# A look back: a change in direction

Many young scientists know the pain of unsuccessfully lobbying an advisor to back an 'impractical' experimental approach, and David Schwartz was no exception. While working on his senior research project at Harvard University in the late 1970s, he had conceived of a method for separating DNA molecules of unprecedented size by electrophoresis, using pulses of a second, shearing electric field to 'unfurl' DNA strands clumped up in the gel matrix, after which reintroduction of the first field could be used to continue size separation. Sadly, his advisor was having none of it. "He told me it was pure trash," Schwartz recalls. "He said, 'Do you know more than Bruno Zimm?'", referring to the chemist whose early work had inspired Schwartz's concept.

In fact, Schwartz continued his studies with Zimm, who proved hardly more receptive: "He looked at me, and he said, 'David, I don't see it.'" With a solid idea in his head—but few resources to make it into a reality—Schwartz jury-rigged a crude prototype device, and saw evidence that his concept might be sound. Personal circumstances brought Schwartz back to the east coast, where he essentially began his graduate studies from scratch with Charles Cantor at Columbia University. Cantor was supportive, giving Schwartz the resources to build and test his first 'pulsed-field gel electrophoresis' (PFGE) apparatus, which alternated its primary current with pulses of a transverse current—and the two were pleased to find that it worked remarkably well, generating diagonal band patterns that nevertheless revealed DNA separation in the megabase range[1].

Their work generated a storm of interest, especially their second paper, which introduced a key step—the lysis of cell samples in agarose plugs, which greatly preserves the integrity of the largest DNA molecules[2]. This fame also brought visitors, who were often surprised when they saw the actual device, which Schwartz had built from a plexiglas pencil holder. "People would come from Europe or wherever, and they're expecting something that looks very sophisticated," recalls Schwartz, "and I'd show them this pencil box with all these electrodes sticking out of it, and they'd go, 'What the hell is that?'"

The method resonated with Washington University investigator Maynard Olson, engaged as he was in efforts to build an accurate physical map of the yeast genome. Existing genetic linkage–based maps were of limited use, he recalls, adding, "We were really desperate for some sort of overview of the genome." He and graduate student Georges Carle developed a PFGE variant, manipulating the angles of the fields to achieve somewhat superior band separation[3]; by incorporating Schwartz's in-gel lysis method, they further improved their technique so that they finally achieved their goal of defining a complete yeast karyotype via 'orthogonal field-alternation gel electrophoresis'[4]. "We could suddenly do almost anything we wanted to do," he says.

As powerful as the technique proved, little was understood about the underlying mechanisms, and both groups went on to more closely explore PFGE. Schwartz built on the work of Mitsuhiro Yanagida to develop methods for visualizing the behavior of individual DNA molecules subjected to PFGE[5], shedding new light on why it works; these findings were in turn extended by similar work from Carlos Bustamante's group[6]. Olson, on the other hand, continued to examine various electrode configurations and was surprised to find that the angle between the two fields is an essential factor for successful PFGE. Obtuse field angles make the technique possible, and Olson found that even a single field that was periodically inverted—producing fields at a 180° angle—could yield separation with nice straight bands[7].

Olson's finding would lead to a key step in the technique's evolution. After discussing his 'field-inversion gel electrophoresis' findings with Stanford researcher Ronald Davis, Davis and biochemist Gilbert Chu developed a system of their own, 'contour-clamped homogeneous electric fields' (CHEF)—a hexagonal configuration of tightly regulated electrodes that generates two fields at an 120° angle to each other[8], allowing remarkably clean PFGE without the bizarre size-independent separation effects that can arise with field-inversion[7]. CHEF essentially became the end of the story—a popular, commercial system that is still found in most molecular biology laboratories.

According to Cantor, PFGE's scientific importance was tremendous: "Without PFGE we probably wouldn't have the genome sequence…if you want to handle large [DNA] fragments, this is the only way." Olson also praises Schwartz's contribution, noting the unusual path of PFGE's development: "I think of it as an invention, like Edison… the people who contributed the most were actually not the theoreticians, but inventors who gradually invented one better solution after another to the essential empirical constraints that the molecular behavior imposed."

**Michael Eisenstein**

1. Schwartz, D.C. *et al.* *Cold Spring Harbor Symp. Quant. Biol.* **47**, 189–195 (1983).
2. Schwartz, D.C. & Cantor, C.R. *Cell* **37**, 67–75 (1984).
3. Carle, G.F. & Olson, M.V. *Nucleic Acids Res.* **12**, 5647–5664 (1984).
4. Carle, G.F. & Olson, M.V. *Proc. Natl. Acad. Sci. USA* **82**, 3756–3760 (1985).
5. Schwartz, D.C. & Koval, M. *Nature* **338**, 520–522 (1989).
6. Gurrieri, S. *et al.* *Biochemistry* **29**, 3396–3401 (1990).
7. Carle, G.F. *et al.* *Science* **232**, 65–68 (1986).
8. Chu, G. *et al.* *Science* **234**, 1582–1585 (1986).