UB Next-Generation Sequencing and Expression Analysis Core
Affymetrix GeneChip Services Document

AFFYMETRIX GENECHIP ARRAY SERVICES

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1) Sample Submission Requirements

An **Affymetrix Services Sample Submission Form** must be completed by the researcher and is required to include the following information for each project:

1) Lab Contact Information – Please provide the email address and phone number of the preferred contacts for the project. This information will be used to communicate with the researcher/technician if we have any questions or problems with the samples.

2) Type of Array – The researcher must indicate the specific Affymetrix GeneChip Array they wish to use for the experiment. Information about all of the Affymetrix GeneChip Arrays can be found on the Affymetrix website at: **http://www.affymetrix.com**.

3) Sample Names – The name provided will be used for all purposes, including scanning of the chip. Verify that each sample has a unique name and that the sample tubes submitted have the correct sample identification clearly written on each tube.

4) Sample Volumes – Accurately measure each sample volume with a pipet.

5) Method of RNA Extraction – Confirm that you have used the recommended Qiagen RNeasy Kit for RNA extraction or supply your alternative protocol and cleanup method.

6) Sample Concentrations (ng/uL) – Measure each sample concentration using a NanoDrop, spectrophotometer, or fluorometer. Accurate measurements are crucial for success of the labeling procedure.

7) 260/280 Ratios – This ratio indicates the purity of the RNA. Please also include the 260/230 ratio when available.

8) Agarose Gel Electrophoresis Image – The image will be used to confirm RNA integrity. If you are unable to provide an agarose gel image of your RNA due to low sample quantity, please contact the UB Next-Gen Sequencing and Expression Analysis Core by email at **cbi-ubnextgencore@buffalo.edu** or phone at (716) 881-7514 to discuss alternative options.

2) RNA Quality and Quantity Requirements

RNA quality is essential to the success of your Affymetrix project. RNA received for Affymetrix Arrays should be of high quality, lack contamination (DNA, protein, organic compounds, etc.), and have no evidence of degradation. We recommend using the Qiagen RNeasy Kit for RNA extraction. Other RNA extraction methods will be accepted if the sample is purified with the Qiagen RNeasy MinElute Cleanup Kit and DNase treated before submission. All RNA samples received by the UB Next-Generation Sequencing and Expression Analysis Core for Affymetrix Services will be run on a RNA 6000 Pico or Nano Chip on the Agilent 2100 BioAnalyzer to assess RNA quality. The samples will also be quantified using the Invitrogen Quant-iT RiboGreen Assay on a Turner BioSystems TBS-380 Fluorometer. We use the data from our BioAnalyzer RNA Chip Run and our Quant-iT RiboGreen Assay as the absolute determinates of sample quality and quantity. If it is determined that a sample does not meet our sample quality and quantity standards, the UB Next-Gen Sequencing and Expression Analysis Core will request a new sample from the researcher. The researcher is still responsible for all costs associated with RNA quality and quantity assessment for any sample that does not meet standards and must be resubmitted. If a sample does not meet our requirements and the researcher still chooses to have the sample processed, the researcher absolves the UB Next-Gen Sequencing and Expression Analysis Core of any liability and is financially responsible for all services regardless of the outcome of the data. **RNA samples received for Affymetrix Services must meet the following quantity and quality requirements:**

1) Sufficient Sample Quantity and Volume – The amount of RNA needed for labeling is dependant on the type of array that you will be using. Please refer to your specific type of array below to determine the concentration and volume of sample necessary for your experiment. We must receive enough sample for quantification and qualification of your RNA and for the labeling process. More sample is always appreciated just in case something needs to be repeated. Keep in mind that we use the quantifications from our Quant-iT RiboGreen Assay as the final determinate of sample concentration if our values vary from those provided by the researcher. This is because most researchers determine the concentration of their RNA based on absorbance at 260nm on a spectrophotomer or NanoDrop, which may overestimate concentration. Absorbance-based quantification methods can be skewed by proteins, free nucleotides, and other contaminants. The Quant-iT RiboGreen Assay is highly sensitive and is not affected by the presence of contaminants. The UB Next-Gen Sequencing and Expression Analysis Core can return unused sample if requested by the researcher, but samples that have not been picked up after a month will be discarded.

a) 3' Expression Arrays - The 3' IVT Express Kit Labeling Protocol requires between 50ng-500ng of total RNA in a volume of 3µL. We recommend providing ≥12µL of sample at a concentration ≥167ng/µL. More sample is always encouraged. The absolute minimum volume of sample that we can accept is 8µL and the absolute minimum concentration is 16.7ng/µL.

