The sequencing shakeup

Deep sequencing technology could soon be competitive with certain array applications. But the jury remains out on which of the myriad platforms will have the greatest impact and broadest application. Amy Coombs investigates.

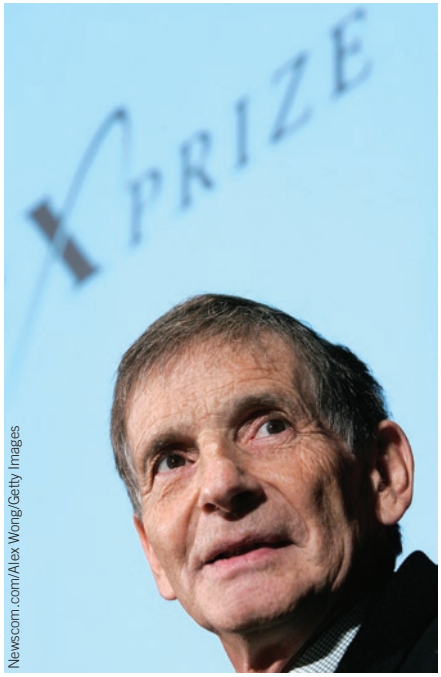
Next-generation sequencing (NGS) is currently taking the research community by storm. This year alone has seen the launch of two entirely new platforms—the Polonator, offered by Salem, New Hampshire–based Dover, and Cambridge, Massachusetts–based Helicos's HeliScope. These, like other NGS technologies, are touted as being faster and cheaper, poised to be both disruptive and revolutionary. Not only will they open doors for unrealized diagnostic and healthcare applications, say analysts, but also they could supplant related technologies such as microarrays. "People are very interested in trying these technologies," says Derik de Bruin, a life science analyst at UBS Bank in New York who follows the sector[1]. "It's almost like a feeding frenzy."

New platforms

Since 454 Life Sciences (now part of the Basel-based Roche) debuted its 454 FLX pyrosequencing platform in 2005, the gene sequencing market dominance of Foster City, California–based Applied BioSystem's (ABI) (3730xl DNA Analyzer) series capillary array electrophoresis sequencing machines has been challenged by two other commercial platforms: San Diego–based Illumina's Genome Analyzer (Solexa) platform (which was launched in 2006) and ABI's own SOLiD system, first sold in 2007 (**Table 1**).

These platforms remain relatively expensive at present and all have sequence read-lengths shorter than those of conventional capillary array electrophoresis. However, the high rate of throughput and base calling accuracy of these so-called 'deep sequencing' platforms is opening up applications in transcript profiling, genome-wide chromatin immunoprecipitation sequencing, copy number variant analyses and metagenomics.

In one recent example, which appeared in the July issue of *Nature Methods,* University of Queensland, Australia researcher Sean Grimmond and colleagues developed a method for sequencing RNA using ABI's SOLiD platform, with which they compared the transcriptomes of mouse embryonic stem cells before and after differentiation[2].



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Company carrot? Stewart Blusson, president of Archon Minerals, speaks during a news conference to announce the Archon X PRIZE for Genomics on October 4, 2006 in Washington, DC. The X PRIZE Foundation announced that the first team to successfully sequence 100 human genomes in 10 days will be awarded $10 million.

"The transcriptomes were characterized with unprecedented depth and resolution," wrote Jay Shendure, of the University of Washington in Seattle, in an accompanying News and Views[3]. "This type of a study would have been prohibitively expensive if conducted with conventional sequencing technologies, but the fast NGS reads makes this type of analysis possible," he notes.

NGS is also proving useful for chromatin immunoprecipitation (ChIP), which is used for mapping sites of protein-DNA interactions along chromatin. In June 2007, Stanford's David Johnson teamed up with Barbara Wold of Pasadena's California Institute of Technology, using Illumina's Solexa platform to develop a high-throughput method for mapping transcription factor binding sites. Applying their technology, they discovered 1,946 binding sites for the neuron-restrictive silence factor on the human genome[4].

As such, the deep sequencing platforms not only represent competition for capillary electrophoresis Sanger sequencing but also for array-based platforms, such as Affymetrix's photolithographically synthesized oligonucleotide chips and Illumina's oligo bead arrays coupled to optical fibers, which are currently the state of the art for genome-wide association studies.

Diversified markets

None of the analysts interviewed for this story believe that NGS will entirely displace microarray technologies. Yet some say some array applications are already facing disruption, and analysts predict NGS technologies used for such applications may bring in as much as $215 million by 2012 (ref. 1). "NGS technology will compete with arrays," predicts de Bruin. "The lower price per data point potentially offered by next-generation sequencing makes routine, large-scale gene expression and transcriptome analysis possible."

