Next-gen sequencing in clinical debuts

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Like an ardent lover, technology sometimes keeps its promises and sometimes breaks them. One technology that has been impressively faithful to its word is next-generation sequencing. NGS debuted in the pages of CAP TODAY just over a year ago, when it was a young research tool, exploring and discovering, but looking ready to settle down into a union with clinical diagnostics. That happy conjunction has been consummated right on schedule, in the view of Karl V. Voelkerding, MD, associate professor of pathology at the University of Utah.

"The first publication on the use of next-generation sequencing for research appeared in 2005," recalls Dr. Voelkerding, medical director of advanced technology and bioinformatics at ARUP Laboratories (Margulies M, et al. *Nature*. 2005;437: 376–380). "Typically it takes five to six years to see a transformative technology move into the clinical arena. So the maturation of next-generation sequencing is fairly consistent with that timeline."

"Isn't this just what the whole Human Genome Project was about?" asks Sherri J. Bale, PhD, FACMG, co-president and clinical director of GeneDx. "It was designed to show how we can use sequence information to improve the health of the population better, faster, and cheaper in the clinical setting. This is exactly where we expected this to go, and it's pretty cool that it's happening."

In its new role as a clinical provider, next-generation sequencing is generating patient benefits in a variety of settings. Let us count the ways.

Emory Genetics Laboratory (EGL) occupies two floors of the building that houses the Division of Medical Genetics; it consists of three contiguous laboratories performing mostly traditional medical genetics testing—biochemistry, cytogenetics, and molecular genetics. In the molecular genetics lab is one striking exception to the "traditional" label. Arrayed on the workbenches are three AB/Hitachi 3730x1 Sanger sequencers and an AB SOLiD next-gen sequencer, all large instruments, and microwave-oven-sized RainDance RDT1000 and Fluidigm target enrichment machines. The newer SOLiD sequencer in combination with either target enrichment machine provides DNA sequence data with a speed and capacity unknown before 2007 in clinical laboratories. Madhuri Hegde, PhD, FACMG, scientific director of EGL, has calculated that a SOLiD v3 can sequence 2200 Mbp per week, compared with 5.6 Mbp for a Sanger instrument. "You would need 392 AB 3730xl's running full time for seven days to generate the same amount of data as a single SOLiD v3," says Dr. Hegde, associate professor of human genetics, Emory University School of Medicine.

In May 2010, EGL became the first academic laboratory to offer NGS as a clinical service, with three multigene panels: X-linked Intellectual Disability (92 genes), Congenital Muscular Dystrophy (13 genes), and Congenital Disorders of Glycosylation (25 genes). As a clinical NGS facility located within the Division of Medical Genetics, EGL offers one model of how this technology can be applied to molecular diagnostics. In Dr. Hegde's view, this is the best setting for NGS because it offers complete integration into genetic medicine. For one, different genetic tests are complementary. "If biochemistry detects galactosemia, 99 percent of the time we find an altered gene," she says. Another feature she likes: "There are 18 genetic counselors in the department and five support our laboratory."

GeneDx, a commercial referencelaboratory that specializes in genetic testing for rare hereditary disorders, is a second model for clinical NGS. In the second half of 2008 GeneDx became the first laboratory of any kind in the U.S. to offer NGS for clinical application when it added an NGS-based cardiomyopathy multigene panel to its menu. Since then it has expanded the hypertrophic cardiomyopathy panel to 18 genes and added a number of other cardiac panels, such as long and short QT syndromes and Brugada syndrome, as well as panels for periodic fever syndrome, retinitis pigmentosum, and mitochondrial and nuclear genes involved in hereditary mitochondrial diseases. "Next-generation sequencing allows us to offer a lot of gene testing for a lot less money than we would have been able to provide with conventional sequencing methods," Dr. Bale says. To do its NGS work, GeneDx has two Illumina Genome Analyzers and a HiSeq. Of the HiSeq, Dr. Bale says, "It has higher throughput, but at about $700,000 it's expensive as heck."