b) Whole Transcript Expression Arrays - The Ambion WT Expression Kit Labeling Protocol requires between 50ng-500ng of total RNA in a volume ≤ 3µL. We recommend providing ≥12µL of sample at a concentration ≥167ng/µL. More sample is always encouraged. The absolute minimum volume of sample that we can accept is 8µL and the absolute minimum concentration is 16.7ng/µL.

c) microRNA Arrays - The FlashTag Biotin HSR RNA Labeling Kit Protocol requires between 0.1µg-3µg of total RNA in a volume ≤ 8µL. We recommend providing ≥12µL of sample at a concentration of ≥125ng/µL. More sample is always encouraged. The absolute minimum volume of sample that we can accept is 10µL and the absolute minimum concentration is 12.5ng/µL. If your sample is highly concentrated we may be able to accept a lower volume of sample, but please contact the UB Next-Gen Sequencing and Expression Analysis Core to make arrangements.

2) High Quality RNA

a) 260/280 Ratio: The $A_{260/280}$ value must be between 1.9-2.1. This indicates purity of the RNA.

b) Agarose Gel Image: The gel image should exhibit the appropriate ribosomal subunit bands (eukaryotic: 18S and 28S, prokaryotic: 16S and 23S). These bands should be sharp and clear and the large subunit band should be about twice as intense as the small subunit band. The UB Next-Gen Sequencing and Expression Analysis Core will not accept partially degraded RNA that has a smeared appearance and lacks sharp, clear ribosomal bands. We will also not accept completely degraded RNA that appears as a very low molecular weight smear.

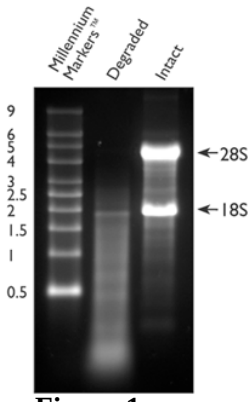

Figure 1.

Figure 1. Intact vs. Degraded RNA. Two µg of degraded total RNA and intact total RNA were run beside Ambion's RNA Millennium Markers on a 1.5% denaturing agarose gel. The 18S and 28S ribosomal RNA bands are clearly visible in the intact RNA sample. The degraded RNA appears as a lower molecular weight smear. Image from **http://www.ambion.com**

c) Agilent 2100 BioAnalyzer RNA 6000 Pico Chip: The electropherogram should have two well defined peaks corresponding to the ribosomal subunits. The ratio between the large and small ribosomal subunits should be about 2:1. There should also be no evidence of degraded products.

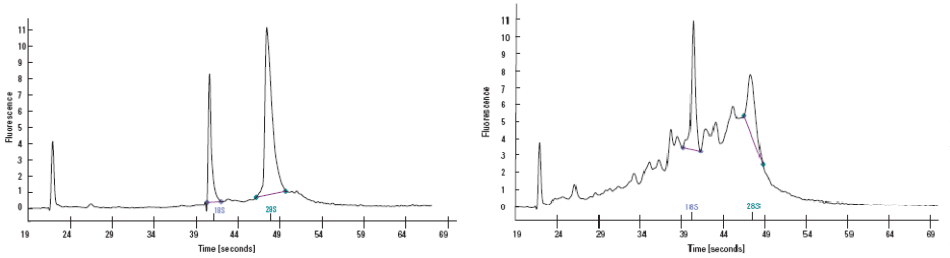

Figure 2. **A** **B**

Figure 2. Agilent 2100 BioAnalyzer RNA 6000 Pico Chip electropherogram for a eukaryotic RNA Sample. Figure 2A is an example of high-quality total RNA. There are two well-defined peaks corresponding to the 18S and 28S ribosomal subunits and the ratio between the 28S and 18S peaks is approximately 2:1. Figure 2B is an example of partially degraded RNA. The 2.1 ratio between the ribosomal peaks is absent and there is a high presence of degraded products. Image from **http://www.agilent.com/chem/labonachip**

3) Data Analysis

The UB Next-Gen Sequencing and Expression Analysis Core provides the initial data analysis, but we do not offer full data analysis services. The researcher is responsible for arranging data analysis services, but the core may be able to help facilitate these arrangements. Please contact us by email at **cbi-ubnextgencore@buffalo.edu** or by phone at (716) 881-7514 for questions regarding data analysis.

4) Service Requests

The first step to requesting Affymetrix GeneChip Array Services from UB Next-Gen Sequencing and Expression Analysis Core is to contact us by email at **cbi-ubnextgencore@buffalo.edu** or by phone at (716) 881-7514 to discuss the project. After the initial contact, the researcher must read the **Affymetrix Services Document** and complete the **Sample Submission Form** prior to sending samples.

