

UHNMAC News

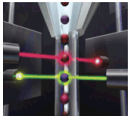
Quarterly newsletter

Winter 2010

Report

Cost-sharing: an inexpensive way to evaluate new platforms

Pages 1-2



Feature Paper

Profiling the mutational evolution of breast cancer at single nucleotide resolution

Pages 3-4

Announcements

*User Group Meetings
Functional Genomics Symposium:
June 14th, 2010*

Page 4

Recent Publications

Page 5

Researcher Spotlight

*Michael Archer &
Amadeo Parissenti*

Pages 5-6

Try it, you'll like it!

Cost-Sharing: An inexpensive way to evaluate the NanoString or BioPlex platforms

The “cost-effectiveness” of high-throughput platforms are evident when a large number of samples are processed simultaneously. At the UHN Microarray Centre, we understand that evaluating new technologies can be expensive and that researchers would prefer to conduct small pilot studies before selecting a platform for the analysis of many samples. Thus, we have launched a cost-sharing strategy for two platforms, the NanoString nCounter™ Analysis System (NanoString Technologies) and the Bio-Plex instrument (Bio-Rad), that will allow researchers to run a small number of samples at a low cost.

For the cost-sharing trial evaluation of the NanoString platform, researchers will be able to profile 10 gene transcripts of their choice, plus an additional 70 transcripts chosen by other researchers. The cost is \$125 per sample with a minimum of 12 samples. Researchers with less than 12 samples are welcome to team up! More information is available on the [nanosttring trial offer webpage](#).

Two different assays have been selected for the trial evaluations of the BioPlex instrument; the Human 27-plex Cytokine assay

(Bio-Rad) and the Human Phospho 5-plex Signal Transduction panel (Bio-Rad). The cytokine assay will cost \$60 per sample and the Human Phospho Signal Transduction 5-plex panel will cost \$25 per sample. More information is available on the [BioPlex trial offer webpage](#).

How these technologies work & why you should try them

Nanostring technology can be used for gene expression profiling of hundreds of genes, or profiling a large number of samples, and is mostly used to validate microarray data and perform biomarker analysis. Nanostrings (Nanostring Technologies) are the fluorescent barcodes that bind to target mRNA for gene expression analysis. The nCounter™ Analysis System measures RNA abundance by tagging transcripts with barcodes and counting individual mRNA molecules using a novel digital technology. This system is able to detect one copy of mRNA per cell, can evaluate up to 550 genes per reaction, and requires 100 ng of total RNA for analysis. Nanostring technology is a cost-effective platform for the validation of 30 to 500 different genes in one sample or fewer genes in many (more than 48) samples.

For more information, please visit www.microarrays.ca or contact us at general@microarrays.ca



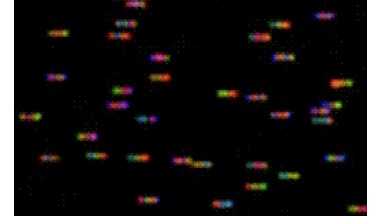
UHN Microarray Centre

The BioPlex (Bio-Rad) instrument, and assays based on xMAP® (Luminex) technology, incorporates several technologies including fluorescently dyed microspheres (beads), flow cytometry, lasers and associated optics, and a digital signal processor. The BioPlex, which can simultaneously analyse up to 100 different biomolecules, is most often used for protein expression profiling, HLA testing, and the detection of cytokines, chemokines, and growth factors. Assays based on xMAP® technology use a liquid suspension array with up to 100 uniquely colour-coded bead sets. Each bead is internally labelled

with a specific ratio of two fluorophores to assign it a unique spectral address. Beads are then conjugated with different biomolecules allowing the capture of specific analytes from the sample. A fluorescently-labelled reporter molecule is then added to the sample in order to detect and quantitate each captured analyte. The beads are drawn through a flow cell where two lasers excite each bead. Fluorescent signals are recorded, translating the signals into data for each bead-based assay.

