynamics has allowed for a new AC Electro-Kinetic method of isolation of these pathogens directly from complex solutions. This array has been used to isolate mCherry red fluorescent tagged T7 Bacteriophage directly from Blood and high salt solutions (0.5x PBS) as well as isolating, lysing, tagging and prepping for sequencing of E. Coli bacteria from complex solutions. *In collaboration with: M.J.Heller, A.Sonnenberg, UCSD; and L.Kumosa, J.McCanna, W.Hanna, J.Lu, E.Tu and D.J.Charlot, Boplogical Dynamics

4:30 **Nanomachine-Based Target Isolation**

Joseph Wang, PhD, Professor, Dept of Nanoengineering, University of California San Diego

The remarkable performance of biomotors is inspiring scientists to create synthetic nanomachines that mimic the function of these amazing natural systems. Particular attention will be given to catalytic nanowire and microtube motors propelled by the electrocatalytic decomposition of a chemical fuel, as well as to fuel-free (magnetically or electrically-driven) nanomotors. This presentation will describe new motion-based bioassays based on the selective capture, sorting and transport of target biomolecules. Microengines functionalized with different receptors will be shown to capture selectively and transporting target DNA and cancer cells from raw complex body fluids. Key factors governing such motion-based target isolation will be discussed. The greatly improved capabilities of chemically-powered artificial nanomotors could pave the way to exciting and important bioanalytical applications and to sophisticated nanoscale devices performing complex tasks.

5:00 **PANEL DISCUSSION:**

Challenges and Opportunities in Advanced Sample Preparation

Facilitator: Michael J. Heller, PhD, Professor, Depts of Bioengineering and Nanoengineering, University of California San Diego

5:45 End of Day One

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Friday, May 4, 2012

8:00 *Registration, Poster Setup, Coffee and Pastries -* ***Exhibit Hall Open***

9:00 Sample-to-Results Using a Fully Automated CARD® System

Richard A. Montagna, PhD, Senior Vice President, Rheonix, Inc.

The Rheonix CARD® technology platform automatically performs all preparative, detection, and readout functions to detect a variety of molecular targets. Without any user intervention, the system can analyze a wide range of clinical or environmental specimens, when the disposable CARD device is placed into either a benchtop (24 assays) or portable, battery-operated (8 assays) version of the Encompass MDx™. Initial sample volumes from 5 microliters to 10 milliliters can be processed.

9:30 The Lab-in-a-Box: Integrating the Macro and Micro Worlds to Achieve True Sample-To Answer Integration

Dennis W. Harris, DPhil, Chief Scientific Officer, IntegenX, Inc.

IntegenX is leveraging MOVe™ valve technology to enable the integration of large volume, real-world samples into microfluidic chip-driven devices that, for the first time allow true sample-in, answer-out solutions for genomics analysis. The underlying technologies and results will be illustrated using two examples: The RapidHIT™ 200 system allows for unambiguous DNA based human identification in under 90 minutes from sample (buccal swab, blood, etc.) to full CODIS profile. The Integrated Sequencing System (ISS™) combines MOVe-based microfluidic sample processing with Next Generation DNA Sequencing to enable whole genome analysis from crude samples in under a day. Such a system could be deployed as a monitoring system to detect bio-agents including genetically engineered micro-organisms.

10:00 Integration of PureLyse® Sample Preparation into Sample-to-Answer Diagnostic Cartridges

