NGS Symposium: Improving Genomics through Collaboration and Innovation

2013

Brought to you by D-MARK Biosciences and Integrated DNA Technologies

March 13-14

NGS Symposium: Improving Genomics through Collaboration and Innovation

Wed March 13, 2013
10:30am – 5pm
5-7pm Poster Session and Social
UBC – Michael Smith Building (MSL)

Thurs March 14, 2013
10:30am – 5pm
5-7pm Poster Session and Social
BC Cancer Research Centre

Integrated DNA Technologies and D-MARK Biosciences are proud to welcome you to this Next Generation Sequencing Symposium, hosted at UBC MSL labs on **Wednesday March 13th** and at the BC Cancer Research Centre on **Thurs March 14th.**

Overview

Next generation sequencing holds great promise for advancing the understanding the genetics of all organisms. Advances in this area continue to come at an increasing rate, and there are now several platforms to choose from. For the uninitiated this can create confusion and make planning for an initial experiment difficult. In order to help demystify NGS as an application, we have assembled a panel of academic and industry speakers experienced in the field. The objective of this symposium is to provide an overview of NGS, move on to a more detailed look at instrumentation and methods, and then hear directly from academics putting local NGS platforms to work. The symposium is open to anyone who is interested in or is already using NGS in their research or diagnostic programs. Each talk will last 20-30 minutes, and ample time will be made available for discussion.

Poster Competition

A poster session will be held from **5-7 PM** on each day following the symposiums, featuring NGS research from industry and academia. Refreshments will be provided, and **prizes** will be awarded for the best 3 posters. Judges will be selected from the main workshop speakers.

If you would like more information, please contact:

- **Jeffrey Seitz** (Jeffrey.seitz@d-markbio.com)
- **Bob Setter** (rsetter@idtdna.com)

Invited Speakers:

Martin Hirst, Ph.D.,
UBC, CHIBI

Dr. Hirst's research is directed at understanding the role of epigenetics in cancer and investigating the therapeutic potential of interventions directed at epigenetic processes. He approaches this from an epigenomic perspective by combining innovative molecular biology and computational techniques with genome-wide detection platforms.

Working with local, national and international collaborators Dr. Hirst was one of the first in the world to develop techniques for performing epigenome profiling utilizing next generation sequencing platforms. Collaborating within a team of scientists and clinicians Dr. Hirst is applying these tools to profile the genetic and epigenetic landscapes of normal and transformed human tissues and cells and has published 81 peer reviewed research articles.

Dr. Hirst is actively engaged in the generation of reference epigenome maps of normal human cells and tissues as part of the CIHR and Genome BC funded Centre for Epigenomic Mapping Technologies and NIH Reference Epigenome Roadmap project. He is a member of the International Human Epigenome Consortium Scientific Steering Committee (ISSC) and the International Cancer Genome Consortium technologies working group.

Reference Human Epigenomes

As part of the NIH Reference Epigenome Project (http://www.roadmapepigenomics.org) and Canadian Epigenetics and Environment Health Research Consortium (http://www.cihr-irsc.gc.ca/e/43734.html) our group is engaged in the generation of comprehensive reference epigenomes from primary disease-free human tissues and cells. Reference epigenomes include six histone modifications (ChIP-seq), selected for their opposing roles in regulating active and inactive chromatin, DNA methylation and hydroxyl-methylation (sodium bisulfite and oxibisulfite shotgun sequencing respectively) and RNA (mRNA and miRNA-seq) profiles. These data are being generated as part of the broader International Human Epigenome Consortium (http://ihec-epigenomes.net/), which aims to generate 1000 reference epigenomes for the research community over the next 5 years. To date our epigenomic mapping centre has generated reference epigenome maps for breast, blood, brain, skin, placental and stem cell types. Future cell types include peripheral and lymphoid B-cells, T-lymphocytes, primitive hematopoietic cells, patient derived iPSCs, as well as thyroid cells and mammary, endometrial, fallopian and colonic epithelium.

