There’s no denying the power and promise of next-generation DNA sequencing (NGS) in the healthcare setting. Among other applications, NGS can pinpoint genetic mutations, diagnose congenital defects, stratify patients in drug trials, and trace pathogenic outbreaks. Since 2005, when the first next-gen instruments hit the market, the field has exploded, and not surprisingly, companies have stepped in to fill unmet clinical needs. Here, we profile five businesses taking five different approaches to clinical NGS.

THE NGS PIONEER

If there can be veterans in a field as young as next-gen sequencing, Ambry Genetics is surely one of them. The company, based in Orange County, Calif., acquired its first NGS instrument, an Illumina Genome Analyzer, in 2007. Since then the company has run “over 100,000-plus” samples, including “tens of thousands” of clinical specimens, says Ardy Arianpour, Senior Vice President of Business Development. “Our vision has always been to apply next-gen sequencing to the clinic to provide actionable products for physicians, genetic counselors, the medical field, to better serve their patients.”

The company’s menu features some 300 tests in all, ranging from targeted panels comprising a handful of genes to a 20,000-gene clinical exome that costs $15,000. Several feature the breast cancer susceptibility genes that became available following the Supreme Court’s 2013 ruling invalidating patents on gene sequences. The first of those tests, a $2,200 assay for BRCA 1 and 2, was introduced the day the court announced its decision in June. The company also offers a $3,300, six-gene panel called BRCAplus and a 16-gene panel called BreastNext ($3,900). Typical turnaround time is two to four weeks.
To run those tests, Ambry has a fleet of five Illumina HiSeqs, three Illumina MiSeqs, and two Ion Torrent PGMs (plus a “bunch” of ABI 3730s), not to mention all the accessory hardware for library prep, sequence targeting, and so on. “We’re technology junkies,” Arianpour says. Ambry is a service provider, like Quest and Labcorp, and its NGS assays are considered laboratory-developed tests, meaning they need not be FDA-approved. Instead, the lab has CLIA/CAP certification, a significantly smoother regulatory path. “We get audited from CLIA and CAP every year, and for the last 14 years we’ve always passed with flying colors,” Arianpour says. Yet clinical NGS isn’t for everyone, he cautions. The technology imposes a significant financial burden. “You have to with flying colors,” Arianpour says. “We get audited from CLIA and CAP every year, and for the last 14 years we’ve always passed with flying colors,” Arianpour says. Yet clinical NGS isn’t for everyone, he cautions. The technology imposes a significant financial burden. “You have to have enough volume in order to justify NGS or else the costs will be tremendous,” Arianpour says. That’s because patients cannot generally wait around for the lab to collect enough samples to fill up the sequencer; they need their test results, stat. That means that, at first, a new NGS lab is likely to lose money, as the costs cannot be amortized over many samples. But if a lab can tough it out and establish a reputation, sample throughput will go up, and so will the company’s profits. Ambry broke through on the NGS front with its first test, an 81-gene panel for X-linked intellectual disabilities. The company was able to easily compete on cost with a much more limited, five-gene assay being offered by the City of Hope. Today, Ambry takes a multifaceted approach to raising brand awareness, presenting at conferences and giving webinars, field presentations, Q&A sessions, and more. Ambry also collaborates with its users. “Half of the time the community tells us that we should be offering [a certain] type of test because we have 100 patients a month that come for this type of disorder and they’re not getting tested,” Arianpour says. With 200-plus employees, Ambry was built on just $500,000 from the company’s founders, family, and friends. “We have zero dollars from venture capitalists,” Arianpour says. “We don’t take the regular corporate route. It’s a more shoot-from-the-hip, let’s put something together, let’s solve the problem, let’s make something, let’s build something, and let’s just go, go, go.”

GOING WHOLE-GENOME

Top NGS vendor Illumina, not surprisingly, has a particularly well-stocked services laboratory. The company won’t share details about the lab’s precise inventory, but according to Marc Laurent, Illumina’s Director of Commercial Services, it has dozens of high-throughput HiSeq systems, and that’s just in their main facility. Illumina’s FastTrack Services lab was originally focused on genotyping – it recently logged its millionth DNA microarray. It began offering human whole-genome sequences in 2009, and it’s now cranking them out at a blistering pace. In the first quarter of 2013, the lab sequenced 4,000 complete genomes for research purposes and shipped its 10,000th overall. Turnaround time is 14 days for RapidTrack™ (“rush”) orders, or under 12 weeks for standard projects.

