GENOMICS

Gene reading steps up a gear

Third-generation sequencing machines promise to make their mark one molecule at a time.

BY HEIDI LEDFORD

"It's super cool, but it's never going to work," genomics guru Eric Schadt responded when a wary investor asked for his opinion about a new DNA-sequencing technology in 2003. A company was creating a machine that it claimed could revolutionize the field by reading over the shoulder of an enzyme as it copied DNA molecules.

Despite his initial scepticism, Schadt touted the method's success last weekend at the Advances in Genome Biology and Technology meeting in Marco Island, Florida. Now chief scientific officer at the company he had once doubted — Pacific Biosciences in Menlo Park, California — Schadt was one of several researchers at the meeting who provided a glimpse of how the company's first DNA-sequencing machines are performing.

All eyes are on these machines. Pacific Biosciences set a high bar for its own success in 2008, when chief technology officer Stephen Turner boasted that the instruments would be able to sequence a human genome in just 15 minutes by 2013, compared with the full month it took at that time. This year, as researchers unveiled data from the first machines to leave the company's campus, the discussion was less about revolutionizing the field and more about niche applications.

After several delays, customers have now been told to expect their machines in the second quarter of this year.

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The machines potentially offer advantages over the 'next-generation' sequencers currently on the market. Users of the new machines last week reported generating sequences an average of 1,500 base pairs long — about ten times the length of those currently produced by the state-of-the-art sequencers from Illumina in San Diego, California. These longer reads make it easier to stitch fragments of DNA sequences together into a coherent genome sequence.

Pacific Biosciences' machines are also fast. In a paper published online in December, Schadt and his team used them to trace the origin of the ongoing cholera outbreak in Haiti by sequencing the genomes of five strains of *Vibrio cholerae* (C. S. Chin *et al. N. Engl. J. Med.* **364,** 33–42; 2011). The team sequenced all five strains in less than an hour. It takes about a week to complete a 150-base sequencing run on an Illumina sequencer.

IN A FLASH

New DNA sequencers watch an enzyme called DNA polymerase as it uses fluorescently tagged bases to synthesize DNA. Each base is identified by a distinguishing colour that flashes as the base is incorporated into the DNA strand.

But for many researchers, the key advance of the Pacific Biosciences machines is the ability to sequence single molecules of DNA. The instruments work by watching as an enzyme confined within a tiny compartment copies DNA, adding fluorescently labelled bases that flash with characteristic colour as they are added to the DNA strand (see 'In a flash'). Leading sequencers on the market instead report an average sequence taken from a population of molecules.

Single-molecule sequencing opens the door to analysing rare sequence variants, and frees researchers from having to amplify DNA samples before sequencing — a step that can introduce errors, and can fail altogether for certain DNA sequences. "Single molecule is the future of sequencing," says Michael Metzker, who studies sequencing technology at Baylor College of Medicine in Houston, Texas. "But it still has hurdles."

Chief among those hurdles has been high error rates. Whereas other methods on the market surpass 99% accuracy, users of the Pacific Biosciences machines last week reported an accuracy rate of about 85%. Schadt argues that this can be overcome by resequencing the same molecule repeatedly.

Nevertheless, because of the cost of its machines (US$700,000 per unit compared with less than $125,000 for the new Illumina sequencer rolling out this autumn) and limits on the number of sequences that can be read during every run, the instruments are unlikely to disrupt the sequencing market in the near future. For now, the machines are likely to be used for tackling regions of the human genome that resisted conventional sequencing. The instruments can also detect some chemical modifications to DNA, which could be useful to the burgeoning epigenetics field. Peter White, who heads the sequencing centre at Nationwide Children's Hospital in Columbus, Ohio, says he is interested in acquiring a machine, but would mainly use it to analyse microbial genomes, which tend to be much smaller than mammalian genomes.

At the meeting last week, Turner did not reiterate his pledge for a 15-minute human genome. But he did emphasize that there is still plenty of room for the current instrument to improve. "We are just at the beginning of this technology." ■

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CORRECTIONS

The News story 'Social science lines up its biggest challenges' (*Nature* **470,** 18–19; 2011) should have said that Nick Nash did his MBA at Stanford University.

The News Feature 'Exoplanets on the cheap' (*Nature* **470,** 27–29; 2011) should have said that the spectrometer on which the comb at the Hobby-Eberly Telescope was mounted came from Pennsylvania State University not the University of Pennsylvania.

The graph in the News Feature 'The End of the Wild' (*Nature* **469,** 150–152; 2011) showing a correlation between rising minimum temperatures in Wyoming and increased survival rates for mountain pine beetles should have made it clear that the beetle data were modelled not measured.