Where there is a commercial laboratory offering a test there must be clinicians ordering it. One of the clinicians who orders GeneDx's cardiomyopathy panel is Elizabeth McNally, MD, PhD, professor of medicine and director of the Institute for Cardiovascular Research of the University of Chicago. "We started doing genetic

testing for cardiomyopathy as soon as it became available, five or six years ago," Dr. McNally says. She sends out samples for this test. "I don't need to do it in-house. I just need it done well and done cheaply," she says. According to Dr. McNally, three commercial laboratories and one academic laboratory (Harvard Partners' Laboratory for Molecular Medicine) offer cardiomyopathy panels. Currently, GeneDx is the only one that uses NGS, though the Laboratory for Molecular Medicine plans to replace its resequencing array with NGS this June.

In addition to sparing clinicians from having to set up, validate, and maintain a complex testing facility, sendout mode should shift the interpretive task from the clinician to the diagnostic laboratory. "Commercial laboratories include the likelihood that a particular variant is disease-causing in their report, based on comparison with databases, some of them proprietary," Dr. McNally says. If a genetic variant has previously been found to be associated with disease, it is reported as likely pathogenic. When a pathogenic variant has been found, Dr. McNally can then offer testing to relatives of the patient using a targeted PCR assay. For variants of unknown pathogenic significance, the task is more complicated since she must then work with genomes of family members to establish a phenotype/genotype correlation.

Elaine R. Mardis, PhD, has taken a different route to clinical application of NGS. Dr. Mardis, associate professor of genetics and molecular microbiology at Washington University School of Medicine and co-director of Washington University's Genome Institute, is a research scientist whose use of NGS for whole genome sequencing in cancer grew out of her work on the Human Genome Project. Last year, in a clinical discovery study conducted with oncologist Timothy Ley, MD, a professor of medicine at Washington University, and several other colleagues, Dr. Mardis sequenced the genome of a patient with acute myeloid leukemia (AML) and a normal karyotype. Such patients fall into the large group who have an "intermediate risk," meaning their cytogenetic profile gives no indication of their long-term prognosis. Sequencing of the AML patient's genome revealed a somatic mutation in the gene DNMT3A, which encodes a de novo DNA methyltransferase. Sequencing of 280 additional patients across DNMT3A revealed that 62 of them also had a mutation, which upon correlation analysis with outcome, was associated with significantly shorter overall survival (12 versus 41 months) (Ley TJ, et al. *N Engl J Med*. 2010;363:2424–2433). Hence, mutation of DNMT3A appears to be a significant predictor of poor prognosis, especially for those difficult-to-predict patients with intermediate-risk cytogenetics.
More recently, Dr. Mardis had the opportunity to apply NGS to clinical diagnosis in real time. An AML patient apparently lacked the t(15:17)/PML-RARA fusion gene by cytogenetics, despite having a pathologic diagnosis of acute promyelocytic leukemia (APL). Patients with this fusion have the M3 (APL) subtype of AML and benefit from the addition of the relatively inexpensive agent ATRA to induction therapy. To clarify her diagnosis, whole genome sequencing by NGS was performed, and analysis to look for evidence of alterations in chromosome 15 or 17 was pursued. Here, Dr. Mardis and her colleagues found a cytogenetically invisible insertion-derived fusion in less than six weeks from sample receipt, roughly coincident with the time needed for a CLIA cytogenetic/FISH workup. To allow that information to be used to guide treatment, specific probes were designed to detect the novel fusion, and PCR was done on the patient's genome in a CLIA-certified laboratory. "We now have FISH probes for this insertion variation," Dr. Mardis says. "In this situation, the main hurdle was to make sure the patient consented so she and her oncologist could receive the information."

To what extent can this mode—use of NGS in a research laboratory to find new variations in a patient, followed by verification in a CLIA-certified laboratory—be used in patient care? "That's a good question," Dr. Mardis says. "Probably initially the hardest cases or late-stage patients will be studied this way." She's enthusiastic about bringing whole genome sequencing and analysis to the CLIA environment: "There are many hurdles, but the benefits to patients are compelling."

