Pacific Biosciences DNA Sequencing Technology Yields New Insights into Virulence and Evolution of German E. coli Pathogen

International Team Publishes Comprehensive DNA Analysis of Outbreak Strain and 11 Related Strains in New England Journal of Medicine

MENLO PARK, Calif.--(BUSINESS WIRE)-- An international team of scientists has successfully employed single molecule, real-time (SMRT™) DNA sequencing technology from Pacific Biosciences of California, Inc. (NASDAQ:PACB) to provide valuable insights into the pathogenicity and evolutionary origins of the highly virulent bacterium responsible for the German *E. coli* outbreak. Published online today in the *New England Journal of Medicine,* the results provide the most detailed genetic profile to date of the outbreak strain, including medically relevant information.

The researchers determined the outbreak strain was a member of the enteroaggregative pathotype of *E. coli* (EAEC) with serotype O104:H4. The outbreak isolates are distinguished from other O104:H4 strains because they contain genes encoding Shiga toxin 2 (Stx2) and a distinct set of additional virulence and antibiotic resistance factors. In addition, the team found that expression of the *stx2* gene was increased by certain antibiotics including ciprofloxacin, suggesting caution should be used before using certain classes of antibiotics to counteract this newly emerged pathogen.

By sequencing the outbreak strain and 11 related strains with the PacBio *RS*, the team concluded that horizontal genetic exchange with the Shiga toxin-producing enterohemorrhagic *E. coli* (EHEC) strain enabled the emergence of the highly virulent Shiga toxin-producing O104:H4 EAEC strain. The genetic analysis also indicates that evolution of this new form was a relatively recent event.

The team identified many virulence factor genes commonly found in EAEC. Furthermore, the exceptionally long sequencing reads that are characteristic of PacBio SMRT DNA sequencing technology enabled the team to also detect larger-scale deletions, insertions, inversions and other structural variation between the O104:H4 outbreak samples and the other O104:H4 EAEC samples that were sequenced. Several of these structurally divergent regions house genes that encode virulence factors. Another feature in which the current outbreak diverges from common EAEC isolates is in the number and nature of SPATE proteases. Taken together, the results provide a possible explanation for the increased virulence of the German *E. coli* outbreak strain.

The authors included scientists in the U.S. and Denmark from Pacific Biosciences, the University of Maryland School of Medicine, the University of Virginia School of Medicine, the World Health Organization Collaborating Centre for Reference and Research on *Escherichia coli* and *Klebsiella*, the Statens Serum Institut, Hvidovre University Hospital, Brigham and Women's Hospital and Harvard Medical School.

"This multi-strain sequencing data and analysis significantly increases the amount of scientific information available for the study of this new deadly form of *E. coli* and has yielded critical insights into its causative agent," said co-author, David A. Rasko, Ph.D., Assistant Professor, University of Maryland School of Medicine, Institute for Genome Sciences and Department of Microbiology and Immunology. "Our results provide the most complete published genome of this strain to date and highlight the importance of DNA sequencing to understanding how the plasticity of bacterial genomes facilitates the emergence of new pathogens."

Whole genome sequencing involves decoding the precise order of nucleotide bases that make up an organism's complete set of DNA and provides more comprehensive information than other analysis methods such as DNA fingerprinting or arrays. With advances in technology and decreasing cost, whole genome sequencing is emerging as the gold standard method for identifying and classifying infectious agents. SMRT technology is the latest advance in DNA sequencing, capable of generating long sequence reads to resolve structural variations and complex genomes at ultra-fast speeds by ‘eavesdropping' on DNA replicating in real time.

Eric Schadt, Ph.D., Chief Scientific Officer of Pacific Biosciences and co-author of the paper commented: "We have reached a new era in which communities of researchers can rapidly share large-scale data sets and analyses vital for public health. Sequencing genomes in hours, as opposed to days or weeks, with unprecedented read lengths is the emerging hallmark of third generation DNA sequencing. The long PacBio *RS* reads enabled a PacBio-only *de novo* genome assembly, a key component of new pathogen characterization, as well as deeper insights into structural variants."

The paper is available at www.nejm.com. The data are available for the bioinformatics community at the PacBio developer's network (DevNet) web site (www.pacbiodevnet.com), where a suite of open source tools and other resources designed for

SMRT sequence data are available to analyze the information. A summary of the sequencing project appears on the Pacific Biosciences website at http://blog.pacificbiosciences.com. More information about SMRT technology is available at www.pacificbiosciences.com.

About Pacific Biosciences

Pacific Biosciences' mission is to transform the way humankind acquires, processes and interprets data from living systems through the design, development and commercialization of innovative tools for biological research. The company has developed a novel approach to studying the synthesis and regulation of DNA, RNA and proteins. Combining recent advances in nanofabrication, biochemistry, molecular biology, surface chemistry and optics, Pacific Biosciences has created a powerful technology platform called single molecule, real-time, or SMRT™, technology. SMRT technology enables real-time analysis of biomolecules with single molecule resolution, which has the potential to transform the understanding of biological systems by providing a window into these systems that has not previously been open for scientific study.

Forward-Looking Statements

This press release contains forward-looking statements. Forward-looking statements may contain words such as "believe," "may," "estimate," "anticipate," "continue," "intend," "expect," "plan," the negative of these terms, or other similar expressions, and include the assumptions that underlie such statements. Such statements include, but are not limited to, statements regarding the Company's SMRT technology. These statements are subject to known and unknown risks and uncertainties that could cause actual results to differ materially from those expressed or implied by such statements, including but not limited to risks discussed from time to time in documents Pacific Biosciences of California, Inc. has filed with the Securities and Exchange Commission, including the risks identified under the section captioned "Risk Factors" in its recently filed Quarterly Report on Form 10-Q. All forward-looking statements are based on estimates, projections and assumptions as of the date hereof. Pacific Biosciences undertakes no obligation to update any forward-looking statements.

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