Innovations

Life after worms
Lynx Therapeutics, Inc.

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Sydney Brenner is a man of many ideas. Asked to rate one of his more recent ones, which has spawned a company called Lynx Therapeutics, Inc., Brenner describes it as "pretty good compared to my previous work." But then, he says, "that is a safe statement to make, because you might think that all the previous ideas were terrible."

Or not. After all, this is the man who co-discovered mRNA and the triplet theory of translation. Then in the mid-1960s he selected the worm *Caenorhabditis elegans* as the next model organism, thus giving birth to an entire field of study. Now, officially retired from his more onerous duties, Brenner constitutes the entire scientific advisory board of Lynx, and is masterminding the implementation of his idea at the company's Hayward, CA, headquarters.

"What Lynx does so uniquely," says CEO Norrie Russell, "is the next and necessary evolutionary step of DNA analysis." From the isolation of single genes, biologists have moved to automated sequencing and DNA microarrays containing a few thousand genes. But still, says Russell, "there needed to come the next step, and the next step is Lynx."

Lynx technology essentially transfers the contents of a genomic or cDNA library onto beads, with each bead containing thousands of copies of one member of the original library. Using those beads, says Brenner, "in one fell swoop we can remove (for analysis) all the pieces of nucleic acid that vary in abundance." Millions of beads can be hybridized, sorted, and sequenced, allowing the company to fish out sequences that are over- or under-expressed, and identify single nucleotide polymorphisms (SNPs) that are associated with a disease. And that, promises Brenner, is just for starters.

Developing a technology

Brenner started thinking about what is now the Lynx technology in the late 1980s in England. He tried to raise some venture capital but failed. "Most people thought it was too risky," says Brenner. "They were pretty much right."

But there was one place, he says, where that risk could be overcome. The risk arose because a unique combination of skills – in molecular biology, chemistry, optics and computing – were needed to produce the final product. "It could only have been done in the [San Francisco] Bay Area where you have all these technologies together," says Brenner.

Sydney Brenner is taking on the challenge of genomics.

The next problem was the money. Luckily Brenner knew Sam Eletr, who was then the CEO of a small biotech company called Lynx. Lynx had been spun out of Applied Biosystems, Inc. (ABI; Foster City, CA) to exploit the possible antisense applications of ABI's phosphoramidate chemistry. But, as Russell puts it, "by 1995 it was becoming apparent that the antisense road is a long and winding road." Technology met company, and by 1998, when Lynx sold off its waning interest in phosphoramidates, the company was focused entirely on making the Brenner technology work.

That process, says Brenner, was "terribly difficult. The one thing I learned was that inventing and implementing technology is much more difficult than doing science. During development you can not change your goals – you have to get to the end."

DNA onto beads

A need for a better DNA sequencing method was what originally inspired Brenner. "By the time I retired to California I had already worked out the sequencing idea," he says. "But to make it work we needed a method for handling lots of templates. Later we came to realize that it was this loading technology that was central, and the sequencing was just one application."

The loading technology, or Megaclone, uses a system of tags and anti-tags. The anti-tags are constructed on 5-μm microbeads by mix-and-divide combinatorial synthesis. The tags are appended to cDNAs during a cloning step. The clones are then amplified to produce thousands of copies of any one cDNA-plus-tag combination. When DNA and beads are mixed, thousands of copies of a given cDNA plus tag hybridize to their cognate anti-tagged bead. A library of millions of cDNAs has been converted to a library of beads, each with thousands of copies of a single cDNA.

Accurate hybridization is crucial if Megaclone is to have any meaning whatsoever. "It really is computing with molecules," says Brenner. In an interesting parallel with his earlier work with mRNA triplets, Brenner says that to make the hybridization work "we had to define a DNA language."

The language must not allow for any significant amount of cross-hybridization. This led the Lynx team to create the tags and anti-tags out of eight four-base "words." Each step in tag synthesis involves the addition of an entire word, so even the closest relative of a given tag will differ by an entire word, not a single base. This reduces cross-hybridization. Additionally, the melting temperature of all correct tag–anti-tag combinations should be identical, as every word forms the same number of A:T base pairs (three) and G:C base pairs (one). The final safety step was to exclude guanine nucleotides from one entire tag strand. This should eliminate self-annealing driven by

self-complementarity, and ensure that no tag contains the type of palindromic sequence required for recognition by most restriction enzymes.