As a first step towards understanding normal epigenomic variation revealed by these data we have undertaken an analysis of reference epigenomes from breast, blood, brain, skin and embryonic stem cells types. Our analysis has revealed that distinct epigenetic states are encoded in the epigenomes of these cell types. Cell type specific DNA methylation patterns both within and outside CpG islands exist and consensus, hypo-methylation regions overlapping with H3K4me1, an epigenetic modification tightly linked to enhancer regions, mark cell-type specific enhancer elements. Cell type specific patterns of both active (H3K4me3) and repressive (H3K27me3) histone modifications in association with transcriptional start sites have also been identified. Overall, our analysis provides insight into the epigenomic patterns that define these cell types, provide a paradigm to prioritize intergenic, non-coding and synonymous variants obtained from whole genome sequencing and lay a foundation for the identification of disease specific epigenomic alterations.

Daniel Evans, B.Sc.
UBC, Centre for Applied Neurogenetics

Dan received his B.Sc. in Computer and Information Science in 2000 from Queen's University in Kingston, Ontario. In 2007, he started working as a programmer and database administrator for Prof. Ronald Beavis at the University of British Columbia. While with Prof. Beavis, Dan participated in algorithm development for investigating proteomics datasets as well as implementing several technical upgrades to the database and software of the Global Proteome Machine, (http://gpmdb.thegpm.org), one of the largest publicly accessible databases of tandem mass spectra of biological samples.

In 2011, Dan joined the lab of Prof. Matthew Farrer at the University of British Columbia, where he has developed analysis pipelines for next generation sequencing data, databases of genetic variation and clinical information, as well as assisted with research on modifiers for Parkinson Disease and the genetics of neurodegeneration. Current projects includes working with geneticists to

identify candidate disease variants from custom capture panels of genes involved in several neurodegenerative diseases on discrete populations, analyzing whole-exome data from family studies, and developing analysis pipelines for use on large computing clusters.

"Drinking from the Firehose"

Genetic analysis has come a long way from the days of using microsatellites and manually generated pedigrees to find disease-linked areas of a chromosome. Current next generation sequencing platforms allow researchers to sequence subsets of genes, the entire coding region or the whole genome of an organism in a matter of days and it will only get faster. Given all this data, what can we do to illuminate the cause and course of genetic disease, and how can we do it?

Turning samples into data

Focused custom capture panels allow researchers to target dozens of specific genes in a large population, with applications in mutation and disease modifier discovery as well as the possibility of informing clinical care. Whole-exome sequencing targets the coding region of the genome, yielding a clear view of the areas that translate into. Using whole-exome sequencing of an affected person and their family members can (and has) lead to the discovery of new disease-causing mutations in the genome: examples are VPS35 and DNAJC13, just in Parkinson Disease. Whole-genome sequencing gives researchers access to everything; applications of whole-genome sequencing include everything listed above, and probably many other things we haven't considered yet. Different platforms, approaches, advantages and disadvantages will be presented.

Turning data into information

How do we handle the gigabytes of information the above approaches generate? Luckily, open access data and software allows for efficient detection of "signal" amidst "noise": mapping and alignment of reads, mapping quality cutoffs and variant calling scores pare the data down considerably, but not enough. Further analysis with additional information is required: how likely is a variant to be real? How common is this variant? Is it seen in some populations, but not others? What effect would this variant have on its protein? Different analysis algorithms for sequence alignment, variant calling and insertion/deletion detection will be discussed.

Turning information into knowledge

Even after all the winnowing down of the data generated by sequencers, hundreds of candidate variants will still exist. How can we determine what is real and what is coincidence? In population studies, is there a significant difference in the frequency of a variant between affected and unaffected? In family studies, does this variant cause disease? Methods of ascertaining significance will be shown.

