

# GENOMICS: FROM CHEMISTRY KIT TO TOOL BOX

Numerous tools have been developed recently that exploit breakthroughs in the understanding of genomic chemistries. From improved sequencing technologies to microRNA detection, advances in basic chemistry – impacting a diverse array of research fields – have been translated into viable products that can aid scientists in achieving their research and clinical goals. Now, as cross-disciplinary communication continues to grow and mature, research at the intersection of chemistry and biology is increasingly exploited to solve fundamental questions in science and medicine. **By David Bradley**

**F**rederick Sanger's original sequencing method is fast approaching middle age and despite instrumentation advances that allow parallel sequencing of several hundred bases, it is the young upstart technologies that promise to fulfill the increasingly demanding requirements of today's research scientists.

## All Fired Up

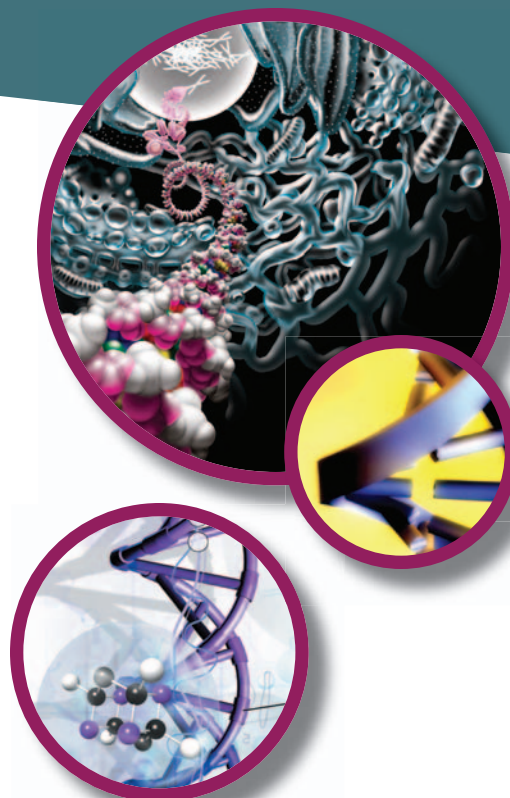
Among the novel approaches currently being developed to do this are sequencing-by-synthesis, which includes pyrosequencing and so-called FISSEQ (fluorescent in situ sequencing), and sequencing-by-hybridization (SBH). The first of these new chemistries to be commercialized was pyrosequencing, by the Branford, Connecticut, company **454 Life Sciences**. In 2005, 454 and **Roche** entered into an exclusive five-year development and distribution agreement for their Genome Sequencing Systems product. A cyclical sequence of reactions is employed, involving sequential addition of deoxynucleotide triphosphates and the generation of a light signal through luciferin that is proportional to the number of bases added. This system is advantageous since no gels or fluorescent molecules are needed and hundreds of thousands of DNA fragments in parallel can be analyzed, making it ideal for whole genome sequencing or identification of single nucleotide polymorphisms (SNPs) in DNA samples from multiple sources.

Timothy Harkins, marketing manager for genome sequencing at Roche Applied Science, believes that the 454 Genome Sequencer is changing science by allowing previously unanswered questions to be addressed, and “at a speed that was unimaginable just a few years ago.” Harkins continues, “This has enabled breakthroughs in several sciences from whole genome analysis to transcriptome analysis to gene regulation studies,” adding, “It's not just the sequencing that has been transformed, but also the front end with regard to sample preparation.” Indeed, the 454/Roche pyrosequencing technology has already been applied successfully to the sequencing of the Neanderthal genome published recently in *Science*.

Another player in this arena, Solexa, Inc. (recently acquired by **Illumina** to round out its sequencing portfolio), has been focusing on driving down costs. Its approach to sequencing-by-synthesis extends Sanger's original four-color tagging but adds a twist that allows massively parallel sequencing to be carried out with novel reversible terminator chemistry, achieving a claimed 99.995 percent accuracy.

In contrast, the FISSEQ technique was developed to enable the localized amplification of single DNA molecules using an acrylamide gel, creating colonies of PCR product called “colonies” which could subsequently be sequenced in situ. It has now been improved and extended by researchers in George Church's lab at **Harvard Medical School** in Boston. Also known as bead-based polony sequencing, this technology – licensed in the US to Agencourt Biosciences, now a part of **Beckman Coulter** – could have significant implications for personalized medicine and low-cost genome analysis. “Our contributions to commercial, next-generation technologies have been focused on interfacing polonies with single-

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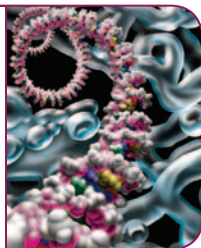
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## Genomics

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tube capture of human exons, yielding an affordable \$3,000 direct assay for causative alleles rather than depending on linkage to noncoding common SNPs,” Church says.

