CC/NUMBER 20
MAY 17, 1993

This Week's Citation Classic

Gubler U & Hoffman B J. A simple and very efficient method for generating cDNA libraries. *Gene* **25**:263-9. 1983. [Department of Molecular Genetics. Roche Research Center. Hoffmann-La Roche Inc.. Nutley. NJ]

A simple and efficient method for the generation of cDNA libraries was described that eliminated the use of S1 nuclease. Very large and therefore representative libraries containing a higher proportion of full length cDNAs could be made from small amounts of mRNA, simplifying the cloning of low abundance cDNAs. [The *SCI*® indicates that this paper has been cited in more than 3,185 publications.]

A Simple and Very Efficient Method for Generating cDNA Libraries

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After finishing graduate school In Switzerland in December 1979, I moved to the US to become a postdoctoral fellow at the Roche Institute of Molecular Biology under the direction of Pat Gage. The next year, my project required the generation of a cDNA library. With a lot of help from Mike Tocci and John Monahan, who worked a few doors down the hall, I more or less successfully overcame all the obstacles associated with this task. However, the necessity to use S1 nuclease as part of the procedure created considerable headaches. The work did result in the publication of a partial cDNA sequence for bovine proenkephalin mRNA.[1]

Subsequently, I moved to a staff position in the Laboratory of Recombinant DNA Research at Hoffmann-La Roche and Beth Hoffman joined the lab as an assistant scientist. We were now faced with the gruesome prospect of generating many more cDNA libraries. Around that time, H. Okayama and P. Berg published their very elegant method for the generation of cDNA libraries that did not require the use of S1 nuclease.[2] It seemed worthwhile, therefore, to try this new approach. But making it work proved to be very demanding. Rumor had it that the ratio of the enzymes used in the second strand synthesis reaction was crucial to the success of the method. Okayama's paper had described two of the three enzymes as obtained from people at Stanford University, and it was obvious that they were much purer than the commercially available enzymes that we had to use. Thus, I prepared unlabeled globin mRNA-cDNA hybrid, using commercially available globin mRNA, and generated labeled second strand by incorporation of labeled nucleotide for the analysis of yields and sizes, using a variety of conditions. It soon became clear that the method was indeed able to generate high levels of correctly sized second strand globin cDNA. Not being formally trained in DNA enzymology, we spent quite some time trying to figure out why the method actually did work. And suddenly, it occurred to me that maybe this was a good way per se to prepare cDNA and circumvent the S1 step all together. Again using globin mRNA as the test, we determined that the method did produce cDNA that could be tailed and cloned into a plasmid. As a next test, Beth generated a cDNA library from bovine adrenal medullar mRNA and recloned a full-length copy of the proenkephalin cDNA.

My first inclination was to not even publish this method, since I saw no sense "in publishing yet another cDNA synthesis method." Somehow, that frame of mind changed—I guess that the lure of a paper was too great. And in retrospect, I am of course very happy about it. Ann Skalka helped a lot with preparing the final manuscript and submitting it to *Gene.* The first two cDNAs that were cloned at Roche using this method before it was published were interleukin-2 and growth hormone releasing factor.[3] Next, we tackled the cloning of the porcine cholecystokininDNAs from brain and gut tissues.[4] While generating these libraries, it became evident that the method was able to produce the large number of clones required for the isolation of such low abundance cDNAs. Anne Chua, who has been in my lab since 1982, subsequently was instrumental in the continued improvement of the technique; our latest modifications were published in 1991.5

Most of the commercially available libraries today are made using the technique, and it also has been incorporated into a number of commercial cDNA synthesis kits. This is probably the best testimonial to the method's ease and usefulness, so I have no problem admitting that the real reason for developing the procedure was my own inability to get the Okayama and Berg method to work successfully.

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2. **Okayama H & Berg P.** High-efficiency cloning of full-length cDNA. M*oL Cell Biol.* 2:161-70. 1982. (Cited 1.050 times.)
3. **Gubler U, Monahan J J, Lomedico P T, Bhatt R S, Collier K J, Hoffman B J, Boehlen** P, Esch F. **Ling** N, **Zeytin** F. **Brazeau P, Poonian M** S *&* **Gage L P.** Cloning and sequence analysis of cDN A for the precursor of human growth hormone releasing factor, somatocrinin. *Proc. Nat. Acad. Sci. USA* 80:4311-4. 1983. (Cited 130 times.)
4. **Gubler U, Chua A O. Hoffman B J. Collier K J & Eng J.** Cloned cDN A to cholecystokinin mRNA predicts an identical prcprocholecystokinin in pig brain and gut. *Proc. Nat. Acad. Sci. USA* 81:4307-10, 1984.
5. **Gubler** U & **Chua A** O. The establishment of cDNA libraries in lambda gt 10. (Brown T A, ed.) *Essential molecular biology: a practical approach.* Oxford, England: Oxford University Press, 1991. Vol. 2. p. 39-56.

Received April 3. 1993