UHNMAC News

Quarterly newsletter Spring 2010

Multiple applications using one versatile technology - Discover the Luminex xMAP® platform

Whether you are studying cytokines, phosphoproteins, or the protein markers of a specific disease, the xMAP® platform has an assay for you! Luminex and its many partners offer assays for protein expression profiling, including assays that evaluate markers for certain diseases such as cancer, heart disease, Alzheimer's, metabolic and endocrine disease, as well as cellular signaling, cytokines/chemokines and growth factors, matrix metalloproteinases (MMP), and transcription factors. Assays are also available for microRNA profiling and genotyping, as well as clinical diagnostic applications.

Applications of xMAP® technology:
In the literature, there are hundreds of studies that have successfully used xMAP® technology for the analysis of various biological samples; however, most of these studies have used this technology to quantify the levels of cytokines (Table 1).

About xMAP® technology:
The xMAP® technology is built on a unique combination of several technologies, including flow cytometry, fluorescently dyed microspheres (beads), traditional chemistry, lasers and associated optics to measure the biochemical reactions that occur on bead surfaces, and a digital signal processor to manage the data. These assays, which are also referred to as suspension arrays and liquid bead assays, involve microspheres that are uniquely colour-coded into 100 distinct sets and then coated with a reagent (ie. protein) specific to a particular bioassay. This fast and reproducible system has small sample requirements and offers greater flexibility than planar arrays.

Luminex Partners include:

- Bio-Rad
- EMD Chemicals (Affiliate of Merck)
- Invitrogen
- Marligen Biosciences (OriGene)
- Millipore
- Panomics (Affymetrix)
- R&D Systems

Table 1. Recent studies using xMAP® technology.

Luminex-based assay used	Study details	Reference
ProCarta® human cytokine assay (Panomics)	Assay used to determine the concentration of growth factors, cytokines and inflammatory mediators in bronchoalveolar lavage fluid (BALF) from patients with acute respiratory distress syndrome to assess the contribution of intra-aveolar neutrophils to the procoagulant properties of BALF in these patients	Kambas *et al.* (1)
Milliplex mouse cytokine panel 13-plex (Millipore)	Assay used to determine the serum levels of various cytokines following *M. tuberculosis* infection; the results from this study identified interferon regulatory factor (IRF) family memeber IRF-8 as critical regulator of host defenses against tuberculosis	Marquis *et al.* (2)
Human cytokine 8-plex assay (Bio-Rad) & 3-plex MMP assay (BioSource, Invitrogen)	Using these assays, the authors found increased levels of lipopolysaccarides and inflammatory cytokines in workers exposed to high levels of LPS at their workplace (facilities that produce bacterial single-cell protein used in animal feed)	Sikkeland *et al.* (3)
Fluorokine® MAP human cytokine panel A assay (R&D Systems)	Assay used to assess the link between the level of circulating chemokines in pregnant women and the risk of miscarriage; results suggest that chemokine ENA-78 may be an early indicator of miscarriage risk	Whitcombe *et al.* (4)
Bio-Plex Pro human diabetes assay (Bio-Rad)	Assay used to measure levels of adiponectin; study examined the way in which glucose kinetics and related factors change after breakfast as a result of colonic fermentation	Priebe *et al.* (5)

UHNMAC Luminex platform service:

At the UHNMAC, we offer the BioPlex™ system (Bio-Rad) which is capable of processing most Luminex-based assays. We offer a plate-reading service, where researchers perform the assay in their own laboratory and submit their plates for reading, and a full service, where our trained technicians carry out the entire xMAP® assay in our laboratory. Our full service customers can take advantage of the discounted pricing agreements we have with several Luminex partners.

Advantages of xMAP platform:

- multiplexed assays save time, money and sample
- assays are quality control tested for accuracy and reproducibility
- straight-forward results provided in spreadsheet format

Not sure if this platform is right for you?

Rather than commit to buying a full assay for preliminary studies, the UHNMAC is currently offering a special promotion that will allow researchers to try out select assays for any number of samples (no minimum required). To learn more about our cost-sharing trial offers, please visit our Luminex technology trial offer webpage.