Digital gene quantification using NanoStrings

Quantify 10 genes of interest to you
As a bonus, an additional 70 genes will be included in the CodeSet



- NanoString assays are highly reproducible and do not involve enzymology or amplification
- NanoStrings can be more cost-effective and time-efficient than qPCR
- mRNA transcripts are detected and quantified individually using a novel digital technology

\$125 per sample

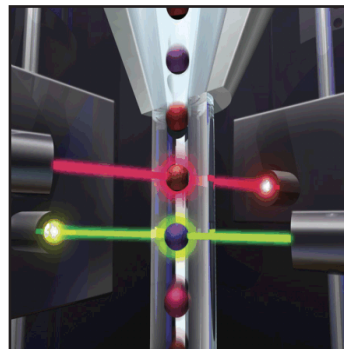
Visit the [UHNMAC NanoString trial offer webpage](#) for more information.

BioPlex platform for multiplex protein identification and quantification

Try the 27-plex Human Cytokine Panel or the 5-plex Human Phospho Signal Transduction Assay

27-plex panel includes:
IL-1β, Il-1ra, IL-2, IL-4, IL-5,
IL-6, IL-7, IL-8, IL-9, IL-10,
IL-12 (p70), IL-13, IL-15,
IL-17, Eotaxin, Basic FGF,
G-CSF, GM-CSF, IFN-γ,
IP-10, MCP-1 (MCAF),
MIP-1α, MIP-1β, PDGF-BB,
RANTES, TNF-α, VEGF

\$60 per sample



5-plex Signal Transduction assay detects:
phosphorylated Akt, ERK1/2,
IκB-α, JNK, and p38 MAPK

\$25 per sample

Visit the [UHNMAC BioPlex trial offer webpage](#) for more information.

Summary of: Shah SP, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. *Nature* 2009, 461:809

Comparing the frequency of somatic coding mutations found in a breast metastasis to the primary tumour

The genome-wide identification of novel single nucleotide variants (SNVs) using next-generation sequencing (NGS), or deep sequencing, is an approach now being used to find new genes associated with cancer (1) and to characterise all somatic coding mutations that occur in the during tumour development/progression (2). In this study, Shah *et al.* compare the frequency of 32 somatic non-synonymous coding mutations found in a breast metastasis to the primary tumour, which arose nine years earlier. This paper also describes a novel Bayesian probabilistic generative model, SNVMix, designed to identify SNVs in NGS data and the probability that each nucleotide is one of three genotypes: *aa* (homozygous with the reference base), *ab* (heterozygous), and *bb* (homozygous for the non-reference base). In addition, this study reports for the first time cancer associated mutations in a subunit of the recently identified augmin complex. Overall, this paper shows the importance of integrating RNA-seq data with tumour genomes in assessing protein variation and the data reveals that single nucleotide mutational heterogeneity can be a property of low or intermediate grade primary breast cancers and that significant evolution can occur with disease progression (2).

In this study, Shah and colleagues re-sequenced the DNA from a metastatic lobular breast cancer specimen and its primary tumour and aligned the reads to reference human genome (NCBI build 36.1, hg18) using Maq: Mapping and Assembly with Qualities (3) software. Of the 32 non-synonymous coding somatic point mutations that were identified in the metastasis, five (in *HAUS3*, *PALB2*, *ABCB11*, *SLC24A4*, and *SNX4*)

were prevalent in the DNA of the primary tumour, six (in *KIF1C*, *USP28*, *MYH8*, *MORC1*, *KIAA1468*, and *RNASEH2A*) were present at lower frequencies (1-13%), 19 were not detected in the primary tumour (possibly a consequence of radiation therapy or innate tumour progression), and two were undetermined (2).

One of the five genes harbouring a non-synonymous coding somatic point mutation in both the metastasis and primary tumour, *HAUS3*, codes for one of the subunits that comprises the human augmin complex (HAUS). The HAUS is an evolutionary conserved protein complex that regulates centrosome and spindle integrity (4). Upon sequencing an additional 192 breast cancers, Shah *et al.* found two specimens that contained two different truncating variants in *HAUS3*. This study reports for the first time cancer-associated mutations in a subunit of the recently identified augmin complex (4-6). Germline mutations in *PALB2* (Partner and Localiser of BRCA2), another gene with a SNV in both the metastasis and primary tumour samples, has been shown to predispose to heritable breast cancer (7-9), presumably through BRCA2-related mechanisms.

