CC/NUMBER 36
SEPTEMBER 6, 1993

This Week's Citation Classic®

Chomczynski P & Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156-9, 1987. [Lab. Biochem. and Metab.. Natl. Inst. Diabetes and Digestive and Kidney Diseases, Natl. Insts. Health. Bethesda. MD; and Lab. Mol. Oncol.. Natl. Cancer Inst., Natl. Insts. Health, Frederick, MD]

The paper describes a new method of total RNA isolation by a single-step extraction with an acid guanidine thiocyanate-phenol-chloroform mixture. The method provides, in a short time and without ultracentrifugation, a pure preparation of unc graded RNA from cell and tissue samples. [The *SCI*® indicates that this paper has been cited in more than 7,330 publications.]

Necessity Breeds Invention: Single-Step RNA Isolation

Piotr Chomczynski
Department of Internal Medicine
Division of Endocrinology/Metabolism
University of Cincinnati Medical Center
Cincinnati, OH 45267-0547

The idea of developing a simple method for the extraction of total RNA evolved in 1982 in Poland. I had just returned from a visit at the National Institutes of Health (NIH) where I had been studying milk protein gene expression in Pradman Qasba's laboratory. There, we had isolated total RNA using a guanidine thiocyanate-CsCI ultracentrifugation method as elaborated by J.M. Chirgwin et al.[1] Back in my lab at the Department of Animal Biochemistry, Warsaw Agricultural University, I soon recognized that to continue my gene expression studies I needed a method of RNA isolation which did not require the use of an ultracentrifuge. At that time, the government in Poland was too distracted by other matters to support research activity and buy ultracentrifuges. To develop an ultracentrifuge-free method, I tried several approaches before combining the traditional phenol extraction with the use of guanidine thiocyanate. To my satisfaction, the very first run of the phenol-guanidine thiocyanate method produced a preparation of usable RNA, especially when the guanidine solution was acidified to pH 4 with a sodium acetate buffer.

In the following months, however, I found an even better way to continue my gene expression studies. I returned to NIH and forgot temporarily about the phenol-guanidine method. At NIH, I was involved in a study of casein gene expression in the laboratory of Yale Topper, a pioneer in research on the mammary gland. Our studies of the muitihormonal regulation of the casein gene were goinr; well. To process the large numbers of samples needed for these studies I had been using Chirgwin's ultracentrifugation method and occupying simultaneously up to three uitracentrifuges. After a while and some rather lively discussions with coworkers, I realized that even at NIH there were limits on the use of equipment. To save both my time and social relationships I resurrected the acid phenol-guanidine thiocyanate method. As in the first attempts, the method appeared to be simple and very reproducible. Later, I asked a friend of mine, Nicoletta Sacchi, to evaluate the method in clinically oriented applications (detection of oncogene transcripts, etc.). She was very positive about the procedure, and we decided to call it a single-step method of total RNA isolation and to publish it in *Analytical Biochemistry*.

Since its publication in 1987, the single-step method has become a method of choice for the isolation of total RNA from a variety of biological specimens. The method has attained its popularity due to a reliable and simple protocol which is especially useful for isolating RNA from large numbers of samples. Also, it allows for isolation of RNA from both small and large biological specimens. Gaining recognition, the single-step method has been included in popular laboratory manuals such as *Current Protocols.*[2] A commercial modification of the method combining phenol and guanidine thiocyanate in a shelf-stable mono-phase solution has been patented.[3] Most recently, I have further improved the single-step method to allow for the simultaneous isolation of RNA, DNA, and proteins from the same biological sample.[4]

1. **Chirgwin J M, Przybyla A E, MacDonald R J & Rutter W J.** Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry— USA* 18:5294-9. 1979. (Cited 13.905 times.)
2. **Chomczynski P & Sacchi** N. Single-step RNA isolation from cultured cells or tissues. (Ausubel F M. Brent R. Kingston R E. Moore D D. Seidman J G. Smith J A & Struhl K. eds.) *Current protocols in molecular biology.* New York: Greene and Wiley-Interscicnce, 1990. p. 4.2.4-4.2.8.
3. Chomczynski P. Product and process for isolating RNA. US patent 4.843.155. 27 June 1989
4. ----------. A reagent for the single-step simultaneous isolation of RNA. DNA and proteins from cell and tissue samples. *BiuTechniques* (In press.)

Received June 27. 1993