Researchers develop new DNA analysis technique using infrared laser heat
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DNA molecules enter into the pore and cause the current signal to decrease. At room temperature, double-stranded DNA molecules remain in the pore for long periods of time, which makes it difficult to analyze a DNA mixture. Focusing a heating laser onto the nanopore speeds up the interaction of the DNA with the pore and yields a far greater number of events that can be analyzed to identify the DNA molecules in solution.

Credit: DNA and nanopore analyzer images reproduced from the PDB: 10.2210/pdb2m2c/pdb and 10.2210/pdb7ahl/pdb respectively

Virginia Commonwealth University researchers have developed a new technique for analyzing DNA molecules. Their new research article, "Infrared laser heating applied to nanopore sensing for DNA duplex analysis," suggests the possibility for improving forensic DNA workflows for more rapid and accurate identification. The article was published in the journal Analytical Chemistry and appeared online on Feb. 19.

"We are interested in increasing the number of parameters that researchers can tune in order to study small DNA molecules," said Joseph Reiner, Ph.D., assistant professor of physics in the College of Humanities and Sciences. "Laser heating has been used in the past, so we applied this methodology to our nanopore technique and found it was effective at discriminating between different sized DNA fragments."

Nanopore sensing allows researchers to learn about the physical and chemical properties of molecules in solution. A nanopore is a little hole with an ionic current. When it's introduced in a solution sample, DNA molecules from the solution drift through it, causing the current to change. Based on the current change and also the length of time the molecule dwells in the nanopore, researchers get information about the molecule. In this case, the researchers were interested in analyzing the size of the molecules.

One problem researchers face with DNA nanopore sensing is that the molecules will sometimes remain in the hole indefinitely. Researchers can speed up the drift of molecules in a number of ways, including heating the sample solution. This controls the interaction time between the DNA and the pore and enables more accurate identification of the types of DNA molecules in a given sample. There are drawbacks when an entire solution is bathed in heat though, including slow heating and cooling times and solution evaporation.

Infrared laser heating solves these problems because the energy is pinpointed at a very small volume of the sample, which allows for rapid and isolated application of heat. "The laser can localize the heat down onto the pore, which gives us much better control over the frequency of DNA events and the time a DNA molecule remains in the pore. By analyzing each of these events, we hope to characterize the number and size of different DNA molecules in a solution," Reiner said.

The current method of forensic DNA profile
development depends on discrimination of the DNA fragment size, according to Sarah Seashols-Williams, Ph.D., assistant professor in the Department of Forensic Science and co-author on the article. The ability to see varying sizes of the DNA fragments known to differ between people means knowing if the sample includes DNA from one or more people.

This information would serve as a prescreening tool for forensic scientists and allow them to accurately decide a course of action for further analysis and workflow. Currently working without a prescreening method, scientists often are required to waste time and resources and run additional tests to understand the DNA found on evidence.

"Researchers are hoping to provide information prior to the manpower and cost of the traditional analysis method so they can better use resources," Seashols-Williams said. "That will also help reduce backlogs because they're able to work more quickly, using only the procedures that will provide information for that sample."


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