OliCheck v1.0.5 - User Guide

Introduction

OliCheck is a program designed to test the validity of potential microarray probes by considering the possibility of cross-hybridization with non-target coding sequences. This analysis can be performed within a single genome or between several different strains/organisms. For more information on the algorithm, see Charbonnier *et al.*, unpublished.

For specific problems/questions, see the Notes section located at the end of this document or send an email to yvan.charbonnier@genomic.ch.

Installing OliCheck

Before you install OliCheck, make sure that your computer system meets the minimum requirements for that product. See System Requirement (page 5) for more information.

To install OliCheck:

Run: setup.exe
Follow the installation instructions displayed on the screen. You can perform a Compact, Typical, or Custom installation. The Compact installation includes only the files you need to run OliCheck (i.e. the software and its manual). The Typical installation includes all the files you need to run OliCheck plus the BLAST executable and example files. Choosing the Custom installation, you can install any combination of the following:
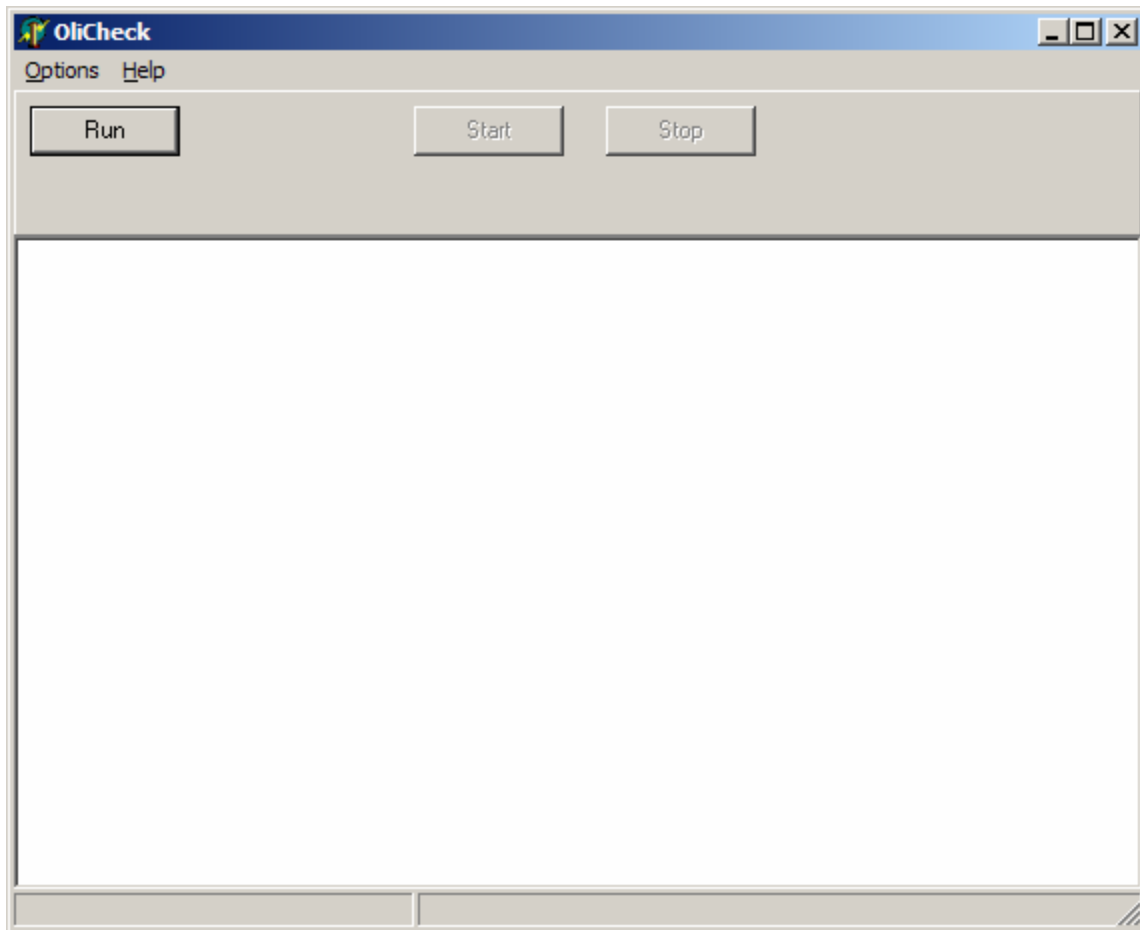
> OliCheck Application
> BLAST Executable
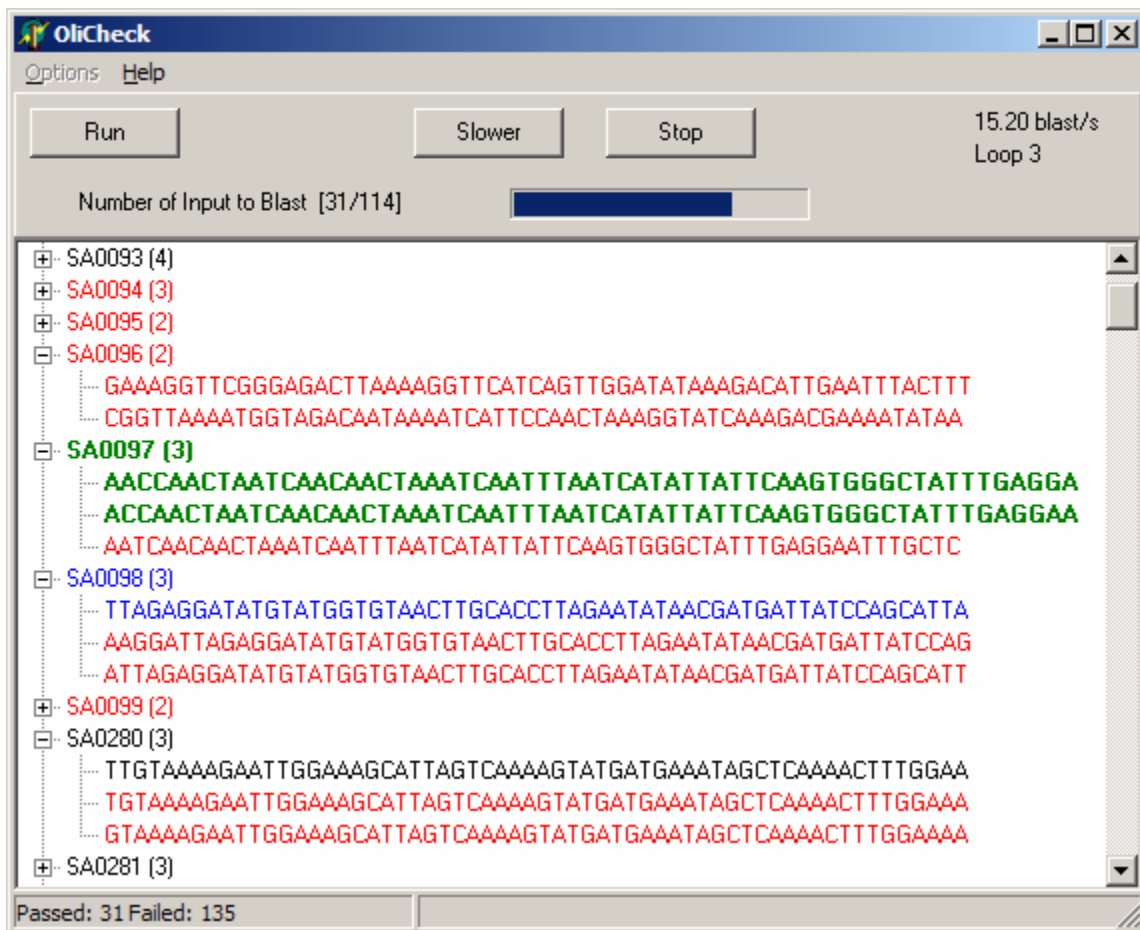> OliCheck Example Files:
> - BLAST Example Database
> - Array Designer™™ Example File

