BIOPHYSICS

Melting trapped DNA

Single DNA molecules stretched in a nanochannel display melting patterns characteristic of their sequence.

When Walter Reisner was a graduate student in Robert Austin's laboratory at Princeton University, he was introduced to the art of stretching single DNA polymers in nanofluidic devices. Now in his own lab at McGill University, single DNA molecule analysis continues to fascinate him.

It has been known for decades that melting DNA yields a heterogeneous pattern of single- and double-stranded regions. Reisner now proposes a fluorescence readout for these melting patterns. Together with his colleagues he devised a simple device, a U-shaped microchannel that leads into nanochannels with small openings for buffer exchange. The researchers bound fluorescent dye to double-stranded DNA, loaded it into the microchannels and raised the temperature.

To their delight, the process worked exactly as predicted. As DNA denatures the dye comes off and single-stranded regions are seen as darker patterns in camera recordings. The first experiments with DNA from the lambda phage showed that the dark bands corresponded to regions of high A+T content. Encouraged by these results Reisner used larger phage DNA and saw characteristic melting barcodes that could be assembled to reconstruct the entire phage genome.

The current resolution of the method is around one kilobase, and Reisner speculates that it may be improved to 100 base pairs but probably not further. He stresses that this will always be a large-scale mapping, not a sequencing, approach.

With a nanofluidic setup—that can capture and lyse single cells and introduce the DNA into the nanochannel, Reisner anticipates the ability to probe structural variations in single human cells. It could also find an application in targeted genome capture. As the theoretical melting pattern for any sequence can be derived, one could train an algorithm to look for a pattern of interest and then release the DNA with the matching pattern for subsequent sequencing.

Another intriguing possibility is to apply the approach to epigenetic studies. "There might be ways to simultaneously do a melting map and a methylation profile," says Reisner. Such a combination of genomic and epigenetic information on a single molecule would be of great value in examining the heterogeneity of tissues and tumors.

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RESEARCH PAPERS

Reisner, W. *et al.* Single-molecule denaturation mapping of DNA in nanofluidic channels *Proc. Natl. Acad. Sci. USA* **107**, 13294–13299 (2010).