### Getting Together

Another approach that could accelerate genomic research is so-called sequencing-by-hybridization. First suggested in the 1990s, it has taken a decade or so to reach commercial reality with Affymetrix and Illumina at the forefront. The process uses differential hybridization of sequence-specific probes on microfabricated arrays to known or unknown DNA samples in order to determine the sequence of the target DNA, technology predominantly used for SNP detection.

Affymetrix spin-out company **Perlegen**, for instance, has produced SBH technology which allows for the simultaneous sequencing of hundreds of millions of individual DNA fragments. The company explains that the driver for this technology is to allow them to quickly identify SNPs for the purposes of understanding genetic disorders and defining the pharmacogenomics of drug reactions in individuals with specific SNPs. While the Perlegen technology has not yet been widely adopted, its biggest advantage is its larger wafer size, meaning more DNAs per chip.

Illumina is also pushing the boundaries of chip density with its latest 1M BeadChip for SNP profiling and detection, an array containing up to 1 million unique probes. Carsten Rosenow, marketing manager for DNA analysis products at Illumina, explains that this new technology “allows researchers to look beyond standard SNPs, giving them the ability to interrogate the so-called unSNPable parts of the genome where we don’t have a uniform probe design.” The 1M BeadChip, elaborates Rosenow, allows integrated analysis of SNPs and novel copy number variations using uniform probe distribution, giving unprecedented whole genome coverage.

### Assembly Line Sequencing Goes with the Flow

Cambridge, UK’s **genapta Limited** is exploiting recent advances in microfluidics and single molecule fluorescent detection to allow the company to pursue an alternative to conventional single strand sequencing systems. “It is possible that up to 30 percent of the fluorophores remain invisible if fixed down to a solid surface,” says genapta’s Julian White. “As a result, so are the molecules to which they are attached.” He suggests that a better approach is to leave the DNA suspended in a flowing medium, allowing the fluorophores more chance to fluoresce and measurements to be carried out continuously. This system is more suited to high throughput methodologies than the inherent batch mode of solid support systems.

“It’s early days,” concedes White, “but despite the simplicity of optics, the technology gives near single molecule sensitivity, which makes us cautiously optimistic that we’re on the right track.” This is an allusion to one of the holy grails of this field: single molecule

sequencing. Although undeniably still in its infancy, much effort and financial resources are being applied to the problem.

### Small, Smaller, Smallest

One of the approaches attempting to directly address the single molecule conundrum involves the use of protein channels or synthetic nanopores. Hypothetically, strands of DNA can wriggle through such pores and their sequences can be read as they emerge.

The concept was first patented more than a decade ago by Church and colleagues John Kasianowicz, Eric Brandin, Daniel Branton, and David Deamer. They demonstrated that individual polynucleotide molecules could be driven through a 2.6 nanometer protein ion channel in a lipid membrane bilayer using an electric field. The passage of each molecule inhibits the ionic current across the channel and its duration correlates with polynucleotide length. The next stage would be to identify exactly how that current fluctuates as each base passes through the channel; each of the four DNA bases being slightly different in size should produce a characteristic measurable fluctuation.

Gregory Timp and his colleagues at the **University of Illinois at Urbana-Champaign** are working on a similar process, in which they punch nanoscopic holes in an inorganic semiconductor material, such as silicon nitride, to create the requisite nanopores rather than relying on biological channels. Timp is confident that with improved control of the transport of DNA through such nanopores they will be able to apply sensing technology at the single molecule scale to obtain the strand’s sequence.

### Seeing the Light

An important component of many genomic technologies is a method for detecting the presence or modification of DNA targets. The conventional labeling molecules used in sequencing have several drawbacks, including photobleaching and interference. David Smoller of **Sigma-Aldrich** in St Louis, Missouri, explains that modern organic chemistry is getting around such problems, enabling the function of genes to be revealed and applications such as drug discovery to be developed. “As companies are looking for novel ways to discover the function of their gene targets, fluorescent chemistries will increase in importance,” he says.

Sigma-Aldrich has worked with **Panomics** (formerly Genospectra) in Fremont, California, to bring together recombinant technology, fluorescent organic molecules, and a novel nanoparticle cell delivery system. This particular combination of technologies allows them to produce biosensors that, once delivered into the cell, can measure the amount, the activity, and the location of endogenous proteins in living cells, Smoller explains. “These biosensors will have great value and application in the fields of high content screening and cell-based assays.”

The system is being developed with David Lawrence and colleagues at Albert Einstein College of Medicine and with Klaus Hahn of the University of North Carolina, Chapel Hill. “There has been wonderful synergy between both companies and our respective research groups,” Lawrence says of the collaboration.