Using OliCheck

OliCheck requires one input file, as described on page 7. This file must be in the format used by Array Designer™, as its standard output (see page 8). All outputs generated by OliCheck will also be provided in this format. The specifications of a run of OliCheck are defined by clicking on the Options menu located in the upper left-hand corner of the program window (Figure below). A detailed review of these parameters can be found on page 3.
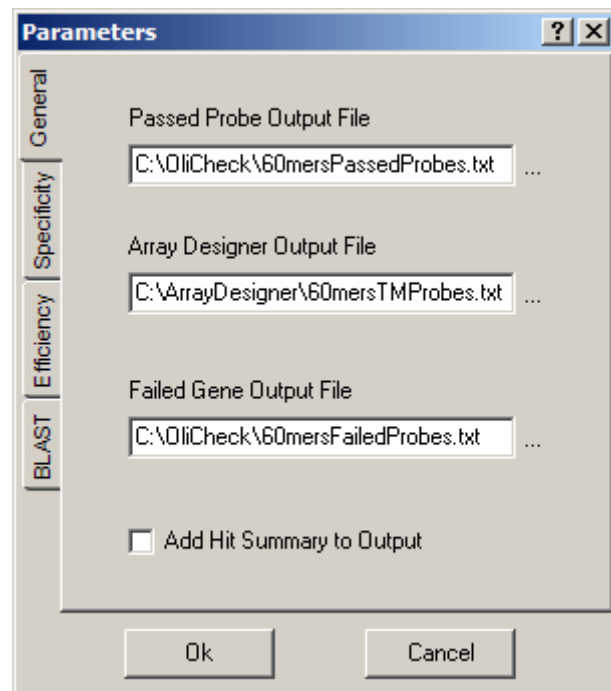
After having completed the setup, click 'OK' on the Parameters box. Then, select 'Run' in the main window. OliCheck will load the desired Array Designer™ file and test the probes according to the selected parameters. The main window provides the user with a variety of information on the status of the process. It displays the current position in the BLAST queue, the number of BLASTs performed per second, the loop number, the number of genes that have probes that passed/failed and the gene names and probe sequences themselves. A gene name/probe sequence is color-coded in the right-hand window depending on whether it has passed (green) or failed (red) the OliCheck process. When a probe is in the process of being BLASTed, it is highlighted in blue. The Typical installation allows the user to run an example illustrating that process.
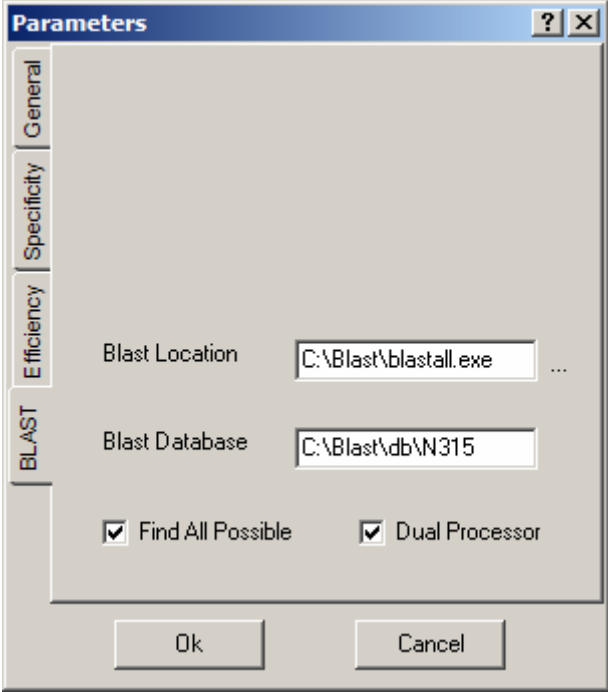
OliCheck Parameters

The first step in initiating an OliCheck test is to select the files using the **General submenu**. The files that OliCheck creates will follow standard formatting conventions. The "passed probe" file will be returned in Array Designer™ format. It will contain the thermodynamics parameters and comments taken from the Array Designer™ input file, to describe each probe that OliCheck validated as being appropriate. The "failed gene" file will contain a simple list of all genes for which no acceptable probe has been found. If the *Add Hit Summary to Output* is checked, the "passed probe" file will contain an extra column with the

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name of the homologous gene(s) that the probe can detect. This will be useful only for checking potential probes against another genome than the one used to generate the probe set.

Using the **BLAST submenu**, the user can select the BLAST database to be used to validate the probe set. The name of the database, as defined by one's local installation of BLAST, should be typed here along with the location of your blast executable. The *Find All Possible box* instructs OliCheck not to stop after having found a probe for a given gene that satisfies user-defined parameters. The *Dual Processor* allows OliCheck taking advantage of BLAST's ability to split its processes on two processors.
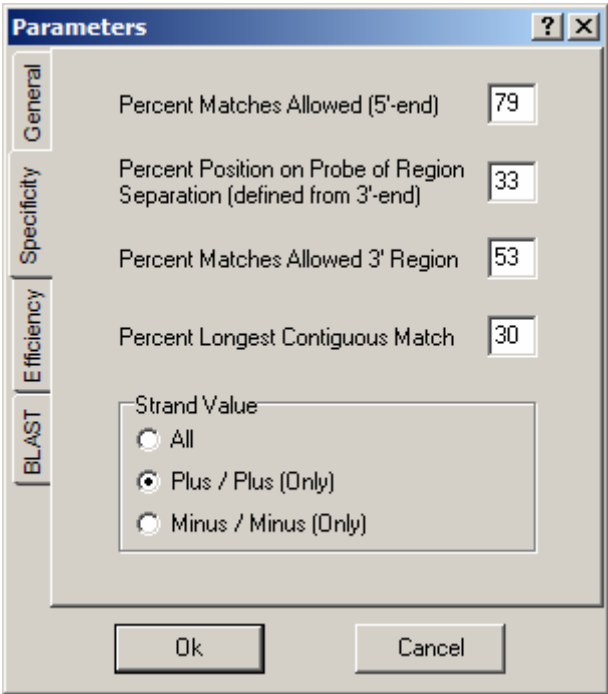
Parameters

General | Specificity | Efficiency | BLAST

Blast Location   C:\Blast\blastall.exe   ...