Hugh Arnold, Ph.D.,
Sr. Scientist, Geospiza/PerkinElmer

Hugh Arnold received his Bachelor of Science in Cellular and Molecular Biology from the University of Washington 1999. He then spent two years at Fred Hutchinson Cancer Research Center where he helped launch the first prostate specific microarray for profiling prostate cell lines and patient tumors. In 2008, Hugh went on to receive his PhD in Molecular and Medical Genetics from Oregon Health and Sciences University while studying the molecular mechanisms underlying the disregulation of the onco-protien c-Myc.

Following two post-docs at the University of East Anglia and Fred Hutchinson Research Center characterizing extra cellular matrix proteins in breast cancer and protein biomarker discovery in prostate cancer, Hugh joined Geospiza in 2009 where he helped support the launch of the Next Generation Sequencing data analysis within GeneSifter Analysis Edition. Currently, Hugh is a Product Manager at Geospiza now part of PerkinElmer where he continues to support GeneSifter Analysis Edition for Next Genation Sequencing and Microarray data analysis.

What Does it Take to Identify Signal from Noise in Molecular Profiling of Tumors?

Cancer is a complex, heterogeneous disease that is driven by continually evolving genomic changes. Our current efforts to identify and the cure or demise of patients has utilized snap shots of DNA, RNA, proteins, and/or protein/nucleic acid interactions among numerous assays. For example, sequencing genomes or exomes distinguishes germline variants from somatic mutations as one step toward identifying nucleotide changes that are truly driving mutations. However, these assays identify very large numbers of variants and substantially reducing the noise requires considering the potential impact of variants (missense, non-sense, synonomous), quality of the call, prevalence of mutations in tumor versus normal cells, and whether a gene carrying a mutation is even expressed. Consequently, molecular profiling of tumors, benefits from data obtained from several different kinds of DNA

sequencing-based assays. Using data from paired tumor and normal samples we will show an example workflow that combines exome and transcriptome sequencing to identify putative driver mutations that display high signal for being impactful in cancer.

Comprehensive Data Analysis Solutions : New Tools for RNA-Sequencing (Seminar & Workshop)

Next Generation Sequencing provides unprecedented access to volumes of genomic information. Discover a better way to manage and analyze RNA-Seq data for Gene Expression, Differential Splicing, Nucleotide Variant, and Gene Fusion data as well as creating New Annotation by leveraging tools such as BWA, GATK, TopHat/Cufflinks, and Chimerascan.

PerkinElmer will be presenting a seminar and workshop focused on RNA-Seq data analysis. Bring your laptop and gain hands-on experience using GeneSifter software to analyze RNA-Seq data sets.

Lin Pham, Ph.D.
NuGEN Inc.

Lin is the Sr. Director of Product Development and Automation at NuGEN Inc. She has 20-years of experience in genomics technology/product development and biomedical research. She has developed and launched a broad range of instruments, reagents and software products in the area of genomic and transcriptome analysis and target resequencing. Dr. Pham brings an in-depth understanding of disease gene mapping and candidate gene research, as well as a passionate approach to researching root causes and genetic basis for common diseases. Previous positions include a Post doc at Children's Hospital Oakland Research Institute and Genentech, Inc., technology startups Sequana Therapeutics in La Jolla and RainDance Technologies near Boston and 15+ years at Applied Biosystems/Life Technologies.

NGS with extremely small, degraded, and irreplaceable specimens

NuGEN has revolutionized sample preparation technologies that enable life science researchers to extract important biological information from extremely small, degraded, and irreplaceable specimens. In this presentation we will present select core technologies developed at NuGEN and show how they enable the analysis of genome, exome, epigenome, and transcriptome. Examples include strand-specific RNA-Seq with elegant, simultaneous removal of unwanted abundant RNA species such as rRNA and globin RNA, methylation studies with ultralow input material, and walk-away automation using microfluidic sample preparation.

