UHNMAC News

The University Health Network Microarray Centre Newsletter – Spring 2009

UHN Microarray Centre

"Excellent group. I particularly value their interest in research and what they add to the research studies we are collaborating on. The Centre provides more than just the techniques. They also engaged in a lot of tweaking of the techniques to apply to the work we are doing."

~Ellen M Greenblatt, MD, FRCSC

Clinical Director, Reproductive Biology and IVF Units, Mount Sinai Hospital
Associate Professor, University of Toronto

Announcements

Illumina iScan System launched in April

Details about services offered on the Illumina platform can be found on our website, http://www.microarrays.ca/services/Illuminaservice.html

Upcoming Events

Microarray User Group Meeting
Friday June 19, 2009
TMDT 4-204, 10-11am
Speaker: John Quackenbush, PhD (Professor of Computational Biology & Bioinformatics at Harvard)

Open House
Wednesday June 24, 2009
TMDT 9-601, 11am-3pm
Everyone is welcome!

This edition of the UHNMAC News summarises the preliminary internal validation of the Illumina iScan System for gene expression studies and provides a brief overview of an article that used the Illumina platform to analyse the differential gene expression between endogenous and reprogrammed β-cells.

The Illumina platform is now available at the UHNMAC for gene expression and miRNA profiling studies. DNA methylation studies will be offered soon. For more information about the iScan System or for a service quotation, please e-mail us at illumina@microarrays.ca.

Internal validation of the Illumina iScan System for gene expression studies

Prior to the launch of the Illumina iScan System at the University Health Network Microarray Centre (UHNMAC), an internal validation of the platform was completed. The purpose of this report is to briefly provide the results of our validation of Illumina's gene expression platform. By repeating an experiment previously performed by the MicroArray Quality Control (MAQC) Consortium,

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The identification of transcription factors that appear to reprogramme differentiated pancreatic exocrine cells

Review of: Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature 2008, 455(7213):627-632

The goal of regenerative medicine is to convert abundant adult cells into other medically important cell types for tissue repair and regeneration (1). The ability to reprogramme differentiated human somatic cells into a pluripotent state would allow patient- and disease-specific stem cells to be generated (2). Cellular reprogramming studies have used a small number of transcription

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Illumina Validation

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we were able to compare our results in order to determine the robustness of the system and to determine the reproducibility of the technology. A growing trend toward the analysis of smaller amounts of RNA has been witnessed (and promoted) by the UHNMAC. As such, this study also looked at the amplification of smaller amounts of total RNA to approximate the lowest starting amount of total RNA required for quality data.

In 2006, the MAQC Consortium published the results of a comprehensive comparison of multiple array platforms (1). The goal of this community-wide study, which involved 137 participants from 51 organisations, was to establish QC metrics and thresholds for objectively assessing the performance of various microarray platforms and to evaluate the advantages and disadvantages of various data analysis methods. Differential gene expression levels between various RNA samples (including Stratagene Universal Human Reference RNA (UHRR), Ambion Human Brain Total RNA (HBTR), and two mixtures: 75:25 mixture of UHRR and HBTR, and 25:75 mixture of UHRR and HBTR) were assessed using seven microarray platforms. The resulting datasets were used to assess the precision and cross-platform comparability of microarrays (1).

Method

For the samples listed in Table 1, total RNA was labelled using the Illumina® TotalPrep RNA Amplification Kit. At least 10 µg cRNA was obtained following amplification (including from 25 ng total RNA input). 1.5 µg cRNA was hybridised to a Human Whole-Genome BeadChip 48K, version 3.0. In some cases, samples were hybridised in duplicate or quadruplicate. Data was normalised (rsn method), quantified in BeadStudio™ and analysed using BeadStudio™ and R-package software applications.

Results

- All 12 hybridisations passed Illumina's internal QC metrics (including labelled hybridisation controls, low and high stringency controls, signal generation (biotin) controls, and negative controls) and all 12 arrays met the Illumina acceptance criteria (greater than 11,000 genes were detected at p-values <0.1); data not shown
- The identification of differentially expressed genes (two-fold up and down) from this validation study was highly consistent with MAQC data, but due to differences in the BeadChip versions, a direct comparison of the data sets was beyond the scope of this preliminary report
- Hierarchical clustering of all samples indicates that the variability between technical replicates is very low and that technical replicates cluster closely together; Figure 1
- Data from brain samples (amplified from 25 ng, 50 ng, and 500 ng total RNA) were highly reproducible as correlation values were >0.99 (excluding one replicate of the HBTR sample amplified from 50 ng, which had a correlation value of 0.96); Figure 2
- Reproducibility and amplification fidelity deteriorate as the amount of starting RNA decreases, however in cases where sample is limiting, relatively robust data can still be obtained from as little as 25 ng of total RNA; Figure 3

Table 1. List of samples used for the internal validation of the Illumina iScan System for gene expression studies.

