
MAQC_Sample_Processing_Overview_SOP.doc
MAQC Sample Processing Overview SOP

1. **Purpose** - The purpose of this procedure is to describe the general steps that test sites will follow for processing RNA samples for MAQC Main Study.
2. **References, abbreviations & definitions**
 - 2.1 SUHRR: Stratagene Universal Human Reference RNA
 - 2.2 HBRR: Ambion First Choice Human Brain Reference RNA
 - 2.3 “MAQC_RNA_Quality_Report_Template.xls”: spreadsheet that specifies the naming convention for samples (tab 1), and provides a template (tab 2) for recording RNA quality data and target preparation information
 - 2.4 Agilent Bioanalyzer User’s Manual
 - 2.5 Agilent Bioanalyzer Reagent Kit Guides (RNA 6000 Nano)
 - 2.6 MAQC: Microarray Quality Control project, representing a consortium of over 30 organizations.

3. **Required equipment and reagents**

- 3.1 **Equipment**

ITEM
Agilent 2100 Bioanalyzer
UV spectrophotometer
-80°C Freezer

- 3.2 **Reagents**

ITEM	1° Source
RNA 6000 Nano LabChip kit	Agilent P/N 50065-4476
RNA 6000 Ladder	Ambion #7152

4. **RNA Receiving and Preparation**

- 4.1 Each site will be provided with one or more sets of 4 tubes of stock RNA, representing:
 - 4.1.1 A Stratagene Universal Human Reference RNA,
 - 4.1.2 B Ambion Brain Reference RNA,
 - 4.1.3 C 25% Brain / 75 % SUHRR
 - 4.1.4 D 75% Brain / 25 % SUHRR.
- 4.2 Carefully inspect the package and contents when it arrives.
- 4.3 If the dry ice has dissipated completely or there is damage to the samples, contact Mike Wilson (phone#: 1-512-651-0200 x6236 or by email: mwilson@ambion.com) for replacements. Do not use the damaged or non-frozen RNA samples.
- 4.4 Store the samples directly into a -80C freezer until ready for use.
- 4.5 Avoid extra freeze-thaw cycles that are not required for the processing of the samples for the MAQC study.
- 4.6 If a platform requires more than 1 set of samples, the appropriate samples (eg A+A+A) should be mixed together before quality assessment procedures are performed.

5. **Procedure**

- 5.1 A naming convention for samples has been established in order to ensure consistency and accuracy across all sites. The convention is described in greater detail in the attached spreadsheet, “MAQC_RNA_Quality_Report_Template.xls”. The naming convention is the first worksheet tab called “Naming convention”.
- 5.2 Assess the concentration of each of the four stock RNA samples.
 - 5.2.1 Use a UV spectrophotometer to measure the OD260 and OD280 values.
 - 5.2.2 Calculate the RNA concentration in micrograms per microliter
 - 5.2.2.1 Use extinction coefficient of 40 (An Optical Density reading of 1 at 260nm = 40ng/ul)

