

Assay Design: Controller of the Genomics Engine

MULTIPLEX PLATFORM OVERCOMES
OLIGONUCLEOTIDE ASSAY DESIGN
BOTTLENECK AND COST



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Assay Design: Controller of the Genomics Engine

Genomics has begun to impact fundamental aspects of everyday life. It is enabling scientists to understand health and disease, to ensure a plentiful and safe food supply, and to generate clean energy. Health, food, and energy are essential to future prosperity.

Appreciated by many is that a key controller of the genomic engine is oligonucleotide assay design. In 1975, Sanger added an oligonucleotide primer to nucleotides, polymerase, and DNA, repeatedly heated and cooled the mixture, and rapidly sequenced a gene. In 1981, Kary Mullis took the Sanger method, pointed a second primer back towards the first, made billions of copies of his target sequence, and called it PCR.

Today, there are hundreds of oligonucleotide methods. Most rely on a PCR amplification step, while others use non-PCR amplification like Rolling Circle Amplification. The newest single-molecule methods require no amplification.

Assay Design Is a Rate Limiting Step

Oligonucleotide assay design has long been a rate-limiting step in genomics research. One reason is that researchers are trained for the biological problem that they are investigating and not oligonucleotide assay design. Design of oligonucleotide assays requires expert knowledge in nucleic acid hybridization, molecular biology, and computational biology, as

well as knowledge of the idiosyncrasies of specific oligonucleotide methods.

Moreover, user-friendly and automated design software has lagged other aspects of genomic technology, such as oligonucleotide manufacture, the science of oligonucleotide assay design, the invention of new oligonucleotide methods, and instrumentation.

Design Alternatives

Befitting its importance, there are many alternatives to the design of an oligonucleotide assay. First, it remains possible to design one manually. However, this method is not practical.

The practical method is to use design software. Assay design requires prediction of hybridization efficiency, cross-hybridization of primers and probes, and off-target amplification. These predictions require expert knowledge and intense computation.

Academics have written a number of software packages. Many of these are now accessible for free over the internet. However, most were written to solve a narrow design problem, and so most have limited utility.

An exception is Primer 3. When it was written in 1995 it had outstanding science, engineering and flexibility. Through its popular web interface, Primer 3 can design for a number of venerable PCR, sequencing, and gene expression methods.

With respect to the well-known commercial packages, there is Oligo 6, Primer Premier, Primer Express, and Visual OMP. A final source is free design software written in-house, such as SciTools (IDT) and Oligo Perfect (Invitrogen). Many in-house tools use Primer 3 as the design engine.

Each of these packages has unique benefits.

Limitations of Alternatives

However, as a group, both academic and commercial assay design software have certain limitations. These include the lack of stable, reliable programming and intuitive user interfaces. Most of the software was written by scientists who did not have sufficient training or talent in software engineering.

Moreover, the software lacked critical features, such as unlimited high-throughput or the ability to configure the software for personal need.

Another limitation is that the stand-alone, commercial software packages listed above are costly. Each requires a single, large, up-front license fee. This cost can be prohibitive to the individual scientist.

Advanced Design Software

Celadon has developed world-class oligonucleotide assay design software that overcomes many of the limitations of earlier systems. This software, ProbelTy Online, allows researchers to focus on solving their biological problem, rather than being frustrated with failed assay design, or being distracted by having to become an expert in an idiosyncratic oligonucleotide method.

Easy To Learn and To Use

In today's fast-paced, interdisciplinary environment, scientists need software that they can learn quickly. ProbelTy is easy to learn because it is easy to use. Many users become productive with only a few minutes of instruction. A sixty minute webinar explains the more expert features.

ProbelTy was made so that a non-scientist can generate an optimal assay design with just a few mouse clicks. Users do not have to be genius biochemists or method-experts. The software was motivated by people like you. No

one has the time to spend hours being frustrated with assay design.

Validated Since 2001

Of course, scientists need to know that others have had success with ProbelTy. In use since 2001, ProbelTy has many satisfied customers. Its core algorithms have been proven time and again.

As an example, during a one week period, the U.S. National Cancer Institute's Core Genotyping Facility designed 700 PCR primer designs for its SNP re-sequencing pipeline, with 98% lab validation.

In another example, Marligen developed three multiplex SNP panels for the Luminex bead platform so as to predict clinical Warfarin dose. The panels consisted of 24 SNPs and the plex sizes ranged from four to eleven.

Finally, in a competition with two leading desktop packages using the TaqMan qPCR method, a leading life sciences vendor found that ProbelTy generated the strongest, cleanest, and most consistent results.

Stable and Reliable

Software serves no purpose if it frequently crashes or hangs. ProbelTy's code base is stable and reliable. It was written under the direction of a highly-talented and skilled MIT graduate.

Using advanced object-oriented design and the latest .NET 2.0 features, the code is split into many small and reusable units. This makes it easier to write, validate, maintain, and upgrade.

In addition, the core algorithms have been perfected through seven years of continuous use. Rarely does a user find a broken or untested path.

Contributions from Both Experts and Novices

There is benefit to having access to the best science and to powerful options. At the same time, it does no good if it takes years of training to understand how to use the options. The last thing a scientist needs is to be confronted with a control panel that looks like the cockpit of a 747.

