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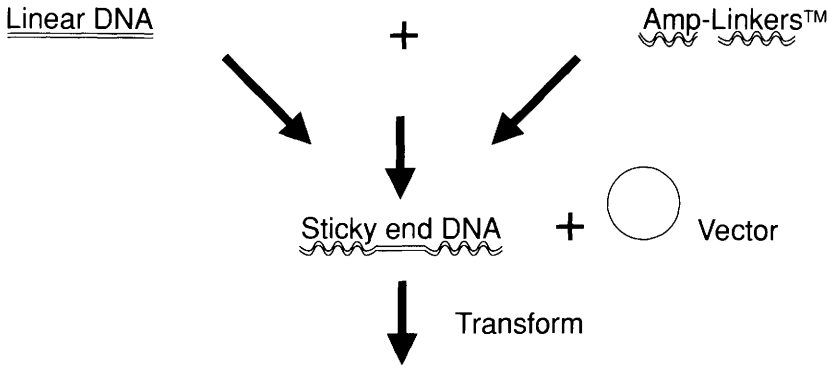

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The PCR process is covered by patents issued to Cetus Corporation.

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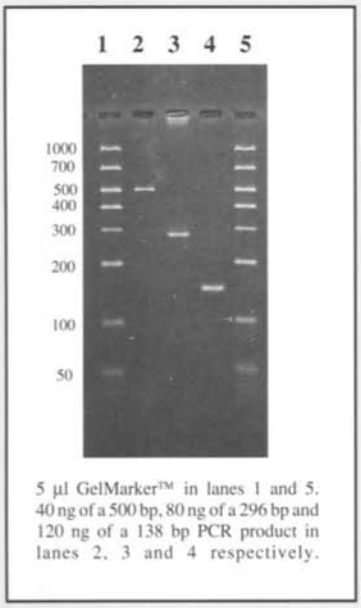

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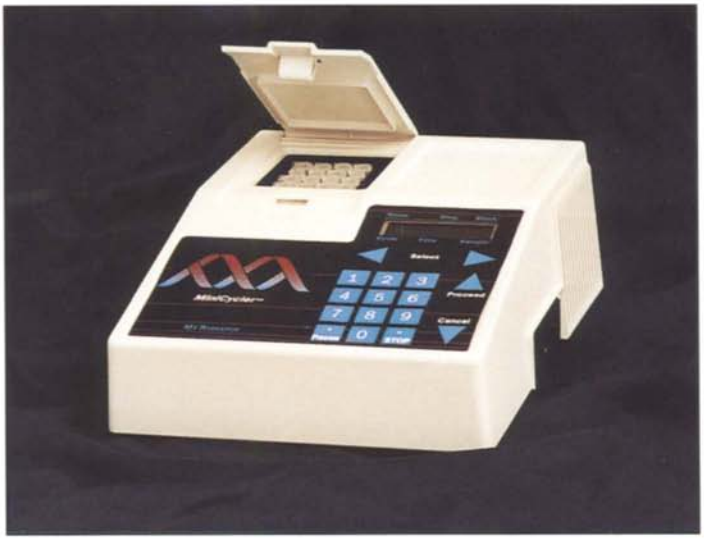

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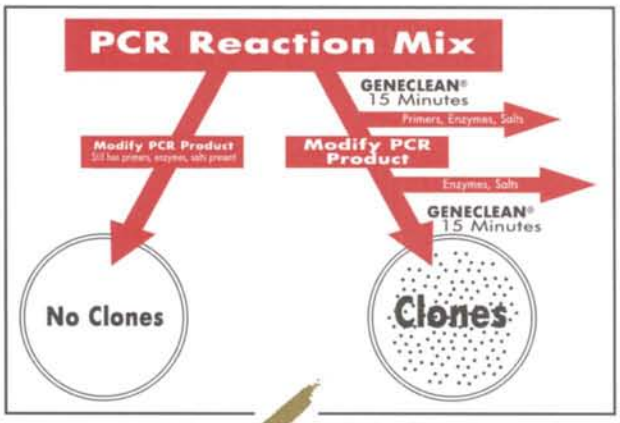

Products of thermostable DNA polymerase reactions contain "ragged ends" - it comes with the territory. "Ragged ends" are caused by non-template nucleotide addition reactions (1) and interfere with blunt-end cloning of PCR products and other amplimers. Consequently, cloning of these products is a universal problem and in most cases must be overcome by "building-in" flanking restriction enzyme sites or by filling in or cutting off the non-template nucleotide additions (2). Ends can also be modified for "direct cloning" (3) techniques. All of these procedures are often inefficient due to excess primers, enzymes, and salts present in reaction mixes causing the frequency of product clones in total transformants to be low or nonexistent. However, by incorporating The DOUBLE GENECLEAN Procedure, without the use of gels, the cloning efficiency of PCR products and other amplimers is increased as much as 100 fold (3,4) .... and transformants contain inserts .... every time ....