Total RNA Sample	Starting amount of total RNA used for amplification
HBTR	500 ng
75% UHRR/25% HBTR	500 ng
25% UHRR/75% HBTR	500 ng
Illumina sample #1	500 ng
Illumina sample #2	500 ng
HBTR	50 ng
HBTR	25 ng

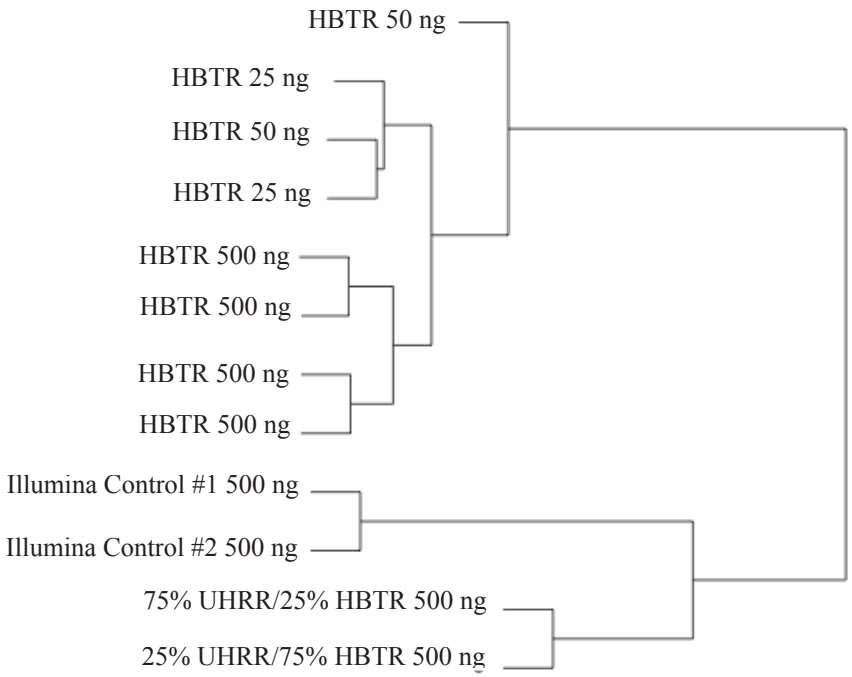

Figure 1. Hierarchical cluster of the 12 samples based on 11068 genes with a sd/mean > 0.1. Technical replicates of the HBTR samples (amplified from 500ng) clustered together, as did the HBTR samples amplified from 25 ng total RNA. When box-plots for each sample were examined, one of the replicates of HBTR amplified from 50 ng appeared more different than the other HBTR samples and thus did cluster as tightly as others. However, all HBTR samples clustered together.

