Case Study:

Multiplexing Algorithm Development & Implementation

Apticraft's molecular biology domain and computational expertise helped transform the clients products into a competitive advantage.

The client is a renowned software vendor in California, specializing in bioinformatics applications to aid researchers in simulating and designing molecular biology experiments. The products include a comprehensive range of applications designed to address challenges in Polymerase Chain Reaction (PCR), Microarray and Cloning.

Market Analysis and Foresight Urged the Need to Include the ability to Design Multiplexing PCR Experiments

Looking at the market need the client wanted to include the ability to design multiplex PCR experiments. Designing normal PCR experiments was in itself a uphill task. Multiplexing would further add to the complexity.

About Multiplex PCR

Multiplex PCR is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction. It is a cost effective technique for biological applications that includes large scale genotyping, gene expression and forensic analysis. It also facilitates in developing diagnostic assays.

Multiplex PCR primer design and optimization is a greater challenge than a single template assay design. The aim of multiplex design is to find primer-probe sets that can be used in a single tube to amplify multiple targets simultaneously. To make this happen, the oligos should be highly specific, should not exhibit cross reactivity and should work under uniform reaction conditions.

The challenge was to design an automated tool that would:

- Analyze all possible primer-probe combinations for each target sequence in the multiplexing set and also analyze for cross linkages, dimerization and melting temperature difference. The analysis requires analyzing millions of combinations.
- Report the most optimum set based on the specified reaction conditions and search parameters in a few seconds.

Looking at the possible large and varied user base, the client wanted the tool to be highly customizable and allow the user to control all their design parameters.

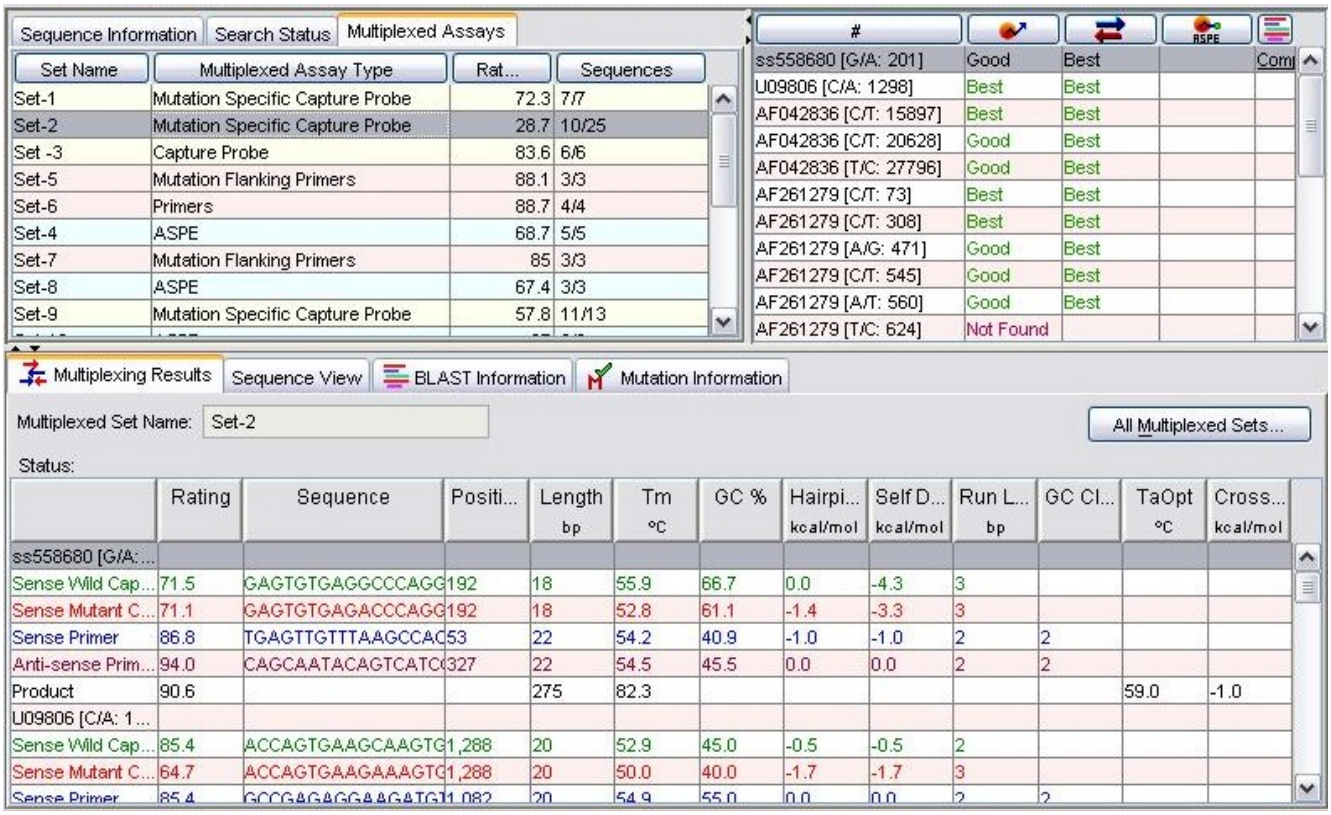
Apticraft's Molecular Biology Domain Experts and Technology Team Helped Transform the Products Into a Competitive Advantage

Having being worked with Apticraft System in the past, the client assigned Apticraft to address this challenge and integrate it with the existing product functionality.