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References:
1. Clark, J.M. (1988) Nucleic Acids Res. 16, 9677.
2. Hemsley, A., et al. (1989) Nucleic Acids Res. 17, 6545
3. Aslanidis, C. and de Jong, P. J. (1990) Nucleic Acids Res. 18, 6069
4. Starr, L. and Quaranta, V. (1991) In preparation

Ordering Information: The DOUBLE GENECLEAN Procedure uses either the GENECLEAN Kit (cat. no. 3105) or the GENECLEAN II Kit (cat. no. 3106). Instructions for the DOUBLE GENECLEAN Procedure are now included with the instructions that come with both kits. If you already have a GENECLEAN Kit and would like an update of the GENECLEAN instructions to include the DOUBLE GENECLEAN Procedure, contact BIO 101 or one of our distributors. Please indicate the lot number of your GENECLEAN Kit and ask for a DOUBLE GENECLEAN UPDATE . . . it's FREE.

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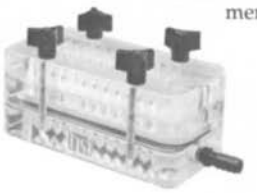

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The GeneAmp PCR process has been patented by Cetus Corporation, Emeryville, CA.

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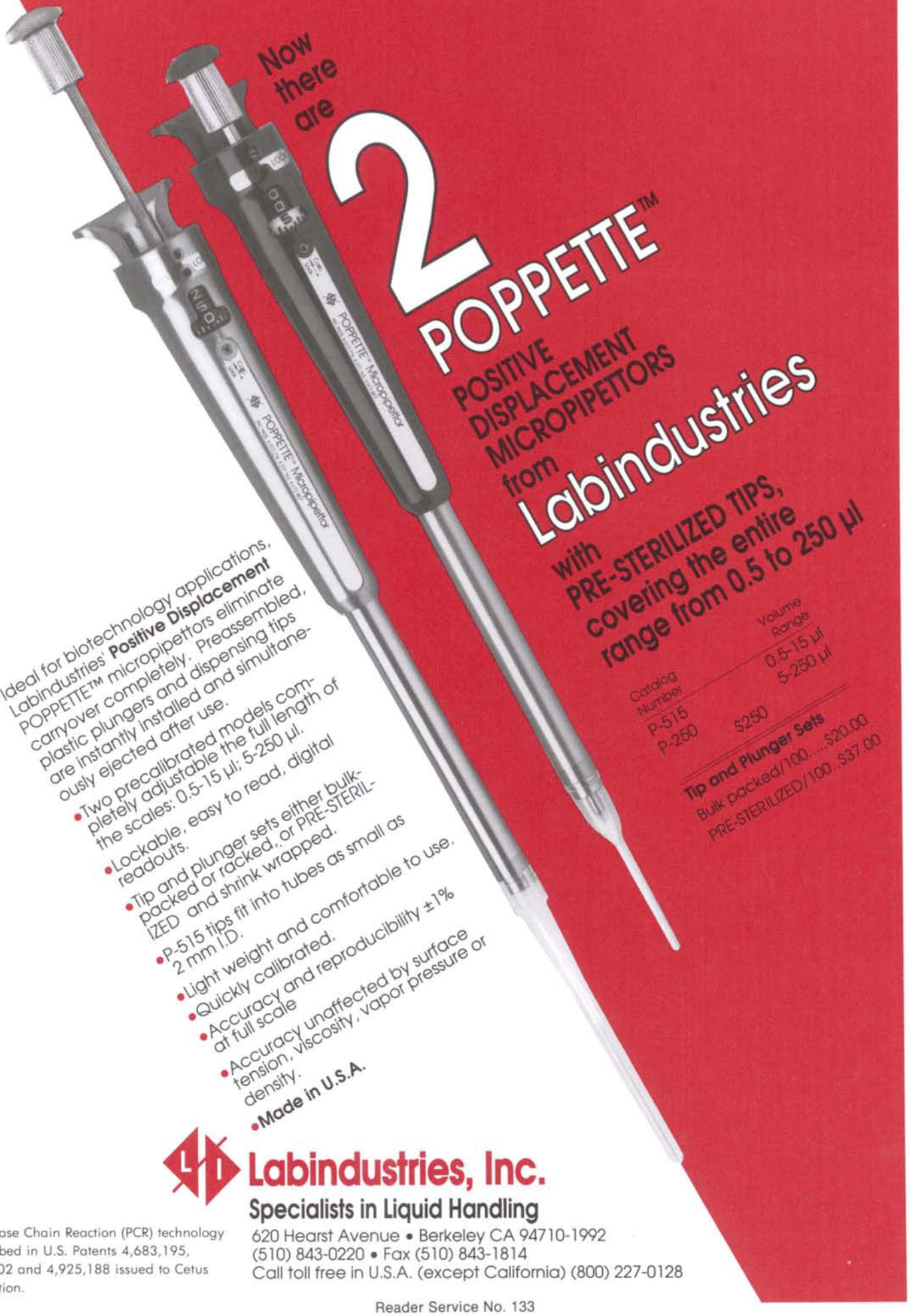
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Polymerase Chain Reaction (PCR) technology is described in U.S. Patents 4,683,195, 4,683,202 and 4,925,188 issued to Cetus Corporation.
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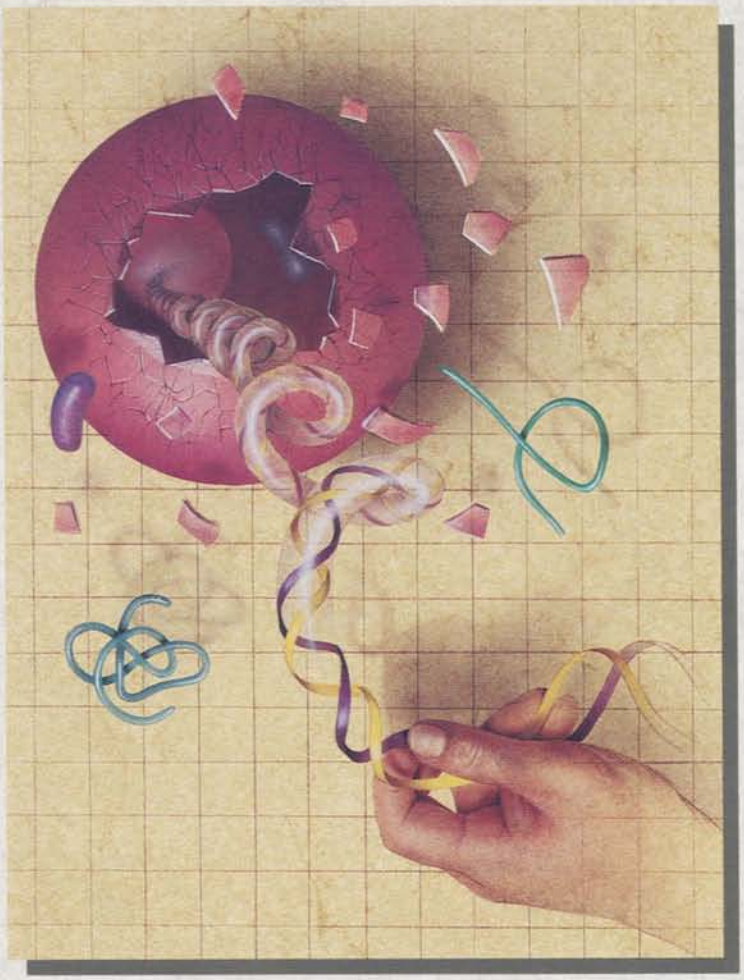

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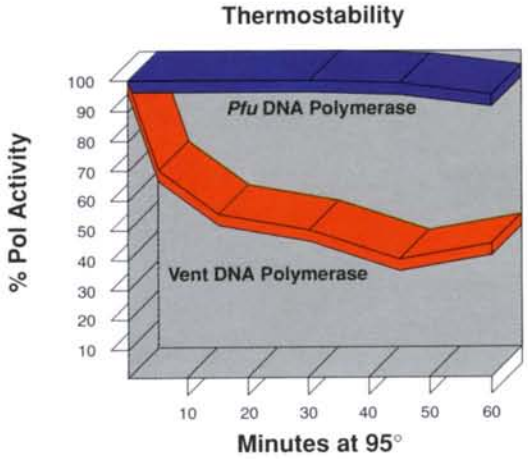

Figure 1: Thermostability of *Pfu* and Vent DNA Polymerases at 95°C.
To determine the thermostability of *Pfu* and Vent DNA polymerases at 95°C, 37.5 units of each enzyme were diluted to a final volume of 150 µl in the recommended reaction buffer and incubated at 95°C. At 0, 5, 15, 30, 45 and 60 minute time points, duplicated 10 µl aliquots (2.5 units) were assayed at 75°C for DNA polymerase activity.