At ARUP Laboratories, Dr. Voelkerding has been working with the sequencing and microarray core laboratories and medical directors involved in genetic testing to develop NGS-based multigene diagnostic panels to add to ARUP's offerings. Initial panels will include Marfan and Marfan-like aortopathies and mitochondrial and nuclear genes that contribute to mitochondrial disorders. "Our timeline for diagnostic launch will be this coming autumn," says Dr. Voelkerding, director of the ARUP Genomics Laboratory. "We first had to establish within our own facility high-throughput sequencing instrumentation and protocols and to validate them and implement them under our CLIA umbrella."

Timothy C. Greiner, MD, professor of pathology and medical director of the molecular diagnostics laboratory at the University of Nebraska Medical Center, is also aiming to implement NGS diagnostic testing. He has been doing genomics research, looking at the methylome in lymphoma, using the Illumina Genome Analyzer in the UNMC genomic core facility. "Moving to NGS for clinical diagnosis presents a challenge, both the large cost of instruments and data storage support," Dr. Greiner says. The introduction of less expensive instruments, in the $50,000 to $100,000 range, will allow clinical laboratories to begin to acquire next-generation capability for a reasonable investment, he believes. Dr. Greiner has suggested the acquisition of such an instrument in the department's capital budget.

Instruments that are less expensive will lower the financial obstacle to wider use of NGS "to a huge extent," agrees Karen Weck, MD, professor of pathology and laboratory medicine and genetics and director of molecular genetics at the University of North Carolina at Chapel Hill. Affordable instrumentation will help to make the technology available for clinical testing and for analyzing the performance characteristics of various platforms, she says. Two of these options are the Ion Torrent at about $50,000 and Roche's GS Jr. at $100,000. Dr. Weck is representative of a large number of experienced molecular laboratory directors who are used to having the latest molecular technology in their laboratories and who are excited by NGS' potential but whose limited financial resources make it difficult to purchase a HiSeq or SOLiD. Assuming the smaller machines prove out, they could allow molecular pathologists to become familiar with NGS and to offer some NGS-based tests. (Dr. Mardis is setting up an Ion Torrent in her laboratory to test that assumption.)

Dr. Weck recently made her foray into NGS by assessing its analytical accuracy using the Roche 454 sequencing platform for detecting mutations in 79 candidate genes associated with primary ciliary dyskinesia in four patients who had been tested in her laboratory by Sanger sequencing. She sent the samples to Roche, which did the sequencing and sent the results back for data analysis. "We found that the platform had excellent sensitivity for detecting single-base mutations but performed less well for detecting indels, especially in mononucleotide tracks, and was not able to detect a whole exon deletion," Dr. Weck says (Berg JS, et al. *Genet Med*. 2011;13:218–229). Assessing the analytical performance of various NGS platforms will be imperative for application to clinical diagnostic testing, she says.

As surely as the first dogwood blooms signal the imminent arrival of spring, these early examples of NGS incorporated into clinical practice herald the arrival of an important new technique in molecular diagnostics. Another signal that attention must be paid to this technology was its ubiquitous presence on the program of the 2010 meeting of the Association for Molecular Pathology. From an early session on Friday at which Dr. Hegde described her work with NGS to a late-afternoon workshop on Saturday at which Dr. Voelkerding presented the basics of the technology (and Steve W. Turner, PhD, of Pacific Biosciences described his single molecule real-time sequencing technology, which is still in development), NGS fairly bloomed, with eight talks devoted to it.

Says Dr. Greiner, a member of the AMP program committee in charge of hematopathology: "The program committee recognized that, after about five years of research information about next-generation sequencing, people were making transitions into the clinical diagnostics arena. We knew our membership was interested in how the field was moving, how fast and where, and that guided our choice of speakers." Notably, the NGS talks at the AMP meeting focused on oncology and genetics but not infectious diseases. "On the research side, finding mutations that help explain diseases and lead to diagnostic applications seems to be moving in two major areas, oncology and hereditary diseases," Dr. Greiner says.