Trial offer pricing:

Mouse 23-plex Cytokine Panel: $60 per sample

Human 27-plex Cytokine Panel: $60 per sample

5-plex Human Phospho Signal Assay: $25 per sample

About Luminex:

Luminex develops, manufactures and markets innovative biological testing technologies with applications throughout the life sciences and diagnostic industries (6). Luminex has two technologies: the open-architecture xMAP® technology allows many bioassays to be conducted and analysed quickly, accurately, and cost-effectively, and the xTAG® technology which utilizes a proprietary universal tag system for clinical multiplex genetic tests. In April 2010, Luminex was ranked in the top 25 fastest growing North American companies by Forbes. Based in Austin, Texas, Luminex also has subsidiaries in Toronto (Luminex Molecular Diagnostics) and The Netherlands (Luminex B.V.).

References:

1. Kambas K, *et al*. **C5a and TNF-α Up-Regulate the Expression of Tissue Factor in Intra-Alveolar Neutrophils of Patients with the Acute Respiratory Distress Syndrome**. J Immunol 2008, 180(11):7368
2. Marquis JF, *et al*. **Disseminated and rapidly fatal tuberculosis in mice bearing a defective allele at IFN regulatory factor 8**. J Immunol 2009, 182(5):3008
3. Sikkeland LIB, *et al*. **Circulating lipopolysaccharides in the blood from "bioprotein" production workers**. Occup Environ Med 2008, 65(3):211
4. Whitcomb BW, *et al*. **Circulating Chemokine Levels and Miscarriage**. Am J Epidemiol 2007, 166(3):323
5. Priebe MG, *et al*. Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. Am J Clin Nutr 2010, 91:90
6. Website of Luminex Corporation. http://www.luminexcorp.com/company/index.html As viewed on April 22, 2010

Feature Paper

Summary of: Qiu J, *et al.* **Occurrence of Autoantibodies to Annexin I, 14-3-3 Theta and LAMR1 in Prediagnostic Lung Cancer Sera.** J Clin Oncol 2008, 26(31):5060

Using protein arrays to predict disease before the onset of symptoms

The identification of autoantibodies against several intracellular and surface antigens in patients with various tumour types has provided evidence for a humoural immune response to cancer in humans (1-3). The detection of certain autoantibodies in prediagnostic sera could one day be used to predict the diagnosis of a certain diseases prior to the onset of symptoms. Dr. Qui and his colleagues have recently published a study that uses a high throughput protein array for the quantitative analysis of serum autoantibodies that could allow for the diagnosis of lung cancer before the onset of symptoms (4). This work builds on previous studies that have used protein arrays to uncover antigens that induce an immune response in patients with lung cancer (5) and other types of cancer (6,7).

"The detection of certain autoantibodies in prediagnostic sera could one day be used to predict the diagnosis of a certain diseases prior to the onset of symptoms"

In this study, protein lysate from the human lung adenocarcinoma cell line A549 was fractionated by anion-exchange high-performance liquid chromatography followed by reverse-phase chromatography. 1824 protein fractions were collected, lyophilised, diluted in printing buffer, and printed on nitrocellulose-coated slides. Using this fractionated protein lysate array, a quantitative analysis of serum autoantibodies annexin I, PGP9.5, and 14-3-3 theta antigens, which were previously found to be targets of autoantibodies in newly diagnosed patients with lung cancer (8-10), was conducted. The arrays were used to determine whether these autoantibodies are found in sera collected from patients in the presymptomatic stage and from matched controls from the CARET high-risk cohort. The Carotene and Retinol Efficacy Trial (CARET) evaluated the daily supplementation of vitamin A and beta-carotene for the prevention of lung cancer involving over 18,000 participants who were at high risk for developing lung cancer due to their history of smoking or asbestos exposure. In Dr. Qui's study, case and control pairs were matched for age, baseline smoking status, and exposure to asbestos, among other variables.

This study reports that autoantibodies to annexin I, 14-3-3 theta, and a novel antigen, LAMRI, were significantly elevated in preclinical sera of patients

with lung cancer, prior to the onset of symptoms and diagnosis of lung cancer, compared with matched high-risk controls who did not develop lung cancer. This study also found that the level of PGP 9.5 in prediagnostic cases was not significantly different from that of matched controls. The findings of this study indicate the value of prediagnostic sera in assessing the significance of autoreactivitiy to particular antigens (1).

