DOI:
10.1038/nrg2238

MILESTONE 18

The next generation arrives

Although Sanger sequencing served researchers admirably for almost three decades, in recent years there has been increasing pressure to produce ever-larger amounts of sequence as rapidly and cheaply as possible. These demands have catalysed the development of a new generation of sequencing technologies.

Sequencing can be made faster and cheaper if a single volume of reagents is used in parallel on thousands or millions of targets. In 1998, Ronaghi and colleagues showed that a method they had recently developed, known as pyrosequencing, could be carried out on a solid support and was therefore suitable for such multiplexing. In pyrosequencing, pyrophosphate — released upon nucleotide addition by DNA polymerase — is converted to ATP. This triggers a luciferase enzyme to produce light, which is used to detect an incorporation event, so that a sequence read can be built up over successive rounds using different deoxynucleotides. Importantly, because nucleotide addition is detected by the emission of photons, this method is well-suited to detection using simple optics and automated data collection.

Another important advance came in 2003, when Mitra and colleagues described an approach that allowed the multiplexing of both template amplification and sequencing. They adapted an existing method, known as polymerase-colony technology, which involves the amplification of millions of DNA molecules by PCR in an acrylamide gel. Because the products are prevented from diffusing away, a spherical colony of DNA — known as a polony — is formed for each target. This paper showed that sequencing can be carried out

on polonies, allowing many reactions to take place in parallel.

In 2005, two papers illustrated the benefits of advances in sequencing technology, describing the rapid sequencing of whole bacterial genomes. Shendure and colleagues used polony-based amplification combined with another new method — sequencing by ligation — which, similar to pyrosequencing, involves successive rounds of detection. Here, a primer is hybridized to a known sequence next to the DNA target. DNA ligase then joins oligonucleotides, which are fluorescently labelled at one position, to the primer. Because the ligase prefers to join molecules when the bases in double-stranded DNA match, the fluorescent signal from the ligated oligonucleotide can be used as a readout of the target sequence, again allowing automated data collection. In the second 2005 paper, Margulies and colleagues described a sequencing system that uses fibre-optic slides with more than 1 million wells. They showed that robust pyrosequencing could be carried out in the resulting picolitre volumes, and sequenced an impressive 25 million bases in a single run.

With high-throughput sequencing now becoming available to increasing numbers of researchers, next-generation approaches are set to bring major advances in genetics and genomics, from the rapid sequencing of new genomes, to the large-scale characterization of genetic variation in populations, to personalized genomes.

Louisa Flintoft, Senior Editor, Nature Reviews Genetics

ORIGINAL RESEARCH PAPERS
Ronaghi, M., Uhlén, M. & Nyrén, P. A sequencing method based on real-time pyrophosphate. *Science* **281**, 363, 365 (1998) | Mitra, R. D., Shendure, J., Olejnik, J., Edyta-Krzymanska-Olejnik, & Church, G. M. Fluorescent *in situ* sequencing on polymerase colonies. *Anal. Biochem.* **320**, 55–65 (2003) | Shendure, J. *et al.* Accurate multiplex polony sequencing of an evolved bacterial genome. *Science* **309**, 1728–1732 (2005) | Margulies, M. *et al.* Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**, 376–380 (2005)

FURTHER READING
Shendure, J., Mitra, R. D., Varma, C. & Church, G. M. Advanced sequencing technologies: methods and goals. *Nature Rev. Genet.* 5, 335–344 (2004)