Scott Rose, Ph.D.
Director of Product Development at Integrated DNA Technologies (IDT)

Dr. Scott Rose was recently appointed Director of Product Development at Integrated DNA Technologies with special emphasis being placed on developing: improved Nucleic Acid Capture and Enrichment technologies, Synthetic Biology tools, RNase H2 based detection technology, and functional genomics. He previously served as Director of Molecular Genetics at IDT under Dr. Mark Behlke, Chief Scientific Officer. He carried out his post-doctoral work at the University of Texas Southwestern Medical Center in the laboratory of Dr. Raymond MacDonald in the department of Molecular Biology and Oncology studying transcriptional based gene regulation using the pancreas as a model system. He received his Ph.D. in Biochemistry from Rice University in 1988 under the supervision of Dr. Susan Berget. His thesis work focused on RNA processing, looking at mRNA splicing and polyadenylation. His undergraduate degree in Biochemistry was from the University of Michigan-Dearborn.

Steve Hallam, Ph.D.,
UBC, Microbiology and Immunology

Steven Hallam is an Associate Professor in the Department of Microbiology and Immunology at UBC and Canada Research Chair in Environmental Genomics. He received his PhD from the University of California Santa Cruz where he studied developmental regulation of neuronal asymmetry and synaptic remodeling in the model nematode Caenorhabditis elegans. Motivated by this experience in complex networks he became a postdoctoral researcher at the Monterey Bay Aquarium Research Institute and later Massachusetts Institute of Technology where he studied various aspects of marine microbiology and environmental genomics. His current research interests include microbial

ecology, genetics and bioinformatics with specific emphasis on the creation of computational tools and combined workflows for taxonomic and functional binning, population genome assembly, and metabolic pathway reconstruction.

"Oceans of Information"

Although the vast majority of microbes in nature resist laboratory cultivation, they represent an almost limitless reservoir of genetic diversity and metabolic innovation. Next generation sequencing technologies are rapidly expanding our capacity to access genotypic and phenotypic information directly from environmental samples. However, to effectively interpret and apply this increasing volume of information, new analytical tools and services must be developed with the end user in mind. Here, I explore problems and solutions in environmental sequence analysis spanning different levels of biological organization from genomes to biomes. I highlight emerging open source tools for integrating and visualizing multimolecular (DNA, RNA and protein) and environmental parameter data and consider the future of data intensive computation as it relates to pathway reconstruction, ecosystem modeling and synthetic ecology.

Carl Vilariño-Güell, PhD
UBC, Centre for Applied Neurogenomics

Dr. Vilariño-Güell started his career with a biology degree from the University of Barcelona and a PhD in molecular genetics at the University of Leicester, England. He held two postdoctoral positions in England, first at the University of Oxford, and then at the Institute of Neurology University College London where he started his career studying the molecular bases of neurodegeneration. In 2007, he moved to the Division of Neurogenetics, within the Department of Neuroscience at Mayo Clinic. Dr. Vilariño-Güell became an Assistant Professor of Molecular Neuroscience in 2009, initiating new research programs in the molecular genetics. In 2010, he relocated to the University of British Columbia for an opportunity to expand his research effort in neurologic and neurodegenerative disease with primary focus on multiple sclerosis and essential tremor.

Dr. Vilariño-Güell is an expert molecular geneticist. He has dedicated the past eight years his career to the identification and understanding of genetic mutations leading to familial forms of neurological disease, and the analysis of complex epidemiological data in ethnically distinct populations, to identify susceptibility variants acting as early precursors of disease development. To date, Dr Vilariño-Güell has published over 50 articles in many prestigious journals including New England Journal of Medicine and Nature Genetics. Highlights of his research include the identification of LINGO1 as the first genetic risk factor common to Parkinson disease and essential tremor, pathogenic MEIS1 mutations for restless legs syndrome, DCTN1 mutations in Perry syndrome; classical linkage analysis for the identification of eIF4G1 mutations in Parkinson disease patients, and more recently, the implementation of next-generation sequencing technologies for the identification of VPS35 and DNAJC13 mutations leading to Parkinson disease.

Dr Vilariño-Güell currently directs an exome sequencing program, and participates in the generation and characterization of recombinant models to assess gene/protein function. He maintains national and international relationships with many leading investigators in movement and memory disorders, and more recently in translational neuroscience. He has a depth of experience in classical genetic approaches, bioinformatics and next-generation sequencing/data validation that focuses on predicting and ultimately preventing brain disorders.