These kinds of results cause analysts to predict favorable outcomes for encroaching NGS technologies. "Displacement is already happening. The question is how quickly NGS technology will penetrate this segment of the microarray market," says de Bruin. "No one can ultimately know what will happen down the road."

The total microarray market was estimated to be $800 million in 2006, 65% of which was dedicated to gene expression analysis[1]. The remaining 35% includes genotyping arrays, and according to Harvard University's George Church, these may also feel the crunch. Resequencing, single nucleotide polymorphisms (SNPs)and copy number variant arrays may be affected. "It looks to me that they are all nearly equally vulnerable," says Church, whose laboratory developed the Polonator.

Still, others predict far less disruption in the microarray space. Even as NGS units become less expensive, researchers may stick by old technologies, say analysts.

"Who knows what the research community will prefer in the end?" asks Jonathan Witonsky, an analyst at the Palo Alto, California-based Frost and Sullivan. "There are still some proof-of-principle experiments that need to be conducted to establish whether NGS is really better," he adds.

Marketing muscle

The success of NGS technologies in the array market may ultimately come down to

advertising and marketing. Research conducted by Frost & Sullivan indicates that established technologies like microarrays remain the platforms of choice for applications, like expression analysis[5]. The company surveyed academics and private sector researchers last summer, and found low levels of adoption for NGS platforms.

"While the research community is paying a lot of attention to these new platforms, and awareness is high, we found that adoption is still very low," says Witonsky.

This may be due to cost. With a median price of $500,000 per instrument, labs have to get a lot of use out of the purchase to justify the investment. Even at the low price of $155,000 per unit, the Polonator might not make sense for small labs. At $150 per chip, microarrays are probably a more affordable option.

The Santa Clara, California-based array provider Affymetrix is paying close attention to competing NGS gene expression applications, according to Jay Kaufman, vice president of marketing. "NGS will impact some array applications more than others, but it will be awhile before there is a 180-degree turn to sequencing in place of arrays for gene expression," he says.

Kaufman also predicts that the Affymetrix platform will remain competitive for whole genome association studies for the foreseeable future. "Due to the sample cohort size that is typically required for association studies and the growing importance of looking at copy number variations in these studies, NGS is probably not best tool for this type of study," he suggests. "It will be a while before sequencing threatens these applications."

This may keep array sales stable, despite the potential competition from the new sequencing market, says Witonsky.

Synergies among platforms

Manufacturer and investor interest may also protect the array market. As Illumina and ABI both have microarray products, it doesn't make sense for them to cannibalize their existing markets with the new sequencing platforms. With its core array business being genotyping, Illumina maintains this will not be replaced by NGS technology. According to CEO Jay Flatley, NGS may be used to selectively replace certain targeted genotyping studies that have previously relied on arrays. Yet, he says that the company's largest array markets will not be affected by NGS.

In June, 2007, Roche strategically acquired the Madison, Wisconsin-based NimbleGen, which markets arrays for selectively capturing genomic subregions for sequencing, and has since bridged the two technologies through its Sequence Capture System (see p. 1101). "Arrays and sequencing are complementary technologies," says 454 Life Sciences CEO Chris McLeod, "This is extremely useful in identifying the elusive causative mutations in disease-associated regions identified by whole genome association."

"It may not be that chip technology and high-speed sequencing are dichotomous, the goal may be to bridge them," says Witonsky. "The companies that will be most successful might approach pairing the two."

Indeed, several possible array-NGS collaboration scenarios exist. For example, high-throughput NGS methods may identify new targets that can then be incorporated into DNA microarrays. "[Most] array experiments are good for looking at gene expression but only in cases where the target sequences are known," says Ilan Zipkin, an investor at the Palo Alto, California-based Prospect Venture Partners. "NGS technology might help identify new targets for array analysis."

Illumina recently developed just such an application. The company used its Solexa-based Genome Analyzer to identify 24,000 bovine SNPs, from which it created a custom array, the BovineSNP50 BeadChip. According to Flatley, "Our customers are using our array and sequencing technologies together more and more frequently."

Affymetrix won't confirm any possibilities of a NGS acquisition or partnership, but Kaufman says the company hopes to leverage all the information coming out of new sequencing platforms. "While sequencing is good for generating a lot of data, this will drive people to want to do array experiments," he says. "This is why I don't think sequencing is going to make arrays obsolete any time soon. There will be a place for both technologies, and there are many ways they can even compliment one another."

