



Frederick Sanger (1918–2013)

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In memoriam

Frederick Sanger (1918–2013)

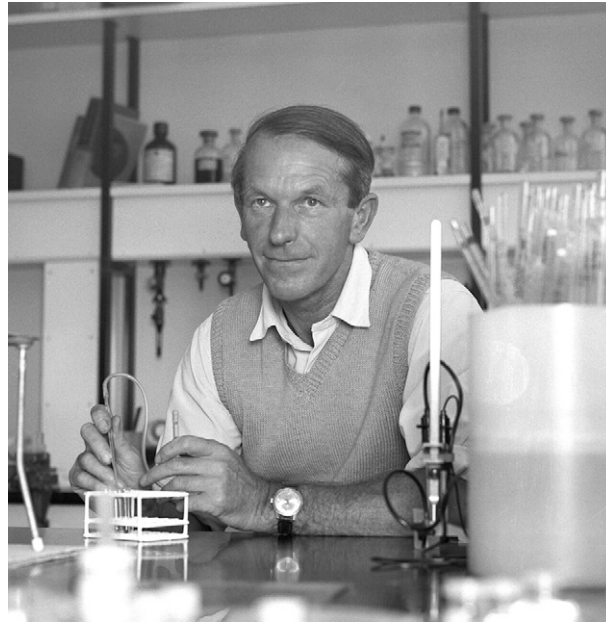


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The scientific community recently lost one of its brightest, imaginative, and humble scientists with the quiet passing of Fred Sanger, at age 95. He is well known for his pioneering work inventing key methods for both protein and nucleic acid sequencing, studies that resulted in his winning two Nobel prizes in chemistry.

Fred's scientific career began as a student at St. John's College, Cambridge University, UK, studying amino acids and their metabolism. While there, he developed methods for protein sequencing that included the "Sanger reagent" and the concept that short, sequenced peptides could be assembled based on their sequence overlap to build large polypeptides. Those studies resulted in completing the primary structure of several proteins, including insulin, which led to his first Nobel Prize in Chemistry in 1958. These groundbreaking studies served as the foundation for our understanding of protein structure and function, and the rules that form the basis of our knowledge of enzymology, as well as non-enzymatic structural proteins. His work also set the stage for developing the concept that the primary protein chain becomes folded to form a three-dimensional protein that can be stabilized by amino acid interactions, whether hydrophobic, hydrophilic, or disulfide bonds. His new and innovative protein structural methods then were extended by others and resulted in automated protein sequencing with instruments that could accomplish in a few days what had previously taken several years of bench work.

In 1962, Fred moved to the newly formed Medical Research Council's Laboratory of Molecular Biology (MRC-LMB), where the faculty at the MRC-LMB had a deep interest in molecular biology. Even though he was the head of the protein chemistry division, his thoughts turned to nucleic acids and he began developing new methods for sequencing RNA. These studies then evolved into new approaches for sequencing DNA, first using the "plus and minus" method followed by the development of the dideoxynucleotide chain termination, or Sanger sequencing method. During this

time, he also improved the methods for DNA fragment separation by introducing the use of very thin slab gels for DNA electrophoresis. Fred also extended his earlier overlapping fragment approach for protein sequence assembly by developing the concept of whole genome shotgun nucleic acid sequencing and by performing sequence assembly by joining overlapping DNA fragments to form larger polynucleotide sequences. This work resulted in the first fully sequenced DNA-based genome, that of phiX174, a 5386 base-pair bacterial virus that earned him his second Nobel Prize in Chemistry. He then proceeded to sequence the 16,569 base-pair human mitochondria and the 48,502 base-pair bacteriophage λ genomes. As the Sanger sequencing method became more widely used, the radioactive labels were replaced by fluorescent dyes. As a result, Sanger sequencing-based instruments soon became available. During the subsequent 25 years, the Sanger sequencing method was used almost exclusively for DNA sequencing, including the completion of the worldwide effort to sequence the 3 billion base-pair human genome, a major portion of which was conducted at the aptly named Sanger Centre (currently the Sanger Institute) in Hinxton, a few miles just outside of Cambridge. Without any doubt, based on his quiet work and significant accomplishments, Fred Sanger is regarded as one of the fathers of both modern proteomics and genomics.

However, Fred preferred working at the bench and encouraged others to do likewise. His Quaker upbringing was revealed by his quiet determination, his modesty, and his focus on excellence. As a result, many of us who were privileged to work in his group gained a renewed outlook to teach by example and question our students using the Socratic method. I remember one time when Fred came into my working area and asked if I could grow and inoculate *E. coli* cells with the M13 phage that he just received from Jo Messing. Since there were strict rules for using recombi-

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In memoriam

nant DNA techniques, I filed the appropriate paperwork to be certified to use the Category 2 room. Once approved, I grew the *E. coli* cells to mid-log and inoculated them with M13 phage for an overnight incubation. That next day Fred followed me to the Category 2 room as I was preparing to add chloroform to “kill” the cells so that they could safely be removed from the room, prior to centrifugation to collect the M13 phage-containing supernatant. Since he was not certified to use the Category 2 room, Fred observed what I was doing from outside the room. Afterward he asked me to explain what I did and why, as he had both an insatiable curiosity and wanted to understand thoroughly this new technique before introducing it into the lab. Shortly thereafter, his lab switched to M13-based sequencing for the mitochondrial genome.

