



Affymetrix Sample Preparation

Sample Requirements

Bioanalyzer

- 50-500 ng of RNA in 1 uL of RNase-free water
- Up to 12 samples can be analyzed at one time.

RNA Labeling

IMPORTANT: The quality of the RNA is essential to the overall success of the analysis.

- A minimum of 5 ug of total RNA at a concentration of >1.1 ug/uL (0.4 ug of mRNA at > 0.4 ug/uL) is required for cDNA synthesis.
NOTE: 10 ug or more of total RNA (1 ug of mRNA) is highly recommended. This allows us extra material for the use of quality control and any necessary repeats. If this is not possible, please consult with CIB personnel prior to sample submission.
- RNA must be pure (devoid of DNA, proteins, etc.) and undegraded. 280/260 ratios of 1.9-2.1 are indications of high quality RNA. Generally, ratios lower than 1.9 indicate potential contamination problems, and ratios higher than 2.1 indicate RNA degradation. Please refer to the Agilent 2100 Bioanalyzer for more information.
- Samples must be in RNase, DNase free 1.5 mL tubes. Please do not submit samples in plates or PCR tubes.
- Please consult with CIB if using prokaryotic RNA.

Hybridization

Please submit labeled samples or hybridization cocktails in RNase, DNase free 1.5 mL tubes.

Naming Samples

- Obviously, less characters is preferable
- Only alpha-numeric characters can be used (0-9, A-Z, a-z)
- The following characters are not allowed: ~ ! @ # \$ % ^ & * () + = { } \ / ; : ' " < > , . ? /

NOTE: Each sample will be given an identification code of two letters and a number. The letters will usually be the initials of the investigator, and the numbers will be sequential in the order provided on the sample submission form. For example, the first twelve samples submitted by John Doe will be given the codes JD01-JD12. The next twelve samples for the same project will continue JD13-JD24. The order will continue indefinitely for each project.

Each sample will be identified on reports and datasets by the code followed by the provided sample name.

Experimental Design

Because of the cost associate with processing RNA samples through Affymetrix GeneChips, good experimental planning is essential so as to prevent the use of unnecessary chips. We would be happy to provide consultation for planning your projects.

Recommendations for RNA Isolation

The following information has been taken from the Affymetrix GeneChip Expression Analysis Technical Manual. **Please refer to CIB sample requirements for further information.**

Protocols are available for preparing labeled cRNA from either total RNA or purified poly-A mRNA. It was found that results obtained from samples prepared by both of these methods are similar, **but not identical**. Therefore, to get the best results, it is suggested to only compare samples prepared using the same type of RNA material.

IMPORTANT: The quality of the RNA is essential to the overall success of the analysis. Since the most appropriate protocol for the isolation of RNA can be source dependent, we recommend using a protocol that has been established for the tissues or cells being used. In the absence of an established protocol, using one of the commercially available kits designed for RNA isolation is suggested.

When using a commercial kit, follow the manufacturer's instructions for RNA isolation.

Isolation of RNA from Yeast

Total RNA

Good-quality total RNA has been isolated successfully from yeast cells using a hot phenol protocol described by Schmitt, et al. Nucl Acids Res 18:3091-3092 (1990).

Poly-A mRNA

Affymetrix recommends first purifying total RNA from yeast cells before isolating poly-A mRNA from total RNA. Good-quality mRNA has been successfully isolated from total RNA using QIAGEN's oligotex mRNA Kit. A single round of poly-A mRNA selection provides mRNA of sufficient purity and yield to use as a template for cDNA synthesis. Two rounds of poly-A mRNA selection will result in significantly reduced yield and are not generally recommended.

Isolation of RNA from Arabidopsis

Total RNA

TRIzol Reagent from Invitrogen Life Technologies has been used to isolate total RNA from Arabidopsis. Follow the instructions provided by the supplier and, when necessary, use the steps outlined specifically for samples with high starch and/or high lipid content.

Poly-A mRNA

Arabidopsis poly-A mRNA has been successfully isolated using QIAGEN's Oligotex products. However, other standard isolation products are likely to be adequate.

Isolation of RNA from Mammalian Cells or Tissues

Total RNA

High-quality total RNA has been successfully isolated from mammalian cells (such as cultured cells and lymphocytes) using the RNeasy Mini Kit from QIAGEN. If mammalian tissue is used as the source of RNA, it is recommended to isolate total RNA with a commercial reagent, such as TRIzol.

IMPORTANT: If going directly from TRIzol-isolated total RNA to cDNA synthesis, it may be beneficial to perform a second cleanup on the total RNA before starting. After the ethanol precipitation step in the TRIzol extraction procedure, perform a cleanup using the QIAGEN RNeasy Mini Kit. Much better yields

of labeled cRNA are obtained from the in vitro transcription labeling reaction when this second cleanup is performed.