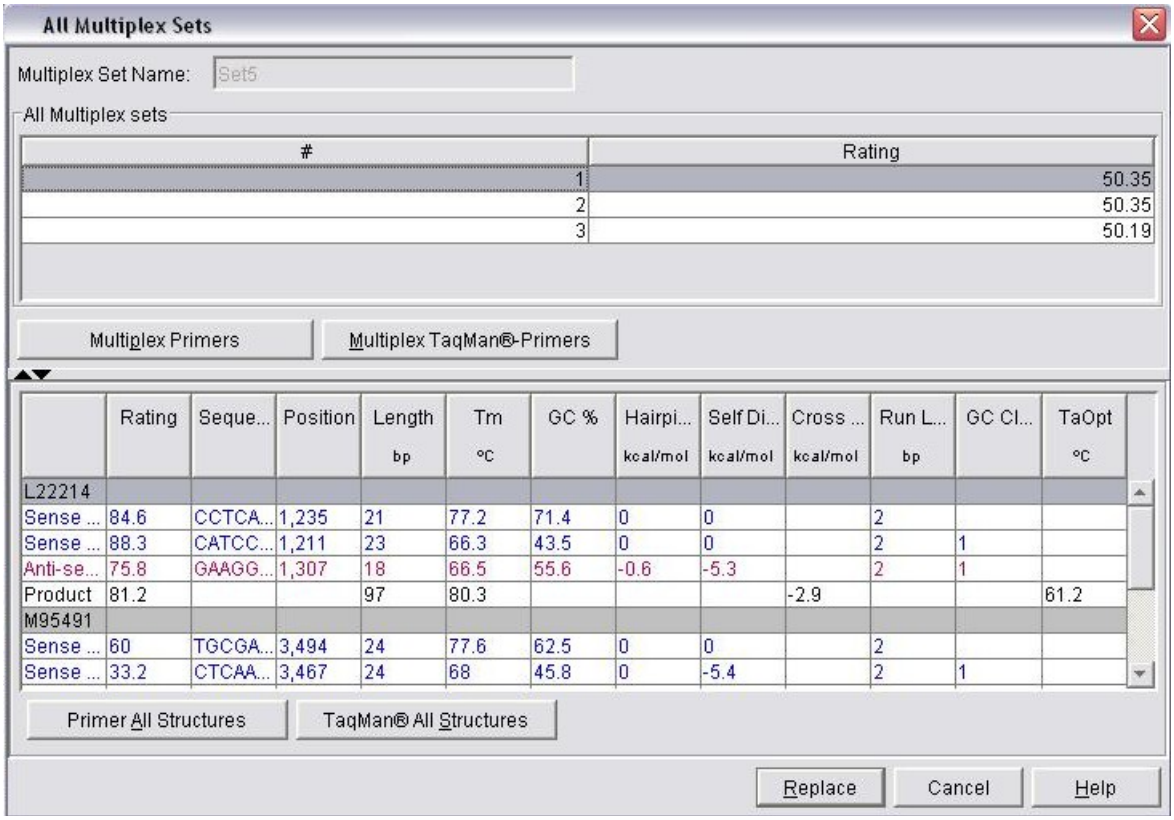
Working closely with the client and the client's clients, Apticraft's domain experts and technology team completed an end-to-end analysis and audit of the requirement. The team also went through research papers and experts working in this domain (clients customer) to gather sample data and to understand the actual pain point of multiplexing.

The team then designed the algorithms to design optimal multiplex sets for up-to 100 sequences in a single search run. Team Apticraft recommended and implemented the following:

- The primer-probe search tool is backed by a rating algorithm that accesses the oligo properties of each set and assigns a rank based on the variation of these properties. This functionality helps users to take an informed decision.
- To improve the efficiency and performance of the application, data structure algorithms were implemented. This helped manage the large quantum of data generated and allowed intermediate sorting of the output.
- Intuitive and user friendly interface to make it easy for customers to use.
- To enable flexibility to alter design parameters and reaction conditions, a user interface was designed.
- Export the design results to a spreadsheet program for use in any downstream application or for storing in a central database.



Multiplex design results



Alternate Multiplex sets

Impressive Results Confirm the Accuracy of the Solution

The client is now the only vendor that offer products support multiplex PCR design. Other products available on the market only help in checking the multiplexing efficiency post primer/probe design. In short, the multiplexing experiment design ability provides unprecedented performance despite the complexity, improved cost savings, user satisfaction and above all competitive advantage.

- Reduced end user overtime cost: 98% reduction in manual multiplex design work.
- The design of sophisticated algorithm ensured that almost all possible primer-probe sets conformed to per user specifications.
- The rating algorithm helped prioritize multiplex sets.
- Quick turn around time helped the client to be the pioneer in the industry.

Technology used

Platform/OS:

- Widows OS (98/2000/NT/XP/Vista)
- Mac OS X

Development environment:

• Languages/Scripts: and Technology	Java, JDBC, Multithreading UML (project design/architecture) XML for data communication on web/internally.
• Editor/ Development Environment:	Eclipse IDE (from IBM), Forte for Java CE IDE (from SUN Microsystems)
• Database:	BORLAND JDataStore
• Native OS specific support:	JNI Lib
• GUI:	JFC/Swing Framework.
• UML Design Tools:	Poseidon (from Gentleware)
• Installer Tool:	InstallAnywhere (MacroVision)
• Deployment Tools:	Java Web Start, Ant
• Repository	CVS