Brief Technical Note

UHN Microarray Centre

Total RNA Isolation Methods

- Methods of total RNA isolation were evaluated
- All methods of total RNA isolation yielded high quality total RNA that was suitable for microarray analyses

Introduction

The goal of this study was to evaluate total RNA isolation methods and determine which of the methods provided high quality total RNA for microarray analyses. Each method was evaluated for its RNA yield, RNA integrity (determined using the Agilent 2100 Bioanalyzer), ease of use, time required, and cost.

Method

Each of the following kits (listed in alphabetical order) was used, following the manufacturer's guidelines, on three separate occasions to isolate total RNA from cultured HeLa cells:

- Absolutely RNA™ RT-PCR MiniPrep kit (Stratagene)
- GenElute™ (Sigma)
- Nucleospin® (Clontech)
- Purescript® (Gentra)
- RNeasy® (Qiagen)
- S.N.A.P.™ Total RNA Isolation kit (Invitrogen)
- SV Total RNA Isolation kit (Promega)
- TRIzol® Reagent (Invitrogen)
- VERSAGENE™ RNA Purification kit (Gentra Systems Inc.)

The RNA yield was determined using a spectrophotometer (Molecular Devices) and the integrity of the RNA was determined using the Agilent 2100 Bioanalzyer (Nano RNA kit). In addition, 10 µg of total RNA was labelled following the standard labelling protocol and hybridised to a cDNA array.

Results & Discussion

All methods yielded high quality total RNA that was suitable for microarray analyses (data not shown).

Methods involving spin columns tended to be faster and simpler to use than other methods. The cost of the kits themselves (per microgram of RNA isolated) were comparable, however, quick protocols provide labour-cost savings.

With all aspects of RNA isolation considered (cost of reagents, time involved, ease of use, etc.), the UHNMAC uses the VERSAGENE™ RNA Purification kit for total RNA isolation from cultured cells. Previously, the RNeasy® (Qiagen) kit was the standard isolation method, however, the new VERSAGENE™ system uses columns that have a significantly higher binding capacity (600 µg compared with 100 µg binding capacity for the RNeasy® column), allowing for the isolation of RNA from more cells in one column.

In addition to using RNeasy® (Qiagen) columns for RNA isolation from cultured cells, the UHNMAC also uses these columns for RNA clean-up. Occasionally

we are required to work with RNA samples that likely contain unknown contaminants that interfere with labelling protocols. In an attempt to remove the contaminants, we perform the RNA isolation protocol (this time in order to clean up the RNA) on these samples. In our experience, 50-70% of the total RNA sample is recovered.

Although this study was not extended to isolating total RNA from tissue samples, the UHNMAC continues to use TRIzol® Reagent (Invitrogen) for such purposes. RNAlater®(Ambion), an aqueous, non-toxic tissue storage reagent that rapidly permeates tissues to stabilise and protect cellular RNA, is also used to store tissues until RNA isolation commences.

Conclusions

All methods of RNA isolation that were evaluated provided total RNA suitable for microarray experiments. In our hands, none of the methods evaluated yielded superior quality total RNA. The UHNMAC currently uses the VERSAGENE™ RNA Purification kit (Gentra), and occasionally the RNeasy® RNA Isolation kit (Qiagen), for total RNA isolation from cultured cells and TRIzol® Reagent (Invitrogen) for total RNA isolation from tissue samples.