



Microarray Core RNA Submission Instructions

Before submitting RNA samples for labeling and hybridization, please follow the procedures below.

I. Total RNA should be prepared and provided in the following manner.

1. Determine the amount of RNA that will be required from the cDNA table below.
2. Isolate total RNA using either Trizol (GIBCO/BRL) or a commercially provided kit, such as those sold by Ambion or Qiagen. Isolation of mRNA is not necessary.
3. Clean total RNA using RNeasy Kit (Qiagen) or a similar spin column based kit. Final solution should be in RNase-free-H₂O. A dry pellet is also acceptable.
4. Measure the optical density (OD) of total RNA samples including OD₂₆₀, OD₂₈₀, OD₂₆₀/OD₂₈₀, and calculate the concentration (µg/µl). Submit your samples with all the values above. Please label each tube of RNA sample carefully.
5. Complete all the details on the:
 - RNA Submission form [volume, concentration, or µg amount (dry pellet)].
 - Hybridization/Experiment Design form.
6. Please ship the forms and the RNA (on dry-ice) to:

Mike Heinz
mail-drop AA8501
4444 Forest Park Parkway,
St. Louis, MO 63108.
Forms can also be faxed to 314-286-1810.

II. Our protocol for RNA Quality Control

To obtain high quality data from your microarray experiment, it is essential to start with high quality total RNA. If RNA is degraded or otherwise unsuitable for microarray application, investigators will be notified immediately.

Spectrophotometric Analysis:

To assess the mass and purity of your total RNA, a small amount of your sample will be assayed with an Eppendorf BioPhotometer. OD 260/280 is calculated to estimate the purity of the RNA. A ratio close to 2 indicates a high percentage of ribonucleotide.

Electrophoretic Analysis:

To assess the integrity of your total RNA, we will test it with the Agilent Technologies 2100 Bioanalyzer Lab-on-a-chip system. This assay is similar to gel electrophoresis in concept, but it is more quantitative, resolved and efficient, and only requires small amount of sample.

In total, we will use 0.5ul of your RNA for quality control procedures.

III. We create cDNA (or aRNA) by a variety of means, depending on the quantity and nature of the total RNA.

These required masses are based on established array platforms. If arrays or species not previously used by core facility, a small hybridization set is recommended to establish total RNA input amounts. Please contact core facility for additional information.

RNA polyadenylated?	RNA mass limited?	Kit	Required mass*	Required concentration range	Comments
Yes	No	Genisphere A350	8.5ug	1.25 to 4.00 ug/ul	~95% the cost of A900
Yes	Yes	Genisphere A900	2.5ug	0.75 to 1.50ug/ul	
No	No	Genisphere A350 MPX (custom)	5.0ug	1.5 to 3.0 ug/ul	Random or GDP priming; ~95% the cost of A900MPX
No	Yes	Genisphere A900 MPX	2.0ug	0.5 to 1.5 ug/ul	Random or GDP priming
Yes	Yes	Ambion MessageAmp aRNA kit	500ng	100 to 500 ng/ul	RNA amplification
No	Yes	Ambion MessageAmp II-Bacteria kit	500ng	125 to 500 ng/ul	RNA amplification

* per sample per chip or per aRNA synthesis

In specific contexts the above-recommended amounts are not adequate (e.g. RNA derived from multiple species present).

