

# Would Fred Sanger Get Funded Today?

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**ABSTRACT** Fred Sanger developed technologies that won him two Nobel Prizes and revolutionized biological research. Yet, in spite of this record, the question has been raised as to whether, in the current scientific climate, he might be unsuccessful in obtaining a grant because of a productivity that would be viewed as too limited. In imagining how a National Institutes of Health study section today might treat a proposal from Sanger to sequence DNA, we can ask whether there are lessons from his career that suggest changes to the grant review process.

FRED Sanger died last November at the age of 95. To many scientists, including me, the two-time Nobel Prize-winner was the most important biologist of the latter half of the 20th century. His ingenious methods for determining the sequence of proteins, RNA, and DNA led directly to many of the achievements of genetics and molecular biology and to the Human Genome Project. Sanger's death has engendered discussion of whether his style of science could survive in today's environment and whether a laboratory pursuing bold but untested ideas and producing a modest number of publications would fail to obtain funding in the current peer review climate. Contrary to the view that he would not win a grant, I will argue that he would receive continuous and substantial funding in the modern scientific world. Nonetheless, that we are having this debate at all indicates that there are fundamental issues with how grant applications are reviewed. By assessing a hypothetical faceoff between Sanger and a National Institutes of Health (NIH) study section, the most common review for an American biologist, we might identify some simple albeit significant fixes.

In a career of a little over 40 years, Sanger published ~70 papers of original research, from "The nitrogen of the potato" in 1942 (Neuberger and Sanger 1942) to "Nucleotide sequence of bacteriophage lambda DNA" in 1982 (Sanger *et al.* 1982). In addition, he was responsible for ~20 reviews, award lectures, and meeting abstracts, as well as a number of papers from members of his laboratory that he did not put his name

on. The claim has been made that Sanger would fail to be funded today because he "published little of import between insulin in 1952 and his paper on RNA sequencing in 1967 with another long gap until DNA sequencing in 1977" (Brenner 2014, p. 262). I take the reference here to "DNA sequencing" to be the famous dideoxy paper of December 1977 (Sanger *et al.* 1977b), given that Sanger had published previous papers both on DNA sequencing methods and DNA sequences since 1973. The idea that the dideoxy publication followed a long gap runs counter to Sanger's own statement that his career had few moments of sudden breakthroughs but many "small and gradual advances" (Sanger 1988). Thus, dideoxy sequencing did not spring fully formed like Archimedes jumping out of his bathtub with the principle of buoyancy. Instead, it was the culmination of steady—yet spectacular—progress in the preceding years.

Let's look at just the 5 years preceding the dideoxy paper as if this were the track record upon which Sanger's next grant application would hinge. I'm reasonably familiar with this literature, as I joined the MRC Laboratory of Molecular Biology in the fall of 1977 as a graduate student with George Brownlee, who himself had been a student of Sanger's in the 1960s and who continued working afterward on sequencing technology. Brownlee moved to Oxford midway through my degree, but I stayed in Cambridge and became loosely affiliated with Sanger's group. In the 5 years before the dideoxy paper, Sanger published nine papers of original research, encompassing a couple of methods and several reports of sequences. Five of these papers appeared in the *Journal of Molecular Biology*, two in *Nature*, and one each in the *Proceedings of the National Academy of Sciences U.S.A.* and *Biochemistry Journal*.

The first of the methods, published in 1973, described the use of DNA polymerase I to synthesize a short fragment of

the single-stranded phage f1 genome by extending an 8-base primer oligonucleotide (Sanger *et al.* 1973). The primer had been designed based on the coat protein sequence Trp-Met-Val, which could be converted to DNA sequence because it encompasses two amino acids encoded by single codons (tryptophan and methionine). The primer acted at a unique site in the phage genome, even though this site ended up not being the expected region of the coat protein gene (and indeed the Trp-Met-Val sequence may have been incorrect). Sanger's challenge was to split the newly made DNA chain into small pieces whose sequences could be determined. He accomplished this task by carrying out the DNA synthesis in the presence of manganese, which allowed the incorporation of a ribonucleotide, along with the three other deoxyribonucleotides, one of which was <sup>32</sup>P-labeled. He first fractionated the polymerized products (Figure 1) by a clever two-dimensional separation method known as "homochromatography," which he had published 4 years earlier. The presence of the ribonucleotide then enabled cleavage of the fractionated products by pancreatic ribonuclease. The use of these approaches along with an analysis of small degradation products resulted in the determination of 50 bases of DNA sequence.