Blast Database   C:\Blast\db\N315

☑ Find All Possible   ☑ Dual Processor

Ok   Cancel

The **Specificity submenu** allows defining values for the parameters used in the Specificity test (see Charbonnier *et al.* for further description). The values listed in the adjacent figure are the defaults used by OliCheck. These parameters are a mathematical interpretation of the experimentally defined specificity parameters in Hughes *et al* (Nat Biotech, 2001; 19(4):342-7). The results from the paper refer to a number of matches/mismatches for 60-mer oligonucleotides. The value used in OliCheck is defined as a percentage to allow dealing with different oligonucleotide lengths. The first parameter indicates the percentage of matches allowed in the solution-end of the probe (5'-end). The second parameter

Parameters

General | Specificity | Efficiency | BLAST

Percent Matches Allowed (5'-end)   79

Percent Position on Probe of Region Separation (defined from 3'-end)   33

Percent Matches Allowed 3' Region   53

Percent Longest Contiguous Match   30

Strand Value
○ All
◉ Plus / Plus (Only)
○ Minus / Minus (Only)

Ok   Cancel

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allows specifying the portion of the probe that will be considered as the 5'-end and 3'-end regions. The third parameter indicates the percentage of matches allowed in the surface-end of the probe (3'-end). A last parameter defines the longest contiguous match allowed. Finally, OliCheck is able to restrict its filtering to the Plus, Minus or Both strands of the BLAST alignment.

The **Efficiency submenu** allows defining values for parameters used in the Efficiency test (see Charbonnier *et al*). Values listed in the adjacent figure are the defaults used by OliCheck. The first parameter specifies the total number of mismatches allowed in the whole BLAST alignment between the probe and its target. The second parameter allows specifying the portion of the probe that will be considered as the 5'-end and 3'-end regions. The third parameter sets the maximum percentage of mismatches allowed in the solution-end of the probe.

Notes

At present, OliCheck contains a memory leak that might cause problems if it is sequentially used to analyze large Array Designer™ files. To avoid this problem, simply quit OliCheck between runs: the program will free all memory associated with it on shut-down thereby eliminating any possible memory leak.

With Microsoft Windows™ XP using graphic themes, user may experience some graphical rendering problems with the vertical tabs. The graphical interface is fully rendered with the Windows™ Classic Theme.

System Requirement

OliCheck requires additional software:

    NCBI Blast provided in the installation executable or freely downloadable on the NCBI ftp server (ftp.ncbi.nih.gov\blast\executables\LASTEST-BLAST)

OliCheck has been tested and validated on Microsoft Windows™ XP. It may also run on Microsoft Windows™ 9x, Microsoft Windows™ NT, or Microsoft Windows™ 2000 (not tested).

The minimal OliCheck requirements are:

Pentium III processor (733MHz)
128 MB RAM
VGA or higher resolution monitor
A mouse or other compatible pointing device

OliCheck is a memory and processor intensive application. As it uses multithreading for BLAST operations, OliCheck may also work with lower memory and processor requirements but this may yield poor performances.

The recommended configuration is the following:
Dual Xeon 1.5GHz processor
1GB RAM
1024x768 (32 bit) resolution monitor
Windows™ XP SP1

The amount of RAM can be scaled according to the BLAST program requirements. The BLAST application loads the sequence databases in memory. When designing probes for larger genomes, the RAM size should be increased accordingly.

Example Files

BLAST Databases File

The NCBI blast uses an internal database format that is generated from a simple FASTA file (See below) by using the `formatdb` tool. This tool generates a set of three files that are used by the `blastall` program as reference database. As an example, the command to generate the N315 database is the following:

```
formatdb -p F -n N315 -i N315.ffn
```

The parameter `-p F` indicate to `formatdb` that the input file contains nucleotide sequences. The `-n N315` parameters allows setting the output name of the database (i.e. the name that will be used in OliCheck BLAST Options). Finally the `-i N315.ffn` parameter setup the input file.

FASTA File

A FASTA file is a simple ASCII file. Every ORF entry is structured in the following way. The description line started by the character ">", it is followed by a free ASCII text. For OliCheck purposes, this text should be the accession name of the corresponding ORF. OliCheck uses this to annotate the output file if the *Add Hit Summary to Output* option is set. The following lines contain the sequence itself. The next ORF is recognized by the next > symbol.

