

# Engineering Droplets for DNA Sequencing

## RainDance's Emulsion-Based Platform Combines Two Critical Technologies

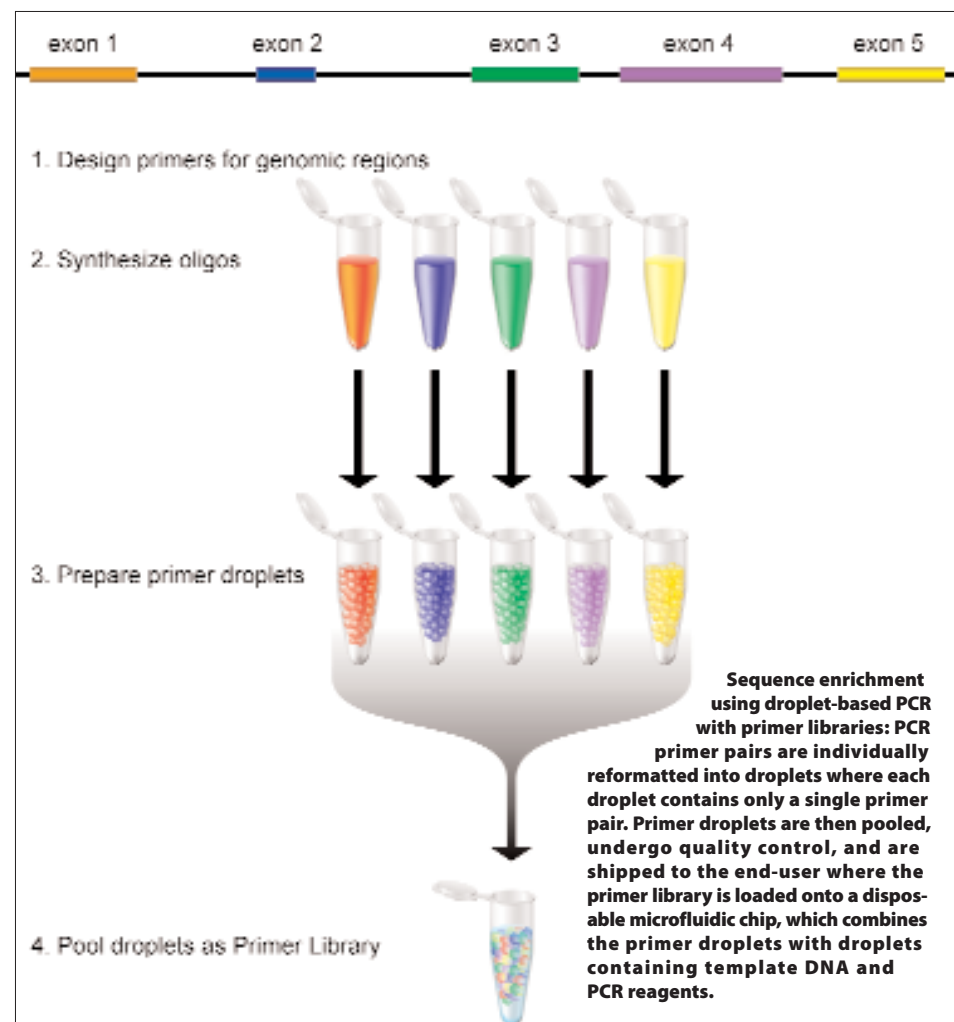
Carol Potera

Scientists once performed experiments in test tubes, then reduced the size of reaction volumes to fit into wells in microtiter plates. Now, RainDance Technologies ([www.raindancetech.com](http://www.raindancetech.com)) plans to miniaturize reactions even more by restricting assays to uniform microscopic droplets in emulsions.

RainStorm™ technology, which performs

assays in picoliter volumes, “is cost effective and fast,” says Steve Becker, vp of commercial operations. The first application of the emulsion technology will be for targeted resequencing of genomic DNA.

RainDance's emulsion-based platform combines microfluidics and droplet technology. Each droplet is the functional equivalent of a well in a microtiter plate. Any substance that researchers can place in microtiter wells, such as reagents, cells,



enzymes, or small molecules, can be encapsulated inside a droplet.

The interior of a droplet holds the aqueous sample that is surrounded by oil and stabilized by biochemically inert surfactants. Then, the emulsion droplets, which serve as individual reaction chambers, are circulated through channels on a microfluidic chip at a rate of 10 million per hour.

Darren Link, Ph.D., vp of research and development, developed the emulsion technology while working in the laboratory of David Weitz, Ph.D., at Harvard University. RainDance licensed the technology from Harvard. In 2004, Link and Weitz cofounded RainDance, along with Jonathan Rothberg, who also founded CuraGen, 454 Life Sciences, Ion Torrent Systems, and the Rothberg Institute for Childhood Diseases. Other cofounders were Andrew Griffiths, Ph.D., an expert in droplet biochemistry at the Medical Research Center in Cambridge, U.K., and Jerome Bibette, Ph.D., an emulsion expert at ESPI in Paris.

