London Regional Genomics Centre: Microarray Facility

Carter, DE[1]; Liu, Y; Robinson, J; Hegele, RA

London Regional Genomics Centre, Robarts Research Institute, Room 4.01, 100 Perth Drive, London, Ontario, N6A 5K8

Introduction

Microarrays are a modern high throughput technology for interrogating RNA or DNA. The probes are immobilized on the array surface and the fluorescently labeled target is hybridized to the array. Results from microarray experiments can provide insights into differential gene expression, or genotyping and copy number variation.

The London Regional Genomics Centre (LRGC) was established through a citywide collaborative initiative by Robarts Research Institute, the University of Western Ontario, London Health Sciences Centre, Child Health Research Institute and The London Regional Cancer Program. The facility provides services on a fee-for-service basis and offers Affymetrix® GeneChip® technologies, spotted microarrays and data analysis.

Sample Process & Data Analysis

Initial Consultation

Before beginning a microarray experiment, an initial meeting with LRGC staff is arranged to discuss experimental design, scheduling and data analysis.

Sample Submission

A completed project and sample submission form must accompany RNA samples. The Agilent 2100 Bioanalyzer is utilized to assess the quality of the starting RNA sample to ensure the highest quality data output. Degraded RNA will result in an incomplete expression profile due to lost message.
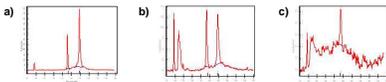

Figure 1: Electropherogram of RNA sample obtained from the Agilent 2100 Bioanalyzer. a) high quality RNA showing 1 marker peak, 2 ribosomal peaks (18S and 28S) and a flat baseline b) partial degraded RNA c) very degraded RNA samples.[2]

Sample Processing & Quality Control

The samples are processed and hybridized to the GeneChips according to protocols from Affymetrix®. Positive controls (spiked in), hybridization controls, house keeping genes and background probes are included on the GeneChip for quality control purposes. The hybridized GeneChips are stained and washed in the Affymetrix® Fluidics Station 450 and scanned by Affymetrix® Scanner 3000 7G.

Figure 2: Affymetrix® Fluidics Station 450 and Scanner 3000 7G[3]

Data Analysis & Biological Interpretation

On-site PC workstations are available for data analysis using various software packages. The LRGC is actively investigating and comparing new analysis solutions, both commercial and open source.

Partek® Genomics Suite™

Partek® Genomics Suite™ provides advanced statistical analysis and interactive data visualization to reliably extract biological signals from noisy data. Partek® Genomics Suite™ is fast, memory-efficient and can analyze gene expression, exon, SNP, copy number and tiling data using easy to follow workflows on a personal computer. [4]
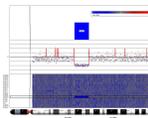
Figure 3: CNV detection using Partek genomics Suite.

Affymetrix® Genotyping Console

Genotyping Console is designed for whole-genome genotyping analysis and quality control for Genome-Wide SNP Array 5.0 and 6.0. Genotyping Console utilizes a novel genotype-calling algorithm, Birdseed to analyze multiple chips for each allele of each SNP. Genotyping Console displays quality metrics and annotation information in either graph or tabular form. [5]

Agilent GeneSpring® GT

GeneSpring GT is capable of analyzing high-volume and high-density genotyping data to discover relationships between genotypes and phenotypes. 1) Genetic linkage tests allows researchers to calculate maximum likelihood and estimate recombination frequencies for millions of variations, based on pedigree information. 2) Linkage disequilibrium tests can analyze potential association between phenotypes and marker loci when pedigree information is not available. 3) Transmission disequilibrium tests can provide high-resolution localization of potential markers when genotypes of many affected individuals and their parents are mapped. 4) Loss-of-heterozygosity analysis is essential to genotyping studies in cancer research. Specialized methods allow researchers to identify and compare homozygous regions across individuals and populations.[6]

DNA-Chip Analyzer (dChip)

dChip can perform samples comparison, hierarchical clustering, expression and SNP data viewing, Loss-of-Heterozygosity (LOH) and copy number analysis of SNP arrays, and linkage analysis.[7]

Ingenuity Pathways Analysis (IPA)

IPA can quickly establish dynamic pathway models and identify key biological insights from the experiment data.

