RNA Sample Requirements for Microarray Analysis

The most important component of a microarray experiment is the RNA template. Please follow the guidelines in this document to produce RNA suitable for hybridization to your microarray.

Sample Collection and Storage Techniques

1) The tissue must be immobilized as quickly as possible after sampling to prevent RNA expression changes due to technical stress and to prevent degradation by RNases.
   a) Snap freeze in liquid nitrogen. Then store at -70C. **Or**
   b) Store in RNAlater (sample must be small so solution can penetrate). Tissues are stored at 4C overnight then moved to -20C or -70C.
   c) Avoid multiple freeze-thaw cycles.

Isolating RNA

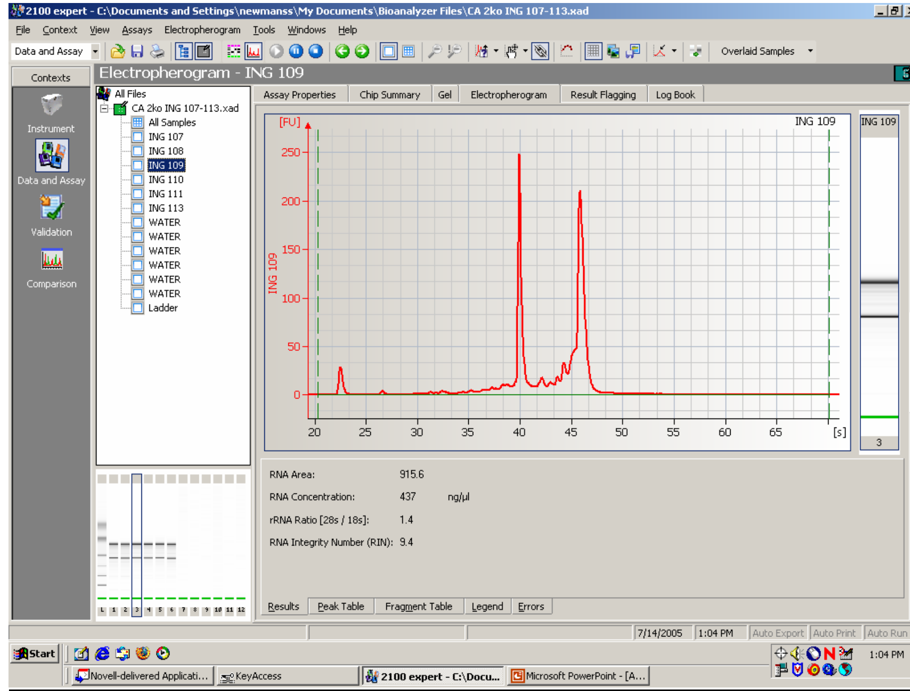
1) Homogenization-Homogenize the minimum time necessary for a complete homogenization.
   a) Liquid nitrogen frozen tissues must be homogenized without thawing the sample. The sample must be immersed in a solution which inhibits RNase activity.
   b) RNAlater stored tissues may be thawed on ice before homogenization.
2) Isolation and Purification of RNA
   a) Trizol and Tri-Reagent are commonly used for RNA isolation but are not recommended for RNA to be used in microarray probe production as they contain inhibitors of the labeling reaction. There is also the risk of genomic DNA contamination. Additional variation in RNA quality is likely due to the competence level of the person performing the extraction. RNA purified by this method may be cleaned later using a solid phase method such as the Qiagen RNeasy column.
   b) Qiagen RNeasy columns have proven to be an extremely reliable method for purification of microarray quality RNA. Special kits are available for processing fibrous or lipid tissues. DNAse treatment is easily performed on the column to eliminate genomic DNA contamination.
   c) Do not use DEPC treated water for resuspension of RNA. DEPC can inhibit enzymatic reactions.
   d) Formazol (used for storage of purified RNA) can also inhibit enzymatic reactions.