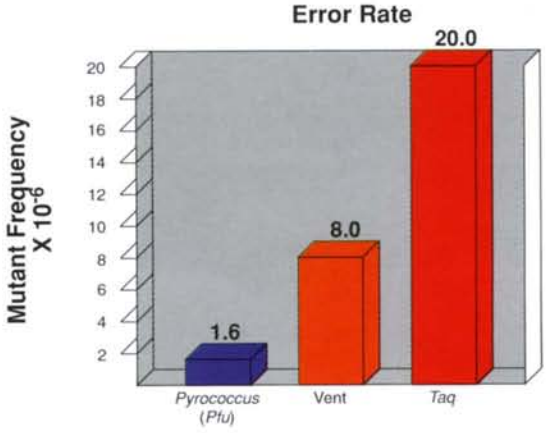

Figure 2: Polymerase fidelity was measured by modification of an assay described by Kohler *et al* (1991) *Pro. Natl. Acad. Sci. USA*, in press. Error rates reflect mutations per nucleotide incurred in the *lacI* gene during DNA synthesis. Vent is derived from *Thermococcus litoralis* and was obtained from New England Biolabs. *Pfu* is derived from *Pyrococcus furiosus* and is sold by Stratagene. *Taq* polymerase is derived from *Thermus aquaticus* and was obtained from Cetus Perkin Elmer.

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1. Bryant, F.O. and Adams, M.W.W. (1989) *J. Biol. Chem.* 264:5070-5079.
2. Fiala, G. and Stetter, K.O. (1986) *Arch Microbiol.* 145:56-61.
3. Eckert, K.A. and Kunkle, T.A. (1990) *Nucleic Acids Res.* 18:3739-3744.
4. Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bac.* 127:1550-1557.

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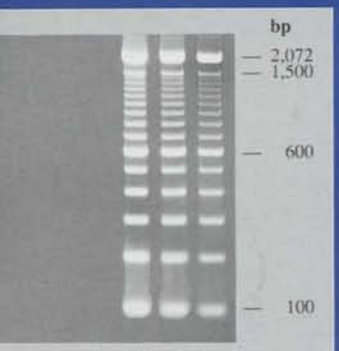

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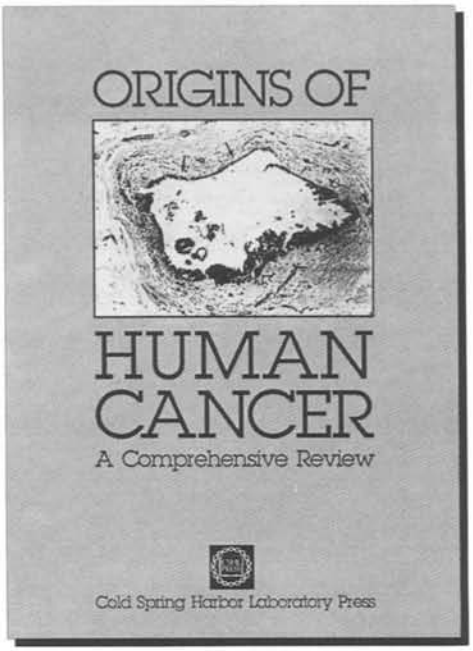

Edited by Joan Brugge, *University of Pennsylvania School of Medicine;* Tom Curran, *Roche Institute of Molecular Biology;* Ed Harlow, *Cold Spring Harbor Laboratory;* Frank McCormick, *Cetus Corporation*

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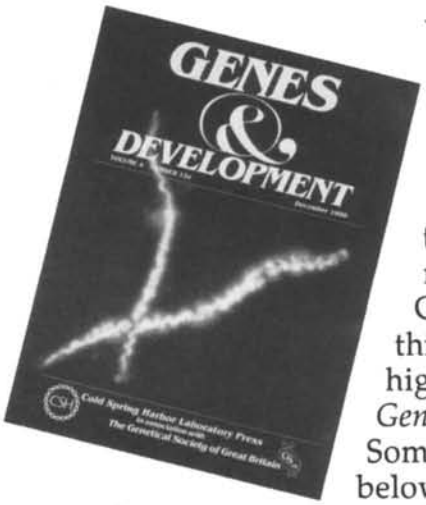

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