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Total RNA and array-based expression monitoring

To the editor:

Mahadevappa and Warrington[1] developed a protocol that significantly improves sample preparation for gene expression using high-density DNA arrays. The method for expression analysis with high-density arrays includes RNA extraction, double-stranded cDNA synthesis, and production of cRNA by in vitro transcription before the sample is ready for hybridization. Until now, the method has required isolation of mRNA as template for the cDNA synthesis. Because of sample loss during the poly(A) RNA extraction, it has been necessary to use large amounts of cells or tissue as starting material. A major advantage of the new protocol is the possibility of using total RNA, instead of mRNA, as the template for cDNA synthesis[1]. By eliminating the poly(A) purification step, sample loss is markedly reduced, making the technique useful for analysis of tissues that are impossible to obtain in large quantities.

For preparation of total RNA, Mahadevappa and Warrington used affinity resin extraction (RNeasy; Qiagen, Chatsworth, CA). In our hands, this method consistently results in lower yield compared with other methods for preparation of total RNA. Furthermore, when total RNA, extracted according to Chomczynski and Sacchi[2], was further purified using RNeasy, we routinely noted a loss of approximately 30% of the RNA. Gel electrophoresis indicated that the loss of RNA mainly was due to loss of small RNAs. We tested the capability of the RNeasy system to process tRNA, which accounts for a large proportion of small RNAs in the cell, and found that more than 80% of the tRNA was lost. This suggests that the RNeasy system relatively selectively extracts mRNA and ribosomal RNA, while most of the small RNAs, including tRNA, which accounts for approximately 20% of all RNA, are lost.

It is well known that the relative amount of mRNA varies greatly depending on the method used for poly(A) extraction. However, previous methods used for extraction of total RNA have had little influence on the relative abundance of specific RNA molecules and thus allowed direct comparison between methods. The work by Mahadevappa and Warrington clearly facilitates gene expression monitoring from a small number of cells. However, it should be clarified that the total RNA used was not comparable to total RNA extracted by other methods.

The altered proportion of different RNAs could be important for several reasons. The specific loss of small RNAs when using the RNeasy method implies that the relative amount of mRNA in the total RNA preparation is increased. Therefore, the amount of total RNA needed for expression analysis may have to be increased if methods other than RNeasy are used. In addition, other parts of the protocol that were optimized by Mahadevappa and Warrington—for example, the ratio of reverse transcriptase to total RNA or the temperature for the first-strand cDNA synthesis—may need to be adjusted depending on the method used for total RNA preparation. It is also possible that the higher amount of tRNA in total RNA prepared by methods other than RNeasy could affect the efficiency of the cDNA synthesis.

In summary, the protocol recently developed by Mahadevappa and Warrington is a significant improvement, and it will greatly facilitate the use of DNA-arrays for expression analysis in small samples. However, before the protocol can be used for total RNA extracted by conventional methods, it may need further validation.

1. Mahadevappa, M. & Warrington, J.A. *Nat. Biotechnol.* **17**, 1134–1136 (1999).
2. Chomczynski, P. & Sacchi, N. *Anal. Biochem.* **162**, 156–159 (1987).

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Janet A. Warrington and Mamatha Mahadevappa respond:

Carlsson, Jernås, Lindell, and Carlsson make good points. In every laboratory, people need to confirm a protocol's performance in their own hands. This is especially important when using a protocol with methods different from those used by other groups. Although we cannot comment on the Chomczynski and Sacchi protocol, we found the Trizol (Life Technologies, Rockville, MD) extraction of total RNA (a relatively routine and standard method) followed by RNeasy treatment to be more than sufficient. We do not experience a substantial loss of total yield by this method, usually less than 10%. In a comparison of total RNA yields obtained from tissue versus cultured cells, we have noted that total RNA extracted directly from tissues using RNeasy with no Trizol step may yield smaller amounts of total RNA than cultured cells treated with RNeasy alone. We can only speculate that the substantial loss of material may be due to incomplete homogenization of the tissue resulting in incomplete absorption on the RNeasy column. This finding led us to routinely use Trizol on tissues.

Finally, Carlsson and colleagues raise a concern regarding size selection or bias in the method. This was a concern of ours also, and at the time of our study, the data were analyzed for loss of information due to size selection, level of abundance, and so on. In our comparison, a very small number (less than 1%) of transcripts reproducibly detected in the total RNA samples were undetected in the poly(A) samples. The minor bias detected resulted from the level of abundance of the transcript, not the transcript length.

Integrated pararetroviral sequences

To the editor:

In the continuing debate over the safety of transgenic plants containing the cauliflower mosaic (pararetro)virus (CaMV) 35S promoter, Cummins, Ho, and Ryan write that pararetroviruses, unlike retroviruses, do not require integration into host chromosomes to complete their life cycle[1]. Although this statement is formally correct, it does not take into account recent data revealing the presence of integrated pararetroviral DNA in plant chromosomes[2–4].

Banana streak (pararetro) viral (BSV) sequences, which could recombine to create infectious virus, have been detected at several sites in the banana genome[2,3]. In another example, approximately 1,000 copies of a previously unidentified tobacco pararetrovirus (TPV) were found integrated into tobacco nuclear DNA[4]. These TPV copies contained frameshifts and other mutations, and therefore, unlike the BSV case, would be unable to produce infectious virus. Nevertheless, the putative TPV promoter has been shown to be active in transgenic constructs reintroduced into plants (W. Aufsatz, M. Matzke, and A. Matzke, unpublished data). TPV-related sequences were also detected in high-molecular-weight DNA of other solanaceous plants, such as tomato, by Southern blot analysis[4].

It is therefore likely that numerous additional examples of integrated pararetroviral sequences will be found in plant chromosomes. Although these results do not reflect on the safety of the CaMV 35S promoter per se, they do demonstrate that viral sequences, including potentially active viral promoters, are not exotic components of edible-plant genomes.

1. Cummins, J., Ho, M.-W. & Ryan, A. *Nat. Biotechnol.* **18**, 363 (2000).
2. Harper, G. et al. *Virology* **255**, 207–213 (1999).
3. Ndowara, T. et al. *Virology* **255**, 214–220 (1999).
4. Jakowitsch, J. et al. *Proc. Natl. Acad. Sci. USA* **96**, 13241–13246 (1999).

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