

**Picky Version 1.0 Tutorial**



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# Picky Version 1.0 Tutorial



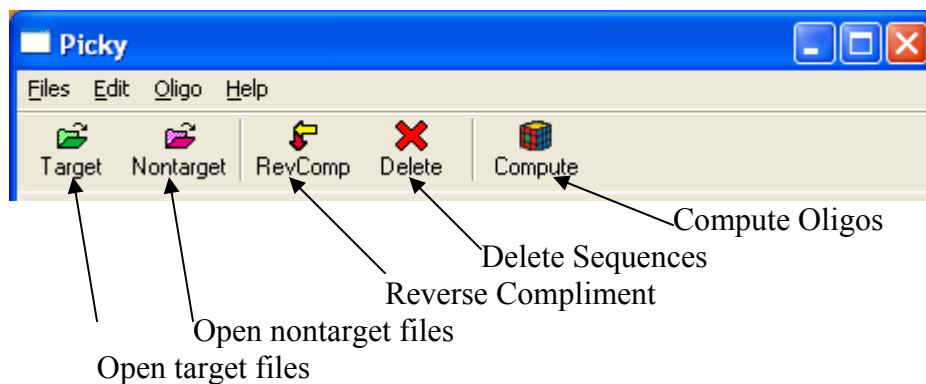
Picky is an oligo design program that alleviates long and monotonous calculations by identifying probes that are very unique and specific to sequence regions. These calculations are based on parameters inputted by the user including optimal probe length, ideal percentage of guanine and cytosine content, salt concentration and the maximum length to which a target sequence matches any non-target sequence. Picky has exceptional computational capability and is user friendly. Macintosh, Unix and PC versions are all available.

## Introduction to Tutorial

This tutorial is designed to give users a basic understanding of the functions of Picky for oligo design. Users should be able to perform the following tasks upon completion of the tutorial:

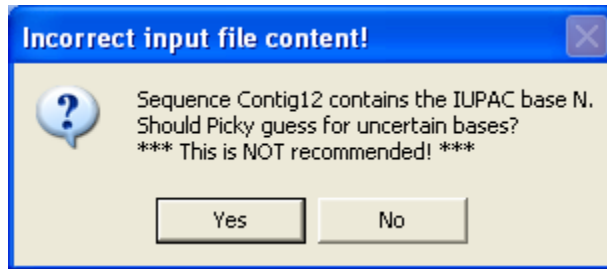
- Load a target gene sequence
- Compute the sequence using parameters defined by the user
- Understand and identify meaning of the various highlighted colors, underlined sequences and text colored sequences
- Pick out a unique sequence based on the program's design

## Guided Tour of Picky



## Loading target files

Picky accepts any sequence of genome as a text file. Simply left click the *Open target files* or select Files > Load target genes from the pull down menu. In this tutorial, we will use the *maize\_est\_contig.seq*, a sequence coming from maize corn. Select the file and choose open. A pop up box will display:



It is always best to input complete base sequences, however, the user may choose whether they want Picky to randomly assign unknown sequences or if they want these unknown bases to remain in the data set. For the purposes of this tutorial, choose *Yes*.

The sequence is now loaded into the program. Use the scroll down menus to view the sequences and numbering.

### Entering Oligo Design Parameters

To specify and compute oligo probes left click the *Compute Oligo* or choose *Oligo > Compute* from the pull down menu. The following screen appears:



Picky allows the parameters to be specified for oligo size, match length, sensitivity, GC content and melting temperature difference. The following is a description of each set of parameters:

