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Polymerase chain reaction automated at low cost

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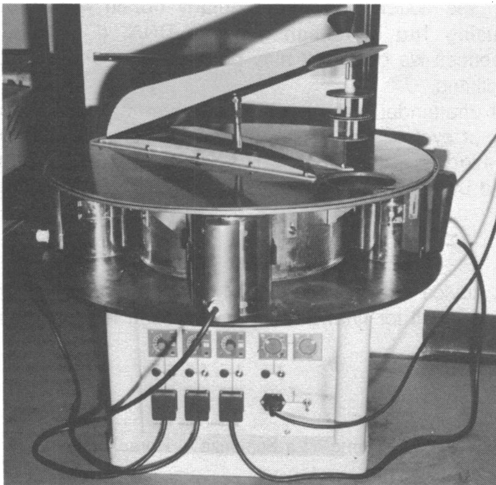
Few methods have become generally established in molecular biology as rapidly as the polymerase chain reaction (PCR; refs.1,2), utilizing the thermostable *Taq* polymerase (3).The repetitive nature of the procedure has created an obvious need for automation and commercial instruments have already been developed in the United States but elsewhere these are not readily available. The recent report in this journal of a simple, low-cost "DNA amplifier" (4) has prompted us to describe our experiences with a different mechanical apparatus that has been used successfully for several months in our laboratory (5).

We have modified a Histokinette, a conventional tissue embedding instrument which has been employed in histopathology departments for some thirty years, for the automation of PCR (Fig.1). A two tier, circular steel rack that can accomodate thirty six 0.5 ml microfuge tubes, was constructed and suspended from the mechanical arm. During one cycle, the arm pivots about the central axis and sequentially dips the rack into the three thermostatically controlled water baths. The tubes are completely submerged in each of the water baths; this reduces condensation and obviates the need for mineral oil. The process is repeated automatically for the required number of cycles.



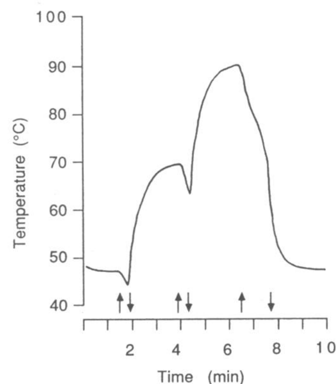


Fig.1 A Histokinette adapted for performing the PCR reaction using *Taq* polymerase (see text). Note the modified control panel. The arm is raised in the process of transferring the tube rack between water baths.

Fig. 2 Temperature profile of one PCR cycle. The graph was obtained by fixing a thermocouple inside a 0.5 ml reaction tube (Sarstedt 0.5 ml, 30 x 8 mm) containing 100 µl of reaction mixture. This probe was linked via an electronic thermometer to a strip chart recorder. The temperatures of the three water baths were 48°C, 71°C and 92°C respectively. Upward arrows denote the point of leaving a waterbath, downward arrows, the point at which the tube is submerged in a water bath.

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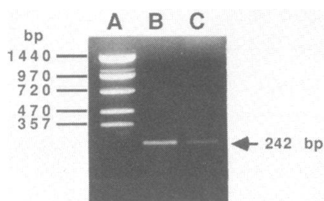

Fig. 3 The automated PCR reaction amplifies exon II of the human glucose 6-phoshate dehydrogenase (G6PD) gene (6). Two 23 mer primers with sequences flanking exon II were used to prime the reaction, which was carried out using *Taq* polymerase obtained from Perkin Elmer Cetus, according to manufacturer's recommendations. For each reaction, 30 cycles were performed by the machine, and 1/10th of the product was analysed by electrophoresis on a 1.5% agarose gel. **A**; *Taq* I digested pEMBL 8 DNA size markers. **B**; 242 bp G6PD exon 2 fragment amplified from 0.1 ng of phage clone DNA containing exon 2 of the G6PD gene. **C**; The same 242 bp fragment amplified from 1 μg human genomic DNA.

To adapt an old Histokinette (Elliots Ltd,Liverpool), the 9 unheated containers were removed, a new gear box mechanism was installed and the thermostatic control of the remaining 3 water baths was upgraded. In addition the mechanical timer was replaced with an electronic timer to allow accurate control of the incubations at each temperature.
Each cycle consists of 2 minute incubations respectively at 48°C, 71°C and 92°C. We have measured the temperature profile of one of the cycles (Fig. 2). We note that during the last two incubations, at the point when the tube leaves the bath, the reaction reaches temperatures one degree below that in the water bath. We regularly obtain $\approx 10^5$ fold amplification from 30 cycle-reactions using 1μg of human genomic DNA. (Fig. 3). To improve the yield of amplified DNA sequences we believe it may be necessary under some circumstances to modify these cycle conditions.
During the reactions, the machine is left unattended and by using an external timer, it can be shut down after the required number of cycles. Any well-equipped workshop should be able to make these low cost modifications to an existing Histokinette. It is perhaps noteworthy that many are being discarded by hospital laboratories in favour of more modern equipment.

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