5) Misc. Information

Sample Drop-Off – The UB Next-Generation Sequencing and Expression Analysis Core Facility is open for sample drop-off Monday-Friday 9:00am-3:00pm. Please call ahead or schedule an appointment to make sure that a technician will be available to accept your samples as we are often busy working on other projects. We will not accept samples unless we have already received the **Affymetrix Services Sample Submission Form** or if the form accompanies the samples. Samples should be brought to the University at Buffalo Next-Generation Sequencing and Expression Analysis Core Facility (B3-123) at the New York State Center of Excellence in Bioinformatics and Life Sciences Building (COE) located at 701 Ellicott Street in Buffalo, NY 14203. There is a receptionist at the entrance of the COE who will be able to let you in the building and contact us to let us know that there is a researcher dropping off samples. Alternatively, samples can be shipped overnight on dry ice to the below address, but please email or call the core to let us know to expect the sample shipment.

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ATTN: UB Next-Generation Sequencing and Expression Analysis Core(B3-123)
State University of New York at Buffalo
New York State Center of Excellence in Bioinformatics and Life Sciences
701 Ellicott Street
Buffalo, NY 14203
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Project Scheduling – Projects are completed in the order in which samples are received. The UB Next-Gen Sequencing and Expression Analysis Core will try to accommodate your deadline if we are provided with sufficient lead time. However, we can not make any guarantees as to when your data will be available. There are a number of factors that influence project completion; such as the number of projects already in the queue, array/reagent shipping, and instrumentation/software issues. The UB Next-Gen Sequencing and Expression Analysis Core does not keep a stock of arrays and reagents, so items must be custom ordered for each project. Orders usually take at about two weeks to receive from the vendor, but can be significantly delayed if an item is on backorder. Please contact the core if you would like to discuss your project completion timeline.

Array and Reagent Ordering - The UB Next-Gen Sequencing and Expression Analysis Core typically orders arrays and reagents after we receive the samples and the **Affymetrix Services Sample Submission Form**. However, the UB Next-Gen Sequencing and Expression Analysis Core is willing to pre-order arrays and reagents before samples are received in an effort to streamline project completion, if a researcher completes the **Affymetrix Arrays and Reagents Pre-Order Form**. This form will authorize the core to order all consumables necessary for the researcher's Affymetrix project and also confirms that the researcher is financially responsible for all items ordered for their project even if the researcher does not end up using the arrays or reagents.

Data Availability – When a researcher's project has been completed we will use an ftp site to send the data. The researcher will be given information allowing access to their data from a password protected ftp site. Please access and download your data as soon as possible. It is highly recommended that the researcher makes a backup copy of their data. Data will be removed from the ftp site 30 days after the data availability email is sent to to the researcher. There will be a $100.00 data recall charge if a researcher requests data to be re-uploaded to the ftp site after the data has already been removed. The UB Next-Gen Sequencing and Expression Analysis Core does not guarantee that data will be able to be recalled after the 30 day data availability period.

Project Costs – The UB Next-Gen Sequencing and Expression Analysis Core are happy to provide a cost estimate for your project if you let us know how many arrays and what type of array you will be using. Our cost estimates include the cost of quantification, qualification, target labeling, fragmentation, hybridization, staining, and scanning. Keep in mind that the cost estimate provided to the researcher is just an estimate. If the researcher makes any changes to the original project plan (number of arrays, type of arrays, etc.) the final cost of the project will reflect these changes.

a) Project Planning for Cost Effectiveness – The UB Next-Gen Sequencing and Expression Analysis Core can process up to 8 samples at a time. The labor cost is the same whether we are processing 1-8 samples, therefore it is most cost effective for the researcher to have samples in multiples of 8 (8, 16, 24, 32, 40, etc). If the researcher's experimental design results in samples that are not in multiples of 8 samples we will make an effort to process samples from another project at the same time to reduce the labor cost for both researchers. However, this is not always possible and if we can not process samples from another project in parallel the researcher is still responsible for the full labor charge.

b) Researcher Financial Responsibility – Once a researcher requests an order the researcher is financially responsible for all arrays, reagents, and shipping costs for items ordered on behalf of their project. Even if the researcher does not use all of arrays and reagents requested for their project they are still responsible to pay for them as we are unable to return unused items to the vendors. Also, if it is determined that at any point throughout the process a sample does not meet quality or quantity control measures the researcher is still responsible for any reagents and labor charges up to the point that the sample failed to pass quality/quantity control standards.

c) Invoices – The UB Next-Gen Sequencing and Expression Analysis Core will send the researcher an invoice for the final cost of their project. Invoices are required to be paid within one month from the receipt of the invoice. University at Buffalo researchers that will be paying from Research Foundation Accounts can pay their invoices with an Interdepartmental Invoice (IDI). UB researchers paying from State Accounts or other types of accounts should let the core know which type of account they will be paying from so that we can send you the appropriate type of invoice. Non-UB researchers will receive a standard UB Next-Gen Sequencing and Expression Analysis Core Invoice and will be expected to pay with a check.