



Pyrosequencing Inventor Building Mini Sequencer That Will Cost Fraction of 454's GS20

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NEW YORK (GenomeWeb News) — As 454's Genome Sequencer 20 enters more academic institutions and companies, pyrosequencing, the chemistry at its core, is becoming more widespread.

Now Mostafa Ronaghi, one of the inventors of this sequencing chemistry, wants to take pyrosequencing to the masses. His group at the Stanford Genome Technology Center at Stanford's Medical School is working on a miniaturized DNA sequencer that will use pyrosequencing but will cost a fraction of 454's instrument, which lists for \$500,000.

"We envision that we could basically enable any laboratory to do genome sequencing," he told *GenomeWeb News* last month. The material cost for the instrument, which will use CMOS sensors instead of a CCD camera, would be a few thousand dollars. "It will basically [cost as little as] a PCR machine, which is used in any laboratory," he said.

In 2004, Ronaghi won a three-year, \$1.8 million grant from the National Human Genome Research Institute to develop a pyrosequencing array for genome sequencing. In October 2006, he presented his work at the International Conference on Genomics in Hangzhou, China.

But his roots in genome sequencing technology run much deeper. As a PhD student in Sweden at the Royal Institute of Technology in Stockholm, Ronaghi developed pyrosequencing and holds eight issued or pending patents on the technology. He also co-founded Pyrosequencing, a Swedish biotech that commercialized the technology. After acquiring three companies, [Personal Chemistry](#), [Argonaut Technologies](#), and [Biotage](#), in late 2003, the company changed its name to Biotage.

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In 2003, 454 Life Sciences obtained an exclusive five-year license from Pyrosequencing to use sequencing-by-synthesis and pyrophosphate-based sequencing in whole genome analysis, and to make and sell instruments and kits that use the technology.

Pyrosequencing retained rights to use the technology for other applications.

Ronaghi also had ties to Illumina, which is about to acquire 454 rival Solexa. Prior to joining the Stanford Genome Technology Center as a senior research scientist in 1999, Ronaghi collaborated with Illumina to obtain a \$100,000 Phase I SBIR grant from the NHGRI in 2000 to combine pyrosequencing with Illumina's technology, which creates random arrays of beads on fiber-optic substrates. However, Illumina decided to abandon the project after the first phase of the grant because it wanted to focus on the genotyping market, according to Ronaghi. Around the same time — in 2000 — 454 Life Sciences was founded to commercialize pyrosequencing for whole-genome sequencing.

Since then, Ronaghi's group at Stanford has been working to lower the cost of pyrosequencing by replacing the CCD camera that 454 uses in its system — and which he said costs approximately \$100,000 — with inexpensive complementary metal oxide semiconductor, or CMOS, sensors, which he said cost as little as \$10 apiece.

Recent applications of CMOS, he said, have boosted their development, and "now comparable or better sensitivity can be achieved with inexpensive CMOS," compared to CCD cameras.

"The same technology has been used in digital cameras," Ronaghi said. "They are very sensitive, but we needed to customize it for chemiluminescence detection."

In addition to lowering cost, the CMOS approach promises to eliminate the optical crosstalk 454's imaging system experiences, a factor that forces the company to use and analyze only a fraction of the wells on its picotiter plate, according to Ronaghi.

454 did not respond to a request for comment last week on how the company is addressing this problem.

Ronaghi's group recently completed a one-megapixel sensor chip with 15 bit dynamic range. "The very key development has been the development of an analog-to-digital conversion unit," he said. "That's the core of the chip."

He is now using this sensor chip to build a "very small DNA sequencer." His lab has already built an automated system and is testing its sequencing capabilities at the moment. So far, they have only sequenced oligonucleotides and PCR products but plan to "do some real sequencing," meaning at least part of a genome, in the coming year.

While the pyrosequencing chemistry is identical to 454's, Ronaghi's group is not using the 454 platform but a homemade device with "a lot of wires and tubing in a black box," he said. Their system integrates a microfabricated well-array on top of the CMOS and uses smaller reaction volumes than 454's instrument.

The aim is to develop a sequencing equivalent of a supercomputer, which has several processors on the same board. "Now we are building a similar device, which we call a superscaler pyrosequencer," Ronaghi said. The objective is to run 400 million sequencing reactions in parallel that can produce between 60 and 100 gigabases of data per run with 200-base reads, "with all inefficiencies included."

With that, you could basically sequence one human genome in a single run with 20x coverage," Ronaghi said. The researchers are optimizing the current system and hope to achieve this goal within three years.

In the next two years they plan to reduce the size of the beads used in the sequencing reactions from 30 microns to 10 microns, and use them with a 20-megapixel sensor they want to develop. After that, they want to put 20 of these chips side-by-side to develop the superscaler pyrosequencer.

Ronaghi is collaborating with an undisclosed California-based startup company to automate the upfront sample preparation of the device. "Sample preparation is probably one of the main bottlenecks for emerging technologies [like this]," he said.

However, he is not planning to found his own company to commercialize the system. "Anyone who would like to license it" can have a go at it, he said. "Probably 454 is in that position," he added. In addition, his group is working on alternative chemistries, possibly those that may enable the firm to avoid 454's exclusive license.

Researchers, he believes, would use the system mainly for sequencing new genomes. "I believe *de novo* sequencing will remain as a market, which will be a bit separate from the

resequencing market," he said, adding that he believes pyrosequencing will have the best chance to succeed in this area.

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