RainDance Technologies first operated out of the Rothberg Institute in Connecticut, before recently relocating to a new Lexington-MA-based, 28,000-sq-ft facility, which was formally dedicated late last month. The move strategically places RainDance in the greater Boston biotechnology corridor and closer to its customer base and life science talent pool. The company has increased its staff from 30 people in May to its current level of 50 people, and plans to be at 60 by year end in order to advance product development and manufacturing operations.

RainDance Technologies received a grant in July 2007 from the National

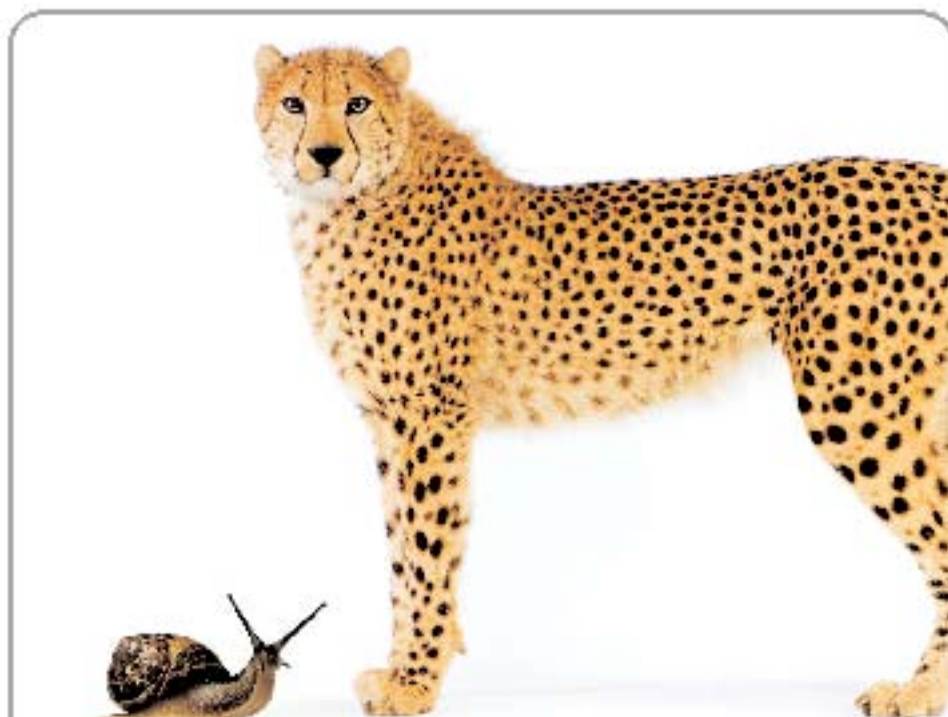
Cancer Institute ([www.cancer.gov](http://www.cancer.gov)) to develop a microfluidic chip to amplify specific regions of genomic DNA in order to sequence thousands of exons simultaneously from a single sample to find regions implicated in cancer.

Using the RainDance technology, genomic DNA is combined with thousands of primer pairs, with each droplet containing only a single primer pair. A single exon is amplified within an individual droplet by PCR. The sample material never touches the walls of the microfluidic channels, eliminating the risk of contamination. One pooled-primer-pair library emulsion can be used to amplify thousands of individual exons for subsequent analysis using any next-generation DNA sequencing instrument.

The same platform underlies RainDance's initial product, called the RDT 1000, which will be introduced later this year. The company will sell reagents and instruments for targeted resequencing of genomic DNA. The instrument and associated reagents will enable high-resolution analysis of genetic variation between individuals and across populations.

Because sequencing of whole human genomes is still financially unrealistic, selecting biologically relevant genomic loci is necessary to maximize the efficiency of second-generation DNA sequencers. The RDT 1000 customers will receive libraries of droplets; each droplet containing an individual primer pair.

The number of unique primer pairs that can be contained in a library is virtually unlimited. The primer droplets are merged



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with droplets containing genomic DNA and PCR master mix. Inside each droplet, individual PCR reactions take place with no competition between primer pairs. The method generates equimolar amounts of amplified material for a virtually unlimited number of targeted loci.

“Our primer design is flexible, and we can target large continuous regions, small dispersed regions like exons, and even repetitive regions of the genome,” says Becker.

The process is carried out upstream of next-generation sequencing and is an open, unbiased system. Additionally, the emulsion technology is cost effective because it uses picoliter amounts of reagents and generates thousands of PCR reactions per second. RainDance’s instrument and reagents are capable of processing a 4,000-member primer library in less than one hour with a single instrument run, according to Becker.

The first customers will be major users of next-generation sequencing equipment, including large programs at genome centers, research consortia, and groups assembling lists of genes that are causative of different diseases.

“Targeted sequencing has most of its life ahead of it,” notes Becker. “RainDance provides a valuable tool for sequence enrichment of the genome.”

#### Simplicity

“Working in droplets generates a lot of data to increase statistical power with a throughput that matches today’s science

workflow,” Becker says. Alternative sequence enrichment methods relying on multiplexing to decrease time face tradeoffs. Although multiplexing generates more data points, it reduces sensitivity and specificity.