"For detecting many single organisms, very good molecular methods are available," Dr. Voelkerding explains. An important area in infectious diseases where people are doing investigative work with NGS is in identifying the emergence of resistant strains of bacteria and viruses. Here, early in the course of infection, resistant clones may constitute only a minor fraction of the pathogen population and may be present at levels lower than the limit of detection for traditional methods, particularly Sanger sequencing. "This is one of several areas where next-generation sequencing may very well have a role in the future in infectious disease," Dr. Voelkerding says. Another area of research with NGS is detecting emergence of novel pathogens (Chiu C, Miller S. *Microbe.* 2011;6:13–20).

Dr. Voelkerding took as the theme of his AMP talk "What you need to know as you contemplate bringing NGS into your lab." [CAP TODAY's coverage of Dr. Voelkerding's presentation can hit only the main points. He has published a detailed description of NGS technology (Voelkerding KV, et al. *J Mol Diagn*. 2010;12:539–551).] Dr. Voelkerding notes that CAP's Personalized Healthcare Committee is looking at how NGS will be introduced into pathology practice. As well, the program at this year's AMP meeting will have a focus on developing next-generation sequencing and translating the technology into clinical diagnostics.

Driving the adoption of NGS in genetic testing is the concept that mutations in multiple genes can result in a single or overlapping clinical phenotype. Cardiomyopathies, mitochondrial disease, and X-linked mental retardation are good examples. "It can be very confounding to sort through that on a genetic level," Dr. Voelkerding said. Options have included Sanger sequencing, which is difficult to scale to many genes, and resequencing microarrays, which are challenging to design and whose sensitivity for detecting insertions and deletions is suboptimal.

In this situation, many have turned to NGS. In this process, the first step is an enrichment for target sequences, either amplification-based (Fluidigm, RainDance) or array-based (Agilent SureSelect, Nimblegen). Each fragment is amplified either in droplet emulsion PCR or on a solid surface; the resulting target-enriched DNA library is sequenced in a massively parallel manner using pyrosequencing (Life Sciences 454), reversible dye termination (Illumina), or sequencing by ligation (AB/Life Technologies SOLiD). Because each base pair is read many times, this process is also called deep sequencing.

Designing and optimizing multiple primer pairs for multi-target gene enrichment is "a huge amount of work to do yourself," Dr. Voelkerding said. Users of RainDance work with that company's laboratory to design optimal primers. RainDance then works with a separate company to synthesize and prepare primer pair libraries for their use.

Array-based enrichment technologies are more scalable than amplification-based approaches. However, they are highly complex, consisting of many steps over a three- to four-day period. They are prone to co-capture of pseudogenes and other highly homologous genomic regions, which can confound sequencing data analysis.

"For certain applications like multi-gene panels, NGS technologies give us more throughput than we need," Dr. Voelkerding said. To leverage that capacity, one can bar code or index two or more patients' DNA libraries with unique six- to eight-base tags and run them in the same flow cell. Sequence reads are deconvoluted with bioinformatic tools.

Quality of NGS reads can be expressed with a logarithmic Q score, which is analogous to the Phred Q score in Sanger sequencing and which quantitates the probability that a specific read is an error. Current NGS instruments have Q scores ranging from 20 (an error probability of one in 100) to the high 30s (probability of error approaching one in 10,000). What is an acceptable Q score? "That is a moving target and can be application-dependent," Dr. Voelkerding says. For the Illumina HiSeq 2000 instrument at ARUP Laboratories, sequence reads with a Q score at or above 30 are used for alignment. (Dr. Voelkerding notes that Q scores for Ion Torrent, which he agrees holds great promise for clinical laboratories, are now in the

Q2O range.) "As chemistries improve and sequencing becomes more robust, Q scores should move higher," he says. With NGS one can compensate in part for lower Q scores by increasing the depth of coverage, which means increasing the read frequency.

