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# Next-gen DNA Sequencers, Changing the Face of Biotechnology

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*—Sanjiv P Karandikar, vice president, Applied BioSystems Division, Lab India Instruments*

Since last couple of years, the rapid progress in several next-generation sequencing technologies is driving down the cost of genome sequencing and re-sequencing at an astonishing rate. It is a writing on the wall that within next two to three years, the era of the $1,000 genome will arrive. And the personalized medicines and early prediction of susceptibility to diseases would be a foreseeable reality in near future.

**Major players in next-gen DNA sequencing**

By virtue of their technological prowess, experience, and funding, today there are four prominent players in the next-gen DNA sequencing space that include Roche (454 Life Sciences), Illumina (Solexa), Applied Biosystems (Agencourt), and Helicos.

Roche (454 Life Sciences)

Roche GS20 is based on pyrosequencing reaction, in which DNA is nebulized into tiny fragments which are subjected to emulsion polymerase chain reaction (PCR). The DNA is then attached to micron-sized beads, which are dropped into octagonal wells etched into micro-titer plates. Then each DNA base (A, C, T and G) is independently washed over the plate. CCD camera records each base added by a pyrosequencing reaction.

In 2005, Roche GS20 managed to demonstrate its high throughput capabilities by publishing ‘Bacterial Whole Genome’ data in Nature. This was the first commercially available next-gen platform in the market. Being able to sequence just over 100 bases with sub-optimal accuracy, it was still costlier than the scientist’s expectations. With every major genome centers craving to acquire first next gene sequencers, early entry in the market was a huge advantage to Roche with virtually no

competition to GS20!
In early 2007, Roche introduced their second-generation system, the GS FLX, featuring several major improvements over its predecessor, including improved read length up from an average of 100 bases to 250 bases. The new system also doubled the number of reads per run from 200,000 to just over 400,000. Most importantly, one system could churn out 100MB data in less than eight hours with greatly improved accuracy of 99.5 percent.
Towards the end of 2008, Roche launched its new titanium series reagents for their existing 'GS FLX systems'. The titanium improved the performance of GS FLX to average throughput of 500MB/Run with the read length of 400 bases with 99 percent accuracy at the 400th base and higher for preceding bases. With this improved performance, despite fierce competition, Roche remains the major player in the field of next-gen sequencing space. It would be interesting to see how it fares on important criteria of 'cost per GB' in the presence of other alternatives like Illumina's GAII and Applied Biosystems SOLiD ver3.0!

## Illumina (Solexa)

Illumina's 1G Genome Analyzer (acquired from Solexa in 2006) is based on massively parallel sequencing of millions of fragments using novel reversible terminator-based sequencing chemistry. In this system, randomly fragmented DNA is attached to an optically transparent surface. Amplification step produces more than 10 million clusters, each containing approximately 1,000 copies of template per square centimeter. These templates are sequenced using a four-color 'DNA sequencing-by-synthesis' technology that employs reversible terminators with removable fluorescence. Short sequence reads of 25-base range were aligned against a reference genome and genetic variants were highlighted using a unique software pipeline. These short fragment reads lend the technology to re-sequencing applications, gene expression, and small RNA analysis. Although the read lengths were smaller than those of 454's, Illumina compensated that by using much smaller beads, producing a roughly 1,000-fold increase in bead density compared to that of 454.
In early 2007, Solexa began shipping its 'groundbreaking' 1G analyzer system which can re-sequence one billion bases of human DNA in two days. It raised lot of hopes to complete human genome at 15X coverage on a single machine in approximately three months. Many scientists were pleased with Illumina's attractive price/GB compared to 454's at that time.
In early 2009, with the intention of taking the technology to its limits, Illumina officially launched its new version of next-gen system GAII. With improvements in optics, flow cell design and chemistry, the performance of GAII is greatly improved. At present GAII performance specifications stands at 10GB/Run with read length of 2x75bp with paired end reads at a price much lower than its earlier variant GI. Illumina has impressive plans to improve its specifications even further in the years to come.
With the continuous development in hardware, reagents and down-stream software pipeline Illumina's GA II is equipped to run wide range of applications such as re-sequencing, gene expression, SNP Genotyping etc. Being a relatively early entrant in the market, Illumina enjoys largest market share in the next-gen sequencing space so far. With strong players like AB, Helicos and others coming out with more options in the market, competition will heat up in near future!

## Applied Biosystems (SOLiD)

Applied Biosystems (AB) was the late entrant in the next-gen sequencing market. After careful due diligence of 40 odd technologies available in the market, in 2006 AB acquired Agencourt Personal Genomics (APG) from Beckman Coulter. By the end of 2007, it introduced its first next-gen DNA sequencing platform SOLiD in the market.
Based on 'sequencing by synthesis' approach, using ligases enzyme coupled with emulsion PCR, SOLiD is designed with several technological advantages. Use of ligases facilitates the interrogation of two bases at a time and reading every base twice leading to 10 times more accuracy than other DNA sequencing platform presently available in the market. With the possibility to increase the density of beads per run, SOLiD is capable of increasing throughput without change in the hardware. With two completely independent flow cells, SOLiD offers much needed flexibility to the scientists.
The first version of SOLiD was introduced with throughput of 3.0GB/Run at 99.95 percent accuracy and most importantly at the lowest cost/GB.
In 2008, AB introduced SOLiD ver2.0 in the market with 'free upgrade' to existing users. The upgrade consisted of change in software and chemistry with no change in hardware. With the introduction of ver2.0, SOLiD throughput went up from three to six GB/Run. That was the highest throughput available in the market at that point in time. This created a positive ripple in the market. No wonder SOLiD system won the prestigious Life Science Innovation of the Year Award in 2008.
In February 2009, AB introduced SOLiD ver3.0 with many more advancements both in terms of automation and unprecedented throughput specifications of 20GB/Run of mappable data within a week. In short, now SOLiD ver3.0 is equipped to map the whole human genome with 15X coverage in less than two weeks at a very reasonable price.
With three major players constantly improving their product range and more companies including Helicos eager to introduce their products in near future, next-gen market space is still in its highly dynamic phase without any one's dominance so far.

**Indian scenario**

Indian scientific community is highly enthusiastic about the next-gen technology available in the market. Running such a state-of-the-art, highly dynamic next-gen sequencing technology will always be a challenge for any research institute unless it is supported well by the suppliers in India. Possibility of having a 'hands on demo' facility along with opportunity to run 'proof of concept' projects would be of great interest to Indian scientists before finalizing the next-gen projects. Needless to say, high quality service and application support along with bio-informatics backup would be the key to successful implementation of next-gen sequencing systems in India.

**Impact of next-gen sequencers systems on other technologies**

Next-gen sequencing systems available in the market can offer 360 degree view of molecular biology. Therefore arguably, these systems have already raised a big question mark on the future of microarrays. With the availability of newer systems to perform hypothesis-neutral evaluation of expressed genes, it also allows researchers to explore the expression of genes without having prior knowledge of DNA sequences of RNA transcripts or genes. When compared to microarrays, the the next-gen sequencers have the added benefit of being capable of high throughput genotyping. Once researchers have identified expressed DNA sequences, they can use the data set from the same experiments to explore mutations and coding single-nucleotide polymorphisms (SNPs) within these sequences. With wide variety of high throughput applications, next-gen systems are set to replace some of the popular technologies like microarrays in near future.