The second method, called "plus and minus" and published in 1975, is the subject of my favorite paper (Sanger and Coulson 1975), not just from Sanger but from any author. Sanger showed that leaving out one nucleotide (*e.g.*, "A") in one of four "minus" reactions of synthesis by DNA polymerase I resulted in products terminating before an A in the sequence. In complementary fashion, providing just one nucleotide (*e.g.*, "A") in a "plus" reaction with T4 DNA polymerase led to degradation of the DNA products from their 3' ends that stopped where an A base was required. Thus, in each of the eight reactions there was a mixture of products terminating either just prior to or just at one of the bases. As creative as this idea was, it was accompanied by the even more striking demonstration that an acrylamide gel run under denaturing conditions could separate a 40mer oligonucleotide from a 41mer, from a 42mer, etc. (Figure 2). Thus, a single radioautograph could reveal a sequence of >70 bases determined simply by reading the positions of the labeled bands in the eight lanes of a gel. Even Sanger, a most modest and soft-spoken man, wrote that "this new approach to DNA sequencing was I think the best idea I have ever had," but then went on to admit that he did not, in reality, remember having the idea (Sanger 1988, p. 22). DNA polymerase I, synthetic primer, labeled nucleotide, one reaction per base, denaturing gel: from there to dideoxy was a relatively short leap.

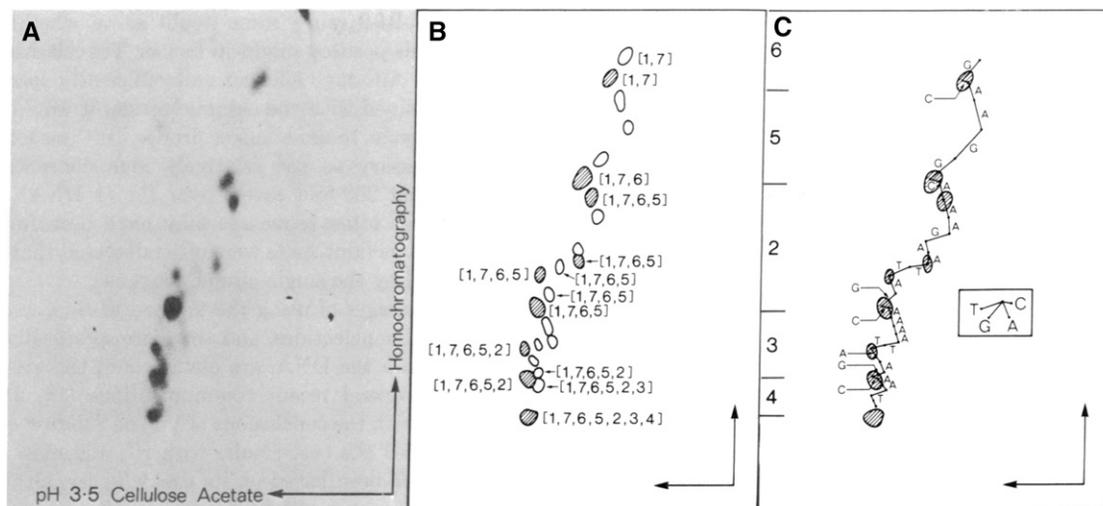
The papers reporting sequences in the 5 years preceding the dideoxy paper included one that showed that the DNA and protein sequences of  $\Phi$ X174 correlated (Air *et al.* 1976). Another paper reported on the amazing phenomenon of overlapping genes in  $\phi$ X174 (Smith *et al.* 1977). Yet another communicated the complete sequence of the  $\phi$ X174 genome, the first DNA genome ever to be sequenced (Sanger *et al.* 1977a). Considering both the methods and the sequen-

ces, I maintain that this second half of Sanger's second period of "little import" constitutes a record that any scientist would look back on with rightful pride.