When NGS identifies probable variants, most laboratories confirm them by Sanger sequencing before reporting them. "I anticipate that will be the practice for some time," he says. While the goal is for NGS eventually to stand on its own, right now, Dr. Voelkerding says, "people who say we don't need to confirm by Sanger are in a minority."

At the AMP meeting, Dr. Hegde spoke about the validation process she carried out to bring multigene panels to clinical status. She has a strong appreciation of the demands of clinical validation, having converted from basic research to clinical work during an ABMG fellowship at Baylor College of Medicine under Carolyn Sue Richards, PhD, and Arthur L. Beaudet, MD, chair of molecular and human genetics.

To choose an enrichment method, Dr. Hegde compared RainDance with SureSelect in 23 patients with known mutations. Both detected all mutations. Fluidigm was also effective. "Right now all three methods are very much competitive," Dr. Hegde said. Fluidigm is good for libraries up to 500 fragments and has higher throughput (48 ∞ 10-plexes); RainDance is good for libraries of more than 500 fragments but has lower throughput per run (eight samples). SureSelect has many steps and cost may be a consideration.

Library design requires highly stringent primers, no primers on SNPs or repeat regions, and no pseudogenes. "This adds a layer of complexity, since five percent to 10 percent of the genes we sequence have a pseudogene," Dr. Hegde says.

For validation of the overall NGS process, Dr. Hegde evaluated SOLiD in 20 patients previously analyzed by Sanger sequencing who had known mutations covering a broad spectrum—97 missense, eight deletions, eight duplication/insertions, and one indel. To achieve greater accuracy, she used a very high read frequency: Coverage/base ranged from 1,821 to 15,566, with an average of 9,870. (For routine clinical work she strives for coverage of 30 to 35.) Of the almost 2 million total reads, 53 percent were mappable or aligned. With this process, 100 percent of gene variations were called, including insertions, deletions, and duplications. There was a 5.5 percent false-positive rate. "We confirm all changes with Sanger sequencing," Dr. Hegde says.

Dr. Hegde's laboratory is CLIA certified and CAP accredited. She collaborated with Greenwood Genetic Center in South Carolina under the NIH Office of Rare Diseases Research CETT (Collaboration, Education and Test Translation) Program to develop the same methodology so that Emory Genetics Laboratory would be able to

exchange samples with Greenwood for mutual proficiency testing. For inspections, she—or any other NGS laboratory director—essentially creates her own standards. Still, basic principles remain the same. Last year one CAP inspector wasn't familiar with NGS, but it wasn't a problem. The inspector asked Dr. Hegde for data establishing basic parameters—specificity, sensitivity, robustness, and reproducibility, which they had obtained during the validation phase.

Dr. Hegde has joined an American College of Medical Genetics workgroup that is developing standards and guidelines for NGS. "It is good that ACMG is taking an active role in the process," she says, "so that laboratories get guidelines to understand how to bring this technology to clinical practice." (The CAP integrates accepted standards and guidelines into its Laboratory Accreditation Program.)

Once the sequencing is done, a major task remains—interpreting the data. "Bioinformatics is a large component in next-generation sequencing," Dr. Hegde says. "We are hiring more bioinformatics people than technicians. They are a very critical component of the laboratory now; they are writing scripts required for automation."

Dr. Voelkerding agrees, saying, "Bioinformatics is something that we in our community will need to tackle." In particular, he says, "Sequence alignment is one of the black boxes associated with this technology, but you want to understand that. While there is commercial software for alignment and variant calling, I would caution not to assume they've worked it all out." He and colleagues have been evaluating both commercial and academically developed programs.

Dr. Hegde evaluated two bioinformatics packages. She chose to work with the SoftGenetics NextGENe program. "During the validation stage we interacted with the company to improve the algorithm," she says.