A truncated example to a FASTA file is shown below:

```
>gi|15925706:517-1878, SA0001 [chromosomal replication initiator protein]
ATGTCGGAAAAAGAAATTTGGGAAAAAGTGCTTGAAATTGCTCAAGAAAAATTATCAGCTGTAAGTTACT
CAACTTTCCTAAAAGATACTGAGCTTTACACGATTAAAGATGGTGAAGCTATCGTATTATCGAGTATTCC
TTTTAATGCAAATTGGTTAAATCAACAATATGCTGAAATTATCCAAGCAATCTTATTTGATGTTGTAGGC
TATGAAGTTAAACCTCACTTTATTACTACTGAAGAATTAGCAAATTATAGTAATAATGAAACTGCTACTC
CAAAAGAAACAACAAAACCTTCTACTGAAACAACTGAGGATAATCATGTGCTTGGTAGAGAGCAATTCAA
TGCCCATAACACATTTGACACTTTTGTAATCGGACCTGGTAACCGCTTTCCACATGCAGCAAGTTTAGCT
GTAGCCGAAGCACCAGCCAAAGCGTACAATCCATTATTTATCTATGGAGGGGTTGGTTTAGGAAAAACCC
ATTTAATGCATGCCATTGGTCATCATGTTTTAGATAATAATCCAGATGCCAAAGTGATTTACACATCAAG
TGAAAAATTCACAAATGAATTTATTAAATCAATTCGTGATAACGAAGGTGAAGCTTTCAGAGAAAGATAT
CGTAATATCGACGTCTTATTAATCGATGATATTCAGTTCATACAAAATAAAGTACAAACACAAGAAGAAT
TTTTCTATACTTTTAATGAATTGCATCAGAATAACAAGCAAATAGTTATTTCGAGTGATCGACCACCAAA
GGAAATTGCACAATTAGAAGACCGATTACGTTCACGCTTTGAATGGGGGCTAATTGTTGATATTACGCCA
CCAGATTATGAAACTCGAATGGCAATTTTGCAGAAGAAAATTGAAGAAGAAAAATTAGATATTCCACCAG
AAGCTTTAAATTATATAGCAAATCAAATTCAATCTAATATTCGTGAATTAGAAGGTGCATTAACACGTTT
ACTTGCATATTCACAATTATTAGGAAAACCAATTACAACTGAATTAACTGCTGAAGCTTTAAAAGATATC
ATTCAAGCACCAAAATCTAAAAAGATTACCATCCAAGATATTCAAAAAATTGTAGGCCAGTACTATAATG
TTAGGATTGAAGATTTCAGTGCAAAAAAACGTACAAAGTCAATTGCATATCCGCGTCAAATAGCTATGTA
CTTGTCTAGAGAGCTTACAGATTTCTCATTACCTAAAATTGGTGAAGAATTTGGTGGGCGTGATCATACG
ACCGTCATTCATGCTCATGAAAAAATATCTAAAGATTTAAAAGAAGATCCTATTTTTAAACAAGAAGTAG
AGAATCTTGAAAAAGAAATAAGAAATGTATAA
>gi|15925707:2156-3289, SA0002 [DNA polymerase III, beta chain]
ATGATGGAATTCACTATTAAAAGAGATTATTTTATTACACAATTAAATGACACATTAAAAGCTATTTCAC
CAAGAACAACATTACCTATATTAACTGGTATCAAAATCGATGCGAAAGAACATGAAGTTATACTAACTGG
TTCAGACTCTGAAATTTCAATAGAAATCACTATTCCTAAAACTGTAGATGGCGAAGATATTGTCAATATT
TCAGAAACAGGCTCAGTAGTACTTCCTGGACGATTCTTTGTTGATATTATAAAAAAATTACCTGGTAAAG
ATGTTAAATTATCTACAAATGAACAATTCCAGACATTAATTACATCAGGTCATTCTGAATTTAATTTAAG
TGGCTTAGATCCAGATCAATATCCTTTATTACCTCAAGTTTCTAGAGATGACGCAATTCAATTGTCGGTA
AAAGTGCTTAAAAACGTGATTGCACAAACGAATTTTGCAGTGTCCACCTCAGAAACACGCCCAGTACTAA
CTGGTGTGAACTGGCTTATACAAGAAAATGAATTAATATGCACAGCGACTGACTCACACCGCTTGGCTGT
AAGAAAGTTGCAGTTAGAAGATGTTTCTGAAAACAAAAATGTCATCATTCCAGGTAAGGCTTTAGCTGAA
TTAAATAAAATTATGTCTGACAATGAAGAAGACATTGATATCTTCTTTGCTTCAAACCAAGTTTTATTTA
AAGTTGGAAATGTGAACTTTATTTCTCGATTATTAGAAGGACATTATCCTGATACAACACGTTTATTCCC
TGAAAACTATGAAATTAAATTAAGTATAGACAATGGGGAGTTTTATCATGCGATTGATCGTGCCTCTTTA
TTAGCACGTGAAGGTGGTAATAACGTTATTAAATTAAGTACAGGTGATGACGTTGTTGAATTATCTTCTA
CATCACCAGAAATTGGTACTGTAAAAGAAGAAGTTGATGCAAACGATGTTGAAGGTGGTAGCCTGAAAAT
TTCATTCAACTCTAAATATATGATGGATGCTTTAAAAGCAATCGATAATGATGAGGTTGAAGTTGAATTC
TTCGGTACAATGAAACCATTTATTCTAAAACCAAAAGGTGACGACTCGGTAACGCAATTAATTTTACCAA
TCAGAACTTACTAA
>gi|15925708:3670-3915, SA0003 [conserved hypothetical protein]
GTGATTATTTTGGTTCAAGAAGTTGTAGTAGAAGGAGACATTAATTTAGGTCAATTTCTAAAAACAGAAG
GGATTATTGAATCTGGTGGTCAAGCAAAATGGTTCTTGCAAGACGTTGAAGTATTAATTAATGGAGTGCG
TGAAACACGTCGCGGTAAAAAGTTAGAACATCAAGATCGTATAGATATCCCAGAATTACCTGAAGATGCT
GGTTCTTTCTTAATCATTCATCAAGGTGAACAATGA
```

