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Rapid Sequencing of DNA Using Micelles

The National Institutes of Health wants the capability to map a person's DNA for \$1,000 or less. That's no small feat considering that the entire human genome was first sequenced in 2003, after 13 years of research and at a cost of \$2.7 billion. Today, reliable albeit slow methods exist for sequencing DNA, like gel electrophoresis, and the push is on to speed up these processes. This challenge has driven James Schneider, a professor in Chemical Engineering and Biomedical Engineering, to spearhead research that aims to rapidly and reliably sequence DNA.

When traditionally sequencing DNA, researchers separate enzymatically derived DNA fragments copied from the original DNA. In an electric field, the fragments are forced through gels, and as the fragments migrate, the sequence can be read base by base. This process is of course automated but it is cumbersome — the gels are injected into capillaries that are about the width of a human hair — and it is slow because the gels

exert considerable friction on the DNA fragments, retarding their mobility. This is why Schneider is replacing gels with micelles, which are tiny oil-like droplets suspended in water made of surfactants.

The goal is to attach micelles onto migrating DNA fragments, and have the micelles pull on the DNA to separate them for sequencing. However, micelles won't bind to DNA on their own, so oily compounds called alkanes are chemically grafted onto the DNA. Like dish detergent attracted to grease, micelles will seek out and temporarily attach themselves for just a few milliseconds to the moving DNA. During the separation process, DNA will interact with hundreds of thousands of micelles during a typical run so that each DNA fragment feels a significant but highly uniform pull.

By replacing the gels, Schneider believes that they will be able to sequence DNA 10 to 100 times faster than traditional methods and provide data that is reliable enough for medical diagnostics. Other research underway involves using larger emulsion droplets to read longer lengths of DNA, which, too, will speed sequencing. — s . s .



James Schneider



MEMS Devices Will Advance Gene Function Research

The fruit fly (*Drosophila*) has long served as an important model for human development and disease. In 10 days, the fly grows from an embryo to an adult, making it a great organism for large-scale experiments. During the past two years, the genome sequences for 12 species of *Drosophila* have become available, and because of this new information, fruit flies, which have been studied for more than 100 years, are back under the microscope – now for extensive, systematic studies on how genes function, how gene expression is regulated and how genes work in concert to create and maintain a complex organism.

An important method for assessing gene function is based on RNA interference or RNAi, a mechanism that allows silencing of specific genes in an organism. (The discovery of RNAi earned Andrew Fire and Craig Mello the Nobel Prize for Physiology or Medicine in 2006.) Researchers inject fruit fly embryos at their earliest stages of development with designed, double-stranded RNA (dsRNA). In cells, the dsRNA triggers an interference mechanism that destroys specific messenger RNA (mRNA) and effectively deactivates a specific gene. Observation of abnormalities during embryonic development indicates when and where the silenced gene is active and what its function might be. However, working with the embryos is not easy. They are incredibly small (their width is about three or four times the width of a human hair) and it takes a great deal of skill to prepare and manually inject them. This time-consuming process is a bottleneck that Stefan Zappe, a professor in biomedical engineering intends to remedy. Early this year, Zappe received a \$400,000 National Science Foundation CAREER award to develop two automated, MEMS-based *Drosophila* embryo injection systems dubbed “Search and Inject” and “Feed and Inject.”

“We have all the genomic data, but we don’t necessarily know what all of the genes do,” says Zappe. “RNA interference gives us a powerful tool for probing an organism. We can turn genes off, look at the effects, and then infer things about the genes,” says Zappe. To help raise these studies to a higher level, Zappe, who has a background in electrical engineering, developed a MEMS device to automate the injection process.

Using a silicon wafer, he created a chip that features a glass reservoir that holds the double-stranded RNA. The substance flows through microfilters and into a tiny injector sitting on top of the chip’s surface. The needle is fabricated with tight geometric tolerances and enables reliable and precise dosing. The injector chip and the embryos that are affixed onto a glass slide are loaded onto motorized stages of an automated system. Using two cameras that look at objects in 3-D space, the system can discern and record the location of the embryos and align them with the needle tip for injection. The system will “search” for and “inject” the embryos. “You repeatedly record 3D images of many injected embryos, one after another, during the entire 24 hours of their embryonic development using an automated, confocal microscope and then your experiment is done,” says Zappe.

Watching specific tissue, e.g. musculature, in a developing embryo is greatly facilitated through the use of genetically engineered flies that express, for example green fluorescent protein (GFP) in a tissue of interest. “But such permanent genetic engineering of flies requires injection of DNA specifically into the posterior end of an embryo. There, special cells are formed that later give rise to the germ cells of the adult fly,” he says, and to help on this front he has designed another injector system that will automate the creation of transgenic flies. “You can design a gene, inject it into a fly embryo, let the embryo become an adult fly, and after rounds of breeding and crossing, you create a line of flies that express the designed gene in all their cells,” says Zappe.

In this MEMS system, again on a silicon chip, the injector will be embedded in a microfluidic channel. Fruit fly embryos will flow from a reservoir, funnel down a channel and self-align with the injector for precise injection into the posterior end of the embryo. After they are injected with the new gene, they will be fed into another reservoir. “In this case you want to keep the embryos happy so they can develop into adult flies,” says Zappe. “You put them down on food after injection. The embryos turn into larvae that eat the food and grow.” The flies are then available for further studies.

Prototypes of both of these systems have been built and the CAREER award will allow Zappe to improve their functionality and in time deliver products that are of vital interest to the *Drosophila* research community. On a broader scale, Zappe’s MEMS-based systems can fuel further development of automated systems for handling of DNA, RNA and other biochemical substances, which is crucial in biological research, biotechnology, drug discovery and medical diagnostics. — S.S.



Stefan Zappe