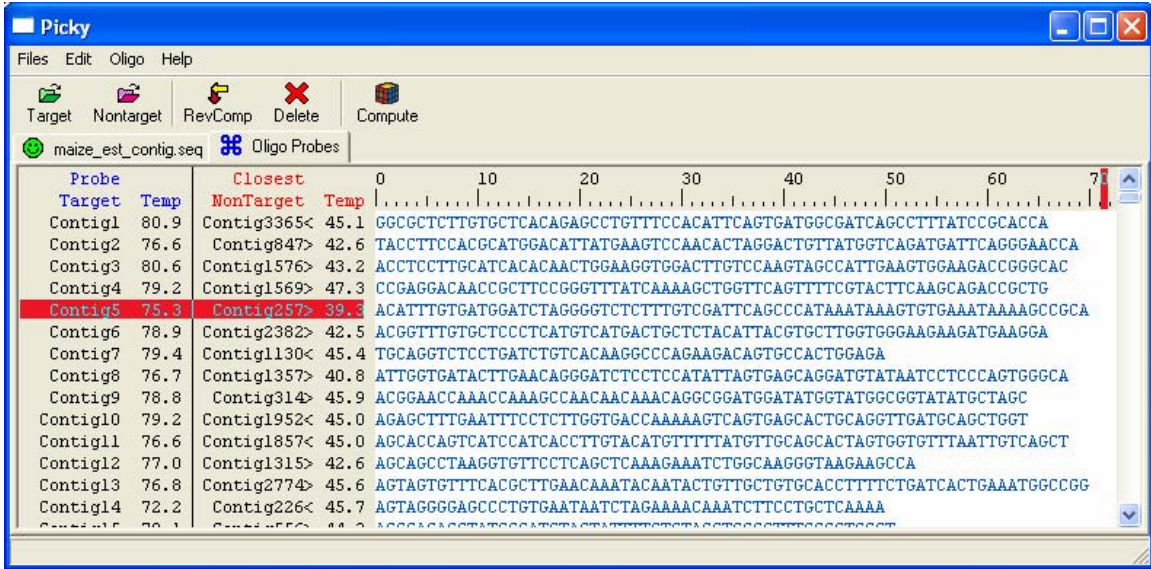
Picky will select oligos based on the constraints specified in terms of length, specificity, sensitivity and temperature performance.

Parameter	Description
<b>Oligo Size</b> Maximum Default: 70 Minimum Default: 50 Ranges: 50 to 90	Picky will provide probes with the base size that lies between the minimum and maximum values.
<b>Match Length</b> Default: 15 Range: 10 to 25	Picky will underline any match that is identical to another sequence by this parameter's given number. These underline sequences will not be contained in any "colored" text probe.
<b>Sensitivity Level</b> Default: 10 Minimum: 5	Picky will color code base sequences that have high similarities with other continuous sequences. Picky is designed to show pair wise alignment that may give a staggered line of similar results. A lower parameter means a significantly longer computational period.
<b>GC Content</b> Default Max: 70 Default Min: 30 Must have a 20-point difference.	Picky will only select probes within the selected parameter of G and C base percentages.
<b>Number of Probe Candidates</b> Default: 100 Range: 50-500	Picky will compare the given number based on match region to all non-target genes for the selection of probes.
<b>Number of Probes per Gene</b> Default: 1 Range: 1 to 5	The number of distinct, non-overlapping oligo probes to be chosen for each sequence.
<b>Minimum Temp Difference</b> Default: 20.0 Range: 5.0 to 30.0	The difference between a probe candidate's target melting temperature and its highest non-target melting temperature that is considered to be safe.
<b>DNA Concentration (nanoM)</b> Default: 1.0 nM Range: 0.001to 1000.0 nM	Picky uses this value to calculate melting temperatures between target and non-target genes and need be given as an approximate value.
<b>Salt Concentration (milliM)</b> Default: 75 mM Range: 0.1 to 3000 mM	Picky will implement this value to calculate hybridization based on melting temperatures.