DNAJC13 mutations in Parkinson disease

Background: The implementation of second generation sequencing technologies in multi-incident Parkinson disease (PD) families has led to the identification of novel genetic determinants of disease.

Methods: Exome analysis was performed in a Saskatchewan family with parkinsonism, followed by genotyping in multi-ethnic case control series consisting of 2,928 patients and 2,676 control subjects from Canada, Norway, Taiwan, Tunisia and the United States.

Findings: A disease-segregating mutation in DNAJC13 (p.Asn855Ser) was identified. Screening of cases and controls revealed four additional affected carriers, two with a family history of parkinsonism. All patients claim Dutch-German-Russian Mennonite heritage, and a common ancestral haplotype harboring the DNAJC13 p.Asn855Ser was confirmed by microsatellite marker analysis. The clinical presentation of carriers is consistent with typical, late-onset PD, and pathologically with brainstem or transitional Lewy body disease, for which DNAJC13 immunoreactivity is noted in the associated Lewy body pathology.

Interpretation: DNAJC13 plays a central role in the uncoating of clatherin-coated vesicles, an essential step to recycle membrane-associated proteins. DNAJC13, with VPS35 and DNAJC6, becomes the third gene harboring pathogenic mutation in parkinsonism directly implicated in endosomal trafficking. With past observations in SNCA, MAPT and LRRK2, this study provides a novel unification for the molecular genetic etiology of PD.

Andre Marziali, Ph.D.
Boreal Genomics Inc., Vancouver, BC,
Department of Physics and Astronomy, UBC

Dr. Marziali received his B.A.Sc. in Engineering Physics from the University of British Columbia in 1989, and his PhD in Physics from Stanford University in 1994. He subsequently worked for several years with Dr. Ron Davis, in the Stanford DNA Sequencing Technology Center, developing instruments for DNA sequencing and sample purification. He returned to Canada in 1998, as an Assistant Professor at UBC in the Department of Physics and Astronomy, where he formed the Applied Biophysics Laboratory. Shortly after his return to Canada, Dr. Marziali formed the Genome BC Technology Development Platform, which he continues to lead as Director. In 2005, he was awarded tenure and appointed Director of the Engineering Physics Program at UBC.

In 2004, Dr. Marziali co-invented the concept of using synchronous mobility perturbations to create divergent velocity fields for selectively focusing nucleic acids. This technology, termed SCODA, is the basis of a spin-off company, Boreal Genomics Inc. founded in 2007 by Dr. Marziali and colleagues to commercialize high performance instruments for DNA and RNA purification. In the last few years he has been awarded the 2003 Killam Prize for Excellence in Teaching, the 2004 BC Innovation Council – Young Innovator award, and the 2005 Canadian Association of Physicists Medal for excellence in teaching.

Multiplexed detection of low abundance, tumor related nucleic acids in the plasma of cancer patients

We present recent data demonstrating multiplexed detection of tumor mutations in plasma with sensitivity as low as 0.01% compared to normal DNA.

Robust detection above background at such low mutation abundances is achieved using electrophoretic separation of DNA fragments containing point mutations from their wild-type counterparts. Commercialized as the OnTarget platform from Boreal Genomics, the technology is able to improve the ratio of mutant to wild-type DNA more than a million fold to improve sensitivity of virtually any genotyping assay.

We have applied this technology to NGS and PCR based detection of low abundance DNA tumor markers in cancer patients' plasma and tissue. With OnTarget enrichment applied to plasma samples, we are able to easily detect and quantify mutated DNA targets at abundances below 0.01% compared to the wild-type allele, and are sensitive to single mutant molecules in the input sample.

In addition, we have demonstrated that this method can be applied over a large number of mutations without sample splitting, and therefore maintains high sensitivity. By enriching for mutants prior to the PCR amplification required for detection of the mutant targets, we have eliminated false positives introduced by sequence errors in NGS or PCR.