Ariadne Pathway Studio

Ariadne Pathway Studio is capable of building pathways and finding relationships among genes, proteins, cell processes and diseases.

Affymetrix® Expression Arrays

3' Gene Expression Analysis Arrays

In 3'-based gene expression arrays, probes are designed to target the 3' end of the mRNA sequence. Currently 34 different species of arrays are available at Affymetrix, including human, mouse, rat, Arabidopsis, soybean, and yeast.

GeneChip® Exon Arrays

Exon arrays perform both gene expression and alternative splicing analysis for the whole genome on a single array. At the exon level, multiple probes for each exon enable the separation among different isoforms of a gene. This exon-level analysis on the whole genome permits the detection of specific alternative splicing that plays a key role in disease mechanism and etiology. At the gene level, multiple probes on different exons are summarized in an expression value of all transcripts from the same gene.[3]
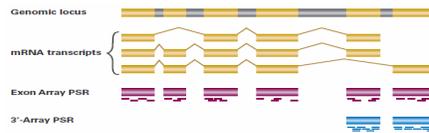

Figure 4: Schematic of Exon Array showing distribution of probe sets across the whole scale of the transcript. Golden regions represent exons and grey regions are introns. The short lines underneath the exon regions for the Exon Array and 3'-Array PSR (probe selection region) represent the individual probes. [3]

	Human	Mouse	Rat
Probe sets	1.4 million	1.2 million	1 million
Exon clusters	>1 million	~1 million	850,000
Supported by putative full-length mRNA	289,961 probe sets	266,200 probe sets	92,038 probe sets
Supported by Ensembl transcripts	306,583 probe sets	266,791 probe sets	195,943 probe sets
Supported by EST	665,175 probe sets	554,003 probe sets	211,451 probe sets
Supported by syntenic mRNA	220,262 probe sets[1]	214,763 probe sets[4]	272,061 probe sets[5]
Supported by gene prediction	883,105 probe sets	835,897 probe sets	875,666 probe sets
Probe selection region	Along the entire length of the transcripts		

GeneChip® Gene 1.0 ST Arrays

Gene Arrays offer advanced and cost-effective gene expression profiling on whole-genome, whole-transcript scale. Approximately 26 probes are distributed across the full length of each well-annotated gene, providing a more complete and accurate view of gene expression than 3'-based expression arrays. All 26 probes are automatically summarized into a single expression value per gene, which enables standard analysis software packages and simple analysis workflows to analyze data. Gene 1.0 ST Arrays are ideally suitable for cost-effective gene-level analysis on whole transcript scale.[8]

	Human	Mouse	Rat
Number of arrays	1	1	1
Array format	169	169	169
Feature size	5 μm	5 μm	5 μm
Oligonucleotide probe length	25-mer probes	25-mer probes	25-mer probes
Total number of distinct probes	764,885	770,317	722,254
Interrogated strand	Sense	Sense	Sense
Resolution (number of probes per gene)	26 (median)	27 (median)	26 (median)
Estimated number of genes	28,869	28,853	27,342
Gene-level probe sets with Ensembl support	28,132	27,543	26,008
Gene-level probe sets with putative full-length transcript support (GenBank and RefSeq)	19,734	19,434	9,916

Tiling Arrays

Human & Mouse Promoter 1.0R Arrays

GeneChip® Human and Mouse Promoter 1.0R Arrays are designed for chromatin immunoprecipitation (ChIP) experiments. The Human and Mouse Promoter Arrays containing over 4.6 million probes tiled through over 25,500 human and mouse promoter regions allow researchers to study the interaction between protein and DNA. The high density of probes and extensive coverage of each promoter region enables comprehensive promoter analysis.[9]

