BRI MicroArray Lab Guidelines Sample Submission of RNA

RNA Sample Requirements

The RNA used in microarray experiments must be extremely clean in order to get high quality results. In our lab, best results were obtained for *C albicans* using the hot phenol method explained previously: **(http://www.bri.nrc.gc.ca/pdf/Candida_RNA_Isolation_Protocol.doc)**. Qiagen Rneasy and Trizol like reagents (e.g. Invitrogen) have also been used successfully for extraction of yeast, human or mouse RNA. For further isolation of mRNA use the Invitrogen Kit (Cat No.K1520-02), following manufacturers instructions (for yeast extend resin incubation time to 2 hours) If total RNA is obtained using the hot phenol extraction method, the RNA must be further cleaned up using Qiagen RNeasy kit. In our experience, the yield of cDNA using mRNA is generally greater than with total RNA, although good results have been obtained using both.

Essential Requirements for direct cDNA labeling:

1. The minimum amount of RNA without sample amplification: is **3-4µg** for mRNA or **20-40µg** or more of total RNA per experiment. If you wish do reciprocal labeling you will need double this amount. We will also need 1 µl extra for internal quality control of the RNA.. The final concentration should be minimum **1 ug/µl** for mRNA, and **4 µg/µl** for total RNA

2. It is possible to use less than 10 ug of total RNA to start the cDNA probe synthesis. In the event that you have limitations in the amount of starting material you can provide us with, we can start with less. However, we will still require you to provide us your starting material at a concentration of **4 µg/µl** plus 1 ul for our quality control checks. If we start a reaction with less than 10 ug and do not obtain enough cDNA to use as a probe , there will still be a charge for the labeling procedure.

3. Measure the **optical density (OD)** of total RNA and mRNA samples including **OD_{260}, OD_{280}, OD_{260}/OD_{280}**, and calculate the **concentration (µg/µl)** of your samples. Submit your samples with all the values above and the **dilution factor** that you used to take these readings.

4. A detailed copy of the method used for RNA extraction mush be provided.

5. The OD 260/280 ratio for the RNA samples must be between 1.8-2.1

6. An agarose gel picture should also be provided.

7. The RNA must be resuspended in RNase free water

8. The quality of the RNA will be checked at our facility upon reception of the samples using the Agilent Bioanalyzer. If low quality is suspected, the procedure will be stopped.

9. Labeling samples : Please download and fill out the attached submission form (http://www.bri.nrc.gc.ca/pdf/ BRI MICROARRAY Lab RNA Submission Sheet.doc) and include it with your samples with your samples at the time of delivery (on dry ice).

Please contact tracey.rigby@cnrc-nrc.gc.ca before starting this process..

On the sample tubes please indicate:

1. Unique sample number
2. Name of responsible researcher
3. Date

4. Concentration

On accompanying RNA submission form

1. Same information as on tube
2. Name of sample
3. Concentration and volume
4. OD 260/280 Ratio

Labeling and hybridization will be done at the BRI MicroArray Lab according to the protocols for labeling posted on this website (**http://www.bri.nrc.gc.ca/pdf/Direct Incorporation Labeling_Protocol.doc**)

Data processing.

You will receive following results:

- Quality report of the RNA samples provided analyzed on the Agilent Bioanalyzer
- Copy of BMP and/or TIFF files of the microarray images
- Data file containing raw array (can be opened excel format)
- Data file containing processed array data and expression ratios (can be opened excel format)
- The data can be sent on a CDROM by courier, or the data can be accessed through an FTP site (data files are too large to send via e-mail)

In order to ensure optimal use of the data we will do everything we can to help you with further analysis and interpretation of your results