Instant DNA Detection
Systems based on electrical signals move from science fiction to reality

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In last year's sci-fi movie "Gattaca," the protagonist, attempting to conceal the fact that he's assumed the identity of a genetically "superior" man, scrubs himself daily to rid his body of loose hairs, skin flakes, and anything that might reveal his true identity through his DNA.

His paranoia is understandable. In the "Gattaca" future, vigilant police and employers carry handheld sensors that instantaneously analyze the DNA in snips of hair, drops of blood, or urine in order to expose the genetically "inferior." Parents receive their newborn babies entire genetic profiles in a few minutes.

Actually, modern-day science is not far behind this technological scenario, though it envisions more humanitarian applications: detecting diseases, monitoring air for biological warfare agents, or checking food-processing plants for bacterial contamination.

Currently, extremely accurate tests for DNA sequences are based on fluorescence signals. The polymerase chain reaction (PCR) multiplies minuscule amounts of DNA into readable quantities. Although these techniques are extremely sensitive and quantitative, they require time, sample preparation, and expensive equipment. And the systems generally aren't portable.

But a number of research groups around the world are now closing in on the technology needed to develop a device that a doctor can use during an office visit or in the field to obtain results within minutes. The key to this technology is electricity.

When a single DNA strand encounters a complementary partner in a sample, it will hybridize. This hybridization can be detected by changes in an electrochemical signal—voltage or current, for example—usually through a redox-active or conducting molecule that behaves differently in the presence of a DNA hybrid than it does in the presence of a single strand.

"If there's a possibility of detecting hybridization in a rapid way using electrical signals instead of fluorescence, that's a tremendous advantage," says David L. Barker, vice president of research and business development at the instrumentation manufacturing company Molecular Dynamics in Sunnyvale, Calif. Electricity can be monitored without regard to the clarity or turbidity of a sample, and it doesn't require excitation lasers or reading by spectroscopic instruments.

"A direct electric reading to analyze DNA sequences appears much more elegant and sensitive," notes Francis Garnier, of the molecular materials laboratory at the National Center for Scientific Research (CNRS) in Thiais, France.

If it were small, battery-operated, and cheap, such a device could be a boon for the diagnostics industry and bring a lot of money to its inventors.

"If they can develop a simple test, it's a huge potential market," says Michael J. Powell, new technologies director at Roche Molecular Biochemical in Pleasanton, Calif.

One company hoping to usher in this new wave of technology is Pasadena, Calif.-based Clinical Micro Sensors (CMS), which unveiled a prototype handheld DNA sensor at the Council on Competitiveness' National Innovation Summit, held at Massachusetts Institute of Technology in March.

CMS was founded by Thomas J. Meade, a chemist at California Institute of Technology, and Jon Faiz Kayem, president of CMS and Meade's former postdoctoral researcher. Meade's lab pioneered research on the fact that double-stranded DNA conducts electricity more efficiently than single-stranded DNA does. CMS is now aggressively pursuing commercialization of the system, tackling the hurdles in this area such as cost, size, and marketing. It is now in pilot production of its handheld DNA sensor.

"Currently, industry can deliver accurate DNA diagnostics, fast DNA diagnostics, convenient DNA diagnostics, and, occasionally, low-cost DNA diagnostics. What it cannot do is combine these attributes into a fast, accurate, convenient, low-cost assay," Kayem says. "That's where many of us believe biosensors will help."

New Mexico State University chemistry professor Joseph Wang, whose lab specializes in sensor research, has also developed a handheld sensor for detecting lead in blood, which he says can eventually be modified to detect DNA.

Garnier's lab, as well as those of Susan R. Mikkelsen, associate chemistry professor at the University of Waterloo in Ontario, Shigeki Takenaka, head of the molecular systems group at Kyushu University, Fukuoka, Japan, and Peter Bauercle, chemistry professor at the University of Ulm, Germany, are also publishing ever more ingenious DNA sensor chemistry, filing patents, and working with medical diagnostics companies.

An electrochemical technique is inherently one that involves surfaces. In all the systems. DNA is bound to—"immobilized"—on a solid electrode. For example, Mikkelsen uses carbon-based material as an electrode because it's easy to generate functional groups on its surface. She then covalently binds single strands of DNA, acting as "probes," to the electrode surface. A solution containing DNA strands, known as "targets," is added. If the targets are complementary to the probes, they hybridize to form a double strand.

Then she adds a re...
Different molecules help signal DNA hybridization

Threading intercalator (top) with ferrocenyl groups at each end inserts itself through DNA. The intercalator dissociates much more slowly in the presence of double-stranded DNA [Takenaka's approach]. Poly(pyrryl)enes, which are highly conductive in water, are functionalized with oligonucleotides (center). They show decreased current intensity when the oligonucleotide hybridizes [Garnier's approach]. Conducting polythiophenes functionalized with a single nucleobase (bottom) bind with the complementary nucleobase and produce a change in conductivity [Baeuerle's approach].

is yes or no, then you have to be very careful about false positives and negatives.” says Mikkelsen. All sources of interference—that is, when a DNA strand matches with something other than its intended partner—need to be eliminated.

Parameters such as temperature can affect selectivity. For instance, some DNA strands could hybridize with a mismatched strand, but they’re much less stable than a strand where all the base pairs are matched. It’s important to find a temperature that makes it difficult for mismatched strands to stay paired.