Dr. Ji Qui (Biodesign Institute, Arizona State University) will discuss his research involving functional proteomics for the discovery of biomarkers at the "Functional Genomics: Present and Future" symposium.

This symposium, hosted by the UHN Microarray Centre, will be held on June 14th, 2010, in the MaRS Auditorium (101 College Street, Toronto). Please visit the symposium website to learn more about this event and to register.

References

1. Stockert E, *et al*. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 1998, 187:1349
2. Gure AO, *et al*. Human lung cancer antigens recognized by autologous antibodies: Definition of a novel cDNA derived from the tumor suppressor gene locus on chromosome 3p21.3. Cancer Res 1998, 58:1034
3. Yamamoto A, *et al*. Detection of auto-antibodies against L-myc oncogene products in sera from lung cancer patients. Int J Cancer 1996, 69:283
4. Qiu J, *et al.* Occurrence of Autoantibodies to Annexin I, 14-3-3 Theta and LAMR1 in Prediagnostic Lung Cancer Sera. J Clin Oncol 2008, 26(31):5060
5. Madoz-Gurpide J, *et al*. Integral protein microarrays for the identification of lung cancer antigens in sera that induce a humoral response. Mol Cell Proteomics 2008, 7:268
6. Nam MJ, *et al*. Molecular profiling of the immune response in colon cancer using using protein microarrays: Occurrence of autoantibodies to ubiquitin C-terminal hydrolase L3. Proteomics 2003, 3:2108.
7. Forrester S, *et al*. An experimental strategy for quantitative analysis of the humoral immune response to prostate cancer antigens using natural protein microarrays. Proteomics Clin Appl 2007, 1:494
8. Brichory F, *et al*. Proteomics-based identification of protein gene product 9.5 as a tumor antigen that induces humoral response in lung cancer. Cancer Res 2001, 61:7908
9. Brichory FM, *et al*. An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. PNAS 2001, 98:9824
10. Pereira-Faca SR, *et al.* Identification of 14-3-3 theta as an antigen that induces a humoral response in lung cancer. Cancer Res 2007, 67:12000

Announcements

Upcoming Microarray User Group Meeting

Thursday, May 6th, 2010
11am - 12:30pm
TMDT, room 4-204
101 College Street

"Extrapolating Genomics Data for Disease Prediction and Pharmacogenetic Applications"

Presented by: Sarah Shaw Murray, PhD
Scripps Translational Science Institute

Functional Genomics: Present and Future

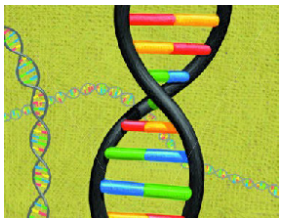
A symposium hosted by the UHN Microarray Centre

Monday, June 14, 2010
8:30am to 5pm
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
  NanoString nCounter as a platform for highly multiplex detection of cancer fusion transcripts in clinical tumour samples
- Elizabeth Edwards, University of Toronto
  Metagenomics and bioremediation: from genomes to solutions
- Rajiv Gandhi, University Health Network
  The pro-inflammatory role of the intra-articular knee fat pad in osteoarthritis
- Ellen Greenblatt, Mount Sinai Hospital
  Application of single blastomere biopsy and microarray for embryo selection in IVF: Towards single embryo transfer
- Troy Ketela, University of Toronto
  Functional genomic screening of cancer cell lines using complex shRNA pools
- Ahmad Khalil, Broad Institute
  A global mechanism for non-coding RNA dependent chromatin formation in mammals
- Amadeo Parissenti, Sudbury Regional Hospital
  Aldo-keto reductases and their role in anthracycline metabolism, localization, and cytotoxicity in doxorubicin-resistant MCF-7 breast tumour cells
- Ji Qiu, Arizona State University
  Functional proteomics for biomarker discovery
- Michael Reedijk, University Health Network
  Identification of novel biomarkers and therapeutic targets in Notch-activated triple negative breast cancer
- Ming-Sound Tsao, University Health Network
  Translational research in lung cancer



Recent Publications by UHNMAC Users

Dellett M, *et al.* **Identification of Gene Networks Associated with Acute Myeloid Leukemia by Comparative Molecular Methylation and Expression Profiling**. Biomarkers in Cancer 2010, 2:43

