



Helicos and Columbia to Test Scripps' Improved Polymerases for Next-Gen Sequencing

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NEW YORK (GenomeWeb News) — Helicos BioSciences and Columbia University sequencing scientist Jingyue Ju have teamed up with researchers at the Scripps Research Institute to improve DNA polymerase, the enzyme that lies at the heart of their respective next-gen sequencing-by-synthesis methodologies, *GenomeWeb News* has learned.

The two groups, which expect to test polymerases with improved properties for sequencing within a year, are following in the footsteps of Solexa, which has already incorporated a DNA polymerase it developed into its sequencing chemistry, used with its 1G Genome Analyzer.

"This is not an exact science and it's a little bit like art or a little bit like cooking."

Making the enzyme better at incorporating modified nucleotides could improve the chances of success of next-gen technologies that employ nucleotide analogs, among them Solexa's and Helicos', according to experts. It could also lower the cost of standard Sanger sequencing.

"For the single molecule sequencing-by-synthesis approaches, polymerases are really the limiting factor," said Floyd Romesberg, a professor of chemistry at the Scripps who developed a polymerase evolution method.

Romesberg this spring won a two-year grant from the National Human Genome Research Institute entitled "Evolving Novel Polymerases for Genome Sequencing." He will work with Helicos, Ju's group, and Scripps' sequencing facility to test polymerases with novel properties in actual sequencing settings.

It's no secret that nucleotides labeled with a fluorescent dye are poor substrates for natural polymerases. "This is an obvious bottleneck for the various 'sequencing-by-synthesis' methods that are in development," Phil Holliger, a group leader in the MRC Laboratory of Molecular Biology at the University of Cambridge told *GenomeWeb News* by e-mail. Therefore, polymerases that can incorporate

such nucleotides better “are obviously going to improve the efficiency of such methods.”

Like Romesberg, Holliger is an expert at using directed evolution to engineer proteins, and originally developed emulsion PCR for evolving polymerases. CuraGen licensed this method for 454 Life Sciences’ sequencing technology, an unrelated use of it.

However, 454 does not use modified nucleotides in its technology, and therefore does not require an improved DNA polymerase. Neither does ABI’s Agencourt technology, which uses ligase instead of polymerase.

According to Romesberg, the aim of the NHGRI-funded project is to evolve polymerases that can more efficiently, and repeatedly, incorporate three types of modified nucleotides: a nucleotide with a fluorophore attached to the base, one with a blocked 3’-end, and one with both modifications.

In order to select for polymerases that can better handle modified nucleotides, Romesberg displays an entire DNA polymerase library, together with the modified substrate, on phage particles. By sequencing the DNA of the phage attached to the enzyme with the desired properties, he can identify which mutations gave rise to the enzyme activity.

Though his lab has used this method successfully to develop polymerases “towards things that seem more challenging,” including synthesizing DNA with unnatural bases, Romesberg stressed that the process is challenging, involving several selection rounds and getting the conditions just right.

“This is not an exact science and it’s a little bit like art or a little bit like cooking,” he said.

What is important for sequencing, he explained, is to make the enzyme not just a few times but several thousand times better at using modified nucleotides. “It’s not sufficient to simply improve something; you have to improve it to a level of efficiency that’s suitable for the application,” he said.

Another challenge is to develop appropriate libraries. “Once you have robust methods to screen through large libraries, it’s not sufficient to just design libraries, [but] figuring out how to design smart libraries,” he said.

So far, he said, his group has built DNA polymerase libraries and is “in the process of just starting our first selections.”

Eventually, Romesberg plans to test the improved enzymes with Helicos, Ju’s group, and the Scripps sequencing facility. Helicos, he said, has “a variety of proprietary approaches that they are interested in.” The company has said it plans to commercialize its single molecule sequencing-by-synthesis platform next year.

Ju’s group uses nucleotide analogs blocked at the 3’ position, and Romesberg said he will “collaborate with him on that methodology.” Ju won an Advanced Sequencing Technology Award from NHGRI in 2004 to develop an “integrated system for DNA sequencing by synthesis.” According to the abstract, his system will use four different photocleavable fluorescent nucleotides with modified 3’-OH groups.

Romesberg also plans to work with the Scripps’ sequencing facility to improve standard Sanger sequencing on ABI machines, although he is not working with ABI.

“One of the most expensive aspects of sequencing by conventional methods is the cost of the fluorophore-labeled triphosphates,” he said. “So if you had a polymerase that incorporated them much more efficiently, you could cut by an order of magnitude the triphosphate level that you had to use, which would cut significantly the cost of sequencing.”

When does Romesberg expect the first results? “That’s what Helicos asks me about every other week,” he said. Despite the technical challenges ahead and the unpredictability of scientific progress, “I am confident that within a year we will have polymerases with improved properties [for sequencing],” he said.

Solexa already developed its own proprietary polymerase that was “engineered specifically to work with its reversible terminators,” according to a company spokesman. That polymerase is protected by both issued and pending patents. This summer, Solexa chose New England Biolabs to manufacture the enzyme, amongst other reagents.

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