“The lab serves both research and clinical users, though according to Laurent, “the vast majority” of samples have been research genomes. Clinical customers can order a so-called “technical” genome, an aligned genome with variants such as SNPs and indels identified, but that don’t code for proteins. And as researchers are increasingly recognizing, many phenotypic variants map outside of coding regions. “There are always things you don’t understand [in the genome],” Laurent says, “but as the literature goes forward we’ll understand more of those things.”

Whole genome sequencing is the future, he thinks it’s what needs to happen for everybody, and we’ll focus on that and we’ll try to enable it,” Laurent explains. Whole genome sequencing offers several advantages over other types of clinical NGS. In particular, they collect every base, even those
It’s really phenomenal,” he says. “To help those customers, as well as their physicians, Illumina has also launched a series of one- and two-day events called “Understand Your Genome” (UYG), which are designed to help patients and doctors better grasp the potential for genetic-based medicine. The sold-out events – each has about 50 slots – allow attendees to have their genomes sequenced, interact directly with the genetic counselors and lab personnel who sequenced and interpreted their genomes, and review their own genome data using an iPad. They also get the opportunity to learn from genetic luminaries like Stephen Kingsmore and Eric Topol. Illumina scheduled three UYG events in 2013, and some 10 more are planned for 2014. The 2014 programs, which cost $5,000 to attend, already have a wait-list of more than 130. Laurent confesses he was skeptical when he first heard about the technology far more widely. The clinical exome workflow already passed the CAP internal inspection in April [2013],” Liu says. Located as it is at a children’s hospital, BGI@CHOP focuses on challenging pediatric mysteries, mostly monogenic diseases. Other smaller gene panels, for instance for childhood cancers, are in development. According to Liu, the lab sequences patient exomes to 100x coverage (exomes for parents of affected children are sequenced to lower coverage, typically). But it doesn’t do so quickly. Some cases can be resolved in eight to 10 weeks, he says; other, more complicated cases, take longer. That’s because at BGI@CHOP, there’s a substantial emphasis on data interpretation. “The sequencing throughput is not a bottleneck, but [it’s] the interpretation,” Liu says. “And it will always be the interpretation.” BGI@CHOP’s clinical exomes are interpreted by colleagues in the hospital’s pathology department. First, the team – which during IExome has process, for which 100 clinical exomes were sequenced, included pathologists, physicians, and research scientists – considers the list of identified variants and the patient’s phenotype and uses those data to narrow down the list of possible suspect genes. That whittles the list of candidate genes from 20,000 to maybe 30. “After that … [the list of genes] has to be manually checked one by one by physicians and … specialists,” Liu says. That can mean literature analysis, but lab work also often comes into play. “If we think that this gene is the causative gene, then for instance in the research lab, they can do some very quick functional studies,” Liu says. Of course, that kind of work takes time, so such follow-up studies are done on a case-by-case basis, he says. Liu says there’s considerable excitement in the Philadelphia medical community regarding the imminent availability of his lab’s exome sequencing service. But the lab is already dealing with logistical hiccups. For instance, with samples dribbling in to the facility, the lab sometimes has to run its sequencers half-empty. Liu is trying to encourage his colleagues to submit samples in batches. Sample quality concerns also occasionally pop up, with degraded samples putting the brakes on the whole workflow. Some physicians, he says, expect this test will represent “the final answer” to their vexing and heart-breaking genetic cases. That’s simply not true. Some diseases have no obvious genetic component. Of those that do, some stem from lesions outside the protein-coding regions exomes scan. And of course, researchers don’t yet understand the function of every protein-coding gene anyway, meaning important mutations may be inadvertently filtered out of their analyses. “Everyone, including me, have to try to deliver the message [that] this may not be the exhaustive, final answer.”

**MAKING NGS ROUTINE**

Much of the excitement surrounding clinical NGS involves dramatic applications in oncology and congenital disease. But these are relatively rare uses. Boston-based Pathogenica Inc. seeks to spread the technology far more widely. Founded in 2009 by Harvard University geneticist George Church and his former postdoctoral fellow, Yemi Adesokan, Pathogenica’s goal is “to apply next-gen sequencing in a way that would be routine rather than an exceptional use,” says Alex Rolfe, the company’s vice president of bioinformatics. (Adesokan is now the company’s CEO; Church is a scientific advisor.) Pathogenica develops NGS-based tests for pathogenic bacteria.
Traditionally, such assays are performed using either bacterial culture or PCR. But the former is slow, while the latter is narrowly focused – each assay tests for one pathogen. Pathogenica’s first marketed test, the HAI BioDetection System, isolates and sequences genetic elements that can both fingerprint and assess the pathogenicity of any of a dozen bacterial species in a biological sample, all in about 12 hours.