Nguyen A, *et al.* **Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain.** FASEB Journal 2010, Epub ahead of print

van Straten EME, *et al.* **The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction.** Am J Physiol Regul Integr Comp Physiol 2010, 298:R275

Vivekanandan P, *et al.* **Hepatitis B Virus Replication Induces Methylation of both Host and Viral DNA**. J Virology 2010, 84(9):4321

Researcher Spotlight

Dr. Susan Done

Investigating genomic alterations in breast cancer

At the University Health Network, Dr. Done's research involves the identification and characterisation of the molecular alterations that lead to the development of solid cancers, particularly breast cancer. By finding and studying the genetic aberrations that are specific to certain cells, the goal of her research is identify potential diagnostic and predictive cancer biomarkers and therapeutic targets.

Using an array-based technique called array Comparative Genomic Hybridisation (aCGH), Dr. Done and her colleagues are able to identify genes and chromosomal regions that are amplified or deleted. Dr. Done and her team have published numerous studies using UHNMAC Human 19K cDNA microarrays for aCGH studies (1, 2, 4). Most recently, a study was published that compared the genomic alterations in primary breast cancers with their sentinel and more distal lymph node metastases (1). This study found that amplification within the 17q24.1-24.2 region was associated with the presence of sentinel or more distal lymph node metastases, larger tumour size, and higher histological grade. Gain on 17q22-24.2 was also identified by them, in a separate study, as a candidate region for further testing as a predictor of invasion when detected in ductal carcinoma in situ (DCIS) and predictor of nodal metastasis when detected in infiltrating duct carcinoma (2). In 2008, Dr. Done together with Dr. Wey Leong and their team published a study that examined the effects of timing of breast tumour biopsies on gene expression profiles. By comparing the expression profiles of breast tumours taken *in vivo* and *ex vivo*, this study found that FOS-related genes, which have been associated with hypoxia and breast cancer development, were differentially expressed before and after surgery (3).

Other recent investigations, in collaboration with Drs. Kristin McLarty and Raymond Reilly, have involved the targeted radiotherapy of cancer and molecular imaging (5-7). One study identified responding and nonresponding human breast cancer xenografts in athymic mice treated with trastuzumab (Herceptin; a HER2 inhibitor) based on changes in the tumour uptake of ^{18}F-fluorodeoxyglucose (5). Another study with Dr. Dan Constantini, also in Dr. Reilly's group, was aimed at the development and preclinical evaluation of radioimmunotherapy of HER2 positive breast cancer using 111In-NLS-trastuzumab.

Dr. Susan Done is an Associate Professor at the University of Toronto in the Departments of Laboratory Medicine & Pathobiology, and Medical Biophysics, and a Pathologist at the University Health Network.

References

1. 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
2. Iakovlev VV, *et al*. Genomic Differences Between Pure Ductal Carcinoma *In Situ* of the Breast and that Associated with Invasive Disease: a Calibrated aCGH Study. Clin Cancer Res 2008, 14:4446
3. Wong V, *et a*l. The effects of timing of fine needle aspiration biopsies on gene expression profiles in breast cancers. BMC Cancer 2008, 8:277
4. Ghazani AA, *et al*. Genomic Alterations in Sporadic Synchronous Primary Breast Cancer Using Array and Metaphase Comparative Genomic Hybridization. Neoplasia 2007, 9(6):511
5. McLarty K, *et al.* ^{18}F-FDG Small-Animal PET/CT Differentiates Trastuzumab-Responsive from Unresponsive Human Breast Cancer Xenografts in Athymic Mice. J Nuc Med 2009, 50(11):1848
6. McLarty K, *et a*l. Micro-SPECT/CT with 111In-DTPA-pertuzumab sensitivity detects trastuzumab-mediated HER2 downregulation and tumor response in athymic mice bearing MDA-MB-361 human breast cancer xenografts. J Nucl Med 2009, 50(8):1340
7. McLarty K, *et al*. Associations between the uptake of 111In-DTPA-trastuzumab, HER2 density and response to trastuzumab (Herceptin) in athymic mice bearing subcutaneous human tumour xenografts. Eur J Nucl Med Mol Imaging 2009, 36(1):81