The sensitivity, simplicity, and speed of this enrichment-based assay make this a powerful tool in oncology for minimal residual disease monitoring, and for non-invasive tumor mutation monitoring for treatment selection.

This presentation will describe the technology, and present both the latest technical performance data of the assay on plasma and FFPE tissue, and the latest clinical data obtained from studies using lung cancer and melanoma patient samples.

Mohammed A. Qadir, Ph.D.
BC Cancer Research Centre

Dr. Mohammad A. Qadir completed his Ph.D. at the Department of Biology, Molecular Genetics Unit, the University of Western Ontario. He then undertook a post-doctoral position at the BC Cancer Agency's Genome Sciences Centre, where he worked on siRNA and their therapeutic applications. Dr. Qadir then moved to the Department of Experimental Therapeutics (BCCA), where he worked on high-throughput large-scale siRNA

screening studies with Dr. Marcel Bally. For the past two years, Dr. Qadir has been involved in next-generation sequencing studies in childhood cancers with Dr. Poul Sorensen. It is there that he co-developed the ChildSeq diagnostic sequencing program for the identification of pathognomonic translocations in childhood cancers.

ChildSeq-RNA: A high-resolution and scalable assay to diagnose childhood cancers

Small round cell tumors (SRCTs) of childhood are a group of predominantly pediatric sarcomas that are extremely difficult to diagnose accurately without the use of a wide range of ancillary methods. The current standard of diagnosis for these tumors is to use RT-PCR or fluorescence in situ hybridization (FISH) based assays to identify pathognomonic gene fusions arising from chromosomal translocations. The two main limiting factors associated with these assays are: (1) their inability to identify these gene fusions at high resolution at the nucleotide level, and (2) their lack of capacity to identify more than one gene fusion simultaneously.

Here, I present ChildSeq-RNA, a highly scalable next-generation sequencing-based diagnostic assay to identify all known pathognomonic translocations associated with SRCTs simultaneously. I will also discuss, ChildDecode, an accompanying bioinformatics algorithm to analyze the NGS data, that operates on a scalable bioinformatics-computing platform.

Ryan Morin, Ph.D.
Simon Fraser University,
Genome Sciences Centre, BC Cancer Agency

Ryan Morin trained at the BC Genome Sciences Centre earning his MSc and PhD degrees under the supervision of Dr. Marco Marra. He has been developing methods for analyzing NGS data since the early availability of RNA-seq and whole genome sequencing. His work has contributed to the identification relevant mutations in numerous human cancer types leading to publications in Nature, Nature Genetics and NEJM.

Using NGS to identify driver mutations and non-invasive biomarkers for non Hodgkin lymphomas

Non Hodgkin lymphomas are a diverse collection of over 30 cancer types that form solid tumours in lymphoid and other tissues. Application of whole genome and transcriptome sequencing (WGS and RNA-seq) to primary non Hodgkin lymphoma samples has facilitated our identification of numerous genes with no previously known role in these or other cancers. A striking observation resulting from our recent work in this area includes the observation of highly recurrent mutations affecting genes encoding histone modifying enzymes including EZH2, CREBBP, EP300, MLL2, MEF2B. More recently, we have identified genes for which mutation status in a lymphoma is individually prognostic. Such highly prevalent and recurrent mutations offer the potential for non-invasive assays that can detect cell-free mutant DNA deriving from tumour cells in the bloodstream. As circulating tumour DNA (ctDNA) can be present at extremely low levels relative to cell-free DNA derived from normal cells, accurate detection and quantification is challenging. We are developing multiple assays utilizing NGS (Illumina) and OnTarget (Boreal Genomics) to enrich and detect ctDNA in the bloodstream of lymphoma patients. We hypothesize that levels of ctDNA will correspond to tumour burden in lymphoma patients and their accurate quantification will offer clinical potential as a novel biomarker for disease progression.