OliCheck Input File

OliCheck typical input file is an Array Designer™ output file (See description on page 8). Moreover, OliCheck can deal with any tab-delimited ASCII text file with the following restrictions. Each probe should be described in one line where columns 2 and 4 contain the accession and the sequence, respectively. The contents of the other columns can be freely filled as it is not considered as relevant by OliCheck. The output file generated by OliCheck can be directly set as an input file to the program, if the probes need to be further annotated.

Array Designer™ Output File

Array Designer™ Output File is a 21 columns tab-delimited ASCII text file with the following form:

A truncated example of an Array Designed output file is shown below:

FileName	Accession	Site	Sense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen	...
NC_002745.fa	SA0280	100	TTGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAA	44.3	50	60	69.63	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0280	100	TGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAA	44.3	51	60	69.63	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0280	100	GTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAAA	43.8	52	60	69.2	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0474	50	ATGATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAAC	60.2	1	60	69.79	33.3	-1.44	-2.62	3	...
NC_002745.fa	SA0474	50	ATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAACGAT	60.1	4	60	70.32	33.3	-1.44	-2.62	3	...
NC_002745.fa	SA0668	100	TATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGA	59.8	54	60	70	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA0668	100	ACTATATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGA	59.8	50	60	70.1	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA0668	100	ATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGAA	59.7	55	60	70.29	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA1414	200	CAGTTAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACC	51.4	158	60	70.41	33.3	-2.93	-2.93	3	...
NC_002745.fa	SA1414	200	TAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACCGTAT	51.3	162	60	70.49	31.7	-2.93	-2.93	3	...
NC_002745.fa	SA1414	200	TTAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACCGTA	51.3	161	60	70.55	31.7	-2.93	-2.93	3	...
NC_002745.fa	SA1220	150	GCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATCACT	51.7	103	60	70.24	31.7	-1.61	-2.62	4	...
NC_002745.fa	SA1220	150	TCAGCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATC	51.4	100	60	70.14	31.7	-1.7	-2.62	4	...
NC_002745.fa	SA1608	350	TGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGGTG	57.6	326	60	72.01	36.7	-1.71	-1.71	3	...

...	Antisense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen
...	TTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACAA	44.3	109	60	69.63	28.3	-2.97	-2.97	4
...	TTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACA	44.3	110	60	69.63	28.3	-2.97	-2.97	4
...	TTTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTAC	43.8	111	60	69.2	28.3	-2.97	-2.97	4
...	GTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAATCAT	60.2	60	60	69.79	33.3	-1.44	-2.62	3
...	ATCGTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAAT	60.1	63	60	70.32	33.3	-1.44	-2.62	3
...	TCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATA	59.8	113	60	70	31.7	-2.06	-2.06	3
...	TCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATATAGT	59.8	109	60	70.1	31.7	-2.06	-2.06	3
...	TTCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTGAGTCTTAAT	59.7	114	60	70.29	31.7	-2.06	-2.06	3
...	GGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTAACTG	51.4	217	60	70.41	33.3	-2.93	-2.93	3
...	ATACGGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTA	51.3	221	60	70.49	31.7	-2.93	-2.93	3
...	TACGGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTAA	51.3	220	60	70.55	31.7	-2.93	-2.93	3
...	AGTGATGGTTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAATGGAAATCGC	51.7	162	60	70.24	31.7	-1.61	-2.62	4
...	GATGGTTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAATGGAAATCGCTGA	51.4	159	60	70.14	31.7	-1.7	-2.62	4
...	CACCTGTTGCTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCA	57.6	385	60	72.01	36.7	-1.71	-1.71	3

OliCheck Passed Output Files

OliCheck output file format is derived from the input file. OliCheck simply copies the line corresponding to the probe in the output file. If the *Add Hit Summary to Output* option is set, all input lines are copied but only the Probes that passed OliCheck filters are annotated. If the probe fails at any stage during the process, the annotation is left blank. Alternatively

if the *Add Hit Summary to Output* option is unchecked, the probes that failed at any stage during the OliCheck process are discarded from the Passed output file.

