Advances in automated DNA sequencing

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Sequencing DNA is a labour intensive and expensive process. At an estimated cost of $3–5 (US) per base pair, it would require about $25 million (US) to complete the sequence of *Escherichia coli*[1].

THIS article looks at the progress in automated DNA sequencing to increase throughput and reliability. New hardware and improvements in sequencing chemistry developed by Applied Biosystems (ABI) are discussed.

Hardware developments

The ABI Model 373A automated DNA sequencing system can run 24 templates simultaneously. One of the rate-limiting steps now becomes performing the sequencing reactions. The risk of pipetting errors and sample mix-ups can increase with this number of samples. A robotic workstation is under development at ABI that is designed to perform sequencing reactions on up to 24 different templates simultaneously (see Fig. 1).

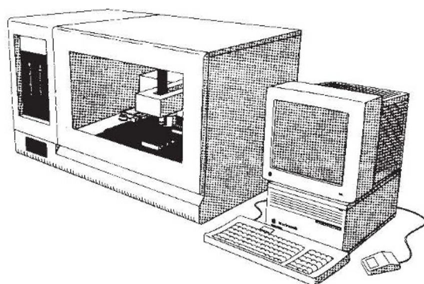

FIG. 1 DNA sequencing robotic workstation.

The worksurface contains refrigerated areas for storing labile reagents such as enzymes and templates, as well as an area for ambient storage. Reagents are pipetted from their storage area to the enzyme reaction module (ERM) using a syringe-based system coupled to a fixed stainless steel probe tip at the end of the robotic arm (see Fig.2).

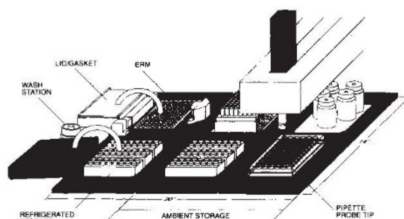

FIG. 2 Details of the worksurface.

An important design criterion of the ERM is evaporation control. This requirement is dictated by the high temperature (95 °C) and small volumes (5 μl) of *Taq* DNA polymerase-based sequencing protocols. An oil 'cap' is effective in preventing evaporation in manual methods, but is not suitable in automated systems. Instead, the ERM has a lid that automatically closes to seal the enzyme reactions. Wells are machined into an aluminium plate, which is then coated with an inert polymer. The well size is matched to the volume of the reaction to minimize condensation. A gasket in the lid seals all 96 wells by compressing on a machined rim. To prevent condensation from occurring on the lid while in the closed position, it is heated to 2 °C above the reaction temperature.

The aluminium plate is a thermal cycler with a temperature range from 2 to 95 °C. The high thermal conductivity of the aluminium ensures rapid and uniform heating or cooling of the samples. To initiate all reactions simultaneously, the robot loads the ERM at 4 °C and then rapidly heats the plate to the desired temperature. This design prevents evaporation and ensures temperature uniformity between wells. The obvious disadvantage of a reusable plate is that it must be cleaned. However, a relatively simple and rapid method for purging the block between reactions has been determined.

Reagents are pipetted using a single stainless steel probe tip coupled to the syringe pumps. A positive displacement effect is achieved by filling the pumps and line with water. The system can detect when the probe tip touches a liquid surface because the stainless steel probe tip has capacitive sensing. This allows reagents to be delivered at the surface of the reaction, which minimizes probe tip contamination. Increased reliability and lower cost are also achieved with this design because disposable tips do not have to be coupled and uncoupled from the system.

The most important design criterion, however, is the ability to pipette sub-microlitre quantities. Prototype instruments are able to pipette 1 μl and 0.1 μl with a variation of one and eight per cent, respectively. This level of performance was impossible with disposable tip systems. Again, the disadvantage is the probe tip must be washed between pipetting steps.

Prototype instruments are able to perform fluorescent primer sequencing reactions using T7 or *Taq* DNA polymerase on 24 samples in less than 4.5 hours. Less than one hour is required to clean the ERM between runs. One robot can easily supply the needs of two automated sequencers, such as ABI's 373A.

With sequencing protocols, contamination between samples is not a problem. The normal ERM and probe tip washing protocols are effective at cleaning the system. The obvious concern is for polymerase chain reaction (PCR) applications. Recent experiments suggest that both the wells and the probe tip can be cleaned of amplified DNA by use of dilute nitric acid. Further work is needed in this area, but it seems likely that a cleaning protocol suitable for PCR applications can be devised.

Chemistry improvements

Although the robotic workstation reduces labour requirements and error, and increases the reliability of sequencing, not all applications require this level of throughput. Improvements in the *Taq* sequencing protocol have reduced both the labour requirement and cost of performing sequencing reactions manually. This has been achieved with linear amplification of signal by thermal cycling with a single fluorescent primer (in contrast to the exponential amplification achieved with PCR). Both labour and error are reduced for manual sequencing because the separate annealing step is no longer needed and a denaturation step is not required with double-stranded templates. Costs are reduced because less enzyme and template are required. The need for less template permits the use of larger inserts for primer-directed sequencing, such as cosmid sequencing in which the mole per cent of real target is considerably less than with M13 and plasmids (R. Kaiser, personal communication). Fluorescent signal level remains high because of the linear amplification. The combination of the robot and the *Taq* cycle sequencing protocol should allow further reductions in the amounts of reagents required. □

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1. Watson, J.D. *Science* **248**, 44–49 (1990).

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