

MicroRNA detection on the cheap: Startup provides rapid, cost-effective microRNA profiling

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Current methods of detecting microRNA (miRNA)—gene-regulating molecules implicated in the onset of various diseases—can be time-consuming and costly: The custom equipment used in such tests costs more than \$100,000, and the limited throughput of these systems further hinders progress.

Two MIT alumni are helping to rectify these issues through their fast-growing, Cambridge-headquartered startup, [Firefly BioWorks Inc.](#), which provides technology that allows for rapid miRNA detection in a large number of samples using standard lab equipment. This technology has helped the company thrive—and also has the potential to increase the body of research on miRNA, which could help lead to better [disease diagnosis](#) and screening.

The company's core technology, called Optical Liquid Stamping (OLS)—which was invented at MIT by Firefly co-founder and [Chief Technical Officer](#) Daniel C. Pregibon PhD '08—works by imprinting (or stamping) microparticle structures onto photosensitive fluids. The resulting three-dimensional hydrogel particles, encoded with unique "barcodes," can be used for the detection of miRNAs across large numbers of samples. These particles are custom-designed for readout in virtually any [flow cytometer](#), a cost-effective device that's accessible to most scientists.

"Our [manufacturing process](#) allows us to make very sophisticated particles that can be read on the most basic instruments," says co-founder and CEO Davide Marini PhD '03.

The company's first commercial product, FirePlex miRSelect, an miRNA-detection kit that uses an assay based on OLS-manufactured particles and custom software, began selling about a year ago. Since then, the company has drawn a steady influx of customers (primarily academic and clinical scientists) while seeing rapid revenue growth.

To date, most of the company's revenue has come from backers who see value in Firefly's [novel technology](#). In addition to a cumulative \$2.5 million awarded through Small Business Innovation Research grants—primarily from the National Cancer Institute—the company has attracted \$3 million from roughly 20 independent investors. Its most recent funding came from a \$500,000 grant from the Massachusetts Life Sciences Center.

Pregibon developed the technology in the lab of MIT chemical engineering professor Patrick Doyle, a Firefly co-founder who serves on the company's scientific advisory board. Firefly's intellectual property is partially licensed through the Technology Licensing Office at MIT, along with several other Firefly patents. Firefly's technology, from OLS to miRNA detection, has been described in papers published in several leading journals, including *Science*, *Nature Materials*, *Nature Protocols* and *Analytical Chemistry*.

Shifting complexity from equipment to particle

The success of the technology, Marini says, derives from an early business decision to focus attention on the development of the hydrogel particle instead of the equipment needed. Essentially, this allowed the co-founders to focus on developing a high-quality miRNA assay and hit the

market quickly with particles that are universally readable on basic lab instrumentation.

"Imagine sticking a microscopic barcode on a microscopic product," Marini says. "How do you scan it? At the beginning we thought we would have to build our own scanner. This would have been an expensive proposition. Instead, by using a few clever tricks, we redesigned the barcode to make it readable by existing instruments. You can write these '[barcodes](#),' and all you need is one scanner to read different codes. To quote an investor: 'It shifts the complexity from the equipment to the particle.'"

Firefly's particles appear to a standard flow cytometer as a series of closely spaced cells; these data are recorded and the company's FireCode software then regroups them into particle information, including miRNA target identification and quantity.

But why, specifically, did the company choose a flow cytometer as its primary "scanner"? Pregibon answers: "To start, there are nearly 100,000 cytometers worldwide. In addition, we are now seeing a trend where flow cytometers are getting smaller and closer to the bench—closer to the actual researcher. We're finding that people are tight for money because of the economy and are trying to conserve capital as much as possible. In order to use our products, they can either buy a very inexpensive bench-top flow cytometer or use one that already exists in their core facility."

In turn, opting out of equipment development and manufacturing costs has helped the company stay financially sound, says Marini, who worked in London's financial sector before coming to MIT. As an additional perk, the manufacturers of flow cytometers have begun "courting" Firefly, Marini says, because "our products help expand the capability of their systems, which are now exclusively used to analyze cells."

The company's FirePlex kit allows researchers to assay (or analyze) roughly 70 miRNA targets simultaneously across 96 samples of a wide variety—including serum, plasma and crude cell digests—in approximately three hours.

This is actually a "middle-ground" assaying technique, Pregibon says, and saves researchers time and money: Until now, scientists were forced to use separate techniques to look at a few miRNA targets over thousands of samples, or vice versa.

Marini adds that if a scientist suspects a number of miRNAs, perhaps 50 or so, could be involved in a pancreatic-cancer pathway, the only way to know for sure is to test those 50 targets over hundreds of samples. "There's nowhere to do this today in a cost-effective, timely manner. Our tech now allows that," he says.

'Over the bridge of validation'

Because miRNAs are so important in the regulation of genes, and ultimately proteins, they have implications in a broad range of diseases, from cancer to Alzheimer's disease. Several studies have suggested these relationships, but the field currently lacks the validation required to definitively demonstrate clinical utility.

With that in mind, Pregibon hopes that Firefly's technology will help push miRNA-based diagnoses "over the bridge of validation," giving scientists the means to validate miRNA signatures they discover in diagnosing diseases such as cancer. "That's where we want to fit in," he says. "With the help of a technology like ours, you'll start to see more tests hitting the market and ultimately, more people benefitting from early cancer detection."

Firefly's aim is to strengthen preventive medicine in the United States.

"In the long term, we see these products helping in the shift from reactive to preventative medicine," Marini says. "We believe we will see a proliferation of tools for detection of diseases. We want to move away from the system we have now, which is curing before it's too late."

Pregibon says Firefly's technology can be used across several molecule classes that are important in development and disease research: proteins, messenger RNA and DNA, among many others. "Essentially, the possibilities are endless," Pregibon says.

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