Annotated Passed File

Annotated passed file adds a new column showing the accession name of the corresponding homologous hit.

A truncated example of an OliCheck annotated output file is shown below:

FileName	Accession	Site	Sense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen	…
NC_002745.fa	SA0280	100	TTGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAA	44.3	50	60	69.63	28.3	-2.97	-2.97	4	…
NC_002745.fa	SA0280	100	TGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAA	44.3	51	60	69.63	28.3	-2.97	-2.97	4	…
NC_002745.fa	SA0280	100	GTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAAA	43.8	52	60	69.2	28.3	-2.97	-2.97	4	…
NC_002745.fa	SA0474	50	ATGATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAAC	60.2	1	60	69.79	33.3	-1.44	-2.62	3	…
NC_002745.fa	SA0474	50	ATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAACGAT	60.1	4	60	70.32	33.3	-1.44	-2.62	3	…
NC_002745.fa	SA0668	100	TATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGA	59.8	54	60	70	31.7	-2.06	-2.06	3	…
NC_002745.fa	SA0668	100	ACTATATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGA	59.8	50	60	70.1	31.7	-2.06	-2.06	3	…
NC_002745.fa	SA0668	100	ATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGAA	59.7	55	60	70.29	31.7	-2.06	-2.06	3	…
NC_002745.fa	SA1414	200	CAGTTAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACC	51.4	158	60	70.41	33.3	-2.93	-2.93	3	…
NC_002745.fa	SA1414	200	TAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACCGTAT	51.3	162	60	70.49	31.7	-2.93	-2.93	3	…
NC_002745.fa	SA1414	200	TTAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACCGTA	51.3	161	60	70.55	31.7	-2.93	-2.93	3	…
NC_002745.fa	SA1220	150	GCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATCACT	51.7	103	60	70.24	31.7	-1.61	-2.62	4	…
NC_002745.fa	SA1220	150	TCAGCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATC	51.4	100	60	70.14	31.7	-1.7	-2.62	4	…
NC_002745.fa	SA1608	350	TGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGGTG	57.6	326	60	72.01	36.7	-1.71	-1.71	3	…
NC_002745.fa	SA1608	350	GTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGGT	57.6	325	60	72.01	36.7	-1.71	-1.71	3	…
NC_002745.fa	SA1608	350	CACAAGGCGTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAG	56.3	317	60	72.27	38.3	-1.71	-1.71	3	…
NC_002745.fa	SA1608	350	CGTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGG	56.3	324	60	72.27	38.3	-1.71	-1.71	3	…

…	Antisense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen	
…	TTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACAA	44.3	109	60	69.63	28.3	-2.97	-2.97	4	SA0499
…	TTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACA	44.3	110	60	69.63	28.3	-2.97	-2.97	4	SA1225
…	TTTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTAC	43.8	111	60	69.2	28.3	-2.97	-2.97	4	SA1221
…	GTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAATCAT	60.2	60	60	69.79	33.3	-1.44	-2.62	3	SA1439
…	ATCGTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAAT	60.1	63	60	70.32	33.3	-1.44	-2.62	3	SA1438
…	TCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATA	59.8	113	60	70	31.7	-2.06	-2.06	3	SA1433
…	TCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATATAGT	59.8	109	60	70.1	31.7	-2.06	-2.06	3	SA1427
…	TTCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAAT	59.7	114	60	70.29	31.7	-2.06	-2.06	3	SA1420
…	GGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTAACTG	51.4	217	60	70.41	33.3	-2.93	-2.93	3	
…	ATACGGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTA	51.3	221	60	70.49	31.7	-2.93	-2.93	3	
…	TACGGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTAA	51.3	220	60	70.55	31.7	-2.93	-2.93	3	SA0883
…	AGTGATGGTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAATGGAAATCGC	51.7	162	60	70.24	31.7	-1.61	-2.62	4	SA0871
…	GATGGTTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAATGGAAATCGCTGA	51.4	159	60	70.14	31.7	-1.7	-2.62	4	SA0869
…	CACCTGTTGCTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCA	57.6	385	60	72.01	36.7	-1.71	-1.71	3	
…	ACCTGTTGCTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCAC	57.6	384	60	72.01	36.7	-1.71	-1.71	3	SA0866
…	CTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCACGCCTTGTG	56.3	376	60	72.27	38.3	-1.71	-1.71	3	SA1611

Simple Passed File

Simple passed file removes the probes that failed OliCheck filters from the input file.