The functions of *ABCB11* and *SLC24A4* are not well characterised but they fall into the class of multi-pass membrane solute transporters and *SNX4*, a sorting nexin, is thought to be linked with endocytic recycling processes (2).

Shah *et al.* also examined how nuclear genomic information was modified by RNA editing. By integrating the transcriptome and genome data of the lobular metastasis, two new RNA-editing events were found for transcripts of *COG3* and *SRP9*. Interestingly, ADAR, an RNA-specific adenosine deaminase, was one of the top 5% of genes expressed.

The results of this study show the importance of sequencing tumour cell populations early as well as late in the evolution of tumours, and of estimating allele frequency in tumour genomes.

References

1. Campbell PJ, et al. Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat Genet* 2008, 40(6):722

“Of the 32 non-synonymous coding somatic mutations that were identified in the metastasis, five were prevalent in the DNA of the primary tumour, six were present at lower frequencies, 19 were not detected...”

2. Shah SP, *et al.* Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. *Nature* 2009, 461:809
3. Li H, *et al.* Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* 2008, 18(11):1851
4. Lawo S, *et al.* HAUS, the 8-Subunit Human Augmin Complex, Regulates Centrosome and Spindle Integrity. *Curr Biol* 2009, 19(10):816
5. Goshima G, *et al.* Augmin: a protein complex required for centrosome-independent microtubule generation within the spindle. *J Cell Biol* 2008, 181(3):421
6. Meireles AM, *et al.* Wac: a new Augmin subunit required for chromosome alignment but not for acentrosomal microtubule assembly in female meiosis. *J Cell Biol* 2009, 184(6):777
7. Erkkö H, *et al.* A recurrent mutation in PALB2 in Finnish cancer families. *Nature* 2007, 446(7133):316
8. Patel KJ, *et al.* Fanconi anemia and breast cancer susceptibility. *Nature Genet* 2007, 39(2):142
9. Rahman N, *et al.* PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nature Genet* 2007, 39(2):165

Announcements

Microarray User Group Meetings
Meetings are held in TMDT room 4-204, from 3-4pm

Thursday, February 25, 2010
"The Genetic Landscape of the Cell"
Presented by: Charlie Boone, PhD

Thursday, March 25, 2010
Guest Speaker: Aaron Wheeler, PhD

Functional Genomics: Present and Future

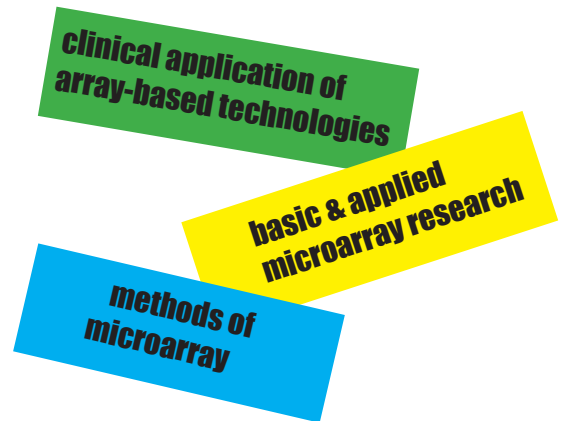
A symposium hosted by the UHN Microarray Centre

Monday, June 14, 2010
MaRS Auditorium
101 College Street, Toronto

Cost: \$50 (includes lunch and coffee breaks)
Registration begins: March 1, 2010
visit: http://www.microarrays.ca/info/symposium_June2010.html for more information

Confirmed Speakers:

Daphne Ang (Memorial Sloan-Kettering Cancer Center) - *Profiling Gene Expression Using NanoString Technology*
Elizabeth Edwards (University of Toronto) - *Environmental Genomics*
Rajiv Gandhi (University Health Network) - *Clinical Application: Arthritis*
Ellen Greenblatt (Mount Sinai Hospital) - *Reproductive Biology*
Troy Ketela (University of Toronto) - *High-throughput Genetic Analysis*
Ahmad Khalil (Broad Institute) - *lincRNA & the Epigenome*
Amadeo Parissenti (Sudbury Regional Hospital) - *Breast Cancer & Drug Resistance*
Ji Qiu (Arizona State University) - *Protein Arrays & Personalised Medicine*
Michael Reedijk (University Health Network) - *Signal Transduction in Malignancy*
Ming-Sound Tsao (University Health Network) - *Translational Research in Lung Cancer*



