

# New technique used to profile anthrax genome

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Scientists at Georgia Tech have used a new approach, known as RNA-Seq, to profile the gene expression of the bacterium that causes anthrax, *Bacillus anthracis*.  
Credit: Nicholas Bergman/Georgia Tech

Scientists at the Georgia Institute of Technology have used a new approach, known as RNA-Seq, to profile the gene expression of the bacterium that causes anthrax, *Bacillus anthracis*. Their study, published March 20, 2009, online by the *Journal of Bacteriology*, marks the first time any bacterial transcriptome—the complete collection of mRNAs produced by a bacterium as it expresses different genes—has been comprehensively defined, and provides a much more detailed view of how bacteria regulate their gene expression.

"Sequencing a [bacterial genome](#) has gotten to be pretty routine, but going to a deeper level and defining the transcriptome has been a much more difficult task," said Nicholas Bergman, assistant professor in the School of Biology at Georgia Tech and senior research scientist in the Electro-Optical Systems Laboratory at the Georgia Tech Research Institute.

"With traditional methods, transcript structure and abundance really have to be determined one gene at a time, and a completely defined transcriptome was out of reach for even the most widely studied species," said Bergman. "The RNA-Seq approach allowed us to get around the limitations of traditional methods so that we can see in a much more detailed way how each of the 5,000+ genes in *B. anthracis* genome is expressed and regulated."

The RNA-Seq approach works by using a technique known as high throughput sequencing, which counts millions of messenger RNA (mRNA) sequences simultaneously. Although the method was used to define the transcriptomes of several [eukaryotic organisms](#) in 2008, applying it to bacteria has been difficult, said Bergman, because bacterial mRNAs have a different structure and cannot be easily separated from the other RNAs in the cell.

To solve this problem, the Georgia Tech team worked with researchers from Life Technologies, a biotechnology tools company, and ultimately developed a set of procedures that can be used to apply RNA-Seq to any bacterium.

In using this approach to study *B. anthracis*, Bergman and colleagues sequenced mRNA samples that were collected from *B. anthracis* cells growing in a variety of conditions. They collected more than 270 million sequence "tags," each of which corresponds to a short fragment of an RNA molecule, and pieced them together using a custom software tool that they developed for the project.

"Once the data were together, it was very easy to see transcript structure across the genome," said Bergman. "We could see clear boundaries between transcribed and non-transcribed regions of the genome, which represent where individual transcripts start and stop. This was really exciting, because transcript boundaries tell us precisely where to find the regulatory sequences that govern

[gene expression](#), and these sequences are extremely hard to find otherwise."

The researchers also found that since RNA-Seq is essentially just a very high-throughput counting technique, it also provides a way of determining how abundant each transcript is in the cell. They showed that this approach is a much more sensitive way of measuring [gene expression](#) than the more conventional microarray-based methods.

"We can very easily see which genes are the most highly expressed, but we were also able to detect very rare transcripts—the ones that are only being produced by 1 in 100 or 1 in 1000 cells—and with this level of sensitivity we can actually get a glimpse of the random events that make individual cells different from one another," said Bergman.

Combining the structure and abundance information for every gene in a bacterial genome allows researchers to take a more rational approach to tasks like antibiotic discovery and microbial engineering, Bergman noted.

"Sequencing-based transcriptome profiling has several huge advantages over array-based profiling," said Bergman. "Right now array-based methods are still a little less expensive, and take a little less effort in terms of the bioinformatics, but I don't think those obstacles will last long. I think we'll see a lot more studies taking this approach in the near future."

Source: Georgia Institute of Technology

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