In Dr. Bale's view, too, the biggest issue in NGS is interpretation, "having enough of the appropriate people who can provide that level of analysis," she says. "We thought it was going to be the technical stuff. But we have great people solving technical problems." She also believes that better reference databases are needed. Dr. Bale is involved in an international effort called the mutaDatabase Project, led by Patrick Willems, MD, PhD, of the University of Antwerp (Bale S, et al. *Nat Biotechnol*. 2011;29:117–118). "We are aiming to get all DNA variation data from all laboratories that do clinical genetic testing into a database," Dr. Bale says. "It should be a huge boon. Clinical laboratories have many times the amount of data in current databases." Previously, data from clinical laboratories didn't get into databases, she says, because they wouldn't accept data unless it was published. "And what clinical laboratory has time to publish?" All but two U.S. clinical laboratories have agreed to contribute to the database. Myriad Genetics and Prevention Genetics are the exceptions.

Dr. Weck says interpreting the clinical relevance of sequence variants is a "huge issue," even with Sanger sequencing. "Finding of novel variants of unknown significance plagues every molecular genetics laboratory involved in diagnostic sequencing," she says. "This will have to be addressed using a variety of methods, including bioinformatics predictions of pathogenicity, family testing, and sequencing of large numbers of unaffected individuals such as the 1,000 Genomes Project."

Dr. Mardis emphasizes the value of the 1,000 Genomes Project, a followup to the Human Genome Project (1,000 Genomes Project Consortium. *Nature*. 2010;467:1061–1073). She and her colleagues recently reported that whole genome sequencing on tissues from a breast cancer patient revealed novel genetic changes in metastatic tissue relative to the primary tumor (Ding L, et al. *Nature*. 2010; 464:999–1005). "In that case the samples came from an African-American woman," Dr. Mardis says. "In our initial analysis we found about 1 million more sites of difference than we do for Caucasian-Americans because African-American SNP identities were poorly represented in the version of the database current at that time. We need to continue to establish the background prevalence of SNPs in populations other than Caucasian-Americans."

In addition to facilitating multigene panels, which are already a reality, other, more sweeping claims have been made for NGS, such as the $1,000 genome and the

entry of whole genome sequencing and exome analysis into the molecular diagnostics laboratory. One can divide these claims into hopeful and hypeful. In the latter category is the $1,000 genome.

"I think there will be a $1,000 genome this year," Dr. McNally says. "I'm not sure we'll know what to do with it. That figure doesn't cover data analysis and interpretation."

"That phrase is good for a newspaper headline," Dr. Greiner says. "The catch is that $1,000 is only the cost of reagents. The bioinformatics analysis and interpretation piece takes time and money that is proportional to the number of genes you are analyzing. That cost is not included in the $1,000."

When asked how she reacts when she hears claims for a $1,000 genome, Dr. Bale laughs and says, "I tend to just laugh. From a technical standpoint, sure, we will get there. But the biggest issue is not the technical aspect of pushing DNA through a machine to get a sequence. The biggest issue is interpreting what comes out. What does it mean in light of the clinical presentation? The more you look at patients' genes, the more you find that hasn't been published. That is really where our time and energy are going—to interpretation."

Whole genome analysis for clinical diagnosis and exome analysis, on the other hand, are topics to take seriously. In fact, exome analysis was the theme of the 2011 ACMG meeting. In a recent review of the literature, Dr. Voelkerding estimated that as of March 2011 about 25 publications had appeared on pathogenic gene discovery through exome analysis.

Dr. Mardis differentiates exome analysis from whole genome sequencing. "Exome loosely indicates coding regions of the human genome," she explains. Coding regions, or exons, are stitched together at the level of mRNA and proteins are translated from the resulting message. To do exome analysis, a whole genome sequence library is hybridized to a subset of DNA that represent coding sequences. "You get a lot more material with whole genome sequencing," Dr. Mardis says, "which gives you the ability to interpret more of the variation in the genome." Exome analysis, on the other hand, is easier to interpret and less expensive and lets you focus on the parts of the genome that are best understood. "But you don't get the full picture," Dr. Mardis says.

