

Rapid, Low-Cost DNA Testing

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Professor Lewis Rothberg of the University of Rochester Chemistry Department received a NYSTAR grant in August 2006 to continue working on a recent discovery by Huixiang Li, a research associate in his group: how to rapidly test DNA to improve our health and make sure we're drinking clean water and eating uncontaminated food. In fact, his new method can be used to help forensics labs identify criminals, test ponds and pools before children swim in them, and identify harmful genetic sequences in medical research, to name only a few applications. Rothberg's innovative procedure quickly and inexpensively identifies genetic sequences in any sample of DNA.

The technology is a novel fluorescent DNA screening assay, which rapidly determines whether specific DNA target sequences are present in an analyte. In simple terms, the analyte contains the DNA target sequences as well as other DNA sequences, and the assay filters out only the targets. Professor Rothberg's assay is based on the electrostatic properties of DNA.

The principle underlying the method is that single-stranded DNA and double-stranded DNA have significantly different affinities for attaching to ionically charged gold nanoparticles. Because ions have electric charges, having gained or lost electrons, they attract their opposites. An anion with a negative electric charge will attract positive charges, a cation with a positive charge will attract negative charges. Single-stranded DNA adsorbs on negatively charged citrate ions on the gold nanoparticles while double-stranded DNA does not. Given that both single-stranded and double-stranded DNA are (nominally) negatively

charged, this proven phenomenon intrigues the research group.

The new assay determines whether a fluorescently-tagged short probe sequence of single-stranded DNA matches a sequence in the target analyte. When it does not, the fluorescently tagged probe adsorbs on a gold nanoparticle and its fluorescence is quenched. If the probe sequence is able to hybridize to the target, it will not adsorb on the gold and its fluorescence persists.

The new method is simple and effective. It costs very little, and it's very quick.

The most widespread and common method of screening DNA is called gel electrophoresis. Each test takes 1 hour and can cost as much as \$1.00. Setting up a lab for gel electrophoresis requires a capital expenditure of \$5,000. By contrast, Professor Rothberg's technique only requires 5 minutes, and it costs approximately \$0.05 (literally five cents) per test. The capital expenditure to set up a lab with the new technique is only \$600.

Here's how Professor Rothberg's procedure is done:

Step 1. Hybridization. This takes 10 seconds and costs \$0.025.

Step 2. Add gold colloid to the hybridization solution. This takes 10 seconds and costs \$0.02.

Step 3. Add salt to the solution. This takes 10 seconds and costs \$0.01.

Step 4. Measure photoluminescence. This takes 1 minute.

It's as simple as that, yet nobody's ever done it before. The method is so new that the University of Rochester filed patents for it in 2004 and

2006. In May 2005, Professor Rothberg created a company called Diffinity Genomics, Inc. with two partners to further study and commercialize his technique.

Professor Rothberg's method is part of a much larger process that analyzes DNA. First, a technician extracts the DNA from the blood, tissue, or food. This typically take up to an hour. Second, there is generally not enough DNA to analyze, so it must be chemically amplified. This also takes apprximately one hour. The new process comes after these two steps, saving a final hour of work for the technician, who ordinarily would be doing gel electrophoresis.

Perhaps more important than the savings in time and money, the new method works to determine single-base mutations in DNA, whereas gels cannot do this without even further processing. Professor Rothbergs concludes, "This could be very important for applications in personalized medicine where a particular DNA sequence will be linked to a prescribed therapy. In fact, we see this happening already."

Source: University of Rochester

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