Bruce Irvine, Chief Technology Officer, Claremont BioSolutions LLC

ClaremontBio has created a novel method of rapid sample preparation that is entirely disposable; it requires no instrument. It provides mechanical cell lysis and simultaneously extracts nucleic acids or proteins. Nucleic acid can be extracted in three to five minutes with two to three steps. The disposable PureLyse® device is comprised of a micro-motor and vane that beat particles at unusually high shear forces. As cells are lysed, nucleic acid binds to the lysing particles. The flow through configuration of the PureLyse® cartridge enables its use for a wide range of sample volumes. This system has been used to lyse and extract nucleic acids from *E. coli, Bacillus subtilis* vegetative cells and spores, *Mycobacterium* bovis, and *Clostridium* difficile. This system delivers high yield RNA preparation as well. Further, unique binding chemistry can select for the binding of RNA over DNA. The miniature disposable nature of this flow-through cartridge lends itself to integration. Our flagship approach to integration is the OmniValve™ fluidic system, where we have embedded the PureLyse® chamber within a valve with up to six ports connecting the lysis and extraction cartridge to other chambers. This facilitates integration of DNA extraction with sample, pre-filters, wash, elution, and amplification. This approach was very successfully applied to an NIH funded SBIR project developing a semi-integrated system for detecting *Clostridium difficile,* resulting in 100% specificity and 96% sensitivity at detecting *C. diff* in previously diagnosed human stool samples.

10:30 *Registration, Poster Setup, Coffee and Pastries -* ***Exhibit Hall Open***

11:00 Microbe Detection in the Open Ocean

James M. Birch, PhD, Director, SURF Center (Sensors: Underwater Research of the Future), Monterey Bay Aquarium Research Institute

Here we describe the Environmental Sample Processor (ESP), an oceanographic, autonomous, *in situ* microbiology laboratory-in-a-can that performs sandwich hybridization assays and qPCR for the detection of bacteria, harmful *algae*, and toxins. The ESP is entirely self-contained and can be deployed for up to 45 days before servicing. Under development at the Monterey Bay Aquarium Research Institute (MBARI), the ESP has been successfully deployed around the world.

11:30 Primary Concentration of Multiple Pathogens and Secondary Concentration of Viruses from Environmental and Drinking Waters

Eric R. Rhodes, PhD, Research Microbiologist, U.S. Environmental Protection Agency

The collection of waterborne pathogen occurrence data often requires the concentration of microbes from large volumes (10 – 1600 L) of water due to the low number of microorganisms that are typically present in environmental and drinking waters. Hollow-fiber ultrafiltration (HFUF) has shown promise in the recovery of various microorganisms and our studies have demonstrated that the HFUF primary concentration method is effective at recovering coliphage, enteric viruses and *Cryptosporidium parvum* from large volume samples. We have also developed an effective secondary concentration method for the recovery of coliphage and enteric viruses from HFUF concentrates.

12:00 Concentrating Pipette for Rapid Sample Prep

David S. Alburty, Chief Executive Officer, InnovaPrep LLC

The concentrating pipette developed by InnovaPrep prepares samples quickly, reducing the final volume to 200 microliters of clean buffer solution. The concentration method used is wet-foam elution, originally developed for integrated biodefense applications. The concentrating pipette's disposable tips prevent sample carryover. The wet foam is now supplied in premixed aerosol cans that are easily loaded into the benchtop instrument. Tips available now include 0.1 micron PES membranes and are applied to concentration of bacteria, protozoa, mold and fungal spores and fragments. Concentration of bacteria from spinach wash water, apple juice, and whole blood will be presented along with efficiency and concentration factor data.

12:30 *Lunch on Your Own -* ***Exhibit Hall Open***

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2:00 **Two Methods for High-Throughput Next-Gen Sequencing Template Preparation from Small Amounts of Degraded Clinical and Environment Samples Without Automation**

John P. Langmore, PhD, Chief Scientific Officer, Rubicon Genomics, Inc.

We have tested 2 kits for high-throughput NGS preparations from picograms of degraded DNA or single cells. ThruPLEX-FD Prep is a 1-tube, 2-hour, 3-step process for preparing 0.5 pg - 20 ng of fragmented DNA for the Illumina MiSeq/HiSeq for accurate and uniform sequencing. ThruPLEX is 10 - 100X more sensitive than conventional NGS preps. PicoPLEX-SC Prep is a 1-tube, 3-hour, 4-step process for single-cell DNA extraction and NGS template preparation. Greater than 95% of single human cancer, sperm, and blastomere cells were reproducibly amplified for NGS-producing sequence fingerprints useful for forensics of human and microbial samples.

