

Directed evolution in Germany: Sloning Biotechnology (Puchheim near Munich, Germany)

# Test-Tube Evolution

Only a few European biotech companies are engaged in directed evolution. One of them is Sloning Biotechnology, a manufacturer of genetically diverse libraries. The young company, close to bankruptcy several times, recently grew in confidence despite the worldwide financial meltdown.

**H**einz Schwer is advancing in a trendy jet-black BMW X5 SUV limousine. To pick up your *Lab Times* reporter at Puchheim S-Bahn station, the founder of Sloning Biotechnology, a small company that develops genetically diverse mutant libraries, got behind the wheel himself. Schwer didn't have far to travel. The 41-year-old biotech entrepreneur based his company, Sloning Biotechnology, just a few steps from the S-Bahn station, some 18 kilometres west of Munich, Upper Bavaria.

"Quite honestly, it was mere coincidence to touch down just here," Schwer recalls, while piloting the company car towards Sloning's premises. He recalls the feverish bio-

tech hype in Germany at turn of the millennium, when start-ups mushroomed. Around Munich, young biotech firms emerged in their dozens between 1998 and 2002. Most of them were clustered in Martinsried, which remains the German Mecca of biotechnology – a place where some 50 firms with a total of 1,100 employees are searching for new drugs and elaborate medical instruments.

## A refinery technician gets chemical

Let's go back in time. How did Heinz Schwer get around to starting his own business? It was, as with most things in life, more or less an accident. Before becoming a biotech entrepreneur, Schwer worked at

an oil distillery in his hometown of Ingolstadt as a refinery technician. Still a young man, he then decided to study organic chemistry in Regensburg. Some years later he obtained a Ph.D. in clinical chemistry for his research on a novel putative carboxylesterase. Thereafter he spent three years as a postdoctoral fellow at Harvard Medical School and the Dana Farber Cancer Institute in Boston, doing basic research on blood platelets and a novel human ubiquitin-specific protease.

Then, in 2000, during an expert conference in Boston, Schwer met a young countryman who had an interesting idea. This colleague, the German molecular biologist



The lion's share of Sloning's workforce poses at their Puchheim workplace (foremost left is co-founder and Chief Executive, Heinz Schwer).

Octavian Schatz, was enthusiastic about a new, sophisticated cloning method using synthetic genes. Not yet thinking of protein engineering, Sloning's current business, both discussed the situation, wrote a business plan, and finally agreed to found a biotech company in Germany.

"I would still live and work as a basic researcher in America if I hadn't take this decision at that time," Schwer says. He returned to Europe at the end of 2000, establishing the company with Schatz as a CEO and himself as a Chief Operating Officer, responsible for business development and pre-marketing activities. Quickly, they picked up investors and, in March 2001, the young Sloning Biotechnology GmbH received €3 million as a first tranche of venture capital funding.

Shortly after, however, an unforeseeable problem emerged. The young start-up had to move out of the Martinsried incubator, IZB, but couldn't find any vacant lab space in Martinsried or nearby. Not even a vacant lumber-room was for hire. Finally, Schwer and his associates found an empty hall in Puchheim, refurbished it to meet their expectations, and settled there.

"It was an act from necessity," Schwer concedes, "but we never regretted it."

Everything seemed running well again, but the young start-up was in fact sliding into its next crisis. It wouldn't be the last one.

### Sudden setback, unexpected rescue

Just when the biotech boom in Europe reached its peak in 2001, the first crashes were approaching. At the end of the year, for example, the Berlin-based genomics company Genprofile went under after only three years. In spring 2002, the functional genomics company Elegene, that had unsuccessfully searched for drug targets, also went bankrupt, becoming the first prominent biotech loser in Martinsried. Just as Sloning had proudly reached its first milestone in 2002, it seemed that the start-up would suffer a similar fate. Lead investor 3i announced sudden plans to end its financing.