### Please SERS

Taking a different approach, nanotech-based chemistries that can detect the tiniest quantities of mononucleotide are [continued](#) ▶

being used to develop label-free assays. This according to scientists at **Queen's University Belfast** who have collaborated with **Avalon Instruments** (now part of PerkinElmer Life and Analytical Sciences).

Steven Bell, director of the innovative molecular materials group at QUB and his colleagues, are exploiting novel silver colloid nanoparticle chemistry to help them obtain surface-enhanced Raman spectra (SERS) of DNA and RNA mononucleotides with high sensitivity. "The main advantage of our SERS approach is that it allows direct, label-free identification in aqueous solution," Bell explains. His approach, which challenges the traditional fluorescence paradigm, can produce spectra at tens of nanograms per milliliter and less. "We are working with large samples, but reducing the sampling volume to a few microliters, which would move the sample down to tens of picograms," he adds. "The ability to skip the labeling steps entirely seems like a significant advantage that could lead to entirely new protocols."

### Pushing Microarray Limits

DNA microarrays have already become widespread, improving expression screening, sequencing, and drug discovery, by virtue of parallel detection. E.M. LeProust, genomics chemistry manager at **Agilent**, comments, "Recent advances in surface chemistry, synthesis fidelity, uniformity and robustness of array manufacture, and inkjet printing technologies have enabled the development of products with sensitivity and specificity not imagined before." In real terms, this means the ability to print designer chips with over 240,000 unique oligonucleotides of any sequence desired, with unparalleled speed and fidelity. "The flexibility of the system combined with the high performance is what makes it unique and powerful," observes L.K. Bruhn, a project manager at Agilent. "This allows researchers to answer questions they previously could not," she continues, "ranging from surveying the whole genome for small deletions with custom designed large arrays to effectively profiling all known human miRNAs using subarrays."

Charles Cantor and colleagues at **Boston University** suggest that microarray use might be extended still further by advances in

spectral self-interference fluorescence microscopy. They recently demonstrated that this interferometric technique can provide precise data on the average location of a fluorescent label relative to a surface and so offer specific information about the conformation of a bound DNA molecule.

The team studied the conformation of both single-stranded (ssDNA) and double-stranded DNA (dsDNA) on glass surfaces using 50- and 21-nt oligonucleotides. The first strand of the DNA was bound to an oxide-coated silicon surface. Applying the interference technique allowed them to estimate the shape of coiled ssDNA, the average tilt of dsDNA, and the degree of hybridization. The research offers a proof of principle for the approach and, says Cantor, offers the possibility of utilizing array technologies to obtain conformation information that would be useful but has not previously been accessible. Applications promise to be widespread and might include both clinical and diagnostic uses.

### To Interference... and Beyond

New chemical technologies are enabling much more than studies on just DNA. Peter Roberts of Danish company **Exiqon** comments that "the postgenomic world is focusing very much on noncoding RNA, what some call the 'dark matter' of the genome." The means of action of this dark matter is RNA interference (RNAi), a mere curiosity for many years, but the subject of this year's Nobel Prize in Physiology or Medicine and a phenomenon with dramatic implications for understanding cellular regulation and treating disease. Understanding RNAi is gradually being facilitated by the new technologies, one of which is locked nucleic acids (LNAs).

"Our LNA is a high-affinity nucleic acid analog that provides higher binding affinities for targets such as microRNAs," Exiqon's Roberts says. LNAs are nucleic acid analogs in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom to the 4'-C atom allowing them to discriminate very effectively between short RNA and DNA targets. Roberts suggests that LNAs, properly incorporated into oligonucleotides, provide a powerful alternative for detection of small RNA targets. "It turns out that LNA-based probes are the perfect basis for studying these short RNA targets, for in situ detection, microarrays, and knockdown," he says. "In situ detection of miRNA on this scale of sensitivity and specificity in this field was entirely enabled by the use of LNA probes," Roberts continues. "It just was not possible previously."

So where are all these diverse new chemical tools taking genomic science? Sigma-Aldrich's Smoller puts it quite succinctly. "Combining the disciplines of chemistry and biology has provided a wealth of innovation," he says. "The innovation has opened many doors into the understanding of human health, biology, and gene function."

Jeffery Schloss, program director for technology development coordination at the **National Human Genome Research Institute** of the NIH, agrees, pointing out that the new genomic and postgenomic chemistries will enable the much-vaunted personalized medicine. "A key factor in the eventual success of these technology development and implementation programs," he says, "is the collaboration among chemists, biologists, physicists, engineers, and computer scientists, to conceive and realize technologies that can be commercialized and produce information of biomedical utility."

*David Bradley is a freelance science writer based in Cambridge, UK.*

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