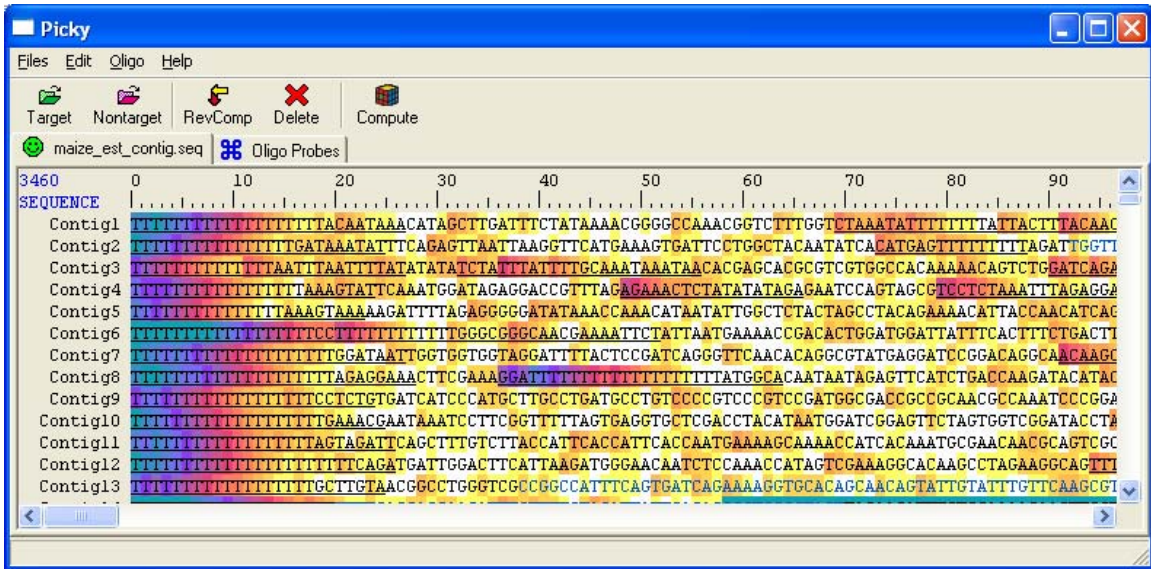
These parameters may have a major effect on the computational time and are also limited to a computer's physical memory. Picky needs roughly 20 times the input data size in order to carry out its computation, e.g., for the Maize input set which is about 2.2M bp, Picky will need about 45M total memory to process this set. Also, when the minimum match length is decreased or when the maximum match length is increased, Picky may require an extended time to run when compared to using the default settings.

Click *OK* on the parameter dialog box.

Two tabs are shown above the output field, one labeled *maize\_est\_contig.seq* and one labeled *Oligo Probes*. Oligo probes for each line appear based on the parameters that have been set. Minimum target temperatures are also shown for each oligo, that is, the temperature in which the probe binds to its complementary strand.



To view the loaded data with color-coded regions, click on the left hand tab of the document field labeled *maiz\_est\_contig.seq*.



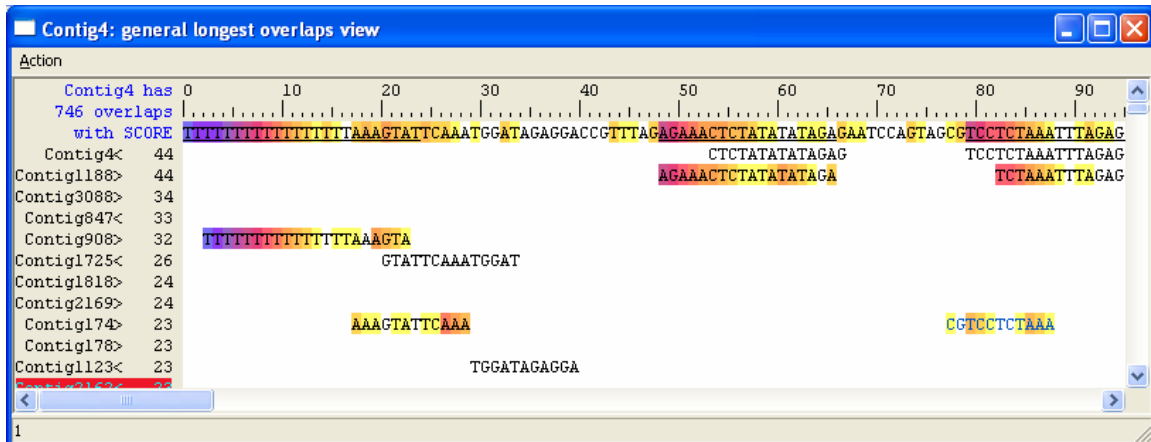
### Analyzing Loaded Data

Red underlined regions represent regions that match non-target regions based on the minimum match length specified in the parameter window. These regions are not used in the probes. Oligos that are candidate for oligo probes are identified in blue text.

Highlighted regions in the field represent degrees of similarity. The dark colors such as

red, orange and yellow of a high degree of similarity among other sequences while the yellow and white have a small similarity between others.

Double click on any row to bring up a new screen that compares the specified sequence with others.



The selected sequence appears in the first row with subsequent sequences listed below, according to specific matching. These sequences were the sequences that appeared with red lines in the previous screen. Use your mouse to roll over any listed sequences. The rest of its sequence appears. You may double-click any sequence again to get another window with the selected sequence at the beginning.

You have completed the Picky Version 1 tutorial. Please stay updated for further versions of Picky at <http://www.complex.iastate.edu>.

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