



The facility offers Affymetrix expression, exon, genotyping, ChIP and tiling GeneChips, spotted cDNA microarray services and RNA quality assays performed on an Agilent 2100 Bioanalyzer. We offer a customized approach to our service.

### **Project Request Form.**

Use this form to initiate a project. We meet with you and you provide us with contact and billing information as well as outlining your experimental plan. This form serves as a mutual planning tool and must be completed before we will order arrays for you, and before you submit samples. We will then schedule your project so that both your lab and our facility can coordinate your sample preparation and our assays.

### **Sample Submission Forms**

Please contact us to arrange sample submission. Use the form that relates to the type of assay. There are forms for **Agilent 2100 Bioanalyzer** only, **spotted cDNA** assays and **Affymetrix GeneChip** assays. Please read the sample submission instructions in this information package. Following these instructions will allow for the best quality data to be generated.

Prior to microarray analysis, each sample will undergo quality assessment with the Agilent 2100 Bioanalyzer. The contact person for the project will be notified for approval to proceed. This is especially important if the RNA is marginal. Excellent quality RNA at the right concentration is the single best factor for success of the assay.

When the assays are complete, all files generated from the experiment will be uploaded to our data server for download, or can be burned to DVD upon request. You will be notified when your data is ready.

### **Data Analysis**

Three computer workstations are available to our users and can be booked for data analysis. Software tools available include:

**Affymetrix Expression Console**  
**Affymetrix Genotyping Console**  
**dChip**  
**Ingenuity Pathway Analysis**  
**ArrayVision 6.0**

**Partek Genomics Suite**  
**Agilent GT**  
**TIGR Mev 3.0**  
**Ariadne Pathway Studio**  
**ArrayStat 1.0**

**Agilent GX (v7 and v10)**  
**EASE**

If you have any questions about the services we provide please do not hesitate to contact David Carter, John Robinson or Yibin Liu for assistance.

Robarts Research Institute  
PO Box 5015, 100 Perth Drive, Room 4294  
London, Ontario, Canada N6A 5K8  
**(519) 931-5246 or 931-5777 x24483**  
[microarray@robarts.ca](mailto:microarray@robarts.ca)

[www.lrgc.ca](http://www.lrgc.ca)



- A Project Request Form must be approved before experiments can commence
- RNA samples are to be submitted in RNase, DNase free water
- Samples submission must be coordinated based on a mutually agreed upon schedule
- Affymetrix GeneChip experiments require one RNA sample per microarray.
- For each sample, two aliquots of RNA must be submitted. Label the lid of the tube with the sample name and either a "**B**" for the sample(s) to be run in the Bioanalyzer, or an "**M**" for the sample(s) to be used in the microarray experiment.
- Concentrations for the "**B**" tube:

Item	Concentration	Volume
Total RNA	200 ng/μl	5 μl
Poly(A)+ mRNA	50 ng/μl	5 μl
Small Sample protocol	33 ng/μl	3 μl
WT (Exon and Gene arrays)	33 ng/uL	6 uL

- Concentrations for the "**M**" tube:

Item	Concentration	Volume
Standard	2 μg/μl	10 μl
Express	500 ng/μl	5 μl
Small Sample protocol	33 ng/uL	6 μl
WT (Exon and Gene arrays)	33 ng/uL	6 uL

- For each Affymetrix Genotyping microarray:

Item	Concentration	Volume
Genomic DNA	50 ng/μl	10 μl



## **Guide to experimental planning and data analysis for Affymetrix experiments**

### **Biological vs. Technical Replicates**

Users are advised that technical replicates are typically not necessary with Affymetrix experiments performed in our facility. Biological replicates will increase the power of your data sets and are recommended. To illustrate, technical replicate studies were performed at our facility using mouse liver RNA. Two aliquots were processed independently by the facility team using the recommended protocol and the resultant MuG74Av2 Genechip array signal intensities were plotted against each other. The Pearson correlation coefficient for this relationship was 0.9917. The next comparison was a triplicate experiment where one aliquot of RNA was processed and the resultant cRNA fragments were hybridized to three MuG74Av2 Genechips. The Pearson correlation coefficients for signal intensities for these three pairwise comparisons were 0.9752, 0.9884 and 0.9883. These represent typical results for our team using existing standard operating protocols from Affymetrix, and indicate that multiple replicates are not necessary for most applications. Technical replicates between operators were carried out and a Pearson correlation coefficient of 0.9727 was found.

### **Data Analysis**

The LRGC has numerous software tools for data analysis. Please contact the LRGC to book an appointment and arrange access. Typical analysis steps includes data QC assessment, normalization (RMA, GC-RMA, MAS5), visualization (PCA, clustering), statistics, (t-test, ANOVA), fold change lists, Gene Ontology and pathway analysis. Software training is provided.

# Expression GeneChip Sample Process

## Standard Protocol

<b>Pre</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>
Agilent 2100 Bioanalyzer	cDNA Synthesis	IVT Labeling Hybridization	Scanning Data QC	Data Analysis

## Two-Cycle Protocol

<b>Pre</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>
Agilent 2100 Bioanalyzer	cDNA Synthesis IVT Labeling	cDNA synthesis	IVT Labeling Hybridization	Scanning Data QC	Data Analysis

# Guidelines for Assessing 3' IVT Expression GeneChip Quality

## Scope

Identify the metrics, and their ranges, in the report file that can be used to assess GeneChip processing quality.

## Noise (RawQ):

A measure of the pixel-to-pixel variation of probe cells on the array. The two main factors that contribute to noise are electrical noise of the GeneArray Scanner and sample quality. A low Noise is desirable.

## Scale Factor:

The unit multiplier needed to make the overall intensity comparable to the Target Intensity. GeneChips in an experiment must have Scale Factors within 3 fold of each other.

## Background:

Average signal intensities of probe cells with the lowest 2% signal intensities. Acceptable average values are between 28 and 150, with a small max. – min. range.

## Percent present:

The number of probes whose signal strength is sufficient to be called present by Affymetrix's MAS5 algorithm. Biological replicate GeneChips in an experiment must be within 10%.

## Number of Outliers

The number of probe cells flagged as outliers in GCOS should be < 500 for 18 um features arrays and < 1000 for 11 um Feature arrays.

## Internal Control Genes

### Housekeeping

Look at the B-actin and GAPDH genes for the specific species. The signal ratio for 3'/5' B-actin and GAPDH for that species should be close to 1.0. Less than 3.0 is considered acceptable for the standard protocol.

### B2 Oligos

creates checkered pattern for grid alignment  
should be equal intensities

### bioB, bioC, bioD, cre

hybridization controls  
ascending signal strengths and called Present

### Spike Controls

#### lys, phe, thr, dap

labeling and hybridization controls  
ascending signal strengths and called Present