Determination of RNA Quality

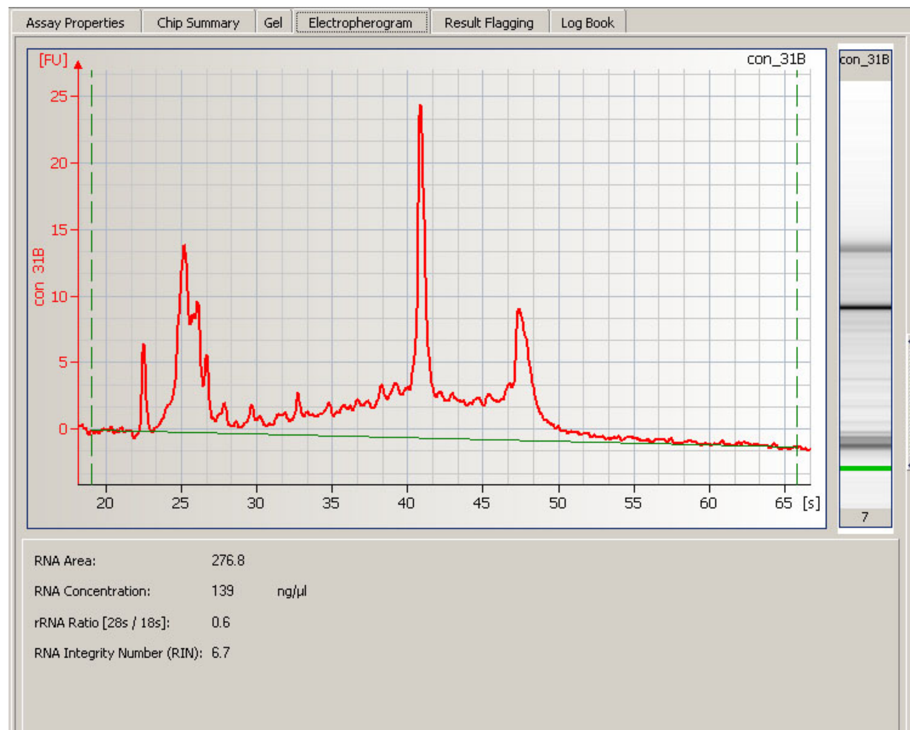
For microarray analysis, perform both Agilent Bioanalyzer and spectrophotometric analysis of your RNA samples.

1) Agilent Bioanalyzer
   a) Quantitation
      i) Dynamic Range-The accurate dynamic range of the RNA Nano 6000 chip is 25ng to 500ng. Dilute or concentrate your RNA as necessary to insure that the sample is within this range when you load 1ul on the chip.
      ii) Quantity- For RNA assays, quantitation is accomplished by comparison with the RNA ladder area. The area under the ladder is compared with the sum of the sample peak areas. The Agilent is more accurate than a spectrophotometer because it does not measure contaminants.
   b) RNA Quality
      i) 18S/28S Ribosomal Ratio-
         (1) Ribosomal ratios will vary according to the species and tissue type as well as according to the RNA extraction method. For a specific tissue, values should be consistent so that after establishing quality criteria for a specific sample type, the ribosomal ratio can be used as a quick check for RNA sample quality.
         (2) A change in the ratio will occur with degradation of the RNA. The 28S ribosomal RNA tends to degrade more quickly than the 18S RNA.
         (3) This ratio is not related to the 260/280 ratio calculated from spectrophotometer readings.
         (4) Do make sure that the software has marked the location of the peaks properly.
      ii) RIN number-The RNA Integrity Number is a measure of the quality of your RNA. A RIN of 7 or greater is necessary for a sample to be used for microarray. Preferably, your RIN should be above 8. The RIN is a measure of the degradation of your RNA sample.