The science and features incorporated into ProbelTy are the joint contribution of numerous internationally-recognized nucleic-acid biochemists, computational biologists, and

oligonucleotide method-experts, both in academia and industry.

Just as importantly, the interface is the result of feedback from many novice users. In combination, this approach has resulted in state-of-the-art science wrapped in a clean, professional, intuitive interface.

Affordable Subscription Service

Scientists have a keen sense of value. And research funding is always tight. Celadon's answer is to offer low-cost subscription plans that fit any budget.

The annual subscription plan is ideal for core facilities, large laboratories and other frequent users. The monthly subscription, which requires no contract, is perfect for small laboratories and individual researchers. Occasional or one-time users will benefit from daily and weekly plans.

Unlimited High Throughput

Given the vast amount of genomic sequence, researchers need to design large-scale experiments. They need unlimited high-throughput. ProbelTy has it.

Users are able to input hundreds of target sequences and ProbelTy will make designs with full automation. Then, ProbelTy saves your design run. You can access it at any time in the future. The system is able to store an unlimited number of design runs.

In multiplex mode, the system is able to make simultaneous designs for up to 200 sequences. This number far exceeds the capacity of most instruments.

Customizable Parameter Sets and Methods

Scientists often need to optimize design criteria, such as to specify the assay conditions that are in use in his or her laboratory. One of ProbelTy's best features is that it provides users with the ability to save an unlimited number of parameter sets.

The ability to save a parameter set also enables a skilled scientist to specify a new method. As an example, it is possible to create asymmetric PCR from classic PCR in three simple steps.

First, clone the classic PCR parameter set (which has equal primer concentrations). Next, modify it so that one primer has high concentration and the other primer has low

concentration. Finally, save your edits. Now, when you initiate a PCR design run, select the asymmetric parameter set.

This is one example illustrating the power of the system.

Accessible Worldwide, 24/7/365

Increasingly, users need to access software anytime of the day and from anywhere in the world. Scientists need it at the lab, from home, from a colleague's office, or to pursue breaking science at an international conference. They need it from their computer, and from the browser that he or she feels comfortable with. Moreover, scientists need access to the latest design science and oligonucleotide technology.

To meet these needs, we made ProbelTy web based, we made it work with all standard browsers, and we located it at a large, secure hosting facility. Because of this, ProbelTy is available 24/7/365 worldwide. All you need is a computer, a standard browser, and an internet connection.

There is never any software to download or to maintain. You always have the latest version. And with your subscription you get all updates and enhancements forever, including new methods and versions.

Secure

Of course, everyone needs to have their data secure. You don't want anyone else to see what you are working on, especially your competitors.

Not to worry. ProbelTy Online is secure. There is no possibility of someone else accessing your data. Not even us.

Moreover, ProbelTy is located behind an industry-leading firewall at a reputable hosting facility. In the remote event that someone maliciously hacks into ProbelTy, the application encrypts your password. There is nothing the intruder can see or do.

Advanced Hybridization Algorithms

In order for an assay to generate a good, clean signal, it must have balanced hybridization at the target site, minimal cross-hybridization among primers and probes, and minimal off-target amplification. Many experts contributed to ProbelTy and its hybridization algorithms are the proven standards in the industry.

To predict the hybridization strength of primers and probes to their targets, ProbelTy

uses the well-known nearest-neighbor model. This model is populated with the latest DNA and RNA thermodynamic parameters.

To evaluate cross-hybridizations, the system counts the number of matched base pairs in the maximal alignments. It does so using the well-known Smith-Waterman algorithm, with focus on the primer's 3' ends. This method yields excellent results.

Optionally, ProbelTy calculates the accumulated thermodynamic energy of all cross-hybridizations. This method has improved accuracy but is computationally intensive.

Finally, to evaluate off-target amplifications, ProbelTy queries GenBank with the PCR primer pair. This method will identify the genomic sequences that the primers' are predicted to amplify. The algorithm that ProbelTy uses is known as reverse electronic PCR.

Ability to Add New Methods Quickly

Developing software for new methods and new algorithms has been prohibitively expensive for all but the largest companies. For example, when Celadon first developed ProbelTy, it took months to add a new method and projects cost in excess of \$100,000.

This is a real problem when there are hundreds of oligonucleotide methods, and scientists invent new ones all the time. Lack of easily-modified and extended software has greatly slowed the pace of life science discovery.

Now, Celadon is able to add new methods to ProbelTy in as little as a few days. Only the most complicated of methods require more than two weeks. With ProbelTy, the time and cost to add new methods has plummeted dramatically.

Automated Design

High-throughput experiments require automated software. For this, ProbelTy provides Batch and Express modes. In Batch mode, you simply input your sequences, submit your design run, and ProbelTy will notify you when it is complete. You are able to shut off your browser, and you do not need to maintain a continuous internet connection.

Express mode is similar to Batch. But, it enables you to have real-time updates on your design run, and to cancel the design run at any time. Express mode requires that you keep your

browser open and to have a continuous internet connection.

