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Epigenomics

To the editor:

The commentary by Stephan Beck, Alexander Olek, and Jörn Walter (*Nat. Biotechnol.* **17**, 1144, 1999) emphasizes the importance of epigenetic information in the human genome, and points out that a European alliance, the Human Epigenome Consortium (HEC), has been formed. This important initiative is to be welcomed, although it is long overdue.

It is now 25 years since it was suggested that DNA methylation is likely to have a role in the control of gene expresssion in higher organisms. Within the past 10 years, considerable evidence for this has accumulated. Yet in the whole history of the Human Genome Project, there has been almost no discussion of the distinction between cytosine and 5-methylcytosine in the genome sequence.

The authors of the Commentary refer to an organism's "epigenotype." However, the true situation is that an organism has the same genotype in all its cells, but different types of cells and tissue have different epigenotypes. The epigenotype is the normally stable and often heritable genotype upon which additional information has been imposed. Thus, organisms have many different epigenotypes in their cells and tissues. In 1995, I wrote an article, "DNA methylation in eukaryotes: 20 years on," which concluded as follows[1]:

> Finally, it is important to consider DNA methylation in the context of the sequencing of the human genome. There are not four bases in human DNA, but at least five, and very likely others[2]. To learn the position of every cytosine, but not to know whether it is methylated or not, will clearly fail to answer many important questions about the human genome. In particular, it will not address the question of those epigenetic controls in human DNA that are due to base modification. The sequence of the genome is one level of information and can be said to comprise the full genotype of our species. The time has come to recognize the importance of the epigenotype, both in the germline cells and in the somatic tissues. When the human genome is finally sequenced there will remain the enormous challenge of deciphering the epigenetic code that is superimposed on the four-base genetic code. This will surely occupy the attention of many investigators in the next 20 years.

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Enzymes by post—restriction enzyme stability

To the editor:

DNA restriction enzymes, with their widespread applications in many diagnostic procedures (e.g., RFLP, PCR, etc), are the very cornerstone of recombinant DNA technology. Unfortunately, their transport and storage are usually carried out at subzero temperatures, greatly increasing the cost per unit activity and limiting availability in many developing countries.

While preservation techniques to extend stability at higher temperatures have been reported[1–4] (e.g., desiccation with trehalose), enzymes are generally unavailable in this format, and storage at –20°C following reconstitution is still recommended. Surprisingly, no one appears to have examined the stability of untreated restriction enzymes as supplied by manufacturers. Our studies suggest that restriction enzymes are in fact quite stable as supplied; some exhibited activity after several weeks' storage at 37°C, and some lost little or no activity after storage for several months at ambient temperatures (J. Clark, J.C. Harrison, and J.B. March, unpublished data). We have even sent enzymes roundtrip between the UK and USA with a marginal loss in activity (Figure 1), a surprising finding given the large fluctuations in temperature likely to occur during such a journey (September 1999).

If applicable to all enzymes, these findings could have major implications. Air transport of dry ice packages is very expensive, restricting their availability in Third World markets; at present, the cost of carriage can easily exceed the purchase price of many enzymes. However, we successfully used inexpensive airmail letters to send restriction enzymes to Tanzania. Upon arrival, they retained activity and were effectively stored in a domestic refrigerator for months. At present, the supply of such reagents involves expensive overland transport on ice from South Africa. By reducing carriage costs and removing the need for refrigerants and insulation materials, simple overnight postage should yield considerable savings while benefiting the environment.

Widespread availability of affordable enzymes in the Third World could have a major impact on both research and diagnostic activities. We would therefore urge manufacturers to, first, investigate the stability of their own enzymes at higher temperatures, and, second, consider the direct supply of enzymes to hitherto uneconomic African and Asian markets. Even if a percentage loss of activity occurs during transport, enzymes are usually used in such excess that compensation can easily be achieved either by recalibrating activity upon arrival or extending incubation periods (e.g., overnight). We have examined more than 20 enzymes (J. Clark, J.C. Harrison, and J.B. March, unpublished data), and all exhibit significant activity after one week at room temperature, while some (e.g., *Hin*dIII and *Tsp*509I) still exhibit activity after storage for six months at 37°C. Although we have only tested restriction enzymes, these findings may well be applicable to other classes of enzymes, particularly thermostable polymerases. A more detailed investigation of a greater range of restriction enzymes by the manufacturers themselves would be beneficial to those in the research community, especially those in countries where enzymes are currently difficult or impossible to obtain because of transportation or storage constraints.

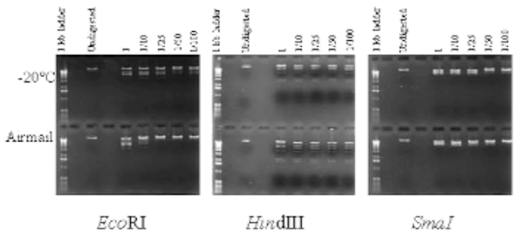

Figure 1. Ethidium bromide-stained agarose gels of restriction digests of 0.5 μg λ-DNA, using 1 μl of serially diluted restriction enzymes (in supplied 1× buffer). *Hin*dIII, *Eco*RI (Boehringer Mannheim), *Sma*I (Promega). All enzymes at 10 U μl^{-1}. In the lower lanes (Airmail), DNA was digested with restriction enzymes that had been sent by conventional airmail in a plain envelope from Edinburgh, UK, to Boston, MA, and returned via the same route. In the upper lanes (-20°C) digestion was performed with samples of the same enzymes stored at -20°C over the time course. The apparent stability of *Sma*I is particularly surprising given that it is considered thermolabile, with an incubation temperature of 25°C often recommended.

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