2:30 **FilmArray® Automated Sample Lysis and Purification**

Stephanie Thatcher, Director of Systems Integration, Idaho Technology, Inc.

The FilmArray system includes automated lysis and nucleic acid extraction for a wide variety of organisms and sample types. It was designed to be flexible for many sample types and sensitive with a reasonable input volume for detection of low level organisms. To date, the system has been developed for respiratory swabs, blood, blood culture, urine, stool and powders for detection of respiratory, sepsis, sexually transmitted, gastrointestinal, and biothreat pathogens.

3:00 **Rapid Nucleic Acid Extraction *(tentative title)***

Speaker to be confirmed, ZyGEM Corporation Ltd., New Zealand

Abstract is not available at time of printing. Please visit www.KnowledgeFoundation.com for the latest Program updates.

3:30 *Registration, Poster Setup, Coffee and Pastries -* ***Exhibit Hall Open***

4:00 **Ambient Biostabilization in the Age of Personalized Medicine - A Paradigm Shift in Biospecimen Management**

Rolf Müller, PhD, President and CSO, Biomatrica, Inc.

Sample Management At Room Temperature (SMART) is an innovative approach to bio-specimen logistics and represents a paradigm shift in sample quality management for biorepositories and biomedical researchers. The technology offers greater sample consistency and dependability for storage and transport while maintaining the highest sample quality for reliable results. Results of scientific evaluations of ambient temperature-preserved RNA and DNA versus ultra-low temperature stored samples have consistently showed that these two storage approaches offer similar levels of sample quality, but ambient storage provides significant cost-savings over cold-stored systems. As nature is often unpredictable, preservation of critical assets such as biospecimens at ambient temperature represent a smart back-up approach in the event of unforeseen disasters like earthquakes, fire, tsunamis, cold-storage system malfunctions and breakdowns, etc. These novel technologies expand the technological innovations required to realize the goals of a globalized health care system that allows access to patient samples from remote locations of the world with significant challenges in social and health care infrastructures. These challenges are overcome with an ambient-based technology that preserves, protects and archives these vital and fragile biological samples from point of collection to labs for clinical analysis, disease diagnosis and disease management.

4:30 **Bioseparation and Preparation of Complex Biological Samples by Microfluidic Chip-Based Dielectrophoretic Manipulation Techniques**

Martin Stelzle, PhD, Head of BioMEMS & Sensors Group, NMI Naturwissenschaftliches und Medizinisches Institut, Germany

Microfluidic device technology provides unique physical phenomena that are not available in the macroscopic world. Here, dielectrophoresis represents a particularly versatile and useful separation technology. These may be exploited towards a diverse array of applications in biotechnology and biomedicine ranging from bioseparation of particulate samples and sensitive diagnostic assays to the assembly of cells into structures that resemble the smallest functional unit of an organ. We have developed a microfluidic system – microPrep – for subcellular fractionation of cell homogenates based on dielectrophoretic sorting. Separation of mitochondria isolated from homogenates of a human lymphoblastoid cell line was monitored by fluorescence microscopy and further characterized by western blot and mass spectrometry analysis. Robust high throughput and continuous long-term operation for up to 60 h of the microPrep chip system with complex biological samples became feasible as a result of a comprehensive set of technical measures: (i) coating of the inner surfaces of the chip with BSA, (ii) application of mechanical actuators to induce periodic flow patterns, (iii) efficient thermal control of the device to ensure integrity of organelle, (iv) a wide channel to provide for high fluidic throughput, and (v) integration of a serial arrangement of 10 dielectrophoretic deflector units to enable separation of samples with a high particle load without clogging. Hence, microPrep yields tens of micrograms of enriched and purified mitochondria within hours. Western blots of mitochondria fractions showed that contaminating endoplasmatic reticulum was reduced by a factor of 6 when compared to samples prepared by state of the art centrifugation.

5:00 **Exhibitors and Sponsors Showcase Presentations**

5:30 **Selected Oral Poster Highlights and Open Discussion**

5:45 *Concluding Remarks, End of Conference*

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