GENOMICS

A 'pore' man's sequencing solution?

A protein pore-based system for nucleotide recognition may help make genome sequencing cheaper and easier.

How much does a genome cost? That depends. The first human genome sequence cost a fortune in financial resources and manpower, but as technology and know-how have improved, this process has gotten simpler and more affordable. But it still is not cheap—and the National Institutes of Health (NIH) are looking to change this with a '\$1,000 genome' initiative, the Revolutionary Genome Sequencing Technologies program, funding research on innovative, budget-friendly systems for rapid genome-scale sequencing.

One promising strategy involves feeding DNA molecules through a pore that carries an electric current, so that fluctuations in the current reveal the sequence of bases. Two groups, Hagan Bayley's at the University of Oxford and Reza Ghadiri's at The Scripps Research Institute in San Diego, are now collaborating to develop such methods, and the two recently showed that after stalling single DNA strands in pores formed from the bacterial protein α -hemolysin, they could distinguish between a trapped adenine or cytosine (Ashkenasy *et al.*, 2005). Unfortunately, the

quality of readings breaks down when the chain is in motion. "The DNA moves through much too quickly for us to distinguish between individual bases," Bayley explains, "and it also moves through in a chaotic way that mitigates against recognition."

More recently, Bayley's team tried a different approach, testing the capacity of a protein pore to identify free nucleotides. They continued to work with α -hemolysin—which is very stable, well-characterized and easy to produce—but constricted the diameter of the pore by adding a cyclodextrin 'adapter'. Using this engineered pore embedded in a lipid bilayer, they found that they could readily distinguish between all four classes of both ribonucleotides and deoxyribonucleotides, with a level of accuracy that approaches 98% (Astier *et al.*, 2006).

The authors see some potential in an exonuclease-driven approach, where single bases are cleaved off of an immobilized DNA molecule and then identified as they pass through the pore, but Bayley and Ghadiri are also continuing to explore methods in which entire chains are fed through the pore. They are now focused on exploring the mechanics of DNA interaction with the pore, with an eye

toward developing methods to better regulate chain movement through the channel. "We've got a lot to learn about the fundamentals of how polymers enter pores and move through them," he explains, "and doing this will drive us toward the goal of DNA sequencing."

Bayley sees several potential benefits for budget-friendly genome sequencing, especially for the rapid discovery of disease risk factors, or for pharmacogenomics research. And although a commercial system may not yet be in reach, Bayley sees promise in pore-based sequencing. "It's a single-molecule technique, so you don't have to clone or amplify your DNA before you sequence it," says Bayley. "So it would be really cheap, and to do a \$1,000 genome might actually be possible."

Michael Eisenstein

RESEARCH PAPERS

Ashkenasy, N. *et al.* Recognizing a single base in an individual DNA strand: a step toward DNA sequencing in nanopores. *Angew. Chem. Int. Ed.* **44**, 1401–1404 (2005).

Astier, Y. *et al.* Toward single molecule DNA sequencing: direct identification of ribonucleoside and deoxyribonucleoside 5'-monophosphates by using an engineered protein nanopore equipped with a molecular adapter. *J. Am. Chem. Soc.* **128**, 1705–1710 (2006).