Once the cDNAs are attached to beads, one cDNA strand is ligated to the tag and the other strand is stripped away. The single-stranded cDNA now acts to detect added, labeled cDNA by hybridization. For example, as in a DNA microarray experiment, cDNA samples from quiescent and cycling cells can be labeled blue and red, respectively, before hybridization. Fluorescence-activated cell sorting (FACS) of the beads then yields cDNAs from blue beads (greater expression in quiescent cells) and red beads (greater expression in cycling cells).

Staring at beads

DNA from FACS-sorted beads can be sequenced conventionally, and used as the raw material for a DNA microarray uniquely suited to your problem of interest.

As an alternative to sorting, beads can be analyzed using Brenner's original sequencing idea. In massively parallel signature sequencing (MPSS) the beads are simultaneously decoded. Exhaustive sequencing has been used for transcript profiling in the past, notably by Incyte Genomics, Inc. (Palo Alto, CA). In theory this could give an exact digital readout of how many copies of each transcript are present in a cell. But standard sequencing technology is too slow and too costly to effect anything approaching saturation coverage, so the end result is vague estimate of relative abundance based on sequencing a few thousand cDNAs sampled from the millions of transcripts present in a cell.

MPSS aims to fulfill the promise of digital precision by increasing speed and throughput. The beads from Megaclone are immobilized in a flow cell, and the comings and goings of fluorescent sequencing probes are monitored by a CCD camera that is trained on the microbeads. Hundreds of thousands of mRNAs can be identified in a few days.

And now for the sequencing

The entire cDNA is not sequenced. Instead, the ingenious sequencing method identifies 16–20 base pair "signature sequences" that can then be used (if genomic information is available) to identify the cDNA. If genomic information is not available for a clone that is interesting because of its abundance, the cDNA must be identified by conventional sequencing.

The signature sequence is identified four bases at a time. Each four bases, present as a single-stranded overhang, is identified by the annealing of one of 256 probes. The probe also contains a binding site for a type IIs restriction enzyme. Although the restriction enzyme binds to the probe, it cleaves within the cDNA, thus exposing the next four bases for decoding.

This procedure not only does away with gels, says Brenner, but "is completely parallel. It is the same if you sequence 1 template or 2 000 000 templates."

The competition – microarrays

To a casual observer it may sound like Lynx technology and DNA microarrays are solving the same problem. Microarrays have gained a substantial commercial foothold thanks to companies like Incyte and Affymetrix, Inc. (Santa Clara, CA). But, says Russell, "I do not think a standard has already been set. I sense more and more that investments have been made (in microarrays) but there is a growing concern of the limitations of the microarrays."

"The arrays have a lot of problems," says Brenner. "If they get too big you are going to face an issue that the noise level will increase" because of closely related sequences. But the Lynx technology, he says, "can be used to explore complete cDNA libraries in complete depth. I think this is going to be **the** tool in exploring function."

Besides, says Brenner, "we do not aim to compete with the chips." He still thinks the best experiment is to use Megasort followed by analysis of what he calls, with apologies to the computing industry, a RISC (reduced instruction set chip). "It is going to be silly to have brain genes on a chip for liver analysis," he says. "Our technology is to define what ensemble of stuff you should use."

Bring on the super-sequencer

Lynx is generating revenue by providing genomic discovery services, and it is gradually increasing its internal biology efforts. The genomics focus is drifting into proteomics, with the development of a liquid-phase 2D electrophoresis system. Meanwhile the work on the basic technology continues, including a Megaclone scheme that accomplishes a genome-wide scan for SNPs.

If Lynx is interested primarily in increasing the applications of Megaclone, Brenner is clearly still attached to his original inspiration – the new, improved sequencing method. The short sequence "runs" of MPSS will not bridge repetitive sequences. But for the increasingly important task of re-sequencing, MPSS may be perfect.

"What we have is workable but it is still the first stage," he says. "My ambition would be to try to sequence 50 bases of 2 000 000 templates and to do that in 1 day. That is the production of the entire Venter factory in one machine."

"It is going to take some work to get to that," he says. "But I think it is within reach." If he can complete the journey from standard sequencing to his proposed high powered system it will be "like going from mainframe transistor computers all the way to integrated circuits," he says. "Perhaps in 5–10 years time we can provide everyone with a super-sequencer on their desktop. That would be the hope."

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