Lest you think I've cherry-picked the single productive period of Sanger's career that did not encompass the famous papers on insulin, RNA, and DNA sequencing, let's instead go back a bit further and consider the 5 years preceding the 1973 phage f1 sequence paper (Sanger *et al.* 1973). During these years, Sanger published eight papers of original research. These included sequences from 16S and 23S ribosomal RNA (Fellner and Sanger 1968), a transfer RNA (Barrell and Sanger 1969), and two phage R17 RNA sequences (Adams *et al.* 1969; Jeppesen *et al.* 1972). It seems worth noting that the earlier R17 paper (Adams *et al.* 1969)—comparing the sequence of a small portion of the phage coat protein to the messenger RNA (mRNA) sequence that encodes it, the first bit of mRNA sequence ever determined—would have solved the genetic code had Marshall Nirenberg not done so a few years earlier by *in vitro* translation of synthetic mRNAs. Other papers described methods, including the aforementioned "homochromatography" (Brownlee and Sanger 1969). Again, despite a relatively modest output of publications, Sanger in this period made groundbreaking contributions to the important field of RNA sequence determination.

Now imagine that Sanger in 1972, or in 1977 with the dideoxy method not yet published, proposes, say, to accomplish something as audacious as the complete sequence determination of the ~50-kb genome of bacteriophage lambda. Would an NIH study section with the mindset of 2014 provide him the necessary grant? Let's consider a comment specific to Sanger's own time, since even back then obtaining funding could be challenging (but Sanger was viewed as a rock star by today's standard). "When Sanger's research plans for undertaking the sequence determination of a bacteriophage genome were reviewed, the referees were generally uneasy about such a large programme, without any precedent and demanding much new chemistry. However, I understand that one American reviewer, consulted because of these concerns, simply replied: 'Fred Sanger continues to bang his head against brick walls. And the walls keep falling down'" (Dodson 2005, pp. 33–34).

I contend that a modern-day NIH study section would give Sanger a highly fundable score for three reasons. First, Sanger had a track record. He had, after all, won a Nobel Prize in Chemistry in 1958 at the age of 40 for determining the amino acid sequence of insulin. Especially when you consider that the average age of principal investigators obtaining their first NIH grant is now ~43, Sanger nearing the age of 55 or 60 at the time of our panel would be viewed as a long-time scientific luminary, if no longer a boy genius. While it may be disingenuous to tell young scientists that their best hope of getting an NIH grant before they're into middle age is first to win a Nobel Prize, we can at least recognize that important prior success paves the way for later favorable evaluations. Sanger would seem a good candidate for a Pioneer or similar award from



**Figure 1** (A) A two-dimensional fractionation of products synthesized by DNA polymerase I. (B) A diagram of these spots. (C) How the direction of each spot from the previous one can indicate the additional residues present. (Reprinted from Proc. Natl. Acad. Sci. U.S.A., Vol. 70, No. 4, F. Sanger *et al.*, Use of DNA polymerase I primed by a synthetic oligonucleotide to determine a nucleotide sequence in phage  $\phi$ 1 DNA, pg. 1211 with permission from MRC Laboratory of Molecular Biology and authors John E. Donelson and Alan R. Coulson.)

the NIH that relies primarily on the qualifications of the investigator.

Second, even the most conservative-minded of our set of reviewers assessing Sanger's progress over a previous 5-year funding cycle would acknowledge its exceptional nature. The publications I cited from 1967 to 1972 or from 1972 to 1977 moved the needle, and certainly the biologists of that time were aware of how far and that RNA and DNA sequencing would change biological research. Rather than "be labeled as unproductive" (Brenner 2014), Sanger would be applauded by our modern-day study section for accomplishing so much while spending comparatively so little.