In late 2007, Affymetrix acquired USB, and at the time, CEO Stephen Fodor predicted rolling out a new chip with longer oligos (75-mers rather than their customary 25) onto which sequencing could be done using enzymes acquired in the transaction.

Follow the money

Whereas most NGS platforms have yet to reach the $100,000 milestone, let alone the $1,000 one (set by the National Human Genome Research Institute in Bethesda, Maryland in 2007), some of the small innovators, like Menlo Park, California–based Pacific BioSciences, VisiGen Biotechnologies of Houston, and the Waltham, Massachusetts–based Intelligent Bio-Systems are promising $1,000 genomes within the next five years. These companies are taking advantage of traditional routes of company funding, such

Box 1 And the winner is…

…Not going to be known for awhile. Archon X Prize Foundation—the group that gave us the $10 million Ansari X Prize for private space flight—has thrown down the gauntlet for human genome sequencers. Sequence 100 genomes in 10 days, for less than $10,000 per genome and you can win $10 million from the Santa Monica, California–based foundation. So far, seven teams have ponied up the $1,000 entrance fee and, not surprisingly, none has yet indicated that they are ready to go. There are two potential start dates per calendar year and a team must indicate its readiness at least four months prior so the other teams can be apprised and join the "attempt" if ready. The participating teams for that attempt will then be provided with 100 DNA samples and the clock starts ticking.

The teams in the competition so far present an interesting array of technologies (so far as it can be known). Entrants from the commercial side include Roche (454 Life Sciences) and VisiGen. In addition, a North Reading, Massachusetts–based startup, ZS Genomics, which is working on an electron microscope–based DNA sequencer, has also recently entered. Several consortia are also taking part: a team assembled by The Foundation for Applied Molecular Evolution in Gainesville, Florida; Reveo, a technology R&D corporation with headquarters in Hawthorne, New York; a team from Warwick, UK, called base4 Innovation; and a group formed around the George Church Polonator technology (the Personal Genomics X team). Unlike the Ansari X Prize, government labs and federally funded labs are eligible in this competition.

According to Michael Timmons, coordinator of the Archon Prize, at the rate that the cost per genome is dropping, some teams may be ready to compete in as soon as two years. And although the panel of judges has not yet been announced, some of the usual suspects—Craig Venter and Leroy Hood, for example—are likely to among them, judging from the X Prize's scientific advisory board. *Laura DeFrancesco*

Table 1 Selected next generation sequencing companies

Company	Platform	Developer	Business	Status (price of instrument)
Applied Biosystems	Bead-based massively parallel clonal ligation based DNA sequencing	Agencourt Personal Genomics, Cambridge, Massachusetts	Public company; revenue from instrument and reagent sales	Launched SOLiD in October 2007 ($591,000)
Complete Genomics	Combinatorial probe-anchor ligation on DNA nanoarrays	Rade Drmanac, Complete Genomics, Mountain View, California	Private company; $46.5 million raised	Launched as a service company this month ($5,000/human genome sequence)
Dover, a Danaher Motion Company	Polymerase colony sequencing by ligation	George Church, Harvard University, Cambridge, Massachusetts	Public company; revenue from instrument and reagent sales	Polonator shipped to first users in February ($150,000)
Helicos	Massively parallel single molecule sequencing by synthesis	Stephen Quake, Stanford University, Stanford, California	Public company; IPO May, 2007	Launched Helicos Genetic Analysis System in February; 2 instruments ordered ($1.35 million)
Illumina	Sequencing by synthesis	David Bentley, Solexa, UK	Public company; revenue from instrument and reagent sales	Launched IG Genome Analyzer in January 2007; ~200 instruments sold ($450,000)
Intelligent Bio-Systems	Massively parallel sequencing by synthesis using proprietary reversible fluorescent nucleotide terminators	Jingyue Ju, Columbia University, New York	Private company	Pinpoint Sequencer under development
Pacific BioSciences (formerly Nanofluidics)	Single molecule, real time sequencing by synthesis	Walter Webb and Harold Craighead, Cornell University, Ithaca, New York	Private company; $178 million raised	SMRT technology under development
Roche	Massively parallel pyrosequencing by synthesis	Jonathan Rothberg, 454 Life Sciences, New Haven, Connecticut	Public company; revenue from instrument and reagent sales	Genome Sequencer (GS) 454 FLX System launched in 2005; ~180 instruments shipped ($500,000)
VisiGen Biotechnologies	Massively parallel real-time single-molecule sequencing	Susan Hardin, University of Houston, Texas	Private company; ABI and Seqwright, Houston made equity investments	VisiGen sequencing system under development

as government grants and venture capital—in July, Pacific BioSciences closed a $100 million Series E financing co-led by the Rosemont, Illinois–based Deerfield Management and the Santa Clara, California–based Intel Capital. And a few intrepid companies have also signed on to the X Prize for Genomics, which will reward the winner with $10 million (**Box 1**).