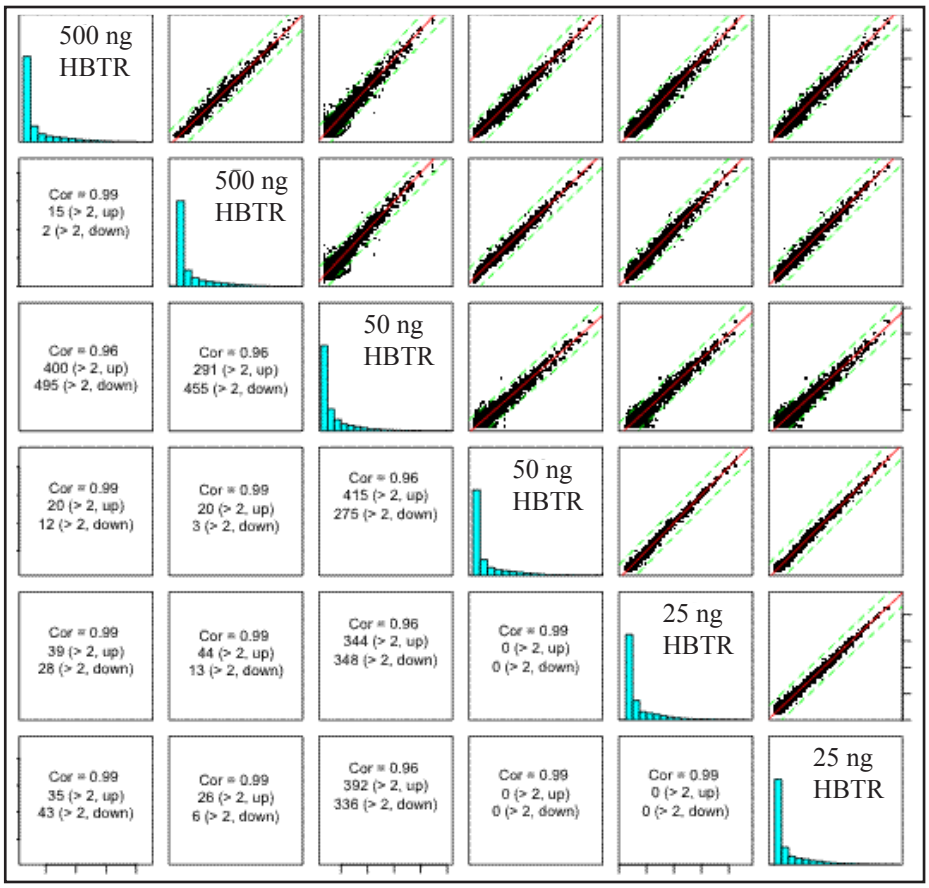

Figure 2. The normalised $\log_2$ signal intensity plots (generated from a random sample of 5000 data points) illustrate the pair-wise correlation of the expression profiles between the following data sets: HBTR amplified from 500 ng, HBTR amplified from 50 ng, and HBTR amplified from 25 ng. The correlation values of the HBTR samples (amplified from 25 ng, 50 ng, and 500 ng total RNA) are between 0.96 and 1. When one of the HBTR replicates is excluded, the correlation value for all sample pairs is 0.99.

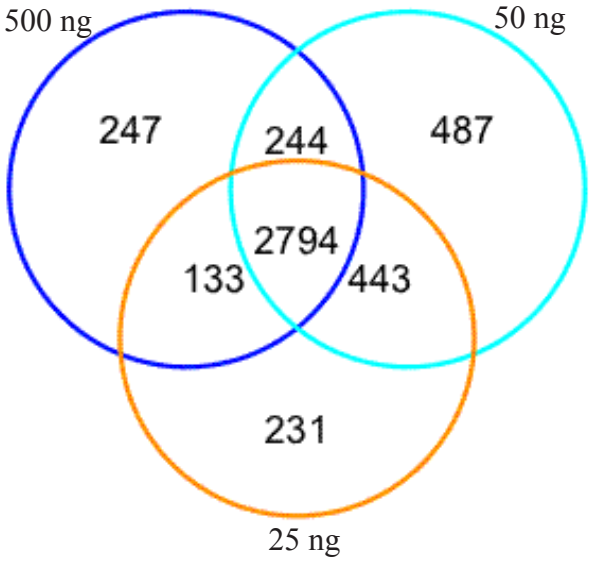

Figure 3. Venn diagram showing the overlap of differentially expressed genes between the 75% UHRR/25% HBTR sample and HBTR samples amplified from 500 ng, 50 ng, and 25 ng total RNA. The number of common differentially expressed genes among brain samples amplified from 25 ng, 50 ng, and 500 ng indicates that robust data can be obtained from as little as 25 ng of total RNA.

Conclusions

- UHNMAC validation data was highly consistent with MAQC data
- The validation data was highly reproducible among technical replicates (correlation >0.96)
- Although relatively robust data can be obtained from as little as 25 ng of total RNA, the UHNMAC requests a minimum of 50 ng of total RNA for Illumina gene expression studies
- This study was one of the preliminary validation tests of the iScan System prior to launching the Illumina service; further testing is ongoing

References
1. MAQC Consortium. Nature Biotechnology 2006, 24(9):1151

Reprogramming

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factors to revert differentiated cells to pluripotent stem cells (called induced pluripotent stem cells or iPS cells) and then redifferentiate the iPS cells into various cell types. Several groups have shown transcription factors Oct3/4, Sox2, Klf4, and c-Myc, when delivered by viral vectors, can reprogramme cultured adult skin cells to iPS cells (2,3). Earlier this year, a group from Mount Sinai Hospital (Toronto) showed that the transgenic expression of the same four transcription factors delivered by PiggyBAC (PB) transposition can reprogramme somatic cells into iPS cells (4). The advantage of the virus-independent PB system is that PB insertions can be removed from established iPS cell lines (4). An alternative method of reprogramming, however, would not require the intermediate step of generating iPS cells but rather convert one cell type directly into another cell type. Zhou cites a couple of examples from the literature that suggest this is possible; one study converts dermal fibroblasts into muscle-like cells (5) and another converts pancreatic cells into hepatocytes by treatment with a synthetic glucocorticoid (6).