Poly-A mRNA

Good-quality mRNA has been successfully isolated from mammalian cells (such as cultured cells and lymphocytes) using QIAGEN's Oligotex Direct mRNA kit and from total RNA using the Oligotex mRNA kit. If mammalian tissue is used as the source of mRNA, total RNA should be first purified using a commercial reagent, such as TRIzol, and then using a poly-A mRNA isolation procedure or a commercial kit.

Precipitation of RNA

Total RNA

It is not necessary to precipitate total RNA following isolation or cleanup with the RNeasy Mini Kit. Adjust elution volumes from the RNeasy column to prepare for cDNA synthesis based upon expected RNA yields from your experiment. Ethanol precipitation is required following TRIzol isolation and hot phenol extraction methods; see Precipitation Procedures for details.

Poly-A mRNA

Most poly-A mRNA isolation procedures will result in dilution of RNA. It is necessary to concentrate mRNA prior to the cDNA synthesis. If going directly from TRIzol-isolated total RNA to cDNA synthesis, it may be beneficial to perform a second cleanup on the total RNA before starting. After the ethanol precipitation step in the TRIzol extraction procedure, perform a cleanup using the QIAGEN RNeasy Mini Kit. Much better yields of labeled cRNA are obtained from the in vitro transcription labeling reaction when this second cleanup is performed.

Precipitation Procedure

1. Add 1/10 volume 3M NaOAc, pH 5.2, and 2.5 volumes ethanol.*
2. Mix and incubate at -20°C for at least 1 hour.
3. Centrifuge at 1,000 x g in a microcentrifuge for 20 minutes at 4°C.
4. Wash pellet twice with 80% ethanol.
5. Air dry pellet. Check for dryness before proceeding. It is very important that all ethanol is evaporated before resuspending the pellet.
6. Resuspend pellet in DEPC-treated H₂O.

The appropriate volume for resuspension depends on the expected yield and the amount of RNA required for the cDNA synthesis.

***Addition of Carrier to Ethanol Precipitations**

Adding carrier material has been shown to improve the RNA yield of precipitation reactions.

Pellet Paint

Addition of 0.5 µL of Pellet Paint per tube to nucleic acid precipitations makes the nucleic acid pellet easier to visualize and helps reduce the chance of losing the pellet during washing steps. The pellet paint does not appear to affect the outcome of subsequent steps in this protocol; however, it can contribute to the absorbance at 260 nm when quantifying the mRNA.

Glycogen

Addition of 0.5 to 1 µL of glycogen (5 mg/mL) to nucleic acid precipitations aids in visualization of the pellet and may increase recovery. The glycogen does not appear to affect the outcome of subsequent steps in this protocol.

Prokaryotic RNA

As starting material for the cDNA synthesis procedure, total RNA can be isolated by using standard procedures for bacterial RNA isolation or various commercial RNA isolation kits.

For *Pseudomonas aeruginosa* and *E. coli*, we have successfully used the QIAGEN® RNeasy Mini Purification Kit. Caution should be used to minimize chromosomal DNA contamination during the isolation, due to the high sensitivity of the assay. It is suggested that no more than 1×10^9 cells are applied to a single purification column. Also, use the lysozyme at a concentration of 1 mg/mL, and not the recommended 400 µg/mL. Additional DNase I treatment may be required to eliminate DNA contamination when the bacterial culture is grown at high density.

We recommend checking the quality of RNA by running it on an agarose gel prior to starting the assay. The 23S and 16S rRNA bands should be clear without any obvious smears. Any indication of the presence of chromosomal DNA contamination (high molecular weight bands or smears on the gel) would require additional DNase treatment before proceeding to cDNA synthesis.

Total RNA Isolation for Two-Cycle

Target Labeling Assay

Several commercial kits and protocols are currently available for total RNA isolation from small samples (tissues, biopsies, LCM samples, etc.). Select the one that is suitable for processing of your samples and follow the vendor-recommended procedures closely since high-quality and high-integrity starting material is essential for the success of the assay.

Quantification of RNA

Quantify of RNA yield can be done by spectrophotometric analysis using the convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA. The absorbance should be checked at 260 and 280 nm for determination of sample concentration and purity. The A₂₆₀/A₂₈₀ ratio should be close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).

The CIB offers the Nanodrop 1000 for easy quantification of RNA/DNA with only 1-2 uL of sample that is recoverable if necessary.

Integrity of total RNA samples can also be assessed qualitatively on the Agilent 2100 Bioanalyzer. Refer to Interpretation of Agilent 2100 Bioanalyzer Data for examples of good-quality RNA.

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