
Ambion Services
MAQC_RNA_Preparation_SOP.doc
MAQC RNA Preparation and Testing SOP

1. **Purpose** - The purpose of this procedure is to describe the processes for preparing the RNA stocks for the 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 Nanodrop User's Manual
- 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
Heat Block or Water Bath set to 37°C
Pre-labeled 0.5 ml, screw cap, o-ring tubes (Axygen part #SCT-060-SS-L-C or equivalent)
Refrigerated Microcentrifuge set to 4°C
Agilent 2100 Bioanalyzer
NanoDrop spectrophotometer or equivalent
Ice bucket trays
Speed-vacuum
Tube racks for 0.5ml screw-caps
Pre-labeled freezer storage boxes
-20°C Freezer (non-frost free)
Ambion, Inc. product boxes

3.2 **Reagents**

ITEM	1 ^o Source
Crushed ice	laboratory
RNA Storage Solution	Ambion, Inc. Catalog # 7000
RNAse-free water	Ambion, Inc. Catalog # 9932
100% Ethanol	
RNA 6000 Nano Assay kit	Agilent # 5065-4476
RNA 6000 Ladder	Ambion, Inc. Catalog #7152
SUHRR	Stratagene, lot #1130623
SUHRR RNAse-free water	Stratagene, Catalog #740000-42
HBRR	Ambion, lot# 055201

4. **Stock solutions to be made**

- 4.1 70% Ethanol
 - 4.1.1 Add 28 ml 100% Ethanol and 12 ml RNAse-free water into a 50-ml conical screw-cap tube.
 - 4.1.2 Mix thoroughly.
 - 4.1.3 Store at -20°C

5. **RNA Preparation**

- 5.1 SUHRR needs to be precipitated, washed and dissolved in RNAse-free water provided with RNA.
 - 5.1.1 Centrifuge all SUHRR tubes in the microfuge at 4°C for 15-30 minutes at ≥12000Xg.

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- 5.1.2 Carefully remove ethanol from precipitated pellet with RNase-free technique and RNase-free tips.
- 5.1.3 Add 1 ml cold 70% ethanol to each tube.
- 5.1.4 Mix thoroughly by vortexing.
- 5.1.5 Centrifuge all SUHRR tubes in the microfuge at 4°C for 15 minutes at $\geq 12000Xg$.
- 5.1.6 Carefully remove ethanol from precipitated pellet with RNase-free technique and tips.
- 5.1.7 Dry remaining ethanol at room temperature open to air for 30 minutes.
- 5.1.8 Dissolve each of the pellets in 190 μ l RNase-free water provided with RNA. Mix thoroughly by vortexing each tube for a minimum of 30 seconds. .
- 5.1.9 Allow at least 30 minutes for the RNA to thoroughly dissolve.
- 5.1.10 Take Nanodrop reading of one tube. The concentration should be 1 ug/ul +/- 10% (0.9-1.1 ug/ul). If the concentration is too low, place the tubes at 37°C for 10 minutes, vortex thoroughly, and test the concentration of another sample.
- 5.1.11 Mix all dissolved SUHRR samples by combining them in a RNase-free, sterile 50ml conical tube with screw cap.
- 5.2 Thaw the stock of HBRR.
 - 5.2.1 Place the sample in a 37°C hot plate.
 - 5.2.2 Manually mix the sample throughout the thawing time to avoid the formation of “warm spots” at the interface with the tube.
 - 5.2.3 When ice is thawed completely, place the tube on the ice bath.
 - 5.2.4 Place tube on the ice for 15-30 minutes. After 5 minutes, vortex for at least 30 seconds to ensure homogenous mixing of the RNA.

6. Procedure

- 6.1 Ensure that all manipulations are performed with meticulous RNase-free pipetting and handling techniques and RNA is stored on ice throughout the procedure. Only adequately trained and qualified personnel will handle the RNA.
- 6.2 Assess RNA concentrations for each stock SUHRR and HBRR using the Nanodrop.
 - 6.2.1 Blank the samples with RNA Storage buffer.
 - 6.2.2 Take at least 3 independent readings.
- 6.3 Calculate the dilution needed to bring the concentration of the more concentrated RNA stock to within 5% of other RNA stock.
- 6.4 Add the volume of RNA storage buffer calculated in step 6.3 to the more concentrated RNA sample.
- 6.5 Repeat steps 6.2-4, if necessary until both RNA stock concentrations are within 5% of each other.
- 6.6 Record the following from the Nanodrop for each RNA stock:

- 6.6.1 OD 260
- 6.6.2 260/280
- 6.6.3 260/230
- 6.6.4 Trace of the wavelength scan

- 6.7 Create mixtures of the HBRR and SUHRR stock RNAs according to the table:

<i>Sample name</i>	<i>SUHRR volume (ml)</i>	<i>HBRR volume (ml)</i>
Mix C MAQC (25% HBRR / 75% SUHRR)	TBD	TBD
Mix D MAQC (75% HBRR / 25% SUHRR)	TBD	TBD

- 6.7.1 Mix the RNA samples thoroughly; store on ice.
- 6.7.2 Verify the expected final volume of each mixture. _____ **volume**
- 6.8 Distribute the RNA into 60 labeled tubes.
 - 6.8.1 Set up the appropriate number of tubes in racks on ice
 - 6.8.2 Separate the tubes for each of the four sample types to be dispensed separately.
 - 6.8.3 Dispense 50 μ l of the appropriate RNA into each of the labeled tubes
 - 6.8.4 Cap tubes securely.
 - 6.8.5 Place all tubes in the appropriate freezer box

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- 6.9** Quality Control assessment of the RNA after packaging
 - 6.9.1** Submit one set of 4 tubes to Ambion for the following testing:
 - 6.9.1.1** Overnight stability testing
 - 6.9.1.2** Nuclease testing
 - 6.9.2** RNA quality assessment on Agilent Bioanalyzer. Record 28S/18S ratio and RIN
 - 6.10** Package sets of four RNA samples in an Ambion product box for shipping
 - 6.10.1** Open freezer boxes of tubes are placed in dry ice for the transfer
 - 6.10.2** Packaged sets are stored in the specified -80°C freezer
 - Freezer _____
 - Rack _____
 - Slot _____
- 7.** Quality Control specifications
- 7.1** 28S/18S ribosomal ratio greater than or equal to 1.0
 - 7.2** Overnight stability: no more than 20% deterioration after overnight storage at 37°C
 - 7.3** Nuclease testing.