In the U.S., the BioDetection system is for research-use only, meaning it cannot be used to diagnose patients. But can be used by hospitals and other healthcare centers to rapidly screen patients and facilities for signs of pathogenic infections, such as MRSA and Clostridium difficile. Pathogenica announced in July that two European hospitals, the University Medical Center Groningen and Amphia Hospital in the Netherlands, are using the test for just that purpose. A CE-marked version of the BioDetection assay was launched in Europe in August, which can be used for diagnostic purposes. The HAI BioDetection CE-IVD kit is “the first sequence-based infectious disease diagnostic kit on the market,” according to a corporate press release.

According to Rolfe, the BioDetection system, which is sold as a kit for on-site use rather than as a service, can help stem the spread of so-called “nosocomial,” or hospital-acquired infections. But sequencers are expensive, as is the expertise to run them, which can complicate the adoption of NGS-based kits. However, says Rolfe, many healthcare centers see such prophylactic screening solutions as worth the prices.

First, clinical sequencers like the Ion Torrent PGM and Illumina MiSeq are certainly within the financial reach of larger healthcare centers, which can also act as central testing facilities for samples collected locally as part of a community-wide infection-management strategy. Furthermore, he says, treatment for nosocomial infections can easily run to tens of thousands of dollars per patient, most of which must be borne by the healthcare provider as insurers generally don’t cover such care.

“Facilities … look at this and they say, well, it’s not cheap to do this, but it’s not cheap to take care of these patients once they’ve acquired this infection in a hospital either.” Rolfe says.

Pathogenica is developing additional tests for tuberculosis, human papilloma virus, and hepatitis C. In one day, the TB test could answer questions that currently can take two months to resolve – namely, is a patient infectious, and what drugs will be effective? TB logged 8.7 million new cases in 2011, according to the World Health Organization, and faster diagnosis and treatment could cut those numbers substantially. The HPV assay could replace or at least supplement Pap tests with data on both the viral strain and the patient’s genetic likelihood of ovarian cancer. Pathogenica will likely sell some of these tests as kits for on-site use rather than a service.
of these tests as kits, others as a service, Rolfe says. But all will advance the goal of making NGS a regular part of the healthcare experience. “There’s a huge potential benefit and there are so many potential uses,” Rolfe says of Pathogenica’s particular application of NGS. “It’s just not what people are thinking about.”

THE DATA-ANALYSIS SOLUTION

Bay Area-based SV Bio concentrates its efforts on another facet of the clinical NGS market: Data interpretation. Like other companies in this space, SV Bio is a service provider:

It can sequence everything from single genes and gene panels to exomes and whole genomes, and do so to clinical-grade, an “absolutely non-trivial” process, says company founder and CEO Dietrich Stephan. Currently, the sequence generation is outsourced, but the company is building its own in-house infrastructure, as well.

But it’s not the sequencing per se that really differentiates SV Bio; it’s translating that information into something a doctor can use and understand. “We, as a medical and healthcare industry, are finally at the cusp of being able to deliver genetic information to physicians at the point-of-care with clinical decision support to really improve outcomes for patients,” Stephan says. “That critical piece of infrastructure is what we’re building at SV Bio.

The problem, as Stephan explains it, is that most NGS analysis tools are research-grade, not clinical-grade. They have low sensitivity and poor specificity, which is good enough for researchers, but not for physicians sitting across the desk from a patient. “We have developed a clinical-grade pipeline that allows the output of next-generation sequencers to be used for diagnostic purposes,” Stephan says.

That pipeline encompasses several steps, including alignment and variant calling, annotation and functional assessment of the resulting variants, automated reporting, and automated assay validation for regulatory compliance. To build it, SV Bio blended existing public tools with proprietary solutions. It started by profiling the available software tools, figuring out their “sweet spots,” and amalgamating them into a pipeline that plays to each one’s individual strength.

For instance, Stephan says, “There’s a solution that’s reasonably good at seeing insertions and deletions of let’s say 25 base pairs or greater, but it’s terrible at everything else. So we’ll benchmark that out. We use that solution, in addition to every other alignment and variant-calling solution, on every patient sample, and we only look at its results in its sweet spot of performance.”

Then, the company put everything together and asked, “Is there an area where all of these solutions fail?” The answer, Stephan says, is yes. For example, if insertions or deletions exceed the sequencer’s read-length. For those cases, the company developed its own proprietary solutions.

All told, the company claims to achieve “99.9-plus-percent” sensitivity and comparable specificity with its patchwork quilt approach and downstream classification engines, a result that’s on-par with the current genetics gold standard of Sanger sequencing. “If the machine calls a variant a pathogenic mutation, chances are it’s real.”