Human Tiling 1.0R Array & Mouse Tiling 1.1R Array

GeneChip® Human and Mouse Tiling Arrays offer the most comprehensive whole-genome analysis for discovering novel human and mouse RNA transcripts or mapping sites of protein/DNA interaction in ChIP experiments.[9]

Other Array Types

Experiments using spotted microarrays printed on standard size microscope slides can also be scanned. Using the BioRad VersArray Chipreader (5um resolution, Cy3/Cy5 filters) cDNA, micro RNA and protein arrays can also be processed.

Affymetrix® DNA Analysis Arrays

Affymetrix® Genome-wide Human SNP Array 6.0 contains more than 1.8 million markers to detect genetic variation, including more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for copy number variation (CNV). The SNP Array 6.0 significantly increases the ability for researchers to design association studies in large quantity of initial scanning and replication.[10]

Affymetrix® Genome-wide Human SNP Array 5.0 provides 500,568 SNPs and 420,000 non-polymorphic probes for CNV.[11]
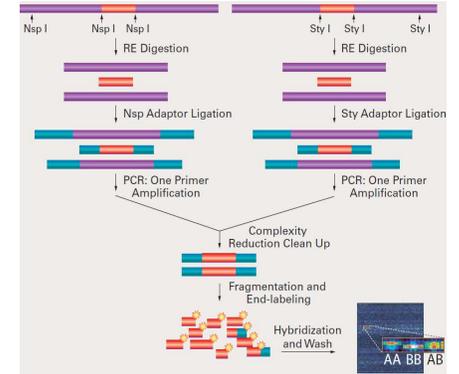

**Figure 5:** Schematic process of Genome-Wide Human SNP 6.0/5.0. [10]

The genomic DNA is digested with restriction enzymes, Nsp I and Styl separately. All DNA fragments are ligated to adaptors that recognize the 4bp overhangs. The adaptor-ligated DNA fragments are amplified using a generic primer complementary to the adaptor sequence. PCR amplification products from two restriction enzyme digestions are combined and purified using polystyrene beads. The purified DNA products are then fragmented, labeled and hybridized to Genome-Wide Human SNP chips.[10]

Microarrays Facility Usage
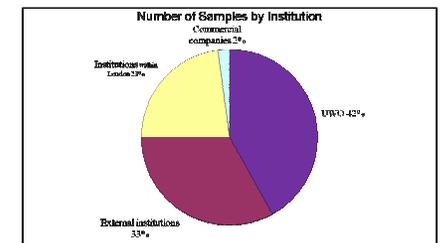
The LRGC has worked with over 165 labs across Canada and our users have published over 50 peer reviewed papers in scientific journals.

Microarray Facility Usage
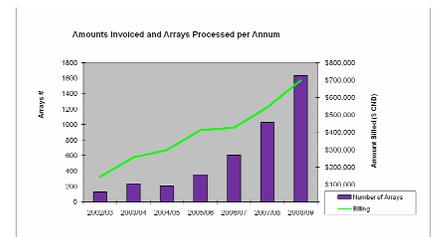
References

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3. Affymetrix® data sheet: GeneChip® Exon Array System for Human, Mouse, and Rat
4. http://www.partek.com
5. Affymetrix® Genotyping Console Workflow
6. http://www.chem.agilent.com/
7. http://biosun1.harvard.edu/complab/dchip/install.htm
8. Affymetrix® data sheet: GeneChip® Gene 1.0 ST Array System for Human, Mouse and Rat
9. Affymetrix® data sheet: GeneChip® Human Tiling Arrays
10. Affymetrix® data sheet: GeneChip® Genome-wide Human SNP Array 6.0
11. Affymetrix® data sheet: GeneChip® Genome-wide Human SNP Array 5.0