DNA 2.0 Newsletter

Q4, 2003

Welcome to **DNA 2.0**'s *Gene Synthesis* & *Protein Engineering* newsletter. The Newsletter keeps you up to date on trends and breakthroughs affecting molecular biologists that are interested in achieving their project goals faster and at lower cost. For more detailed gene synthesis and protein engineering information, log onto our web site at **www.dnatwopointo.com**.

1455 Adams Dr. Menlo Park, CA 94025 November 2003

FOUR REASONS TO OUTSOURCE

Outsourcing of non-core competence needs is a well established way to: 1) Save significant money, 2) Increase flexibility and stability, 3) Increase speed, and 4) Access technology and domain knowledge outside of your own corporation. A vivid example is how quickly custom oligonucleotide suppliers supplanted all in-house oligonucleotide synthesis efforts. Just a few years ago every lab doing molecular biology had an ABI 392 DNA synthesizer sitting in a corner. Today Qiagen, IDT, Sigma-Genosys, Invitrogen and other specialists have completely replaced them.

Gene synthesis is next.

In today's challenging financial environment for biotechnology, it has become critical to properly manage a company's resources. As the biotech marketplace is evolving, it is increasingly expensive and inefficient to build and maintain infrastructure that is not directly associated with a company's core competence. Accordingly, outsourcing is sweeping through the biotechnology industry today as it already has in more mature industries.

Many biotechnology companies still have their own IT departments, in-house DNA sequencing and media labs. Despite biotech's reputation as being quick, nimble and technology driven, it is only slowly coming to terms with the advantage of outsourcing for improved efficiency, lower cost and access to external technologies. These are advantages most other industries have taken for granted for many years.

Despite the recent excitement in company boardrooms for outsourcing, it is certainly not a new phenomenon. The advantage of outsourcing was first extensively analysed at the English pin factory in the 1776 classic "The Wealth of Nations" by Adam Smith. More than 200 years after its initial publication, many of the conclusions from Smith's book are still excellent fiscal guidelines for national macroeconomics and cornerstones of twenty-first century corporate culture. In essence, the outsourcing proposition is that we can all specialize in what we do best and exchange our products in the marketplace for those that are more efficiently produced by others. In other words; ***Outsourcing (or 'Division of Labour' as Adam Smith called it) is the main driver for increased productivity and value building***.

The last two years have seen more layoffs in biotech than ever before. Many biotech companies have shrunk considerably within their new financial constraints. The reduction in employees can pose a significant challenge to managers trying to deliver on milestones and commercial goals without the same in-house manpower to do so. At the other end of the scale are relatively few expanding biotech companies. Such cash-rich companies can use outsourcing to reduce risk and grow rapidly without taking on extra fixed costs.

1. Cost is often the initial driver in accepting outsourcing. Even the least fiscally conservative biotechnology companies back in the days of the NASDAQ bubble would outsource basic necessities. As access to venture capital has dried up, technologically challenging services that benefit from dedicated expertise and economies of scale can be more easily and economically managed by an external provider. Laboratory media and DNA sequencing are examples of expensive and non-core technology processes that are ripe for immediate outsourcing to save cost and increase efficiency. When overheads, salary and benefits are accounted for, the cost of a basic DNA sequencing reaction done in-house can rarely compete with that of most specialized DNA sequencing providers. **DNA 2.0** is moving custom gene synthesis into the ranks of these services; with prices as low as **$2.45/bp**, it is difficult for a corporate in-house facility to compete. Substantial savings can be made very rapidly in most biotech companies by exploring outsourcing options for every non-core competency within the company.

2. Flexibility AND Stability. An added accounting benefit is that the cost of outsourcing is directly tied to the service, resulting in instant scale-up or scale-down when needed. In comparison, the time and resources needed to hire and train specialist employees are a significant investment, which may be difficult for a company to justify. Conversely, outsourcing often also leads to more stability. If an external provider is having problems with their process, it is usually trivial to switch to a competing provider. If on the other hand your in-house process is facing significant downtime, correcting the problem can be both time consuming and expensive to the entire organization.

3. Speed. An external service provider is typically set up to deliver their product as quickly as possible – their cash flow relies on the fastest possible turnaround. Making a 1kb synthetic gene can easily take a month or more even for an experienced scientist. **DNA 2.0** typically can have the gene synthesized and sequenced in **only 10 business days**, a turnaround that is hard to beat.

4. Access to technology and domain knowledge. Outsourcing allows immediate access to technologies that could take years and significant resources to build up in-house. Such in-house investment must compete with other priorities that include development of a company's own expert domain knowledge, which is its competitive advantage. By outsourcing to an external gene synthesis or protein engineering provider such as **DNA 2.0**, a company can immediately access cutting edge technology without sacrificing speed or developments of technologies important for long-term corporate success. Gene synthesis can replace many mind numbing gene manipulation efforts (cDNA cloning, mutagenesis, codon optimization etc) allowing in-house scientists to focus on the research instead of creating the tools to do research.

THE NEW DNA 2.0 PRICING STRUCTURE FOR CUSTOM GENE SYNTHESIS

Recent changes in how we make longer genes have reduced our cost, while maintaining our exceptional turnaround and quality. We of course want to pass those savings on to you and have modified our pricing table accordingly:

500 - 830bp	**$2.95/bp**	**10 business days**
830 - 1,000bp	**$2,450 Flat rate**	**10 business days**
1,000 - 1,675bp	**$2.95/bp**	**10 business days**
1,675 - 1,800bp	**$4,950 Flat rate**	**15 business days**
1,800 - 3,000bp	**$2.75/bp**	**15 business days**

We also ***guarantee*** the turnaround. Genes shorter than 1,675bp within 15 business days, genes between 1,675bp and 3kb within 20 business days OR YOU ONLY PAY 80% OF LIST PRICE.

