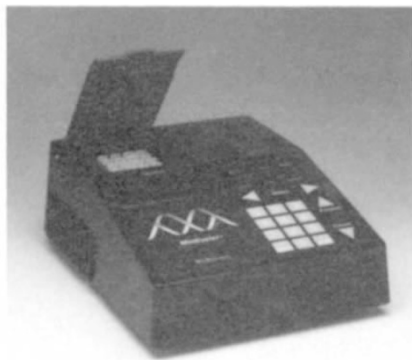


# PCR AND OTHER AMPLIFICATION TECHNIQUES.



### MiniCycler.

From MJ Research (Watertown, MA) comes a new generation of Peltier-effect thermal cyclers, with twice the thermal homogeneity, three times the speed, and a substantially lower cost than earlier generations of the technology. The heart of the machine is a thermoelectric heat pump that uses an electronic process to release or absorb heat to/from a precisely machined aluminum sample block. The unit has a thermal range of -9 to 100°C, it can cycle speeds up to 2.4°/sec, and the whole apparatus weighs just 3 kilograms.

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### Tips.

Vanguard (Neptune, NJ) introduces a filtered tip system for pipetting applications, especially polymerase chain reaction (PCR) applications. The aerosol-free pipette tips are fitted with a patented hydrophobic filter designed to eliminate the aerosol effects associated with DNA amplification techniques, manipulation of clinical samples, and radioisotope labeling. This filter will not "wet-out" when in contact with aqueous solutions, thereby eliminating the chance of aerosol or liquid contact with the pipettor. The tips are supplied sterile and racked.

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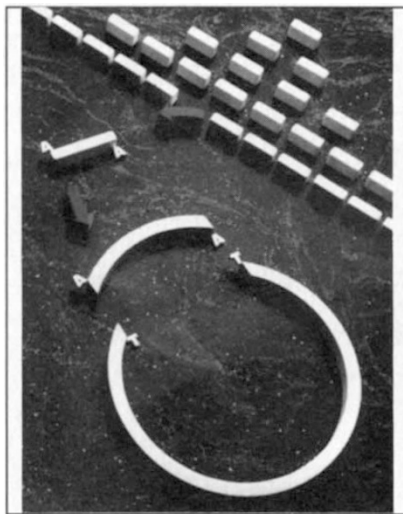


### pUCAmp.

From ILS (London) comes a new kit designed to take advantage of existing PCR

technology. The first phase of the kit allows users to prepare large amounts of plasmid samples or glycerol stock cultures without the tedious processes of cloning/plasmid preparation, restriction enzyme digestion, and insert purification. Once prepared, the amplified DNA insert can also be used as a substrate for a second phase of reactions.

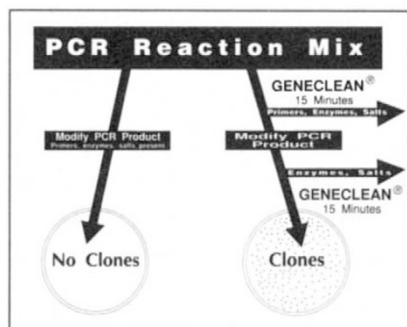
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### Cloning Kit.

From Invitrogen (San Diego, CA) comes the TA cloning kit that allows direct cloning of PCR products without the use of restriction or modifying enzymes. Thermostable polymerases consistently add a single A residue to amplified nucleic acids, allowing direct ligation into the TA cloning vector, which has been specially prepared to contain a single T overhang. The TA cloning kit eliminates the need for incorporation of restriction sites into PCR primers, and does not require purification of PCR products prior to ligation.

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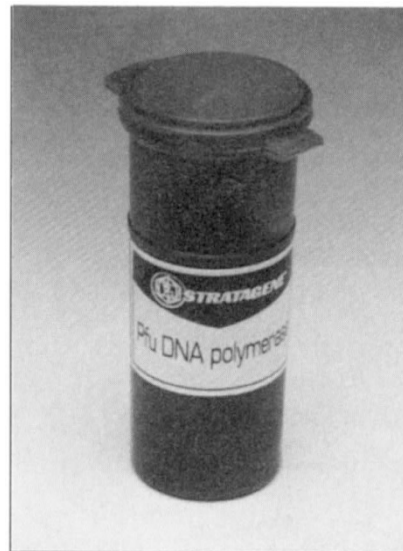


### Double GeneClean.

From BIO 101 (La Jolla, CA) comes the double geneclean procedure for PCR cloning. Products of thermostable DNA polymerase reactions contain "ragged ends" due

to non-template nucleotide addition reactions that interfere with blunt end cloning. Consequently, cloning of these products can be a significant problem. These problems can be overcome by incorporating the double geneclean procedure, without the use of gels. The cloning efficiency of amplified products is increased as much as 100 fold.

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### Polymerase.

From Stratagene (La Jolla, CA) comes *Pfu* DNA polymerase, a multi-functional, thermostable enzyme that possesses both 5' to 3' DNA polymerase activity, and 3' to 5' exonuclease proofreading activity. During DNA synthesis, the 3' to 5' exonuclease activity associated with *Pfu* DNA polymerase will excise mismatched nucleotides from the 3' termini of the primer. The polymerase contains greater than 95 percent activity following a 1 hour incubation at 95°C.

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### First Strand cDNA Synthesis.

cDNA templates suitable for subsequent amplification in a PCR can now be generated from limited quantities of unpurified mRNA using the First Strand cDNA Synthesis Kit from Amersham (Little Chalfont, U.K.). Based on AMV reverse transcriptase, the first strand synthesis system can be used to synthesize first strand cDNA from as little as 0.5-1.0 µg of unpurified total RNA using a single temperature, 40-minute incubation. When combined with PCR amplification, sufficient quantities of cDNA for cloning and analysis are generated.

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