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A. M. Smith
DNA Seq.

Dr. J. Lederberg
The Rockefeller University
New York, NY 10021
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Dear Dr. Lederberg:

Thank you for your very kind remarks on my article in American Scientist on the sequence of ØX174 DNA. Of course the sequence determination is a tribute to Fred Sanger's intuitive genius.

With regard to specific mutagenesis, I enclose a reprint of the first paper on transition mutations, and a manuscript on the induction of specific transversion mutations. I am particularly satisfied with the latter because even without targeting it hasn't been possible to program this class of mutation. Also, it was pleasing that the method worked well even when the mismatch involves a purine-purine pairing. In unpublished studies we have been able to make mutations with close to theoretical efficiency (50%) using oligonucleotides as short as nine long. We also have been able to develop a strategy for enriching, in vitro, the mutant fraction of a mixture of a mutant and wild-type DNA so that the mutant constitutes 75-100% of the DNA. This will be useful for studies on mutations where there is no means for selection or screening other than direct DNA sequence determination. We are now trying to see if we can induce specific single nucleotide insertions and deletions to round out the complete list of types of point mutation.

In terms of extending the methodology to more complex DNAs, we envisage doing the mutation on DNA cloned in bacterial plasmids. The reason for this is that one requires a circular DNA in order to ligate in the synthetic oligonucleotide at both ends. Without ligation, repair mechanisms starting at the gap will edit out the mismatch. Also, the low genetic complexity of the recombinant DNA (5,000-10,000 nucleotide pairs, approximately) means that an oligonucleotide as short as nine long can be used. As you know, statistically the length of a unique oligonucleotide, n, is 4^n = nucleotide pairs in the genome. Thus, whilst 6 or 7 should be long enough for a genome of the size I mention, other factors limit the efficiency of the method. Firstly, the length of oligonucleotide required to give a stable Watson-Crick structure to act as an efficient primer even though there is a mismatch, is significant. Equally, or more significant is the editing by the 3'-exonuclease which is part of the DNA polymerase. Hence I think 9 is close to the shortest length of oligonucleotide

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which will work efficiently. Once the recombinant DNA has been mutagenized it could be introduced back into its parent genome by recombination or it could be introduced into the organism as a stable minichromosome. We hope to test out these ideas in yeast because we have been working on the cytochrome c gene (see enclosures). In terms of biohazard, I'm not sure what to say. I don't think that synthetic oligonucleotides by themselves can present a hazard, as they do not act as mutagens unless they are ligated into a DNA at both ends before being introduced in a cell. Thus, marker rescue does not work with DNA fragments less than about 30 nucleotides long, again presumably because of editing from the ends.

Thank you for sending me the copy of your Nobel Prize lecture. I'm sure I read it at the time (I was a postdoc in Gobind Khorana's lab) but I had forgotten how perceptive you were in anticipating what I would be doing in 1979.

Yours sincerely,

Michael Smith

Michael Smith
Professor

MS/sf
P.S. In saying that marker rescue doesn't work with polynucleotides shorter than about 30, this refers only to E. coli. I don't think that people who have used DNA fragments for marker rescue on SV40 have done any studies on the relationship of efficiency versus polynucleotide length.