Recent Publications by UHNMAC Users

Bouchard L, *et al.* **Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction.** *Am J Clin Nutr* 2010, **91**:309

House B, *et al.* **Acid-stress-induced changes in enterohaemorrhagic Escherichia coli O157:H7 virulence.** *Microbiology* 2009, **155**:2907

Moretton A, *et al.* **Gene expression patterns in heterozygous Plk4 murine embryonic fibroblasts.** *BMC Genomics* 2009, **10**:319

Wang C, *et al.* **Genomic alterations in primary breast cancers compared with their sentinel and more distal lymph node metastases: An aCGH study.** *Genes Chromosomes Cancer* 2009, **48**(12):1091

Researcher Spotlight

Dr. Michael Archer

Identifying genes and dietary factors involved with carcinogenesis

One aspect of Dr. Archer's research programme is to understand the genetic basis for the differences in susceptibility to breast cancer induction of certain rat strains. Wistar-Furth (WF) rats develop multiple mammary adenocarcinomas following treatment with a mammary carcinogen, whereas Copenhagen (Cop) rats are completely resistant to the development of mammary tumours. Dr Archer and his colleague Dr Yaacov Ben-David from Sunnybrook Health Sciences Centre isolated cell lines from tumours induced in resistant Cop x WF F1 rats by infusion of a retrovirus harboring v-Ha-ras directly into the main mammary ducts. Some of the cell lines were able to grow in soft agar but a significant number did not display anchorage-independent growth. They hypothesised that the anchorage-dependent and -independent cell lines may recapitulate the resistance and susceptibility of Cop and WF rats, respectively, to mammary carcinogenesis and could facilitate the identification of breast cancer susceptibility genes. They compared by microarray analysis using the UHNMAC Gene Expression Service (Agilent platform), genes that are differentially expressed in these cell lines (1). The expression of *IL-24* and *β4 integrin* was highly correlated with the inability of cells to grow in soft agar. Ectopic expression of *IL-24* in anchorage-independent cells inhibited their growth in monolayer culture, in soft agar and in nude mice *in vivo*, and inhibited their ability to migrate and invade in *in vitro* assays. Furthermore, growth suppression by IL-24 was associated with the transcriptional up-regulation of *p27^{kip1}* via the activation of Stat3. They showed for the first time, that *β4 integrin*

is a downstream target of IL-24. However, $\beta 4$ does not play a direct role in regulating the proliferative capacity the rat mammary tumor cells. Overall, their results show that IL-24 suppresses the growth of rat mammary carcinoma cells and may play a role in the resistance of Cop rats to mammary carcinogenesis.

A Professor in the Departments of Nutritional Sciences and Medical Biophysics, Dr. Archer also investigates the molecular targets of dietary factors involved in the development of breast and colon cancer. He has published a number of studies that focus on the role of fatty acid synthase (FAS) in tumour development. Most recently, he has shown that the transcription factor Sp1 regulates both *de novo* lipogenesis and proliferation in cancer cells, and he has proposed the concept that Sp1 coordinately regulates multiple biological processes that are essential for the survival, growth and progression of cancer cells (2). He has also recently shown that FAS is over-expressed in aberrant crypt foci, the earliest identifiable lesions in colon cancer development (3). Finally, in a series of studies in knock-out and mutant mice, Dr. Archer and his group have unraveled the complex interactions linking obesity, type II diabetes and colorectal cancer. He has shown that insulin and IGF-I are the key factors (4).