In this study, Zhou developed a strategy to identify adult cell reprogramming factors that could convert adult pancreatic exocrine cells into insulin-producing β-cells. Zhou focused on re-expressing multiple embryonic genes, as previous studies have shown that dedifferentiation of adult cells to progenitors is accompanied by reactivation of embryonic regulators (7,8). In addition, Zhou et al. previously screened more than 1100 transcription factors and identified groups of

transcription factors with cell-type-specific expression in the embryonic pancreas (9). Of the 20 transcription factors that were expressed in mature β-cells and endocrine progenitors, 9 were selected for initial reprogramming experiments. Mature exocrine cells of the adult pancreas were chosen for reprogramming because, like β-cells, they are derived from the pancreatic endoderm (1). The transcription factors were delivered to pancreatic exocrine cells by adenoviral vectors as adenovirus preferentially infects pancreatic exocrine cells rather than islet cells. Although the adenoviral method is transient and minimizes the potential for insertion mutagenesis, it does not allow for maximal reprogramming efficiencies (4). However, reprogramming experiments were conducted in vivo so that induced β-cells would reside in a native environment, thus promoting survival and also allowing for the direct comparison of endogenous and induced β-cells.

Using Illumina Sentrix BeadChip Arrays (MouseRef-8 v1.1), the expression profile analysis of the endogenous β-cells and reprogrammed cells revealed a strong overlap of endocrine-enriched genes. The expression analysis also found that the reprogrammed cells did not express exocrine genes such as amylase, nor pancreatic hormones such as glucagon or somatostatin, indicating that the reprogrammed cells did not exhibit any expression characteristics of an exocrine cell. This study concluded that the combination of three transcription factors, Ngn3, Pax1, and Mafa, are required for reprogramming differentiated pancreatic exocrine cells in adult mice into cells that closely resemble β-cells.

This study is significant as it describes how a specific combination of transcription factors appears to directly reprogramme differentiated exocrine cells to cells that closely resemble β-cells in adult mice. Although this study cannot formally exclude the possibility that partial dedifferentiation of the exocrine cells may occur, the results indicate that extensive reversion to a dedifferentiated state for an appreciable time does not occur (1).

... a specific combination of transcription factors appears to directly reprogramme differentiated exocrine cells...

As the UHNMAC strives to meet the needs of the UHN research community, we have recently launched the Illumina array-based platform. This platform can be used for gene expression profiling, miRNA profiling, and DNA methylation studies. This review of the publication by Zhou et al. illustrates the usability of the Illumina platform for studies involving gene profiling.

References

1. Zhou Q, *et al*. *In vivo* reprogramming of adult pancreatic exocrine cells to β-cells. Nature 2008, 455(7213):627-632
2. Takahashi K *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007, 131(5):861
3. Park IH, *et al*. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008, 451(7175):141
4. Woltjen K, *et a*l. piggyback transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009 Mar 1 [Epub ahead of print]
5. Choi J, *et al*. MyoD converts primary dermal fibroblasts, chondroblasts, smooth muscle, and retinal pigmented epithelial cells into striated mononucleated myoblasts and multinucleated myotubes. PNAS 1990, 87(20):7988
6. Shen C-N, *et al*. Molecular basis of transdifferentiation of pancreas to liver. Nature Cell Biol. 2000, 2:879
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8. Tanaka EM. Cell differentiation and cell fate during urodele tail and limb regeneration. Curr Opin Genet Dev 2003, 13:497
9. Zhou Q, *et al*. A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell 2007, 13(1):103

Illumina iScan System

- Bead-bound 50-mer oligonucleotides
- Probes with high feature redundancy
- Handles partially degraded (FFPE) samples
- 6-, 8-, and 12-plex formats are cost effective
- Catalogue arrays for Gene Expression: Human, Mouse, & Rat; custom arrays available
- Catalogue arrays for miRNA: Human & Mouse
- Whole Genome SNP and CNV also available

Illumina Service

Whole Genome Expression: starts at $300/array
RefSeq Expression: starts at $250/array
MicroRNA Profiling: starts at $250/array

Please check our website for volume discounts
http://www.microarrays.ca/services/Illuminaservice.html

Standard Service Price includes:

- cost of the array
- quality check of RNA sample
- generation of biotin-labelled cRNA by IVT
- hybridisation, washing, and staining with streptavidin-Cy3
- scanning of BeadChip
- feature quantification

Data analysis is optional

UHNMAC Value-added Services

- Experimental Design
- Bioinformatics consultation
- Secure User Portal Access
- Data warehousing, analysis, and mining
- Pilot Studies and Protocol Development
- Quality checks that ensure successful results

For information about the Illumina Service, please call 416-581-7623 or e-mail us at: illumina@microarrays.ca

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