

In Sequence

The Inside Read on Genome Sequencing



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Hoping to Cut Reseq Costs, Transgenomic Devising Enrichment Method for Variant DNA

TRANSGENOMIC said last week that it is developing a new method to enrich DNA that differs between a reference and a test genome. It promises to reduce the amount of sequencing required, and thus the cost, of detecting all small-scale variations in a genome.

The technology, which is still at least two years away from commercialization, generates so-called Surveyor Endonuclease Adaptor-ligated Libraries, or SEAL, that can be analyzed by second-generation sequencers. An extension of the company's Surveyor nuclease technology, SEAL is designed to recognize SNPs and small insertions and deletions across a genome with high sensitivity.

According to Omaha, Neb.-based Transgenomic, the technology is being designed to reduce by at least 90 percent the amount of sequencing required to analyze a genome for such variations.

"It's a very elegant technology that would enable you just to sequence where there is change," Transgenomic CEO and President Craig Tuttle told *In Sequence* last week.

SEAL "bridges the current technology gap between haplotyping of known SNPs and deep high-throughput DNA sequencing," Transgenomic said in a statement last week.

Generating a SEAL library starts with mechanically fragmenting either genomic DNA from both test and reference genomes, or selectively amplified regions of

interest within those genomes, according to the company.

Researchers then mix and hybridize fragments from both samples, with a proportion of them forming heteroduplex DNA that contains one strand from the test genome and one from the reference sample. Mismatches occur in regions where the two samples differ.

Transgenomic then treats the DNA with Surveyor nuclease, a mismatch-specific endonuclease that cuts heteroduplex DNA at sites where a SNP, a small insertion, or a small deletion is present.

Next, the company ligates an oligonucleotide tag of known sequence to the ends of the cleavage products and uses a biotin-streptavidin bead to isolate the tagged DNA to produce the SEAL library.

Scientists can sequence the library using high-throughput or traditional sequencing systems, Transgenomic said.

SEAL OF APPROVAL

The concepts for SEAL and the Surveyor nuclease technology were developed by Gary Gerard, vice president of the molecular biology research and development group at Transgenomic's laboratory in Gaithersburg, Md.

Transgenomic currently sells a number of mutation-detection and sequence-validation kits that are based on the Surveyor technology. However, these kits, which usually assay between one and six PCR products at a time, rely not on high-throughput sequencing-based genome-wide analysis, but use instead standard gel electrophoresis, fluorescent capillary electrophoresis, or Transgenomic's WAVE system.

So far, Transgenomic has been validating each step in the SEAL process using several model gene systems and has filed a patent application on the overall technology, according to Tuttle.

The next step will be a proof-of-principle project to analyze differences between microbial strains. For this, Transgenomic, is currently in continued on next page



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discussions with undisclosed collaborators that have at least one type of second-generation sequencing system, which Transgenomic does not have in-house.

Besides academic centers, Tuttle said the company plans to approach vendors of second-generation sequencers, including Roche, Illumina, Applied Biosystems, and Helicos Biosciences, to co-develop SEAL.

"If one partner wants to gain an exclusive [partnership] and develop the technology thoroughly, we would participate with them in accelerating the development of this technology, and then partner with them to sell it," he said.

Over the next two to five years, Transgenomic plans to develop a variety of applications for the technology, including in clinical and industrial microbiology, for SNP discovery, and for surveying subsets of the human genome such as the kinome, the oncogenome, or regions associated with complex diseases.

Tuttle pointed out that one advantage of SEAL will be its sensitivity, estimating that the technology will be able to detect small genomic differences such as a mutant allele that is present at 0.1 to 1 percent of the level of the wild-type allele. According to Tuttle, this ability could be useful, for example, for analyzing tumor samples.

Despite the continuing slide in sequencing costs, he believes that "there will still continue to be a very strong market" for SEAL, which itself promises to cut by 90 percent the amount, and thus the cost, of sequencing required for a genome-wide project.

Because more than 99 percent of DNA between any two given genomes is identical, "the downstream processing, analysis, and archiving of this redundant DNA will be a considerable drain on resources and time," according to Eric Kaldjian, Transgenomic's chief scientific officer.

SEAL has received interest from infectious disease

and oncology researchers at undisclosed pharmaceutical companies who "see the benefit in their discovery programs," according to Tuttle. He added that Transgenomic has submitted an application to the National Science Foundation for a Small Business Research Innovation grant to further develop the technology.

Developing SEAL would expand the use of Transgenomic's Surveyor nuclease technology and enhance the company's current portfolio of mutation detection technologies.

A major part of the company's business comes from its WAVE systems for mutation discovery, which relies on denaturing high-performance liquid chromatography. WAVE systems, which can detect differences

between alleles from a test and a reference sample, have been used to screen for a variety of disease-related mutations, according to Transgenomic. For example, labs in Europe and Asia use the platform to analyze BRCA genes, according to Tuttle.

Transgenomic has installed approximately 1,500 WAVE systems worldwide, he said.

Almost three years ago, Transgenomic, which is publicly traded, launched two service businesses: a molecular clinical-reference laboratory that focuses on detecting mutations associated with mitochondrial diseases, and a pharmacogenomics research services laboratory that supports pharmaceutical companies for clinical trials and performs a variety of assays, including methylation analysis, DNA sequencing, RT-PCR, and cytogenetics.

In 2007, Transgenomic had approximately \$23.2 million in sales, a loss of about \$2.1 million, and a headcount of about 130 employees.

During the first quarter of 2008, Transgenomic generated net sales of \$6.3 million, and had a net income of \$122,000. The two laboratory services contributed about \$1 million to revenues.

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