# LETTERS

## Misuse of PCR

Polymerase chain reaction (PCR) seems set to become a universal technique in DNA analysis and manipulation. Invented for tasks not manageable by other means, PCR was promptly applied to make standard procedures easier and quicker. Now it is even used to facilitate trivial tasks such as whole genomic DNA isolation and subcloning. However, the speed and versatility of the new technique has its price: the polymerase makes errors, amplifies them and introduces new errors in each cycle (for Taq polymerase mostly A.T to G.C transitions), resulting inevitably in 0.3–0.8% accumulated mutations after the usual 20–30 cycles[1,2]. In addition to generating point mutations, PCR is known to promote formation of artificial recombinants by co-amplifying different DNA fragments[3,4]. The high error rate does no harm when the whole product is analysed on a gel, used as a hybridization probe or even sequenced. The danger begins with cloning of PCR products, by which a randomly picked fragment from the mixture of mutants is conserved. Only a minority of researchers recognizes this critical point and keeps in mind the initial PCR amplification through the whole 'life-span' of the recombinant clone. It may later be sequenced, perhaps in another laboratory, and if the mutagenic PCR treatment is not clearly indicated, the sequence will be submitted to a database without any warning note. The consequences could be fatal. Comparisons of alleles conferring different phenotypes with their wild-type counterpart, for instance, will be totally misinterpreted. A polluted sequence in a database may mislead generations of researchers.

What should we do about this horror? No one has to renounce PCR, but we should be aware that a clone obtained by PCR is not a full-value clone equivalent to classical cloning products, simply because amplification of DNA *in vitro* is five to seven orders of magnitude less accurate than replication of plasmids and phages in *E. coli*. In agreement with Fordham-Skelton and co-workers[5], nucleotide sequence of a PCR clone should be regarded only as a confirmation of its identity as a probe for screening DNA libraries, not as an authentic gene sequence. If the PCR product is to be cloned and the sequence is important, Taq polymerase can be replaced by T4 DNA polymerase[6], which makes less errors due to its proof-reading activity. Several independent clones must be sequenced in any case. If the initial DNA material consists of only a few molecules or if it is highly damaged (ancient samples), these clones should preferentially originate from different PCR reactions, since mutations introduced in the first few cycles will be found in the great fraction of molecules at the end.

There are tasks which cannot be carried out in any other way than by PCR. But the benefits of PCR should be carefully weighed against its drawbacks when its product is to be cloned. The use of PCR to amplify genomic fragments for cloning in cases where sufficient amounts of DNA can be easily isolated, to amplify sequences from a standard cDNA library (instead of amplifying and screening the library) or even to facilitate subcloning(!) between recombinant phages and plasmids[7] is hardly beneficial to anyone else than to companies producing PCR kits and temperature cyclers.

Davies and Pugsley[8] complain that we are in danger of becoming a 'kit generation' of biologists who fail to understand the simplest principles of the techniques they use, since commercial kits (instruction leaflet included) guarantee success without thinking. Is the misuse of PCR technology a manifestation of this phenomenon? The editor of one of the PCR Bibles seems to admit it quite frankly. He says about molecular biology[9]:

> Its spectacular success over the last 30 years suggests that significant progress can be made even in the absence of an established theoretical foundation ... Given the ease and simplicity of PCR amplification, it can be said to allow the practice of molecular biology without a permit.

Let us hope that he is wrong and that, eventually, intelligent application will succede seductive, uncritical utilization of this powerful technique.

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## Errors in representing protein and nucleic acid structures

In his recent Textbook Error article, Edison[1] implies that the representation of β-sheet structures drawn with D-amino acid residues, rather than the natural L-amino acid residues, is an error that has lain undiscovered for 29 years. This is not so!

Day and Ritter discussed this 'mistake' in a paper published in 1967[2]. Their paper also gives examples of errors in the representation of the sense of DNA and protein helices, and describes simple ways of distinguishing right- and left-handed helical forms. As they point out, these diagrams are not necessarily 'wrong', but it is unfortunate that many textbooks do not present these structures as they appear in nature.

**References**

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