

## In increasingly competitive DNA sequencing market, Pacific Biosciences argues its low cost tools meet the need for more detailed information

Adoption of new micromanufacturing technologies is bringing dizzying change to the DNA sequencing business. The first of this new generation of high speed, low cost systems hit the market in 2011, only to be followed already in early 2012 by announcements of still faster, cheaper models. Pacific Biosciences now faces more competition, but stresses it offers fast turnaround of more detailed information for practical applications.

**S**hrinking government budgets for funding basic research—and the daunting difficulty of finding actionable information in the vast complexity of interrelated genetic data—have slowed the market for high volume DNA sequencing tools. Suppliers are instead starting to target the emerging market for clinical applications, where the lower cost tools enabled by micromanufacturing innovations can offer faster results, at least for smaller volumes of data from small genomes or select parts of genomes.

Sales of the new breed of microsystems-based sequencers started to shake up the market in 2011. Pacific Biosciences says it's sold 55 of its MEMS-based units to date, since introducing its commercial product in April, taking in revenues of ~\$21 million for the six months through September at last report.

But the competition is heating up. Ion Torrent has made a bigger splash since launching its semiconductor-based product at the end of 2010, reporting sales of some \$41 million for the nine months through September 2011. Its technology cuts costs by electrically sensing hydrogen ions given off by distinctive base reactions, eliminating the need for optical markers, and the company has shown it can scale the semiconductor technology to more sensors per chip to increase throughput, moving from 1 million to 12 million sensors per chip in the second generation last year, and then recently announcing plans for 165 million sensors in the second half of this year, and 660 million by 2013. The company's claim that it can sequence the full human genome in a day for \$1000 on a \$150,000 tool has been met with considerable skepticism, but it's clearly making progress. And it now plans to submit its smaller first generation tool for approval for clinical diagnostics. Market leader Illumina also announced its own new genome-in-a-day sequencer for later this year, albeit at closer to \$700,000.

### More detail from direct observation of single molecules in real time

Pacific Biosciences says it's not playing the same high volume data game. The company's MEMS-enabled solution for ultra high resolution microscopy can see the DNA replication one molecule at a time in real time. This eliminates the time consuming step of amplifying the DNA first, and allows reading of a longer segment of a strand at one time for faster results and simpler assembly of the analyzed segments into a connected whole genome sequence afterwards. The low cost tool gives fast and detailed information but for relatively small genomes, like those of bacteria and viruses or targeted parts of human genome. "It's not the high throughput workhorse for repeat sequencing," notes CTO Steve Turner. "It's more expensive for the cost per base, but gives higher level information that they can't get any other way, and complementary with the second generation machines." In contrast, the mainstream high volume, high throughput sequencers are more efficient in sequencing the whole 3 billion bases of the full human genome, by looking instead at many nominally identical amplified molecules at once, one reaction step at a time, with pauses between steps to wash away one reactant and introduce the next.

Turner says they stumbled upon the unusual nanoscale behavior that enables 1000x higher resolution imaging of the polymerases scooting along the DNA strand when looking into using nano-temperature near field scanning microscopy. His research group discovered that a metal film with tiny holes got stronger axial confinement of observation inside the bore of the hole, than outside it-- the conventional (and intended) mode of using them. Inside, the light is attenuated in as little as 10-20nm, keeping the light from propagating upwards, allowing



SMRT Cell 8Pac  
(Courtesy of Pacific Biosciences)



**Dr. Turner**  
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He was awarded a Ph.D. in Physics by Cornell University in 2000, where he worked with Prof. Harold Craighead to study the behavior of biomolecules in nano-fabricated structures. His work contributed to the establishment of the Nanotechnology Center at Cornell. Dr. Turner's undergraduate work was at the University of Wisconsin, Madison, where he received a Bachelor of Science in Applied Mathematics, Electrical Engineering and Physics. He is listed as the inventor on nine U.S. patents and more than 20 published patent applications.

imaging down to the zeptoliter ( $10^{-21}$  l) range allowing operation up into the micromolar region, high enough resolution to see the polymerase replicating each base, and about half of the enzymes in nature, most of which can't be seen at the single molecule level by other methods.

MEMS houses helped develop the initial manufacturing technology on standard tools. Improvements to optical lithography over the years allowed researchers to move from e-beam writing of holes in a metalized glass plate, to optical lithography with improved performance and higher throughput. An optical MEMS paraboloid reflector below each tiny hole—a gumdrop-like structure in the glass with a reflective inner surface—folds the light into a lower angle to reduce losses.

### Counting on more information in targeted areas

Looking at the continuous process across many steps of replication of one strand of DNA also provides information on the time of response of the chemistry, which turns out to be very useful in distinguishing other, epigenetic, base variations like methylation that turn genes on or off and significantly impact traits, for changes not explained by just the paradigm of mutations in the genome. "We see from the different tempo and rhythm that some Cs are changed into something else," says Turner, noting that these and other epigenetic traits essentially create an alphabet with more than 20 different bases that explains things the 4 main bases alone cannot, from how poor diet in pregnancy changes the epigenome of the next generation, to what changes make bacterial strains become more virulent. "Researchers have looked hard, but haven't been able to find many common individual letter changes that can usefully inform healthcare," he notes. "It's to a huge degree determined by evolution that the paradigm of common single-letter changes didn't give us the gains we hoped for."

Beyond the first lab users doing sequencing and genomic assembly, Turner says the company is now seeing interest particularly from the microbiology field who want a genome, but at the lower cost per run of the company's smaller runs. It's also seeing adoption for validation of cancer research, for checking the accuracy of results. Though some critics say Pacific Biosciences' tool is less accurate than others, Turner argues that its longer read lengths simplify matching sequence patterns to assembly the complete genome from the longer parts, and its errors within or between runs are random, so repeat runs on the same strand quickly produce very accurate consensus data, while errors from the high throughput sequencers are more likely to be systematic and thus repeated.

The company got to show off the advantage of its fast detailed analysis of short bacterial genomes with the cholera outbreak in Haiti. Turner says it completed all the sequencing in five hours, but was already able to see after just 45 minutes by comparing deletions in the genome to those of others that the strain had originated in SouthEast Asia. With the German e-coli outbreak, Ion Torrent sequenced the bacteria first, but Pacific Bioscience followed up by quickly sequencing that and 11 other e-coli examples, and then using its longer read lengths to identify patterns indicating areas where exchanges with other bacteria apparently created more virulent toxins and antibiotic resistance.

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