Although many of these technologies are still developmental, investors say there will be an explosion of new applications as platforms continue to hit the market. Last month, for example, Complete Genomics launched its hat into the ring, announcing the first commercial human genome sequencing center at its headquarters in Mountain View, California. The business is a pure service play—no instruments or reagents for sale—but the price is unbeatable in today's marketplace: a complete human genome for $5,000. The only other company currently offering human genome sequencing as a service is Cambridge, Massachusetts–based Knome, which charges $350,000 (see p. 1105).

"The potential is unimaginably large, and there is enough room for many players," says Zipkin, who is an investor in Complete Genomics. These new applications are predicted to generate the bulk of future NGS revenue, as the potential to create new, unimagined markets is powerful. "No one could have predicted the many applications for PCR and recombinant technology, and the scope of NGS stands to be just as revolutionary," says Witonsky.

Sales of conventional instruments are also on the rise, which is a positive sign for NGS markets. Illumina's combined instrument revenue grew from $25.4 million in the first quarter of 2007 to $43.2 million in the second quarter of 2008, says Flatley. Although the company doesn't release instrument-specific sales figures, it attributes this 70% growth to demand for the Genome Analyzer II.

After years of sharp decline, the global market for DNA sequencing has also begun to stabilize. This is primarily because the cost of conventional genomic sequencing has itself stabilized, after declining over the past few years. Today's high performance DNA sequencers are 200 times faster than those used by the Human Genome Project in the late nineties, and the price of sequencing an entire genome is nearly one-thousand times cheaper. These improvements in cost, coupled with a slight decrease in demand, caused global DNA sequencing revenue to fall from $1.2 billion in 2003 to $885 million in 2006.

Since ABI carries 60% of the total high-speed sequencing market, analysts say its revenue trends are indicative of larger market forces. For the fiscal year ending June 30, ABI reported a 6% increase in revenues over the previous year and in June, the company announced plans for a $6.7 billion merger with the Carlsbad, California–based bioscience company Invitrogen.

Next, next-generation?

Investors say there is no way to tell which platforms will be the most competitive. Illumina and ABI are currently directly competing for market share with their short-read technologies, whereas 454 is focusing on applications for long reads, including most of the traditional Sanger market. Illumina's recent acquisition of the minipyrosequencing company Avantome, a spin out from Stanford University's Genome Technology Center, suggests that the company may be putting technology in place for increasing read lengths, though they are mum on their plans.

"Each of the technologies on the market has a drawback in speed, accuracy or cost," says Zipkin. "If you go with a cheap platform, it will take a year to sequence a genome. If you go with a faster technology, you may compromise accuracy. You have to choose the lesser of two evils, and decide which parameter you want to give up."

When it comes to business models and market competition, the companies developing

new platforms have substantial difficulties to overcome as well. NGS markets have a high rate of technology turnover, making it difficult to stay ahead of the game. Just as the platforms capable of a $100,000 genome are becoming established, faster, cheaper technologies are being introduced.

For example, Illumina has replaced its Genome Analyzer I with Genome Analyzer II, 454 is about to roll out its titanium upgrade to original GS FLX, and ABI will be releasing its third-generation sequencing platform this month, called the SOLiD 3 System.

The key to success will hang on the ability to position new instruments on the market effectively—a strength of established companies in the NGS market, such as ABI, Illumina and Roche. "I think being bigger is going to play a huge role in how these technologies are positioned, especially when applying them as a healthcare tool," says Witonsky.

Large companies with diverse product lines that can generate revenues to insulate them from the high costs of launching and marketing new, unproven products will also likely be at an advantage.

As for the $1,000 genome, it's possible the market will have to wait for nanopore methods to mature, according to Charles Cantor, chief scientific officer at the San Diego–based Sequenom. "Nanopores offer much faster data acquisition times, and have potential for other high-throughput applications beyond sequencing," says Cantor. However, he is quick to caution that nanopores are still a few years off. These so-called "next, next generation" sequencing technologies hope to compete in a market environment that views the $1,000 genome as a base-line standard. Sequenom is working on nanopore methods as well as a variety of complementary technologies, but Cantor concurs with the view that future sequencing strategies will likely combine results from two or more methods.

The industry may look back at the current growth cycle as a foundational event—much like the commercialization of PCR. "No one knows exactly what will happen. We have to wait and see," says de Bruin, "but given the pace of technology advancement, the future market will be a drastically different place than it is today."

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