Stephen Yip, MD, PhD, FRCPC
Vancouver General Hospital, UBC

Stephen Yip, M.D., Ph.D. is a board-certified neuropathologist and is clinically appointed at Vancouver General Hospital and is an assistant professor of medicine at the University of British Columbia. He is also trained in molecular genetics pathology and has an active research interest in brain tumour genomics. He is very interested in the practical adoption of next generation sequencing technology in clinical pathology and as an accessible investigative tool in research.

Dr. Yip completed his combined M.D.-Ph.D. training at the University of British Columbia and completed four years of neurosurgical training at VGH prior to switching to neuropathology. He obtained his FRCPC certification in 2006 and then completed two years of research fellowship with Dr. David Louis at the Massachusetts General Hospital. He then underwent an additional year of molecular genetic pathology training in Boston prior to returning to Vancouver. Initially he performed mainly neuropathological consultations for brain tumour cases at the BC Cancer Agency and has since moved to Vancouver General Hospital to take on full clinical duties in neuropathology. He maintains his brain tumour research at the CTAG lab at BCCA and also works closely with Dr David Huntsman on

applying next generation diagnostics in pathology. He also collaborates with Dr Marco Marra at the Genome Sciences Centre which lead to the identification of recurrent somatic mutations in the CIC gene in oligodendrogliomas, a form of glioma characterized by recurrent co-deletions of 1p and 19q and IDH mutation.

Panel- based deep sequencing on the Ion Torrent PGM platform – The Complex Ecosystem of Cancer

Massively parallel sequencing or "deep sequencing" allows for deep interrogation of the genomic and epigenomic make-up of biological samples. This technique is especially useful in dissecting the complex ecosystem of cancer. For example, deep sequencing can provide critical information on allelic frequency, presence of rare tumour clones, and permits the study of spatial as well as temporal evolution of "driver" clones. This presentation will explore the major challenges, technical as well as practical, of implementation of deep sequencing in the pre-clinical setting – especially pertaining to the differences between whole genome/transcriptome versus focused amplicon sequencing approach. These include quality/quantity of sample material, time, costs, downstream analytical pipeline, and ultimately the "translatability" of results to affect clinical decision. This talk will also discuss our experience on using panel- based deep amplicon sequencing with "off- the- shelf" as well as "customized" panels on the Ion Torrent PGM platform.

Kaston Leung, Ph.D.
UBC

Kaston received his B. Sc. in electrical engineering from Queen's University, followed by his M. Sc. in electrical engineering at the University of Alberta, where he specialized in integrated circuit design and testing. He then went on to complete his PhD in electrical engineering, specializing in programmable microfluidic device design and its application to single-cell analysis.

Kaston is currently a post-doc in Steven Hallam's lab, while also working in Carl Hansen's lab, where he is developing microfluidic methods for single-microbe genetic analysis.

Microfluidic single-cell whole genome amplification in nanolitre-volume droplets with the lowest representational bias reported to date

From biological systems ranging from unculturable single-celled microbes to aberrant growth in diseases such as cancer, it is now appreciated that the genetic heterogeneity between individual cells, undetectable by conventional averaged measurements, plays a crucial role. To understand and exploit this diversity, the genomes of large numbers of single cells must be analyzed. Whole genome amplification (WGA) is a method by which sufficient DNA quantities can be generated from single cells for analysis. However, current single-cell WGA methods are impaired due to both the high cost of reagents required to amplify large numbers of single cells in conventional microlitre-volumes, and the high representational bias of the WGA reaction, which significantly increases the sequencing effort required to obtain useful genome coverage.

Here, we describe work that addresses both of these limitations. We previously developed a versatile droplet-based microfluidic device, which enables phenotype-based sorting and isolation of single cells and programmable formulation of any multistep reaction protocol in nanolitre-volume droplets (1). In this work, we applied the device to the WGA of 60 single Escherichia coli cells by multiple displacement amplification (MDA) in 60-nL droplets. Quantification of the representational bias of single-cell MDA in microfluidic, combined microfluidic/conventional, and conventional reactions by next generation sequencing indicated that the microfluidic reaction had the lowest representational bias, achieving 99% coverage of the reference genome at only 7x mean coverage depth. We demonstrate that the ultra-low bias of the microfluidic reaction is due to improved reaction kinetics in a nanolitre-volume. This result, to our knowledge, represents the highest genome coverage and lowest representational bias obtained from a single-cell WGA reaction reported to date.