Hands-On Design

Even with the flood of genomic data, there remain times when a scientist needs to focus on a particular assay design. Method-experts sometimes need to generate assays for difficult sequences, to optimize parameter sets for their laboratory protocols, and even to configure new methods.

ProbelTy excels at stepping a scientist through the design process. Custom mode is easy to learn and intuitive. In Custom mode, you are able to scroll through all available primers and probes, to re-sort them based on biophysical properties of your choice, to make individual selections, to modify parameter sets on the fly, to return to a previous design step, and more.

Multiplex

Scientists always want to generate more data, faster, and for less cost. One way to achieve this is to perform multiple experiments in a single chamber. Because of their utility, the number of multiplex methods, reagents, and instruments is increasing rapidly.

To meet this need, ProbelTy has multiplex design for all methods. Celadon's algorithm is remarkably efficient, given the enormous computational challenge of evaluating fine-grained cross-hybridizations among many assays.

It takes just a few minutes to generate a gene expression assay that is compatible with a standard reference assay. It takes just a few more minutes to generate SNP panels of ten and twenty-plex.

Bead Compatible

Scientists are purchasing bead-based systems such as Illumina and Luminex's xMAP. These systems have the advantage of processing much higher multiplexes than traditional optical thermal cyclers. ProbelTy is compatible with both Illumina and Luminex.

Widely-Used Methods

Assay design is not useful unless it is for the method that you want. ProbelTy has a large number of widely-used methods, such as

TaqMan, PCR, Allele-Specific Primer Extension, Single Base Extension, Mass Extend, Amplifluor, amplicon walking, sequencing, and more.

If there is a method that you need, but ProbelTy doesn't have it, call us. The cost for adding it is modest.

Compatible with Most Instruments and Reagents

The genomics market boasts a large number of reagents and instruments. Each combination may require unique design criteria.

No matter which instrument or reagent you use, ProbelTy is able to design for it. This is because it enables users to adjust and store an unlimited number of personal design criteria.

Modified Chemistries Overcome Limitations of Native DNA and RNA

Native DNA and RNA provide astonishing functionality in natural organisms. However, native DNA and RNA have limitations when it comes to biotechnology applications.

In particular, scientists often need to study a particular SNP, exon, splice site, regulatory element, or some other genomic feature. In order to study the feature, they need to place an assay in that exact sequence location.

However, the more constrained is the location, the more likely that the available sequence will be poor for assay design. This is where modified nucleic acid chemistries are most beneficial.

Modified nucleic acid chemistries include Peptide Nucleic Acids, Locked Nucleic Acids, Phosphorothioates, 2'-O-Methyl RNA, and Morpholinos. Other compounds, such as Minor Groove Binders, offer similar enhanced function.

The benefit of these chemistries is that they have altered thermodynamic profiles. They

provide greater specificity and flexibility in design. It is often the case that traditional assays will fail in a given location, while assays that incorporate a modified chemistry will succeed perfectly well.

Celadon is familiar with all of the modified chemistries, and it is a world-leader in researching the best use of Locked Nucleic Acids. It has written a number of modules that incorporate modified nucleic acid chemistries.

Security behind Your Firewall

Many institutions have a business need to locate all data and applications behind the corporate firewall. We understand this need.

To meet this need, we created ProbelTy Private Install. ProbelTy Private Install has all of the same great features as ProbelTy Online. In fact, it is the exact same application.

The only difference is that it is installed on your institution's computers, behind the firewall. Your institution has unlimited, private use of it. Administering the application, such as creating and managing users, is a snap with the built-in administration tools.

Assay Design as a Sales Tool

Genomic vendors want to solve their customer's entire problem. This is why more and more vendors are providing integrated platforms of instruments, reagents, software, and methods. Included in this is assay design, tailored to instruments, reagents, and methods.

ProbelTy Commerce is the ultimate assay design software to promote sales. In fact, ProbelTy began as ProbelTy Commerce. Because of this, its web pages are pre-configured to be private labeled with your company's look and feel.

The application can be integrated easily with your shopping cart, including usernames and passwords. Call us today!

The World's Best Scientists Use Celadon Software

Founded in 1999, Celadon Laboratories has established itself as the leading developer of web-based software for the design of nucleic acid diagnostic assays. As part of this commitment, Celadon conducts pioneering research for determining thermodynamic design rules for modified nucleic acid chemistries. In both of these key areas, Celadon has the same goal: to cure disease through the design of more effective research and therapeutic oligonucleotides.

In 2003, the U.S. National Cancer Institute commissioned Celadon to further develop its state-of-the-art, multi-method platform. In total,

Celadon has earned two SBIR Phase I/II Fast Track contracts with the U.S. National Cancer Institute, a Phase I SBIR contract from the U.S. Center for Disease Control, and research grants from the State of Maryland.

Since 2001, Celadon has licensed its software to leading vendors of proprietary genomic analysis methods, to provide fast, easy-to-use, web-based software that facilitates customers' assay design and purchasing process. These vendors include Illumina; Qiagen; Epoch Biosciences (now Nanogen); Serologicals; Biosearch Technologies; Proligo (now Sigma); and Thermo Hybaid.