

## **1.5. New Features and Enhancements:**

(Fragment from the Oligo 7 Manual)

Oligo 7 has been re-written from scratch, to make it fully compatible with ever-changing operating systems. Most of the source code is written in Java, and processor-intensive sections were written for specific hardware to maximize speed of calculations. Compared to the version 6, OLIGO 7 has several changes. The major enhancements are listed below.

### **1.5.1. New interface.**

Starting up the program shows only one but more informative window. The Tm graph shows the entire sequence. Position of up to 4 (previously 2) oligos is graphically displayed. This window also displays primers Tm, PCR product, strong loops in DNA (RNA) template, potential loops in oligos, palindromes, features read from annotations, and other data.

### **1.5.2. New thermodynamic parameters**

Oligo 7 implements unified nearest-neighbor thermodynamics assembled by John SantaLucia (1, 2). Values for single mismatches and dangling ends are incorporated. This complicates expression of the melting temperature values: Oligo reports standard melting temperatures (without mismatches & dangling ends) as  $t_m$  and with mismatches and dangling ends as  $T_m$ .

### **1.5.3. More primer/probe options.**

Introduced the ability to select 4 oligos for analysis. Oligo 6 only handled 2. This was done to enable searches for TaqMan Probes and nested primer sets. Oligo also can search automatically for molecular beacons and siRNA. A series of user-editable design constraints have been added, so you may search for oligos with certain bases or sequences built in.

### **1.5.4. New search criteria and the entire search algorithm.**

Oligo 7 is no longer constraint to a fixed length of a probe/primer during a single search. You may now select a range of lengths for your primers. The number of sub-searches was increased, so you may restrict not only 3'-stability but also 5'-end stability to aid in siRNA selection. In addition, a search for strong hairpin loops in the template was added. This does not include primer design by itself but with this search you may avoid hard to amplify regions. The sequence constraints option lets you find primers with certain bases at the 3' and 5'-ends. There is also an option to restrict the number of guanosines in your primers that greatly enhances possibility of multiplexing.

### **1.5.5. The quality of oligos and pairs are scored in a single number**

Many Oligo users requested this option because it is trivial to find the best primer

that way. Unfortunately, different applications require different approach and emphasis on different parameters or features of a primer/probe. This led to a fairly complex and user-customizable scoring system. In most cases, however, it

is not essential to modify the definitions.

### **1.5.6. Enhancements to the search for primers & probes protocol**

The primer and probe search protocol allows the user to "lock" every parameters such as, Tm range or 3' stability ΔG settings, so that the automatically change stringency setting, which incrementally relaxes each parameter, is more controlled during a search. In addition, the user can choose to balance PCR Primers according to the priming efficiency number rather than Tm which has been shown to provide better amplification results. The priming efficiency algorithm has been slightly modified.

#### **1.5.7. Automatic OLIGO updating**

In the Help Menu, the item "Check for Update" has been added. This enables automatic installation of new versions from the MBI's server. In all previous versions this was a manual cumbersome process.

#### **1.5.8. Open reading frame**

This new analysis window provides information on all ORF, and gives basic information on translated proteins, including molecular weight and pKa.

#### **1.5.9. Homology analysis**

Besides the false priming sites check you may also view homologous sites.

#### **1.5.10. Batch processing**

Now searches for primers and probes can be performed on large number of sequence files at a time. Results of this type of search is automatically stored in a results text file.

#### **1.5.11. Database functionality**

Has been greatly improved. The main change is a possibility of fully automatic multiplexing. Besides single oligos storage the Database can store also primer sets. You may also check oligos for dimerization directly from the Database menu.

#### **1.5.12. Improved features**

All previously existing windows have been re-designed. They have more pleasing graphics and usually show more information than in Oligo 6. Some windows have greater functionality. For example, you may read consensus sequences from the list of primers (Analyze-Selected Oligonucleotides window) or read the info on four, instead of two, primers from the PCR window.