The detection limits of many electronic methods are currently hovering around the femtomole level—and CMS can now detect at the attomole level—but researchers are trying to lower that. “Attomole detection is acceptable for many applications, but we’d like to get down to 100 molecules to better compete with PCR,” Kayem says. Labs are also looking at ways to amplify the tiny electrical signals generated by each molecule.

Researchers use many different approaches to design a DNA sensor system. In many systems, an electroactive group that facilitates electron transfer—such as cobalt or a ferrocene complex—is attached to a molecule that preferentially binds to duplex DNA. These molecules can include intercalators, which slip between base pairs of DNA, or molecules that bind to the minor groove of DNA.

Takenaka, in collaboration with chemistry professor William David Wilson at Georgia State University, Atlanta, has developed a “threading” naphthalene diimide intercalator that actually slides through a space between DNA base pairs and out the other side. This property is useful because typical intercalators pop off, or dissociate, from a DNA strand very quickly and easily, which can make it difficult to make measurements. Takenaka’s intercalator, with two bulky ferrocene groups at each end, dissociates from double-stranded DNA much more slowly and yields greater sensitivity.

Some labs are functionalizing conducting molecules with DNA. For years, Garnier’s lab has studied conducting polymers with the goal of designing “intelligent materials” that sense chemicals such as enzymes and antigens or physical quantities such as photons or electric fields.

In a recent study, he grafted a 13-base oligonucleotide onto a poly(pyrrole), which in addition to being conductive, also shows a high degree of electroactivity in water. He found that hybridization of the oligonucleotide caused a decrease in current intensity, which he attributes to conformational changes along the polymer backbone [J. Am. Chem. Soc., 119, 7388 (1997)]. The sensitivity of their system is now $10^{-15}$ M. Garnier is working on improving sensitivity to $10^{-17}$ M. A French biomedical company is developing prototype devices based on Garnier’s results, which “in terms of sensitivity, appear even more promising than expected.”

Baeuerle and graduate students Andreas Emge and Alexander Meyer, who had seen changes in electrical signals when crown ethers attached to a polythiophene were complexed with alkali ions, at first studied the effect of single nucleoside-functionalized polythiophenes [Adv. Mat., 3, 324 (1998)]. They are now developing polythiophenes that are more electroactive in water and plan to do experiments with 15- and 16-base DNA strands. “We are very optimistic to see pronounced changes in the electrochemical response of the modified polymer,” Baeuerle tells C&EN.

Margaret Harding and Sally Lucas, of the Australian Membrane and Biotechnology Research Institute (AMBRI) at the University of Sydney in Australia, have patented a lipid membrane biosensor in

Molecular ‘wire’ connects DNA complex to electrode in the CMS system

which the conductance or impedance of the membrane depends on the presence or absence of a nucleic acid sequence. Ambri Pty Ltd., which commercializes Ambri technology, is "currently considering the commercial development" of a sensor based on their work, says Stephen Conlon, business development executive at Ambri Pty Ltd. in Australia.

Wang's lab is introducing some new concepts that he hopes will enhance selectivity and sensitivity. Peptide nucleic acids, which can base-pair to DNA, can be used as probes. Because of its neutral, peptide-like backbone, peptide nucleic acid recognizes specific DNA strands even more selectively than DNA, Wang says (J. Am. Chem. Soc., 118, 7667 (1996)).

"This is crucial for obtaining mismatch discrimination, as needed for detecting point mutations," Wang says.

Wang is also developing an indicator-free sensor, based on the fact that the nucleobase guanine is easily oxidized. Hybridization can then be detected directly by an electronic signal of guanine oxidation [Analyst, 121, 965 (1996)]. These detection schemes have been combined with single-use microfabricated electrodes and handheld analyzers, Wang says.

Also, binding highly branched DNA dendrimers—rather than a single strand—to an electrode can increase sensitivity, Wang says. "So far, we have over 10-fold signal enhancement and greatly improved linearity." However, he says, so far, they have used dendrimers with only 30 arms. "It is possible to design dendrimers with hundreds of arms," he adds.

The CMS system tackles the whole picture, from DNA immobilization to manufacturing. The CMS team saves preparation steps by having the label, which is usually added after hybridization, already covalently bound to the probe DNA. Samples containing whole cells and viruses can be added directly to the device, where they are split apart with heat or guanidinium thiocyanate.

The CMS system avoids the problem of the gold electrode interacting with other redox species floating in solution by coating the electrode with a self-assembled insulating monolayer of alkane thiols. The DNA-label complexes are connected directly to the electrode by phenylacetylene molecular "wires," and they push through the "lawn of insulator material like dandelions," Kayyem says.

Because of the protective layer, "we can do measurements directly in very dirty environments, including blood," he adds. In addition, no washing is needed, eliminating another time-consuming step. The sensitivity of their device is on par with any current electrochemical system, Kayyem says.

The CMS system is made possible in part because of advances in DNA chip technology, where large arrays of nucleotides are attached to supports using lithography techniques. To make its electrodes, CMS buys custom-made circuit boards and immobilizes the DNA on them. Other labs are also looking into DNA chip technology. Takenaka’s lab is submitting a patent on its model system with two electrodes.

In the next few years, DNA detection technology may bring the technology of “Gattaca” closer, as researchers continue to pursue the ideas that may find their way onto the DNA chips of the future.

"All ultimately may play a role in DNA diagnostics," Kayyem says.