Good RNA Quality



Poor RNA Quality



2) Spectrophotometer

c) Quantitation

i) Dynamic Range-The accurate dynamic range may vary from spectrophotometer to spectrophotometer. It is important to determine this prior to measuring your RNA sample. Usually absorbance values should fall between 0.1 and 1.0. Solutions that are outside this range cannot be measured accurately. Generally the greatest error occurs at lower concentrations.

ii) Because this method does not discriminate between RNA and DNA, it is advisable to first treat RNA samples with RNase-free DNase to remove contaminating DNA. Other contaminants such as residual proteins and phenol can interfere with absorbance readings, so care must be taken during RNA purification to remove them.

iii) Calculation of quantity-1Absorbance Unit at A_{260} = ~40ug RNA

d) Measures of Sample Quality

i) $A_{260/280}$ and $A_{260/230}$ ratios determine the purity of the RNA sample. Good quality RNA will have an OD 260/280 ratio of 1.8 to 2 and an OD 260/230 of 1.8 or greater. Nucleic acid is detected at 260 nm, whereas protein, salt and solvents are detected at 230 and 280 nm. High OD 260/280 and OD 260/230 ratios therefore indicate that you have extracted RNA devoid of any of these contaminants. Ratios of greater than or equal to 1.8 for 260/280 and greater than or equal to 2.0 for 260/230 generally perform better for microarray than samples with lower ratios. It is recommended that samples with ratios <1.8 for either ratio not be labeled for microarray.

ii) Effects of pH on A_{260}/A_{280} Ratio.

BLANK/DILUENT	A_{260}/A_{280}RATIO
DEPC-treated water (pH 5-6)	1.60
Nuclease-free water (pH 6-7)	1.85
TE (pH 8.0)	2.14

iii) Causes of low 260/280 Ratio <1.8

(1) Residual organic solvents in the RNA (phenol, chloroform).
(2) Sample not homogenized with sufficient TRIZOL Reagent.
(3) pH of diluent is acidic.
(4) A260 or A280 outside the linear range.

Quantity of RNA required for Labeling for Microarray Analysis

Remember that you will need additional RNA for validation of your microarray data with real-time PCR.

Applied Biosystems RT Labeling Kit- 40ug total RNA or 2-5ug mRNA

Applied Biosystems NanoAmp Labeling Kit- For a single round of amplification use 500 ng of very good quality RNA. Increase to 1-2ug if the quality of the RNA is low. Label low quality RNA only if there is no other alternative. If you have a very small amount of RNA, you may use two rounds of amplification. Use 5 to 100 ng of RNA for first round amplification.

Reference RNA may be purchased from Stratagene or Clontech. Label the reference RNA alongside your sample RNA as a labeling control.

Qiagen

Total RNA purification	**Binding Capacity**	**Product**
Animal cells and standard tissues, bacteria, yeast, and enzymatic reactions	100 ug RNA	RNeasy Mini Kit 74104
	1 mg RNA	RNeasy Midi Kit 75142
	6 mg RNA	RNeasy Maxi Kit 75162
Animal cells and tissues, with gDNA Eliminator spin columns		RNeasy Plus Mini Kit 74134
Fiber-rich tissues	100 ug RNA	RNeasy Fibrous Tissue Mini Kit 74704
	1 mg RNA	RNeasy Fibrous Tissue Midi Kit 75742
Fatty tissues	100 ug RNA	RNeasy Lipid Tissue Mini Kit 74804
	1 mg RNA	RNeasy Lipid Tissue Midi Kit 75842

Sigma-Aldrich

Tri Reagent RNA Isolation Reagent

Product Number	Product Description	Product Size
T9424	Tri Reagent RNA, DNA and Protein Isolation Reagent	25, 100 and 200 ml
T3809	Tri Reagent BD	25, 100 and 200 ml
T3934	Tri Reagent LS	25, 100 and 200 ml
B9673	1-Bromo-3-Chloropropane (BCP)	200 ml

Ambion

#7020: **- RNA*later*® (100 ml)**
#7021: **- RNA*later*® (500 ml)**
#7024: **- RNA*later*® (250 ml)**
#7022: **- RNA*later*® (50 x 1.5 ml)**
#7023: **- RNA*later*® (20 x 5 ml)**
#7030: **- RNA*later*®-ICE (25 ml)**
#7031: **- RNA*later*®-ICE (10 x 25 ml)**