This work has important implications for future single-cell genomic studies in a plethora of applications. Our microfluidic single-cell WGA method has a thousand-fold reduction in reagent consumption relative to conventional methods and the dramatic reduction in representational bias allows for useful genome coverage with greatly reduced sequencing costs. These order of magnitude improvements thus have the transformative potential to enable currently intractable genomic studies of large numbers of single cells.

Robin Coope, Ph.D.
Genome Sciences Centre

Dr. Robin Coope received his BASc in Engineering Physics in 1993 and a MASc in 1996 from the University of British Columbia. His studies focused on scanning tunneling microscopes with Dr. Tom Tiedje and total internal reflection-based display technologies with Dr. Lorne Whitehead. A unifying theme in these experiences was the development of instrumentation. This led to a PhD with Dr. Andre Marziali on physics related to capillary electrophoresis, completed in 2006.

Following his PhD, Dr. Coope joined Canada's Michael Smith Genome Sciences Centre, heading a new Engineering technology development group. The Group's mission is to enhance the productivity of the GSC's high throughput pipelines with new devices and processes. Dr. Coope co-directs the Genome BC Technology Development Platform with Dr. Marziali.

Automation Challenges in Library Construction for Next Gen Sequencing

The relentless rise, albeit now slowing, in next-gen sequencing capacity has meant that for about the last two years, the main challenge has been in constructing libraries. The BCGSC for example, made about 12,500 libraries in the last 12 months using numerous different protocols. This brings numerous challenges to the lab in creating robust robot programs to carry out biological protocols at the scale required. The rise of clinical sequencing brings a further challenge balancing "random access" against "massively parallel" pipelines. This talk will present some of the issues and solutions as seen at the BCGSC.

About the sponsors:

D-MARK Biosciences

www.d-markbio.com

D-MARK Biosciences specializes in providing solutions to Molecular Biology and Protein Biology laboratories. The company was founded in 2008 by the former Canadian General Manager of a top 15 Multinational Life Sciences supply company, with an emphasis on PCR/qPCR and sequencing. D-MARK works with small manufacturers of advanced solutions for molecular and protein biology, allowing cutting edge research to be performed faster, more accurately, and at a lower cost.

D-MARK Biosciences is the premier Canadian distributor of advanced solutions for Molecular Biology. It has a unique focus on Genetic Technologies surrounding key applications such as Next Generation Sequencing, Microarrays, qPCR, PCR, nucleic acid preparation and Sanger Sequencing. Using a solution focused consultative approach; we will exceed your expectations and build a long lasting scientific relationship with your laboratory.

For more info, please contact Jeffrey Seitz: Jeffrey.seitz@d-markbio.com

IDT DNA

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Integrated DNA Technologies (IDT) is a leader in manufacturing and developing products for the research and diagnostics life science market. IDT serves the areas of academic research, biotechnology, and pharmaceutical development. IDT was founded by Dr Joseph Walder in 1987. Since then, its development has been guided by an uncompromising approach to quality, a belief in the value of good service, and a determination to minimize consumer costs.

Serving over 80,000 life sciences researchers, IDT is widely recognized as the industry leader in custom oligonucleotides due to its capabilities in:

Analytical Sophistication – IDT pioneered the use of high throughput quality control (QC) methods and is the only oligonucleotide manufacturer to offer purity guarantees and 100% QC. Every oligonucleotide is analyzed by mass spectrometry and purified oligonucleotides receive further analysis by CE and HPLC.

Design Engineering – IDT maintains an engineering division dedicated to advancing synthesis, processing technology, and automation. An in-house machine shop provides rapid prototyping and custom part design/control.

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For more info, please contact Bob Setter: rsetter@idtdna.com