



# Bio-IT World

Indispensable Technologies Driving Discovery, Development, and Clinical Trials

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## Pacific Biosciences Preparing the 15-Minute Genome by 2013

BY KEVIN DAVIES

Feb. 12, 2008 | Marco Island, FL — Midway through this year's "Advances in Genome Biology and Technology" conference, Pacific Biosciences sponsored a beachfront fireworks display to promote its name and celebrate its emergence from years in stealth mode. Perhaps the 600 or so attendees were intended to imagine the exploding multi-colored fireworks as a metaphor for the captured fluorescence at the heart of the company's novel DNA sequencing technology.

But it turns out that Pacific Biosciences didn't really need to burn money on pyrotechnics after all. The closing talk, by company founder and Chief Technology Officer Stephen Turner, was all the delegates could talk about.

"How cool was that?!" purred Washington University's Elaine Mardis, following Turner's talk. Writing in his Tree of Life blog, evolutionary geneticist Jonathan Eisen said Turner's talk "really did blow my mind ... The long reads, coupled with many molecules per run, plus the high speed, this technology is the first I have seen that has shown some results and that could really lead to the \$1000 human genome." And Yu-Hui Rogers, scientific director of the J. Craig Venter Joint Technology Center, hailed the technology as "potentially revolutionary."

Such sentiments were echoed afterwards by many other academics and, interestingly, by staffers from most of the current crop of next-generation companies, no doubt girding for extra competition a few years down the road.

Turner confidently predicted that within five years, his technology will be able to produce a raw human sequence in less than three minutes, and a complete, high-quality sequence in just 15 minutes. Genome scientists have heard similar hype before, but Turner's preliminary data left most of the audience believing that this technology has the potential to be truly disruptive.

The company's CEO, Hugh Martin, a former telecommunications executive, told Bio-IT World that a commercial instrument won't be ready until 2010 or 2011 at the earliest. PacBio has raised almost \$80 million thus far, and is looking to raise a lot more to finance commercialization. But that didn't stop Martin from confidently proclaiming to The New York Times last weekend, "When we're ready, we're just going to win the X Prize."

### In the Light

PacBio was founded in 2004, but the technology dates back to Turner's days as a grad student and post-doc at Cornell University. The SMRT (single molecule real time) system monitors the real-time procession of a DNA template as it interacts with a single DNA polymerase enzyme. Using four fluorescently tagged nucleotides, the system images each nucleotide as it is bound by the enzyme. The polymerase is tethered to the bottom of a zero mode waveguide (ZMW) — a sub-microscopic, 20-zeptoliter well that the company claims is "the world's smallest detection volume." All this happens at a speed of about 10 bases/second (in nature, the polymerase moves 50-75 times faster).

Using the ZMW concept that Turner and his former Cornell colleagues, physicists Harold Craighead and Watt Webb, published in Science in January 2003, the PacBio method ingeniously illuminates the area around the tethered enzyme, while leaving the unincorporated fluorescent bases floating in the dark. The light generated by each nucleotide held by the enzyme is recorded by a CCD camera through a prism, to identify the color and corresponding identity of each nucleotide.

Turner likens the principle to the small holes in the mesh screen door of a microwave oven, which do not allow the longer microwave radiation to pass through. Here's how he described it to Bio-IT World:

"If you shrink both the radiation wavelength and the holes down to the nanoscale, so the wavelengths go to visible light of 500 nm, and the holes are just a few tens of nanometers in diameter, the result is that if you illuminate the hole through the transparent substrate that's holding it, light that impinges upon the circular aperture of the hole can't pass through the hole. So all of these nucleotides, while close at hand and accessible to the polymerase, are in the dark and don't contribute to the background noise."

Meanwhile, the enzyme sits at the bottom of what Turner colorfully calls "the evanescent decay zone of the device," allowing his team to specifically detect the emanating fluorescence from the enzyme.

Turner pointed out several key advantages of the SMRT system. Each nucleotide carries its fluorescent tag at the very end of the molecule, such that the tag is cleaved away and not incorporated into the growing DNA strand. And because the system is a close facsimile to in vivo DNA synthesis, the read lengths will be comparable to Sanger sequencing — hun-

dreds or thousands of contiguous bases — thus avoiding the bioinformatics challenges of assembling very short reads. Moreover, there are no moving parts, aside from the polymerase itself, once a run is started.

Turner presented preliminary data on synthetic DNA templates. He presented CCD images showing a grid of 1000 ZMWs on a chip smaller than a pinkie fingernail, which burst into fluorescent life when all the necessary ingredients were presented to the enzymes sitting in each well. That's a throughput of 36 megabases/hour. (The video had to be slowed down, because the human eye wouldn't be able to register the images in real time.) "No-one's ever seen 1000 polymerases making DNA before in real time," says Martin.

Although the SMRT system is far from perfect, Turner presented readable sequence traces from known DNA templates, as well as the ability to derive consensus sequences by using circular templates — a method that will presumably be exploited down the road. Turner says PacBio is launching its first genome sequencing project, after which it will have a much better sense of the accuracy of the system. That will be improved by directed evolution of the polymerase enzyme, work already in progress.

Although PacBio is still a couple of years at least from debuting its instrument, Turner outlined several future enhancements that will, he predicts, deliver the 15-minute human genome. First, produce a chip with 1 million ZMWs (no bigger than the current prototype, which only uses 0.1 percent of the available real estate). Second, increase the speed of DNA polymerization from 10 to 50 bases/second — the limiting factor here is not the enzyme but the detection capability. And finally, use a 20-megapixel CCD camera with on-chip magnification to make it single-photon sensitive. Both of these technologies exist now, he said, they just have to be brought together.

The projected output with these enhancements, in about five years' time, is 100 Gigabases/hour. "This is what is required to get genome sequencing into routine medical practice," says Turner. "It is disruptively faster than current next-generation technologies. Instead of being hours per base, it's bases per second." ●



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