OPTIMIZE GENES FOR EFFICIENT EXPRESSION IN HETEROLOGOUS SYSTEMS

A cornerstone in modern biotechnology is the ability to express functional proteins in heterologous hosts. Unfortunately, many heterologous proteins are difficult to express, expressed at low levels or expressed in a misfolded state resulting in inclusion bodies. Common solutions to expression problems include changing the genetic background of the expressing cells or the growth conditions; reducing growth temperature or even removing glucose from the media has solved many expression problems.

With the advent of **DNA 2.0**'s rapid and low cost custom gene synthesis, it is now also possible to redesign the entire gene sequence to maximize the likelihood of high protein expression. Common design features that often significantly improve protein expression and facilitate purification of the protein of interest include:

- Codon optimization. Codon frequencies vary significantly between different species and between proteins expressed at high or low levels with *E coli* being a notoriously picky host. The preferred solution for codon optimization of heterologous genes is to use the same codon distribution profile as that of the host organism. This is different from simply taking the most common codon for each amino acid and using it exclusively throughout the gene. By using a frequency weighted codon distribution, the risk of depleting the host cell for particular isoacceptor tRNAs is minimized. Local or global isoacceptor tRNA depletion can lead to starvation signaling and immediate down regulation of ribosomal translation and synthesis. Using only one tRNA chain for each amino acid can also lead to decreased translational fidelity through increase of both substitution errors and frameshift errors.
- Avoiding repetitive elements and mRNA secondary structures. Repetitive DNA sequences can be genetically unstable, and secondary mRNA structures may result in decreased mRNA half-life and lowered translation levels as it can stall ribosomes that shield the mRNA from RNAses. The redundancy of the translational code provides many nucleotide sequences to encode any amino acid sequence. We can perform rapid computational searches to help select those sequences that circumvent repetitive or structure forming DNA
- Generation of tags (cleavable or not) that can be used for purification, labeling, or as folding nucleus. The ribosome has been shown to be prone to abortive translation during the first 10-20 amino acids. Once the ribosome switches from initiation mode to elongation mode, it seems to be much more resistant to premature stalling/termination. N- and C- terminal tags of everything from His-tags to Maltose binding protein has been shown to not only be helpful in streamlining the purification process, but sometimes also acts as a ribosomal start point and folding nucleus.
- Sequence modification. Removal or insertion of restriction sites, internal splicing sites and premature polyadenylation can all be effected by custom gene design and synthesis. Additionally gene variants can easily be constructed for different purposes. Gene homologs from different organisms can be compared, splicing variants can be synthesized and tested, point mutations and combinatorial variants can be created for protein engineering purposes.

DNA 2.0 offers free assistance with gene design using a combination of in-house algorithms and public domain information. We can help you with testing many different variants in as an information rich dataset as possible. Please let us know how we can be of assistance.

Some of **DNA 2.0**'s favorite references on protein expression include:

1. Hale RS, Thompson G. Codon optimization of the gene encoding a domain from human type 1 neurofibromin protein results in a threefold improvement in expression level in Escherichia coli. (1998) Protein Expr Purif. 12(2):185-8.
2. Elf J, Nilsson D, Tenson T, Ehrenberg M. Selective charging of tRNA isoacceptors explains patterns of codon usage. (2003) Science. 300(5626):1718-22.
3. Sinclair G, Choy FY. Synonymous codon usage bias and the expression of human glucocerebrosidase in the methylotrophic yeast, Pichia pastoris. (2002) Protein Expr Purif. 26(1):96-105
4. Feng L, Chan WW, Roderick SL, Cohen DE. High-level expression and mutagenesis of recombinant human phosphatidylcholine transfer protein using a synthetic gene: evidence for a C-terminal membrane binding domain. (2000) Biochemistry. 39(50):15399-409.
5. Li Y, Chen CX, von Specht BU, Hahn HP. Cloning and hemolysin-mediated secretory expression of a codon-optimized synthetic human interleukin-6 gene in Escherichia coli. (2002) Protein Expr Purif. 25(3):437-47.

CRAIG VENTER IN THE NEWS

Just as we were about to send the newsletter to the printer, Craig Venter proudly announced that he had used $3M of DOE funding to build a 5.4 kb plasmid – the circular genome of bacteriophage ΦX174. Here at **DNA 2.0** we are somewhat puzzled by all the hoopla. As much as we admire Dr. Venter for his contributions to genome sequencing, his synthesis of 5.4 kb would be a rather routine gene order for us. For approximately $15,000 (5,400bp x 2.75/bp) we would have delivered the completed bacteriophage to the Department of Energy in 20 business days. Readers of the PNAS paper may also notice that only 1 in 10^6 of Dr. Venter's synthetic phage have the designed sequence. Only by using a selection scheme followed by a significant amount of DNA sequencing was it possible to identify the one-in-a-million phage with the correct sequence. Here at **DNA 2.0** we routinely get 1 out of 2 correct.

Dear Department of Energy, please let us know next time you need a genome synthesized.

We Love Referrals!!!

If you refer a customer to us who orders gene synthesis or protein engineering from DNA 2.0, we'll send you a bucket of our favorite chocolate.