

# UHN Microarray Centre Guidelines for Preparing and Sending RNA For Analysis on the Agilent 2100 Bioanalyzer

## Introduction

The Agilent 2100 Bioanalyzer is a device that is used to monitor the quality of total RNA prior to performing a labelling reaction for microarray analysis. The Bioanalyzer is a microfluidic electrophoresis device that produces an electropherogram for each sample. The electropherogram can be used to assess RNA degradation and to approximate the quantity of RNA. The Bioanalyzer software also calculates a RNA Integrity Number (RIN) to allow users to easily assess the quality of their RNA samples. We have found a strong correlation between the quality of RNA (as judged by this method) and the quality of the resulting array image.

The UHNMAC currently offers the Nano RNA and Pico RNA assays as part of its Bioanalyzer service. This document outlines how to prepare and send the RNA samples for analysis. Please follow the guidelines carefully to ensure an accurate/successful reading on the Bioanalyzer. A checklist and work order form is also provided. RNA samples will not be processed until the completed checklist and work order form has been received.

## Quantity of RNA Required

We request that you send *at least* 2  $\mu\text{L}$  *total* RNA of each sample. For the Nano RNA kit, each sample concentration should be between 100 and 200  $\text{ng}/\mu\text{L}$  in RNase-free water. For the Pico RNA kit, each sample concentration should be between 5 and 10  $\text{ng}/\mu\text{L}$  in RNase-free water. Currently, mRNA and amplified RNA samples are only assessed using the Nano or Pico kits (not the mRNA kit), thus no RIN is available for these types of samples.

## Extraction and Purification of RNA

The quality of the RNA samples is critical for microarray experiments. We have evaluated many commercially-available RNA extraction kits and found that all yield high quality RNA, provided the protocols are followed. Often, the RNeasy kit (Qiagen) is used for RNA isolation from cultured cells and many labs use TriZOL (Invitrogen) for RNA isolation from tissue samples. Regardless of the purification protocol, please resuspend total RNA RNase-free water prior to Bioanalyzer analysis.

## **Sending RNA**

RNA eluted in RNase-free water should be sent frozen on dry ice. If you are hand delivering the samples, you should ensure that the RNA samples remain frozen during transit and that the samples are handed directly to one of the members of the microarray lab. Please have the lab member that receives your samples sign the "RNA RECEIPT" and retain this receipt for your records.

If you are shipping RNA samples, they should be sent by overnight courier to ensure they arrive frozen. They should be sent such that they will arrive at our laboratory between Tuesday and Thursday. Do not ship a package on Friday, as we do not work weekends. RNA should be sent to the following address:

UHN Microarray Centre  
MaRS Centre, TMDT  
101 College St. 9-601  
Toronto, ON, Canada  
M5G 1L7

Include in your shipment a completed copy of the checklist and the work order found at the end of this document. Also include a spreadsheet containing designations and concentrations of each sample with your shipment. You should send an email to [geneservice@microarrays.ca](mailto:geneservice@microarrays.ca) informing us that you have sent a shipment. Please provide the tracking number for the courier and your contact number/email address such that we can confirm that your sample has arrived safely.

**PLEASE NOTE THAT ANY LEFTOVER RNA SAMPLES WILL BE DISCARDED.**

## **Turnaround Time**

Typically, samples are analyzed within two business days; however, larger numbers of samples may require more time. We will email your results when they are ready.

Data is provided in a PDF format. This includes a gel-like picture, electropherogram of each sample, and an estimation of sample concentration.

## **Questions?**

If you have any questions about the information contained in this document please contact [geneservice@microarrays.ca](mailto:geneservice@microarrays.ca)

## UHN Microarray Centre RNA Checklist

Please completely fill out this checklist and submit it with your RNA samples.

Orders that do not include this checklist will not be processed. Please check the appropriate boxes and initial the line at the bottom of the form.

**NAME:** \_\_\_\_\_ **PHONE NUMBER:** \_\_\_\_\_

**EMAIL:** \_\_\_\_\_

### RNA Quantity

1. There is at least **2  $\mu\text{L}$**  of each total RNA sample.
2. Each sample is at the appropriate concentration.   
(100-200 ng/ $\mu\text{L}$  for the Nano kit; 5-10 ng/ $\mu\text{L}$  for the Pico kit)

### RNA Purification

3. RNA samples have been purified.
4. RNA purification was performed by (Check all that apply):
  - A. Qiagen RNeasy
  - B. TriZOL
  - C. Other \_\_\_\_\_
5. Samples are dissolved in RNase-free water.

### RNA Quality Assesment

6. Quality and mass of each sample was estimated by UV ( $A_{280}/A_{260}$ )   
(A list of sample concentrations should accompany samples)

Initials confirming that all steps have been completed: \_\_\_\_\_

# RNA Sample Analysis using Agilent Bioanalyzer 2100 Work Order Form

**Cost per sample analysis is \$10.00 (Cdn)**

We require at least 2  $\mu\text{L}$  of total RNA **per sample** at a concentration of 100-200 ng/ $\mu\text{L}$  (Nano kit), or 5-10 ng/ $\mu\text{L}$  (Pico kit), in RNase-free water. Samples must be provided in a tube clearly labelled with a unique sample designation on top and the researchers name printed on the side, and must be kept frozen during transport.

***Clearly fill out the following***

**P.I. Name:** \_\_\_\_\_

**Billing Address:** \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Preferred method of contact (circle one): phone or e-mail**

**Phone Number:** \_\_\_\_\_

**E-mail:** \_\_\_\_\_

**Number of samples for RNA Nano kit:** \_\_\_\_\_

**Number of samples for RNA Pico kit:** \_\_\_\_\_

**Please provide list/spreadsheet of Sample Designations/concentrations**

\_\_\_\_\_  
Researcher's Signature

\_\_\_\_\_  
Date

# RNA RECEIPT

Date: \_\_\_\_\_

Samples delivered by: \_\_\_\_\_

Samples received by: \_\_\_\_\_

Number of samples received: \_\_\_\_\_

Were all samples frozen when received?

YES

NO

\_\_\_\_\_  
Recipients (UHNMAC) Signature

**Please note that any leftover RNA samples will be discarded following analysis on the Agilent Bioanalyzer.**

**University Health Network Microarray Centre**