While reviewing Fred’s background for writing this tribute, I noticed that Wikipedia has a page called the Cambridge Reference Sequence where it states, “A group under Dr. Fred Sanger at Cambridge University sequenced the mitochondrial genome of one individual of European descent during the 1970s...” and have heard others indicate that this was Fred’s mitochondrial DNA. Let me set the record straight here. First, although Fred was a Fellow at Kings College of Cambridge University at that time, he worked at the MRC-LMB. Second, the sequenced mitochondrial genome was not from one individual of European descent but from several sources. Initially, human placental mitochondria DNA was isolated in Fred’s laboratory and then, after restriction enzyme digestion, Jacques Drouin cloned the fragments into pBR322 (Drouin 1980). However, one region of the circular genome was not present in this clone collection. Therefore, to complete the circular mitochondrial genome, we sequenced the missing portions in part from HeLa cell mitochondrial DNA supplied by Professor Giuseppe Attardi at Caltech, as well as mitochondrial DNA isolated from human liver obtained from cadavers studied by medical students at the New Addenbrooke’s Hospital across the street from the MRC-LMB. Therefore, the final “human” mitochondrial genome sequence often referred to as the “Anderson” sequence or Cambridge Reference Sequence actually was a composite of human placental and human liver mitochondrial genomes from anonymous individuals of unknown ethnicity as well as from HeLa cells.

There is one related remembrance that reinforces Fred’s modesty and humility: Fred’s decision to alphabetically list the authors on the Anderson et al. *Nature* paper describing the sequence of the human mitochondrial genome (Anderson et al. 1981). At some point I asked Fred why the author list was alphabetical. Ever soft-spoken, he answered that he believed it was a group effort and that he did not want to have anyone, especially himself, singled out as the senior author.

Fred taught by example and asked critical questions that serve as the foundation for a greater understanding of molecular processes. Reinforcing these traits that are critical to learning about the natural world had a significant impact on my own career and those of my students. Because of this, I was asked by the *Genome Research* editors to mention some of Fred’s academic “children” and “grandchildren” as examples of those influenced, directly or in-

directly, by Fred. Over the years, many outstanding scientists worked in Sanger’s laboratory. Several who were involved in nucleic acid sequencing immediately come to mind. George Brownlee and Bart Barrell began working with Fred in the early 1960s developing methods for RNA sequencing. George Brownlee also developed cDNA-based methods for studying the structure and function of immunoglobulins and the immune response before moving to Oxford, where he had a successful career studying blood clotting and hemophilia. Although Bart Barrell initially was a technician with Fred, his RNA sequencing studies earned him a PhD. He remained in Sanger’s lab playing a significant role in sequencing numerous viral genomes. Alan Coulson was the technician who worked with Fred developing DNA sequencing methods. When Fred retired, Bart and Alan moved to the Sanger Institute where Bart established the highly productive microbial sequencing group and where Alan worked with John Sulston sequencing *C. elegans* and *C. briggsae* as well as a number of human chromosomes and the mouse genome. Jacques Drouin, who, as mentioned, originally cloned fragments of the human placental mitochondrial genome in *E. coli*, has had a highly successful career focused on hormone biochemistry at the Clinical Research Institute of Montréal.

It is difficult for me to select only a few of the 40 PhD students who earned their degree from work completed in my laboratory by carrying on Fred’s tradition of critical questioning and teaching using the Socratic method. However, I will try. Rick Wilson and Elaine Mardis, now codirectors of The Genome Institute at Washington University in St. Louis, joined my lab as PhD students soon after my sabbatical and move to the University of Oklahoma. Both follow Fred’s approach to science and, because of that, have contributed to numerous genome projects, the most recent being their pioneering studies sequencing cancer genomes. The list of Fred’s “grandchildren” also includes Ellson Chen, who helped develop a dideoxynucleotide sequencing kit while at Bio-Rad Laboratories and later established the sequencing group at Genentech. Stephanie Chisoe, who contributed to sequencing the *BCR* and *ABL* genes involved in the Philadelphia chromosome and sequencing human chromosome 22, now serves as the Director of Genetics at GlaxoSmithKline. Feng Chen leads the Advanced Sequencing Group at the Joint Genome Institute (JGI), while Judy Crabtree is teaching at Louisiana State University Medical School where her research is focused on understanding endocrine tumor disorders.

There should be no doubt that Fred’s novel contributions to both protein and genomic science and his influence on those who knew him were monumental. Although his work led to numerous prestigious awards, I believe he wished to be remembered simply as a scientist whose efforts resulted in new discoveries.

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