A truncated example of an OliCheck passed output file is shown below:

FileName	Accession	Site	Sense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen	...
NC_002745.fa	SA0280	100	TTGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAA	44.3	50	60	69.63	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0280	100	TGTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAA	44.3	51	60	69.63	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0280	100	GTAAAAGAATTGGAAAGCATTAGTCAAAAGTATGATGAAATAGCTCAAAACTTTGGAAA	43.8	52	60	69.2	28.3	-2.97	-2.97	4	...
NC_002745.fa	SA0474	50	ATGATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAAC	60.2	1	60	69.79	33.3	-1.44	-2.62	3	...
NC_002745.fa	SA0474	50	ATTCAAGCATACTTAGGATTAGGTAGTAATATTGGTGATAGAGAAAGCCAGTTAAACGAT	60.1	4	60	70.32	33.3	-1.44	-2.62	3	...
NC_002745.fa	SA0668	100	TATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGA	59.8	54	60	70	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA0668	100	ACTATATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGA	59.8	50	60	70.1	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA0668	100	ATTAAGACTCAAACGAAACTAACAGTTCAAGTTGTTGGTATTGATAATCTGCTGATAGAA	59.7	55	60	70.29	31.7	-2.06	-2.06	3	...
NC_002745.fa	SA1414	200	TTAAGTTAGTAGACAAAGCTGCTCAAAGTAATTTAATACATTCAAACAAAGCTGACCGTA	51.3	161	60	70.55	31.7	-2.93	-2.93	3	...
NC_002745.fa	SA1220	150	GCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATCACT	51.7	103	60	70.24	31.7	-1.61	-2.62	4	...
NC_002745.fa	SA1220	150	TCAGCCGATTTCCATTTTAACAACACTAGGTATATTAATCACATTGCTTTTAGAAACCATC	51.4	100	60	70.14	31.7	-1.7	-2.62	4	...
NC_002745.fa	SA1608	350	GTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGGT	57.6	325	60	72.01	36.7	-1.71	-1.71	3	...
NC_002745.fa	SA1608	350	CACAAGGCGTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAG	56.3	317	60	72.27	38.3	-1.71	-1.71	3	...
NC_002745.fa	SA1608	350	CGTGGATAAAGCATTAGAGTATCGTGATAAAGATAGCGAAGAAGAAATTGAAGCAACAGG	56.3	324	60	72.27	38.3	-1.71	-1.71	3	...

...	Antisense Probe	Rating	SeqPos	Length	Tm	GC%	Hairpin dG	SelfDimer dG	RunLen
...	TTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACAA	44.3	109	60	69.63	28.3	-2.97	-2.97	4
...	TTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTACA	44.3	110	60	69.63	28.3	-2.97	-2.97	4
...	TTTTCCAAAGTTTTGAGCTATTTCATCATACTTTTGACTAATGCTTTCCAATTCTTTTAC	43.8	111	60	69.2	28.3	-2.97	-2.97	4
...	GTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAATCAT	60.2	60	60	69.79	33.3	-1.44	-2.62	3
...	ATCGTTTAACTGGCTTTCTCTATCACCAATATTACTACCTAATCCTAAGTATGCTTGAAT	60.1	63	60	70.32	33.3	-1.44	-2.62	3
...	TCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATA	59.8	113	60	70	31.7	-2.06	-2.06	3
...	TCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAATATAGT	59.8	109	60	70.1	31.7	-2.06	-2.06	3
...	TTCTATCAGCAGATTATCAATACCAACAACTTGAACTGTTAGTTTCGTTTGAGTCTTAAT	59.7	114	60	70.29	31.7	-2.06	-2.06	3
...	TACGGTCAGCTTTGTTTGAATGTATTAAATTACTTTGAGCAGCTTTGTCTACTAACTTAA	51.3	220	60	70.55	31.7	-2.93	-2.93	3
...	AGTGATGGTTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAAATGGAAATCGC	51.7	162	60	70.24	31.7	-1.61	-2.62	4
...	GATGGTTTCTAAAAGCAATGTGATTAATATACCTAGTGTTGTTAAAAATGGAAATCGCTGA	51.4	159	60	70.14	31.7	-1.7	-2.62	4
...	ACCTGTTGCTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCAC	57.6	384	60	72.01	36.7	-1.71	-1.71	3
...	CTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCACGCCTTGTG	56.3	376	60	72.27	38.3	-1.71	-1.71	3
...	CCTGTTGCTTCAATTTCTTCTTCGCTATCTTTATCACGATACTCTAATGCTTTATCCACG	56.3	383	60	72.27	38.3	-1.71	-1.71	3

OliCheck Failed Output Files

The failed output file is a list of accession numbers for which no probes have been found valid according to OliCheck filter parameters. This file is not generated if the *Add Hit Summary to Output* option is checked.

A truncated example of an OliCheck failed output file is shown below:

```
SA1248
SA1245
SA0498
SA0496
SA0494
SA0493
SA1240
SA0489
SA1236
SA1235
SA0487
SA1234
SA0486
SA0485
SA1233
SA0484
SA1231
```