One Small Step for DNA Sequencing

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DNA Sequencing

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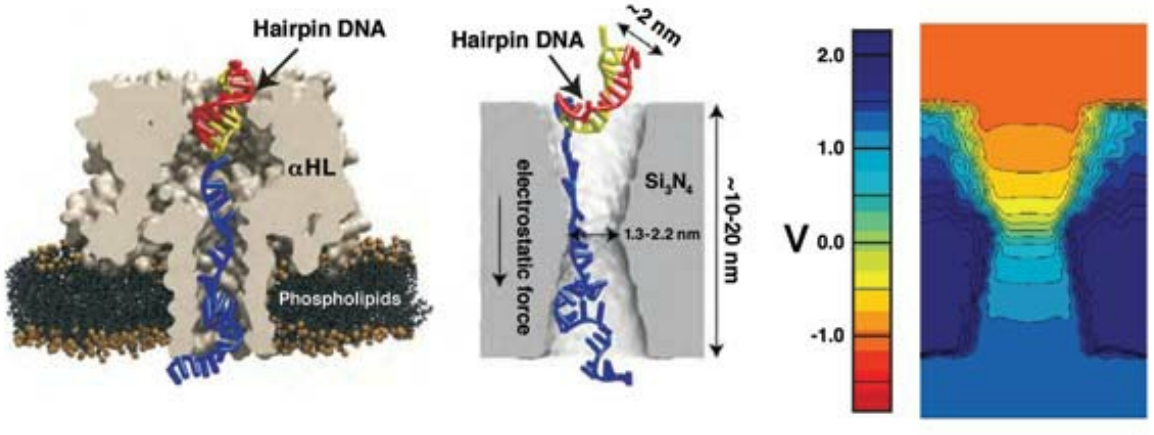
Since Watson and Crick first discovered the molecular structure of deoxyribonucleic acid (DNA), researchers have been building on their findings to try to unlock the mysteries of life. The Sanger method of DNA sequencing, which provided the first draft of the human genome, also transformed genetics and biology, but it is now being replaced by next-generation sequencing (NGS) technologies that deliver faster and more accurate genomic information. The challenge with NGS comes from the enormous volume of data produced — in some cases more than one billion short reads per instrument per day. Taking NGS one step further, a Notre Dame-led team has developed a revolutionary concept that can sequence one molecule of DNA using a single nanopore. In short, the team is offering a giant leap in sequencing sensitivity that provides equally reliable outcomes but with a smaller price tag and a smaller sample size.

Except for a few viruses, DNA is the biological road map of every living organism known to man. Understanding how it affects one generation to the next not only answers “Why Tommy has green eyes” but may also identify if he is prone to a genetic disorder. DNA sequencing, determining the order of the adenine, guanine, cytosine, and thymine bases in a molecule of DNA, has been instrumental in identifying and better understanding genetics and the human genome. This knowledge has been applied to basic biological research, as well as specific fields such as diagnostic and forensic biology. However, the sequencing technologies currently used are not without their challenges.

Gregory L. Timp, the Keough-Hesburgh Professor of Electrical Engineering and Biological Sciences, and the DNA sequencing team — Utkur M. Mirsaidov (Singapore National University), Aleksei Aksimentiev, Jean-Pierre Leburton, and Klaus J. Schulten (University of Illinois), and Andrew P. Feinberg and Winston Timp (The Johns Hopkins University) — have developed what they believe to be the next logical step in sequencing technology: single-molecule DNA sequencing.



Using nanopores fabricated within thin membranes, Gregory L. Timp and team are sequencing deoxyribonucleic acid (DNA) on the smallest of scales. With a pore diameter of 1.3 nanometers, the graphics above show a cross section of the DNA as it travels through the process … through a solid-state nanopore in a silicon nitride membrane, being trapped in the pore, and the distribution of electrostatic force in the pore, before the DNA is present. By applying an electrical field to the pore, the team is able to force individual molecules to move through the pore in sequential order, much like threading a needle. The electric signatures captured by each DNA base provides quick results and easy sequencing with a minimal amount of primary samples (DNA) needed from patients.

According to Timp, single-molecule DNA sequencing extracts the maximum amount of information (it offers very long read lengths, >1 kbp) from a minute amount of material. The low material requirement coupled with quick results offers easier sequencing of samples from human patients, allowing doctors to look for rare and hereditary diseases much earlier, before symptoms would typically appear.

The team began studying nanopores in solid-state membranes as an alternative technology because of the flexibility in their fabrication and ease of integration into a sequencing platform. Preliminary results have shown that with careful control of the dimensions of the nanopore and the shape of the electric field applied to the DNA molecule, control of DNA translocation through the pore is possible. In addition, discrimination between different base pairs of DNA may be feasible.

Thus, using a nanopore in the sequencing process promises inexpensive, reliable, high-throughput sequencing, which could thrust genomic science into the realm of personal medicine because the time and cost would be much more accessible. For example, the Sanger method of DNA sequencing provided a draft of the human genome at a cost of approximately $2.7 billion. The third-generation technology Timp and team are working on could help Tommy's parents to identify or rule out a genetic disorder for around $1,000.

Suggested Reading

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