GENOMICS

Light-speed genomics

A pico-scale reaction system serves as the basis for a new generation of high-throughput sequencing machines that promise to bring genome-center power down to the laboratory level.

Sanger sequencing has had an astonishingly good run, remaining virtually unchallenged during nearly three decades as the sequencing technology of choice for everybody from individual lab technicians all the way up to multi-institutional genomic analysis consortia. Nonetheless, genome-scale analysis with existing Sanger-based fluorescent sequencing technology is expensive, labor-intensive and time-consuming, and many would say that a new sequencing technology is well past due.

One alternative to emerge in recent years is pyrosequencing, a so-called 'sequencing by synthesis' strategy. As each base is added to a primer-template hybrid, a luciferase-coupled enzymatic reaction allows light-based, real-time detection of successful nucleotide incorporation (Ronaghi *et al.*, 1998). This technique forms the foundation of a new generation of high-throughput sequencing machines developed by 454 Life Sciences, and described in a new article in *Nature* (Margulies *et al.*, 2005). In this system, genomic DNA of interest is fragmented and ligated to polymer beads under conditions that favor one fragment per bead. The bound fragments are subjected to emulsion PCR, a process by which simultaneous amplification is performed on hundreds of thousands of such beads within the confines of individual aqueous droplets in oil. Single beads are then deposited into picoliter-capacity wells on a precisely engineered slide, and subjected to automated pyrosequencing via a fluidics system, while the results for each well are read and analyzed by a computer connected to a charge-coupled device camera.

As an initial trial, the authors demonstrate the successful sequencing of the *Mycoplasma genitalium* genome in 24 hours; the present system requires exclusion of repeat sequences, but the team nevertheless obtained 96.5% genomic coverage with an accuracy of 99.96%. Michael Egholm, a lead author on the study and vice president of Molecular Biology at 454, attributes the system's success to the fruitful collaboration of scientists from a broad range of scientific disciplines, and describes their system as an important breakthrough for genomics. "We've democratized sequencing," he says, "in that we allow your average research laboratory to do entire organism sequencing… [and] within the next two years, comfortably, we'll be able to do routine, whole human sequencing." Similar sequencing systems are now under development by several other groups as well, offering potential competition to the 454 system, but Egholm believes that low cost, efficiency and high speed will give their approach a strong edge. Whatever the outcome, all of this innovation represents an important first step beyond the constraints of an aging technology. "What we have commercialized now is just the beginning of a new cycle," says Egholm.

Michael Eisenstein

RESEARCH PAPERS

Margulies, M. *et al.* Genome sequencing in microfabricated high-density picolitre reactors. *Nature*; published online 31 July, 2005.

Ronaghi, M. *et al.* A sequencing method based on real-time pyrophosphate. *Science* **281**, 363–365 (1998).