"3i's decision to get out was a real disaster for us," Schwer remembers. At that time, Sloning had a staff of seven and, to avoid bankruptcy and unemployment, a new lead investor had to be found very quickly. By chance, Schwer met in Munich with Jack Objeski, who then was a top dog at Genentech. Objeski exposed the young German entrepreneurs to Swiss venture capital firm HBM Bioventures. In 2003, Sloning performed an increase of capital stock and

got additional financing. The company was able to carry on.

### Working in Moscow

Octavian Schatz left the company after another crisis in 2005, and Schwer became Chief Executive. Since then, staffing levels at Sloning Biotechnology have risen to nearly 30. The premises at Zeppelinstrasse 4, Puchheim, are filled with personnel, high tech instruments and robotics.

*"Gene synthesis doesn't pay off in Europe. That's a pity, because there is a lot of tricky brainchild hidden in a synthetic gene."*

Schwer resides in Moscow, judging from the doorplate that is attached to his office door. Asked about it, he smiles. "Well, that's the brainchild of our marketing folks." Nearly every room at Sloning's premises has its own city name: the S1-Lab, for example, hides behind a safety door with the inscription "Madrid"; the production of Sloning's mutant libraries is done in "Vancouver"; the research and development team resides in "Havanna"; and contaminated test tubes are purified, well, in "Kairo."

"Our primary business idea was to replace traditional cloning by automated gene synthesis," Schwer comes back to the topic. Founding the company, he and Schatz felt confident that gene synthesis would soon emerge as a cheaper "cloning" method. From today's point of view, they were right – and wrong, too. The complete *de novo* production of structural genes (aka "gene synthesis") has indeed become a business in demand, while more and more research groups cease classical in-house cloning and mutagenesis procedures. But gene synthesis has got its drawbacks, too.

Modern gene synthesis hasn't a lot in common with the classical method of Nobel prize winner Har Gobind Khorana, who in 1970 synthesised a short artificial tRNA gene for the very first time. Meanwhile every commercial supplier has its own murky manufacturing method, treated by most as a state secret. The basic idea, however, the annealing based connection of oligonucleotides, is always the same. A set of individually designed oligonucleotides is connected by annealing and ligation or polymerase reactions, performed by thermostable enzymes. Typical advancements are, for example, the ligation of phosphorylated overlapping oligonucleotides, a modified form

of ligase chain reaction, and diverse PCR assembly approaches using overlapping oligonucleotides.

Sloning's patented approach is similar. Synthetic genes are synthesised from short universal double stranded oligos that are produced by solid phase technique in 96-well plates. According to Schwer, this meth-



Photo: Köppelle

Heinz Schwer has experienced many a company crisis at Sloning Biotechnology, but never thrown in the towel.

od leads to significantly less defective material, while, on the other hand, the expenses for automation are bigger.

He speaks from his own experience. Sloning's next company crisis, around 2005, was provoked, well, by automation. "We didn't find an appropriate partner to develop our robotics. To make bad things worse, our financial situation was poor – and that nearly broke our company's neck again."

### Shifting the preferences...

The commercial gene synthesis market is divided into an estimated hundred companies. The most prominent are Genearth and Mr Gene (both Germany), Genscript, Blue Heron and DNA2.0 (USA) and Eurofins MWG Operon (France/Germany). All these manufacturers, however, have been suffering for years from a dramatic decline in prices that has now reached 40 cent per base pair. According to Schwer, it is hardly possible to produce profitable synthetic genes at such low prices in Western countries.

"I am sure that every competitor who is manufacturing its genes in Germany doesn't even cover its costs," he argues, suggesting

that cheap genes are generally produced in China or elsewhere in the Far East. "Gene synthesis still implicates a lot of handiwork that doesn't pay off in Europe. That's a pity, because there is a lot of tricky brainchild hidden in a synthetic gene."

"Anyway, for us," Schwer says, "gene synthesis has shifted to a niche business." After five years of technology development and perpetual financial difficulties, Sloning has shifted its preferences to directed evolution (or, in other words, the optimising of biomolecules by randomised methods). "Since January 2009, directed evolution is to be Sloning's major business area."

