

RNA PURIFICATION FOR MICROARRAY

It cannot be stressed enough that the quality of the starting 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 recommended that you use 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.

Isolation of Total RNA from Mammalian Cells or Tissues

Affymetrix suggest using the RNeasy Mini Kit from QIAGEN for isolation of high-quality total RNA from mammalian cells (such as cultured cells and lymphocytes). If mammalian tissue is used as the source of RNA, Affymetrix recommend that total RNA is extracted using a commercial reagent, such as TRIzol. In our experience it is beneficial to perform a second cleanup on the total RNA before submitting samples for processing. After the ethanol precipitation step in the TRIzol extraction procedure, perform a cleanup using the QIAGEN RNeasy Mini Kit. Much better yields of labelled cRNA are obtained from the *in vitro* transcription labelling reaction when this second cleanup is performed.

NB. If you are working on microRNA please ensure that you use an appropriate isolation kit for small RNA molecules.

Isolation of Total RNA from Yeast

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). It may be beneficial to perform a second cleanup on the total RNA before submitting samples for processing. After the ethanol precipitation, perform a cleanup using the QIAGEN RNeasy Mini Kit, as much better yields of labelled cRNA are obtained from the *in vitro* transcription labelling reaction when this second cleanup is performed.

Isolation of RNA from Arabidopsis

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.

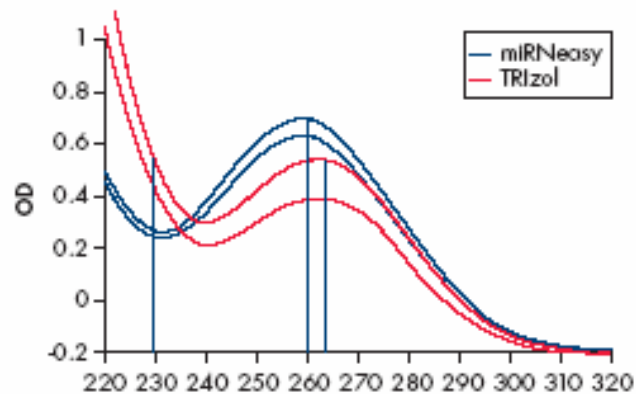
Storage of RNA

For long term storage at -80°C , store RNA in absolute ethanol. For medium term i.e. around 6-8 months, TE at pH 7 is fine. As the presence of salts can interfere with subsequent reactions it is important to re-precipitate the RNA and re-suspend it in RNase-free water.

CHECKING THE QUANTITY AND PURITY OF TOTAL RNA

At the Centre we use the NanoDrop spectrophotometer to assess the quantity and purity of RNA.

The NanoDrop spectrophotometer gives a spectral profile of your sample from 220 to 320nm. It calculates quantity of RNA and gives A260:A280 and A260:A230 ratios. Pure RNA should have a A260:A280 ratio between 1.9–2.1 and a A260:A230 ratio of 1.8–2.3. Low A260:A280 ratios indicate contamination with protein and low A260:A230 ratios indicate contamination with reagents used in the extraction such as phenol or salt. The figure below is an example of a NanoDrop absorbance spectra. The sample in blue has a maximum absorbance at 260nm indicating pure RNA. The sample in red has a maximum absorbance >260nm indicating contamination with phenol. Ignore contamination at your peril as it will interfere with labelling.



(Figure taken from QIAGEN website.)

CHECKING THE QUALITY OF TOTAL RNA

We assess the integrity of total RNA on the Agilent 2100 Bioanalyzer. An example of a bioanalyzer electropherogram of good quality RNA is shown below. The Bioanalyzer software generates an RNA Integrity Number (RIN) a quantitation estimate, and will calculate the ribosomal ratios of the total RNA sample. The maximum RIN for RNA is 10. The RIN cut-off is tissue dependent therefore the researcher is advised to do a preliminary experiment to decide the cut-off for their sample. For example, for mouse heart a RIN of 8 yields good microarray results. In comparison, for human blood a RIN of 7 is good, anything below this would compromise data analyses. You can consult Agilent's online RNA Integrity Database (RINbd) to compare the RIN for your sample to other records in the database.
<http://www.chem.agilent.com/rin/rinSearch.aspx>

Agarose gels may be used to check the quality of RNA but they are not as informative as a Bioanalyzer trace. It is highly recommended that you run your RNA on the Bioanalyzer.