Researchers who use multiplex assays spend a lot of time optimizing reactions to prevent competition and ensure specificity. The simplicity and speed of the RDT 1000 should serve researchers better than more complex systems, the firm reports.

“Working in droplets, where each droplet represents a single reaction, gives greater control,” says Becker.

The droplet-based technology “is a natural evolution of liquid-handling processes,” says Becker. The learning curve for new end-users should be relatively flat, because the easy-to-use system is nearly “walk-away” once it is set up, he adds. Customers will benefit from the enhanced throughput with easy sample handling.

RainDance’s business model is to sell instruments and consumables for specific solutions. The microfluidic channels can be preconfigured to perform a number of laboratory tasks. “We believe the technology is portable and we’re looking for other applications in the areas of proteomics and cell-based assays,” Becker states.

Future potential applications could include drug screening, gene expression, cell sorting, and biomarker detection. **GEN**

## At-a-Glance

### RainDance Technologies

#### Location

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#### Phone

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#### Website

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#### Principals

##### Chris McNary

president and CEO

##### Darren Link, Ph.D.

vp, research

##### Steve Becker

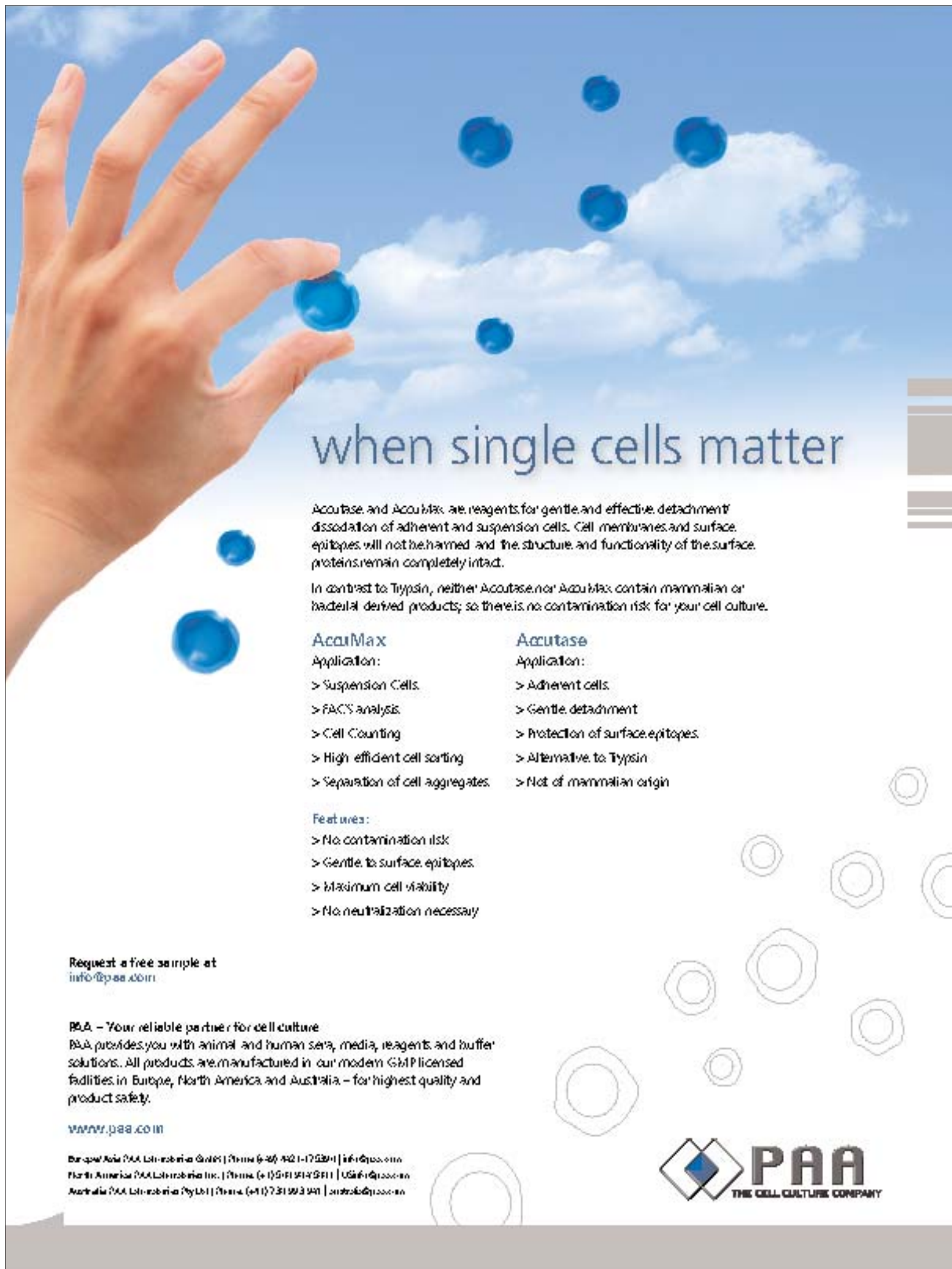
vp, commercial operations

#### No. of Employees

50

#### Focus

RainDance Technologies provides microdroplet-based solutions for human health and disease research.



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