A look back: providing means to an end

By the mid-1980s, the cloning era was entering full bloom. cDNA synthesis techniques were finding widespread use, and many researchers were taking advantage of the newly obtained ability to generate immense cDNA libraries for the identification of novel genes. "The field, the way it was in the eighties, was a lot of fun," recalls Michael Frohman, at the State University of New York at Stony Brook. "You would clone a gene and figure out the sequence and do some *in situ* hybridization and see what the gene expression pattern was, and then the next part was really hard—you had to do something to figure out what the function was—so most people just gave up at that point and said, 'well, there are lots more genes to clone!'"

Of course, cloning wasn't always just fun and games—conventional cDNA cloning techniques often didn't make it all the way to the 5′ end of a given transcript, and many scientists found themselves frustrated in their efforts to obtain full-length cDNA sequences from long or scarce transcripts. Frohman encountered this as a postdoc in the lab of Gail Martin, at the University of California at San Francisco, where he was struggling as a molecular biology neophyte to clone the full-length engrailed gene. "It was a typical story for the time," recalls Frohman. "Everybody was making cDNA libraries and screening for low-abundance genes; they were difficult to clone, you would maybe get a couple of hits after plating out twenty huge plates of phage, and frequently it wasn't full-length, and you'd have to start over. It was a frustrating thing, and people would spend six months to a year, to two years, unable to do anything else because they couldn't get a full-length gene product."

Then, in 1985, the molecular biology world underwent a seismic change, when scientific renegade Kary Mullis and his colleagues at the Cetus Corporation introduced the research community to a new and surprisingly simple—but soon indispensable—technique: the polymerase chain reaction (PCR)[1,2]. Within a few years, PCR was being used in a wide variety of applications, and Frohman would draw on it as well, using it as the foundation for a new strategy for characterizing full-length gene sequences. The rapid amplification of cDNA ends (RACE) requires only a little advance sequence knowledge, for the development of an internal primer for the first stage of reverse transcription from an mRNA of interest. This first strand is then enzymatically tailed with poly(deoxyadenosine), providing the priming site for second-strand synthesis. Subsequent cycles of amplification with these two primers then generate adequate copies of even low-abundance transcripts, allowing the analysis of the furthest 5′ sequences in the transcript[3].

As is often the case with good ideas, other members of the research community were simultaneously and independently developing related techniques, and at least two other articles would be published over the next six months describing amplification and cloning techniques similar to RACE[4,5]. Nonetheless, RACE would become the most widely used and accepted of these. In the early days after his paper's publication, Cetus, the PCR patent holder and manufacturer of *Taq* polymerase, persuaded Frohman to travel throughout the west coast to lead seminars on RACE, paying him with free polymerase. "Today, I'd say, 'Hey, where's the cash?'" he jokes. "But at the time, enzyme was like gold!" At first, such training was often essential, as the learning curve could be steep for scientists new to PCR; but as more efficient enzyme variants became available and new and enhanced versions of RACE emerged, the technique became more or less standard practice.

In some ways, the golden age of frenzied cloning has passed, giving way to the rush to structurally dissect and assign appropriate functions to the many thousands of cloned but uncharacterized genes, and one might assume that there is less demand for a technique like RACE. This is not the case, however. "I was kind of tickled the other day to note that it's still being used," says Frohman. "You know, once the genome was published, I thought OK, that's it—all the 5′ ends are known now. But we just had a symposium on RNAi… and three of the eight speakers all described using RACE to clone their microRNAs! [So] it's still out there—people are still using it." RACE is also a popular tool for characterizing genes from species with incompletely sequenced genomes or for analyzing transcripts from genes with multiple alternative start sites or splice variants—and with nearly 500 articles citing Frohman's work in the last four years, it clearly remains an important technique for modern molecular biology.

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1. Saiki, R.K. *et al. Science* **230**, 1350–1354 (1985).
2. Mullis, K. *et al. Cold Spring Harb. Symp. Quant. Biol.* **51**, 263–273 (1986).
3. Frohman, M.A. *et al. Proc. Natl. Acad. Sci. USA* **85**, 8998–9002 (1988).
4. Loh, E.Y. *et al. Science* **243**, 217–220 (1989).
5. Ohara, O. *et al. Proc. Natl. Acad. Sci. USA* **86**, 5673–5677 (1989).