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Innovations

Pathogenica: Diagnosing by DNA

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In 2009, a small biotech company, Pathogenica, was founded in Cambridge, Massachusetts, aimed at applying the advances made in next-generation DNA sequencing technology to the clinical diagnostics arena. Thus, Pathogenica became one of the first companies poised to take advantage of the burgeoning wealth of genomics data by devising clinical testing kits for detecting sample pathogens, drug resistance genes, and toxins. "We have 20 years or more of genomics research pushing us towards clinical applications, and a great weight of new genomics data emerging every week," says Adeyemi Adesokan, Ph.D., Chief Executive Officer. "Both patients and clinicians are now increasingly genomics literate and demanding ever more accurate and useful diseases diagnostics. This combination of drive and demand is ripe for the correct application. The new generation of DNA sequencers is the most accurate tool we have for disease identification. The technology has evolved to the point where it can now be applied in clinical labs".

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Pathogenica's non-PCR approach is a variant of universal molecular inversion probe (MIP) technology. With MIP, specific regions of a target genome are captured using single-stranded DNA molecules as probes. The probes are complementary to the genome target. Their scientists have performed selective sequencing of key genomic markers within pathogenic genomes and developed a library of probes, called DxSeq probes, for use in clinical testing. "We do not sequence the entire pathogen, just the portions that are necessary to specifically identify the organisms, toxins, or drug resistance genes of interest," explains Alex Rolfe, Director of Bioinformatics. "A key advantage of this approach in the clinical space is the optimization of sequencing bandwidth for quantitation of target nucleic acids, rather than sequencing greater than 99.9% unwanted/nonuseful reads as might be the case for other approaches."

Probes are then deployed in sample genomic DNA extracted using standard tissue extraction protocols. If the probes find a genetic match in the sample, a definitive diagnosis is made. "We select a region of sample DNA with probes that are homologous to the surrounding region," explains Rolfe. In many cases, the sample DNA does not have to be replicated or amplified, such as with qPCR methods, reducing some of the steps required with other protocols. Fewer steps hopefully will translate into faster turnaround time. "Our DxSeq technology greatly improves the time taken to turnaround sequencing results. In combination with third-generation platforms such as the Ion Personal Genome Machine (PGM) Sequencer, we can achieve greater than 12 hour turnaround from clinical tissue samples," Rolfe adds.

Next-generation sequencing has obviously been fantastically useful in academic settings. "I am excited about its potential uses for clinicians and patients," adds Graeme Doran, Chief Scientific Officer. "Pathogenica is one of the first companies really pushing next-generation sequencing into the clinical space." While no tests are FDA-approved yet, Pathogenica currently provides research use only (RUO) and service-based products to the research community. Its long-term goal is to license and sell diagnostic kits to hospitals and commercial clinical testing laboratories.

Some of the initial research leading to Pathogenica's probe technology was done by Adesokan, while he was a postdoctoral fellow in the laboratory of Harvard Medical School's George Church, a member of Pathogenica's scientific advisory board.

Lower Cost Multiplex Testing

Molecular DNA sequencing is vastly more informative and accurate than a culture-based test. And compared to a qPCR test or culture sequencing, Pathogenica's technology allows highly multiplexed tests reaching as low as $10 per test. Its DxSeq probes are designed to detect multiple strains or species with a single probe. What gives the testing protocol extra power is the ability to deploy multiplex probe sets that can capture dozens to hundreds of strains or species in a single reaction and from a single sample. "This efficiency of sample usage contrasts favorably with other highly parallel PCR technologies that perform many individual PCR reactions, but require a lot more sample to populate each of these reactions," says Adesokan. "We can detect multiple pathogens from one test and adding new pathogens adds relatively little cost. For qPCR, cost would scale much more rapidly with the number of target loci and organisms."

"The use of next gen sequencing for multiplex identification of microorganisms is a very promising idea," says Timothy O'Leary, President of the Association for Molecular Pathology and Deputy Chief R&D Officer at Veterans Health Administration. "In principle, with next gen sequencing it is possible to look for many more microorganisms at the same time and to characterize them and quantitate them. I think that is very very interesting and potentially extremely powerful for something like infectious disease diagnosis." The ability to multiplex thousands of loci simultaneously gives Pathogenica an advantage in broad target coverage, while maintaining target resolution and sensitivity through near single-molecule nucleic acid sequencing. Says Adesokan, "I'm not aware of any

other technology that can boast the same strengths and perform individual patient diagnostics in this price range in under 12 hours.''

PCR has been the reigning molecular diagnostic technology, thus a comparison with Pathogenica's technology is inevitable. Adesokan explains that the DxSeq probes have significantly reduced cross-reactivity and mispriming, allowing the multiplexing of thousands of probes in a single sample lane and a modular approach to combining probe sets targeting different types of pathogen.

Infectious Disease and Biosurveillance

Pathogenica's first clinical applications are for test panels in viral disease including HPV, HCV, and HIV. ''We like to point out new highly interesting reports implicating viral infection in as many as 40% of cancer types,'' says Doran. The company is also developing probe panels for MRSA, respiratory diseases, *E. coli*, and food-borne diseases, both for diagnostic and surveillance uses. ''Essentially any pathogen detection problem can be addressed with sequencing,'' adds Doran. ''The core advantages of next generation sequencing are the breadth of target coverage (tens of thousands of loci can be genotyped), unmatched specificity provided by single-nucleotide resolution, near single-molecule sensitivity, and accurate population profiling—which is important in viral quasispecies analysis or detection of low abundance tumor-causing mutations.''

Doran explains that a rapid, low-cost DNA sequence-based diagnostic technology would have widespread appeal. ''Our initial utility is in diagnosing, genotyping, and detecting pathogenic drug resistance,'' he explains. ''All of this information is useful for influencing a clinician's decision-making in terms of therapeutic strategy.''

While it grows its clinical application, Pathogenica is branching out with its RUO service offerings. In July 2011, the company teamed with GenomeQuest, a company that provides data analysis tools integrating next-generation sequencing into everyday data analysis workflows. ''One specific application is for human and pathogen marker profiling,'' adds Doran. ''We partnered with GenomeQuest to offer their analysis tools to customers who are interested in targeted sequencing of human loci.''

While the dynamics of the clinical diagnostics marketplace are always changing, the future is likely to include an expanded portfolio of sequencing-based products. Pathogenica, as its name implies, hopes to be among that first of the next-generation of service providers.

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