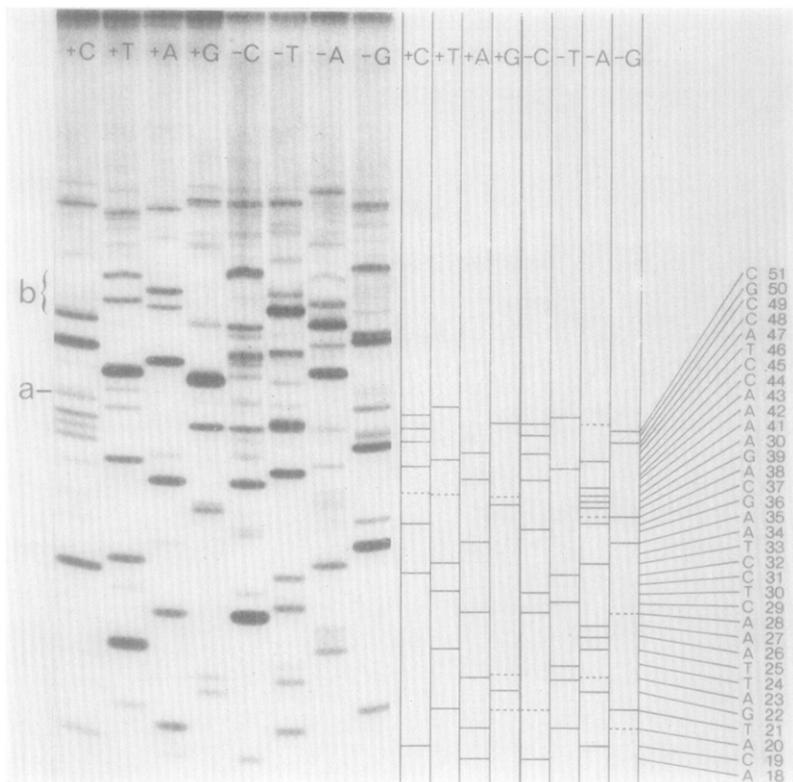
Third, at least some members of our panel would read Sanger's proposal and appreciate its boldness and its ingenuity. Rather than condemn it ("This research is too risky") or dismiss his expertise ("This applicant has never sequenced that much DNA before") or find fault with the details ("The control in figure 2B is not adequate"), our hypothetical study section would consider the potential payoffs of the research to easily balance the risk.

So Sanger would get his NIH grant, stay funded throughout his career, and go on to win Nobel Prize number 2. I was always amused by Sanger's comment in his 2001 interview on Nobelprize.org ([http://www.nobelprize.org/nobel\\_prizes/chemistry/laureates/1980/sanger-interview.html](http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/sanger-interview.html)) that "It's much more difficult to get the first prize than to get the second one, because if you've already got a prize, then you can get facilities for work, and you can get collaborators, and everything is much easier." It seemed to me that if anyone with one prize was forced to work in a tiny garret lab with a minimum of collaborators and a scant amount of funding (none of this, of course, was true for Sanger) and told to win a second, it would be Fred who would be up to the task.

Most of us, however, don't manage to win our first Nobel Prize by the age of 40, and convincing you that Fred Sanger would succeed in the current American grant system doesn't necessarily provide relevant lessons for the rest of us. But by examining the trajectory of Sanger's career, we can learn much that is applicable to mere mortal scientists. And the debate over Sanger's fundability suggests some major policy prescriptions that could ensure that the next generations of biologists are not shut out of the system before they get a chance to make their major contributions.

That a scientist be accorded credit for having already demonstrated creativity and perseverance in solving a tough problem should be a given, even if the solution was not Nobel-worthy; it almost never is. Those who have proved themselves capable of imaginative science should get more of a boost than they currently do in an NIH review system where the quality of the investigator is merely one of five criteria. The lesson here is that funding for people—especially those who knock down brick walls—and not just for their projects, pays off, often spectacularly. Given that there are plenty of unimaginative people who manage to publish prolifically and continue receiving grants, someone with a track record of brilliance but limited recent progress should be afforded basic funding.

The NIH has moved a little in the direction of funding people through programs like the New Innovator, Pioneer, Transformative Research and Early Independence awards, but these few grants go largely to current and rising superstars. The total budget of \$123 million in 2013 for these awards (<http://www.nih.gov/news/health/sep2013/od-30.htm>) constituted ~0.4% of total NIH spending. Perhaps this fraction could be boosted 10-fold or more so that a thousand creative investigators are given the freedom to pursue their ideas.