Stephan says what really distinguishes SV Bio from its competitors in a crowded marketplace is quality and scalability. Indeed, the company recently struck a deal with the Mayo Clinic and Mayo Medical Labs as a genomic medicine provider. “To have passed muster there gives you a sense of what’s under the hood,” he says.

The company currently focuses on monogenic diseases, and germline cancer risk testing is on
Dietrich Stephan, Founder and CEO, Silicon Valley Biosystems (SV Bio)

“I think [genomic medicine] is now an exponential slope that’s going of its own volition with momentum. I think there’s no stopping it at this point.”

Still, Stephan – an industry veteran who founded consumer genetics firm Navigenics – figures those problems will be worked out in the next two years or so, and anticipates SV Bio will come out on top.

“I think [genomic medicine] is now an exponential slope that’s going of its own volition with momentum,” he says. “I think there’s no stopping it at this point.”

Managing it is no easy feat for the IT organizations tasked with doing so. Most IT organizations supporting clinical NGS applications share similar data-management challenges. They must operate within designated capital and operating budgets, adapt IT resources rapidly to customer demand, provide a variety of end-users access to data, and enable analytics workflows.

“IT’s reimbursement reform.” Stephan – an industry insider, says. “So everything is in flux.”

“I think [genomic medicine] is now an exponential slope that’s going of its own volition with momentum. I think there’s no stopping it at this point.”

Still, Stephan – an industry veteran who founded consumer genetics firm Navigenics – figures those problems will be worked out in the next two years or so, and anticipates SV Bio will come out on top.

“I think [genomic medicine] is now an exponential slope that’s going of its own volition with momentum,” he says. “I think there’s no stopping it at this point.”

SIMPLIFYING NGS DATA MANAGEMENT

As these five examples show, next-generation sequencing (NGS) platforms are playing an increasingly prominent role in the clinical and diagnostic healthcare arenas. But the resulting data threatens to overwhelm existing infrastructure. It won’t be long before sequencing service organizations will have the capacity to sequence a million cancer genomes, and the data generated from those genomes could consume 100 petabytes (PB) of storage capacity. (1) Whole-genome sequences (WGS) for all 4.3 million U.S. newborns would require 100 PB annually, and whole-genome sequencing for everyone older than 50 in 2012 would require 10 Exabytes. (2, 3) This is Big Data, and the job of interpreting the test – which, if done incorrectly, could result in misdiagnosis – is no easy task. What’s more, the resulting data threatens to overwhelm existing infrastructure. It won’t be long before sequencing service organizations will have the capacity to sequence a million cancer genomes, and the data generated from those genomes could consume 100 petabytes (PB) of storage capacity. (1) Whole-genome sequences (WGS) for all 4.3 million U.S. newborns would require 100 PB annually, and whole-genome sequencing for everyone older than 50 in 2012 would require 10 Exabytes. (2, 3)

This is Big Data, and the job of interpreting the test – which, if done incorrectly, could result in misdiagnosis – is no easy task. What’s more, the resulting data threatens to overwhelm existing infrastructure. It won’t be long before sequencing service organizations will have the capacity to sequence a million cancer genomes, and the data generated from those genomes could consume 100 petabytes (PB) of storage capacity. (1) Whole-genome sequences (WGS) for all 4.3 million U.S. newborns would require 100 PB annually, and whole-genome sequencing for everyone older than 50 in 2012 would require 10 Exabytes. (2, 3)

By the horizon, SV Bio also provides data analysis services to other molecular diagnostics companies that can generate the sequence but lack the skills to analyze it. Therein lies perhaps the biggest short-term issue the company faces, Stephan says, and it has nothing to do with technology. “It’s reimbursement reform.”

“It’s unclear as to whether people will get paid for just interpreting the test versus running and interpreting the test – which, if you think about interpreting an archived genome, becomes a really important point,” Stephan says. “So everything is in flux.”

Managing it is no easy feat for the IT organizations tasked with doing so. Most IT organizations supporting clinical NGS applications share similar data-management challenges. They must operate within designated capital and operating budgets, adapt IT resources rapidly to customer demand, provide a variety of end-users access to data, and enable analytics workflows.

“It’s reimbursement reform.” Stephan – an industry insider, says. “So everything is in flux.”

References
1. “Genome and Transcriptome Standards for Clinical Use”, Christopher E. Mason, Weill Cornell Medical College, Hanson-Wade WGA Meeting, 11.28.2012.
3. Assume 100 GB per whole genome, compressed.