References

1. Xuan *et al.* Interleukin-24 Induces Expression of $\beta 4$ Integrin but Suppresses Anchorage-Independent Growth of Rat Mammary Tumor Cells by a Mechanism That Is Independent of $\beta 4$. *Mol Cancer Res* 2009, **7**:433.
2. Lu & Archer. Sp1 coordinately regulates de novo lipogenesis and proliferation in cancer cells. *Int J Cancer* 2010, **126**:416
3. Lau & Archer. Fatty acid synthase is over-expressed in large aberrant crypt foci in rats treated with azoxymethane. *Int J Cancer* 2009, **124**:2750
4. Ealey & Archer. Colon carcinogenesis in liver-specific IGF-I deficient (LID) mice. *Int J Cancer* 2008, **122**:472

Dr. Amadeo Parissenti

Multidrug resistance in breast cancer and PKC signalling

Identifying the mechanisms by which tumour cells acquire resistance to chemotherapy drugs will enable the development of effective strategies to reverse or minimise drug resistance in cancer patients. Dr. Parissenti and his colleagues have identified genes that may play a role in the onset of resistance to the taxane and anthracycline classes of chemotherapy drugs in breast cancer cells. Using UHNMAC cDNA arrays, Dr. Parissenti's team has published a study that identified distinct drug-specific gene signatures of resistance to paclitaxel and doxorubicin in breast tumour cell lines (1). Using tumour core biopsies from patients with locally advanced/inflammatory breast cancer enrolled in a recently completed clinical trial by the National Cancer Institute of Canada (MA.22), his group is now examining by microarray and quantitative PCR the utility of these genes (and others) to predict response to epirubicin/docetaxel chemotherapy. Another recent study by his group concluded that resistance in breast tumour cells to anthracyclines and taxanes is acquired at a certain threshold drug concentration and that the onset of drug resistance is not always correlated with the induction and activity of drug transporters (2). Rather, a variety of mechanisms appear to be at play at or before the acquisition of drug resistance.

Dr. Parissenti is also looking at the possibility that aldo-ketoreductases (AKRs) may confer anthracycline resistance and that the inhibition of AKRs may be a new approach to combat drug resistance. AKRs convert anthracyclines like doxorubicin and epirubicin to less potent hydroxy metabolites and may also play a role as scavengers of reactive oxygen species generated by anthracyclines and other classes of chemotherapy agents. His group recently published data that used UHNMAC arrays to identify and characterise the role of AKR1C2, AKR1C3, and other drug dose-dependent genes in the acquisition of anthracycline resistance (3). This study found that the expression of certain genes (such as those coding for AKRs and other proteins involved in drug transport, drug metabolism, redox reactions, cell

signaling, transcription, cell proliferation, apoptosis, and immune response) correlated with the onset and magnitude of anthracycline resistance in breast tumour cells. Surprisingly, solely by inhibiting the 1C family of AKRs, the sensitivity to doxorubicin in doxorubicin-resistant cells was almost completely restored.

Dr. Parissenti and his colleagues are also studying the role of Protein Kinase C (PKC) in the control of cellular growth and the development of cancer. While examining the structure-function relationships for PKC, his group has identified regions within the PKC regulatory domain (outside of the pseudosubstrate sequence) that are important for inhibition of the catalytic domain. Dr. Parissenti's team is currently elucidating the precise mechanism by which PKC inhibits cell growth and how the cytoskeletal protein calponin induces autophosphorylation of PKC. His group is also investigating agents like calphostin C, a PKC inhibitor, which can effectively kill drug-resistant breast tumour cells.

Dr. Amadeo Parissenti is a Professor in the Department of Chemistry and Biochemistry, Laurentian University (Sudbury) and is a Professor in the Division of Medical Sciences at the newly established Northern Ontario School of Medicine. He is also Chair in Cancer Research at the Sudbury Regional Hospital. In 2007, he was appointed a member of the Ontario Institute for Cancer Research's Clinical Investigation Advisory Board (CIAB).

References

1. Villeneuve DJ, *et al.* cDNA microarray analysis of isogenic paclitaxel- and doxorubicin-resistant breast tumor cell lines reveals distinct drug-specific genetic signatures of resistance. *Breast Cancer Res Treat* 2006, 96:17
2. Hembruff SL, *et al.* Role of drug transporters and drug accumulation in the temporal acquisition of drug resistance. *BMC Cancer* 2008, 8:318
3. Veitch ZW, *et al.* Induction of 1C aldo-ketoreductases and other drug dose-dependent genes upon acquisition of anthracycline resistance. *Pharmacogenet Genomics* 2009, 19(6):477

Dr. Parissenti will be presenting his recent research at the Functional Genomics: Present and Future symposium on Monday, June 14, 2010