### ... towards protein engineering

Is protein engineering, the developing of useful and valuable proteins, really more lucrative than gene synthesis? "By all means," Schwer affirms, "after the change of our venture focus in 2008, our sales increased significantly."

In general, man-made protein engineering is performed according to two strategies: rational design and directed evolution. While it is necessary to have detailed knowledge of a protein to make the desired changes, the rational design approach is usually inapplicable. So the industry has focussed on directed evolution, mimicking natural evolution. Random mutagenesis is applied to genes (respectively proteins) and those variants with the desired qualities are picked out. Then these steps are repeated several times in a high-throughput approach.

If successful, a somehow "better" protein (better when it comes to longevity, reaction speed, thermal stability etc.) is the result. Take an ordinary amylase, present in every washing powder, that is suddenly able to remove not only blood but also ink stains; an amylase that works just as well at lower water temperatures; an amylase that works well at uncomfortable pH-values and so on. Or take an improved therapeutic protein that causes fewer side effects.

Sloning offers the genetic tools to create such novel amylases (or other improved proteins). The evolutionary processes mentioned above are performed with genetically diverse DNA libraries. Schwer maintains that these "SlonoMax" libraries, "contain the complete set of all possible mutants and can be produced with a precisely predefined ratio of the selected codons," in contrast to traditional methods like error-prone PCR, gene shuffling or site directed mutagenesis." Schwer calls this "intelligent libraries."

***"The current crisis could even be an opportunity for biotech companies, if pharma companies will outsource other projects to external suppliers."***

tion, and, if we are reliable and deliver high grade on time, we can charge better prices than to academic customers." In Schwer's opinion, Sloning's brand logo, an elephant, meets these expectations. "An elephant is a synonym for trustworthiness."

Is it a synonym for affluence, too? If everything goes well, Sloning will turn a profit from 2010, Schwer hopes. He opens his laptop and starts a Power Point presentation. A lengthy list of companies appears, each name followed by a six- or seven-digit sales figure. Given Sloning's 2008 revenue of a single digit million euro, it cannot be a list of current customers. It must be a list of potential customers.

### Ban on taking photos in Hong Kong

Well, it seems there's still a lot to do for Thomas Waldmann, responsible for Sloning's marketing and business development. Waldmann is sitting in "Hong Kong", and is someone who takes charge of nearly everything. On that day, he takes charge of the doorplates. "You shouldn't take photos of them!" he unsmilingly declares. "They still



Photo: Köppelle

Protein engineering usually requires huge robots. In this picture, Heinz Schwer presents Sloning's "elongation robot", designated to synthesise short DNA fragments of up to 18 bp long.

have our old corporate design." Quaking in his boots, your *Lab Times* reporter puts the camera down.

At the moment, Sloning is on the lookout for new personnel. To expand its SlonoMax libraries for the development of therapeutics and the selection of enzymes for industrial applications, Schwer is looking for additional technicians and biologists that are skilled in molecular biological techniques and protein expression.

Throughout the interview, Schwer radiates confidence, and indeed, given the current global economic crisis, seems rather optimistic. "It's quite obvious that there is a worldwide crisis," he states, "especially in the United States. And it's absolutely essential for the biotech industry that the financing situation isn't worsening even more." Even so, Schwer hasn't yet noticed concrete consequences, "we never had so many enquiries from potential customers." Thus, Sloning hasn't reduced its marketing expenses, Schwer says.

### Crisis as an unexpected opportunity?

The current crisis could even be a big opportunity for small biotech companies, he hopes. "It would be possible that big pharma companies like Dupont or Bayer consider budget cuts, implying that they would discard a number of planned projects and that they would outsource other projects to external suppliers such as Sloning."

Wishful thinking? Maybe, but, "strangely enough," Schwer adds, "just in the recent two or three months several venture capital firms addressed us in order to invest in Sloning Biotechnology."

"That's a somehow odd thing," he ponders, "we had severe problems to get funding for years, and just at the moment, with this worldwide financial turmoil around, the situation changes for the better for us."

WINFRIED KOEPELLE