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Modified acid guanidinium thiocyanate – phenol – chloroform RNA extraction method which greatly reduces DNA contamination

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The most common and consistently successful methods for isolating intact RNA are modifications of the Guanidinium Thiocyanate method of Chirgwin *et al.* (1). One widely used modification of this method, developed by Chomczynski and Sacchi (2), involves extraction of the guanidinium thiocyanate homogenate with phenol–chloroform at reduced pH. The Acid Guanidinium Thiocyanate–Phenol–Chloroform (AGPC) method is very rapid and does not require ultra centrifugation making it ideal for isolating RNA from multiple samples.

During our study of gene expression in the mouse macrophage cell line, RAW 264.7, by reverse transcriptase–polymerase chain reaction (RT–PCR) we observed that the AGPC method would often yield considerable amounts of genomic DNA contamination. RT–PCR experiments can often tolerate small amounts of DNA contamination by using PCR primers designed to span one or more introns such that efficient PCR amplification is only achieved from cDNA or so that the PCR products derived from genomic DNA and cDNA can be distinguished on an agarose gel. However this is not possible for genes that lack introns or genes or which the genomic structure is unknown. Some investigators have found it necessary to treat the RNA with DNase-free RNase (3).

We have made a simple modification of the AGPC method which greatly reduces DNA contamination. The modification involves the addition of a brief RNA selective precipitation step following lysis of the cells with the guanidinium thiocyanate solution. This is achieved by the addition of 1/3 volume of 95% ethanol and incubation of the mixture on ice for 5 minutes. Following centrifugation for 15 minutes the supernatant is removed and the RNA pellet dissolved in 1/2 of the original volume of guanidinium thiocyanate solution. We also reduce the time of the isopropanol precipitation from 1 hour to 30 minutes.

Figure 1 shows a comparison of RNAs isolated from equal numbers of RAW 264.7 cells on a non-denaturing agarose gel. RNAs obtained with the original and modified AGPC methods are shown in lanes 2 and 3, respectively. As seen in Lane 2, an abundant band of genomic DNA which co-migrates with the largest of the λ–*Hin*dIII size markers is apparent when the original AGPC method was used. By contrast, very little genomic DNA is visible when the modified AGPC method was used (Lane 3). The yield of 28s and 18s rRNA was identical for the two

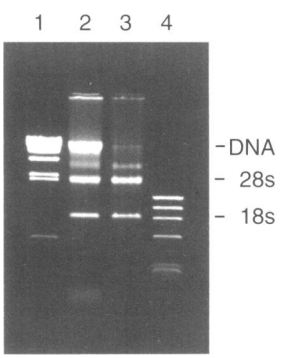

Figure 1. Non-denaturing gel electrophoresis of RNA isolated with the original and modified AGPC method. Lane 2: RNA was isolated from approximately 0.5×10^6 mouse macrophage RAW 274.1 cells using the original method. Lane 3: RNA isolated as in Lane 2, except that, after cell lysis, RNA was differentially precipitated by adding a one-third volume of 95% ethanol and incubating the mixture on ice for 5 min. Following a (15 min) centrifugation, the supernatant was removed and the RNA pellet dissolved in one-half of the original cell lysate volume. Equal cell equivalents of RNA were isolated on the gel Lanes 1 and 4: λ-*Hin*dIII and φX-174 *Hae*III DNA size markers, respectively. Samples were run on a non-denaturing 1.0% agarose/EtBr gel. On this neutral agarose gel, the 28s and 18s rRNA bands comigrated with the 2.0-kb and 870-bp DNA size markers, respectively.

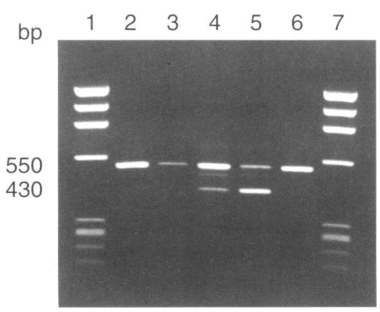

Figure 2. RT–PCR analysis of RNA isolated with the original and modified AGPC method. RNA was isolated from mouse macrophage RAW 274.1 cells, as described in Figure 1, using either the original method (Lanes 2 and 4), or the modified method (Lanes 3 and 5). Lanes 4 and 5: cDNA was synthesized from 1 μg of RNA utilizing MMLV reverse transcriptase and an oligo (dT) primer. Lanes 2 and 3: reverse transcriptase was omitted from the cDNA synthesis reaction. Lane 6: RT–PCR products obtained from 12.5 ng of mouse genomic DNA. For all amplifications, the PCR primers used were specific for mouse *c-fos*. 30 cycles of amplification were performed. PCR products were run on a 1.8% agarose/EtBr gel.

methods. We were unable to clearly detect the presence of genomic DNA contamination of the two preparations when the RNA was examined in denaturing formaldehyde agarose gels (data not shown).

Figure 2 shows an RT−PCR analysis of RNA isolated with the original and modified AGPC methods. cDNA was synthesized from 1 μg of RNA utilizing M-MLV reverse transcriptase and an oligo (dT) primer. In some reactions the reverse transcriptase was omitted so that no cDNA was made, and only genomic DNA would be amplified. PCR was then performed using primers specific for the mouse c-fos gene. We designed the primers such that they spanned one intron, thus making the product generated with cDNA 432 bp and the products generated with genomic DNA 570 bp. Lanes 2 and 3 contain PCR products from cDNA synthesis reactions using RNA prepared with the original and modified AGPC method respectively, but lacking reverse transcriptase. RNA obtained from the original AGPC method (Lane 2) generated a very strong band of 570 bp which co-migrated with PCR products generated from pure genomic DNA (lane 6). In contrast, very little of this genomic DNA product was generated using the modified AGPC method (Lane 3). Lanes 4 and 5 are the same as Lanes 2 and 3 except that the cDNA reactions contained reverse transcriptase. The modified AGPC method generated a single strong cDNA amplification product of 432 bp and very weak genomic band (lane 5). In contrast, a weak band of 432 bp along with a very strong genomic DNA product was generated with the original AGPC method (lane 4). The reduction in the amount of 432 bp product in Lane 4 is probably due to competition between cDNA and genomic DNA templates in the PCR reaction.

The amount of contaminating genomic DNA in the modified AGPC RNA method is greatly reduced. The addition of the brief ethanol precipitation step does not lengthen the time required for the procedure because the time needed for the isopropanol precipitation step is reduced. If desired, further removal of genomic DNA contamination can be accomplished by performing an additional ethanol precipitation step (data not shown). The modified method, which circumvents the need for DNase digestion, will be very useful for RT−PCR studies of genes lacking introns or genes for which the genomic structure is unknown.

REFERENCES

1. Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry* **18**, 5294−5299.
2. Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.* **162**, 156−159.
3. Grillo,M. and Margolis,F.L. (1990) *Biotechniques* **9**, 262−268.