**Figure 2** The first published radioautograph in which a DNA sequence can be read directly by comparing bands in adjacent lanes after polyacrylamide gel electrophoresis. Note the tremendous increase from 2 years earlier in both the speed of analysis and in the number of bases that can be determined in one experiment. (Reprinted from the *Journal of Molecular Biology*, Vol. 94, F. Sanger and A. R. Coulson, A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase, pp. 441–446, with permission from Elsevier.)

Many biologists might submit a grant application that documents a productivity of one or two papers per year in their previous grant period. If these were important papers, regardless of which journal they appeared in, they should serve as an adequate basis to establish that a lab is doing good work. Papers well in excess of this total sometimes represent work that was rushed into print to boost the record for a grant review—not exactly the ideal recipe for reproducible science. The lesson here is to assess scientists not by counting their publications or by using the names of the journals in which their papers appeared as proxy for the work’s significance.

Sanger’s development of thin acrylamide gels for sequencing (Sanger and Coulson 1978), a simple innovation that revolutionized the field for over a decade by vastly increasing the speed and resolution of separating DNA fragments, appeared in *FEBS Letters* (2012 Impact Factor 3.6). The “plus and minus” and other methods appeared in the *Journal of Molecular Biology* (2012 Impact Factor 3.9). Let’s ask grant applicants to annotate their five key publications and let’s ask study section members, who put a lot of their time into the review process, to look specifically at these five publications. By reading a limited number of papers rather than counting them all or dismissing the ones that don’t appear in some tiny subset of journals, a study section will better assess an applicant’s true impact. It’s more critical to produce a few papers that, even if they don’t shake the world, at least jiggle it, than a slew of papers that describe methyl-ethyl science (“you showed it for the methyl derivative so I will show it for the ethyl derivative”) or a single gleaming one in a “high impact” journal whose luster might not stand the test of time.

Distinguishing an idea that is truly ingenious from one that is merely crackpot is not always obvious. But reviewers should be able to see when a novel approach might make a major difference. If they can’t make this judgment, it may be that the group lacks any scientists who can assess a creative proposal and counterbalance critical reviewers who get bogged down in minor details or niggling complaints. When I first submitted grant applications to the NIH, the list of members in the study section that reviewed them was replete with the leading lights of the time. Alas, they didn’t find my proposals—which in retrospect weren’t that exciting—worthy, but at least I was turned down by people qualified to judge me (I can say now that they were qualified; at the time I used other adjectives). Today I am sometimes hard-pressed to recognize more than the occasional name on a study section roster. The lesson here is that more of the senior members of our community need to serve on review panels. Many have suggested that receipt of an NIH grant should constitute an obligation to serve when asked, a suggestion that should be formalized by the funding agency.

Biologists are often hesitant to move away from the field of their expertise to tackle problems in other areas, feeling that they will fail to convince a panel of their ability to contribute in a new area. But those who switch fields—as Sanger did most notably from proteins to RNA to DNA—should be appreciated, not penalized. Dorothy Hodgkin, herself a Nobel Prize-winning chemist, thought that Sanger had made a mistake and was wasting his talents by leaving the field of protein chemistry to head off into the realm of nucleic acids (Dodson 2005). The lesson is that study sections should

acclaim boldness and ambition in tackling a problem in a useful new way, whether it comes from a long-time player in a field or a just-arrived newcomer.

Fred Sanger was a remarkable scientist who left a legacy of superlative achievements that continued throughout his career, not punctuated brilliant insights interspersed with long periods of reflection and inactivity. Regardless of the vagaries of current NIH peer review, Sanger would have flourished with these achievements. But in our fears that even a Sanger might no longer be fundable, are we also expressing a wistfulness that the days of the lone scientific genius might be gone? In an era of large collaborations, multi-authored papers, and enormous datasets, is there still room for the single creative idea that proves to be a game-changer? I for one surely want to believe there is still room. Many of us went into science in search of those rare moments of discovery when we alone comprehend some sliver of the physical world that was unknown a moment earlier. If creative biologists directing small laboratories no longer can obtain the funding to continue that search, science will be a bit the poorer for it. But if there is no place even for the next Sangers to carry out their studies, humanity will be much the poorer for it.

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