Exome analysis works only as long as the genome behaves itself. In cancer the genome doesn't behave—there are many changes to chromosomes, especially translocations. "When genes get fused together by translocation or deletion, exome capture won't work in that region," Dr. Mardis says. Disruptive changes occur at the germline level in genetic disease too, but they are less extensive.

Dr. Mardis says it's time to start talking about whole genome sequencing as a diagnostic application, at least for understanding atypical cancer cases. She points to Translational Genomics Research Institute (TGen) in Phoenix, a CLIA-certified laboratory that performs whole genome sequencing on genomes of end-stage cancer patients in an attempt to identify mutations that can be targeted by a specific therapeutic agent. Of course, as Dr. Mardis acknowledges, "End-stage cancer is fundamentally different from our newly diagnosed patients."

Discovery by exome sequencing is moving fast, Dr. Hegde agrees. However, for clinical purposes, she says, "Exome analysis can be a daunting exercise in a clinical laboratory. You can't interpret that data in the clinical setting yet." When a novel variation such as a missense change is found in patient DNA, the laboratory needs to show that it affects gene function (Nelson D, *Gibbs R. Nat Genet*. 2009;41[5]: 535–543). Dr. Hegde says, "Clinical laboratories are not set up to do functional testing to show that a new nucleotide change is actually causing disease."

Dr. Hegde is directing an exome research project among patients who are negative on XLID or metabolic panels, such as Congenital Disorders of Glycosylation. "In our research laboratory we are sequencing about 30 exomes looking for new genes. Exome sequencing in the research laboratory will help us understand and prepare for translating it to the clinical laboratory," she says. It can take a year to find a new gene this way, so exome sequencing as a first step isn't always sensible. "If exome

analysis becomes a clinical reality, the focus will be on interpretation and delivering the results within an acceptable turnaround time."

Dr. Bale is more optimistic. "I see exome sequencing being a clinical test soon, probably less than two years," she says. "Whole genome sequencing is a bit different. There is a lot of intron crap and no one knows what to do with that." A drawback to exome analysis is that it is more difficult to capture "all the sequence you might want" than with multigene panels. An alternative is to sequence the whole genome and mask out data from genes that don't bear on that patient. "If another gene becomes important," Dr. Bale says, "you go back and unmask the sequence of that gene, which is a neat feature."

Will pathologists be doing some version of NGS in their molecular diagnostics laboratories? Dr. Greiner predicts that the large reference laboratories and perhaps

one-fourth of the major academic genetic diagnostic laboratories, like Dr. Hegde's, will be using next-generation sequencing in one to five years. "In more general laboratories that's harder to predict," he adds. "I'd say maybe more around 10 years."

"I can see myself doing it in my lifetime," Dr. Weck says, "even in the next few years." But before NGS becomes widespread, she says, the cost of instruments needs to drop well below the $500,000 price of a SOLiD or $700,000 for a HiSeq.

"There are still only a handful of laboratories actively working with next-generation sequencing," Dr. Voelkerding says. Many more would like to and are trying to identify the finances for the necessary capital investment. Clinical laboratories are well positioned to learn the bench technology associated with NGS, he says, but many will be working to find individuals to assist with data analysis and bioinformatics.

"I think what will accelerate translation over the next one to three years will be lower-throughput, less-costly platforms coupled with lower cost per instrument run," he predicts. "Right now high-throughput instruments require enough samples to justify the high per-run reagent cost." This barrier will be reduced with the newer instruments such as Ion Torrent, Illumina's MiSeq, and the SOLiD 5500, which, while having a high purchase price, can run in both high- and low-throughput modes.

That said, Dr. Voelkerding sees only a minority of clinical laboratories doing NGS in the near future. "Clinical laboratories are being economically conservative right now," he points out. "Only a subset of larger academic and reference laboratories will continue to be at the forefront of moving next-generation sequencing into clinical diagnostic implementation."

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