The AiO Version 8 Handbook

by

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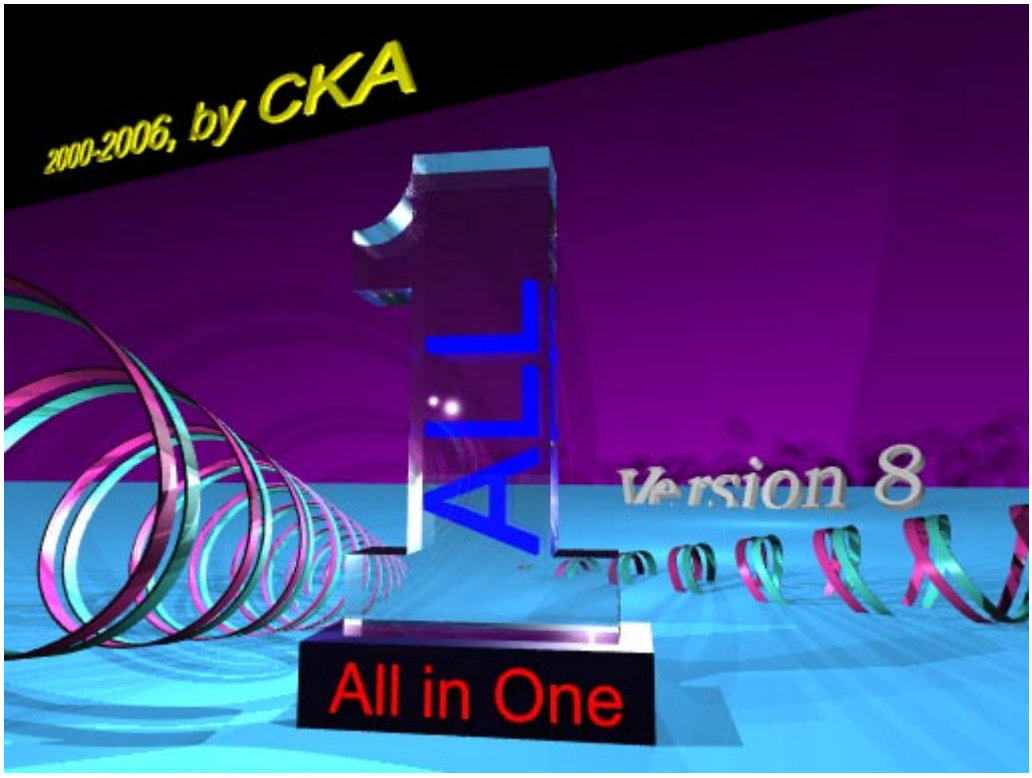
2006



This is the handbook for the AiO (All in One) freeware. For more info see:

Web http://132.187.163.191/aio/
Forum http://132.187.163.191/cgi-bin/E-Guest_show.pl

If you use this software for your scientific publications please cite:

Karreman C., (2002), AiO, combining DNA/protein programs and oligo-management., *Bioinformatics*.,**18**: p 884-885.

General Introduction

Description

AiO is a package that incorporates Database (DB) programs and DNA/protein related programs.
Even though, AiO is a multi-group/multi-user program it is completely capable of running on one computer for one user.

History

A large part of AiO was built around the idea of sharing Oligo related information between the members of the same group. Other parts were programmed to visualize plasmids or to compare sequences. In AiO all these parts have been connected.
With AiO you can design and test oligos, order them on-line, manage them in DBs and subsequently visualize them in DNA programs.
Not only oligo DBs are part of AiO, it also contains other DBs. There is a restriction enzyme DB, updated automatically every month, an oligo-manufacturer DB and a DB for structural DNAs that contains information about genes and how they are to be represented.

Data Bases

A number of other DBs are the result of the fact that AiO is a multi-group/multi-user program. It can handle the information of several groups that all have a large number of members. All group share some DBs - like the restriction enzyme DB - but others like the group's oligo DB are kept separate. Some DBs are even user specific and can only be accessed by one individual.

Some things you should know

-This *handbook* is linked to AiO. The help function will open it in the Adobe Reader. If you press F1 the handbook will be opened on the relevant page. So if you are aligning some DNA files and press F1, the page describing the Align subprogram will be displayed.
- *Shortcuts*: the normal shortcuts linked with the clipboard will work in AiO e.g. Ctrl-C=Copy, Ctrl-X=Cut and Cntrl-V=Paste. As most of the files used will contain DNA the following shortcuts are defined in AiO: Cntrl-D=Copy the reversed sequence, Cntrl-Y=Cut the reversed sequence, Cntrl-W=Paste the reversed sequence, Cntrl-R=Reverse the sequence and Cntrl-B=Capitalize the sequence. These function will work only on *selected* text.
- *Restriction enzymes*: AiO knows all REs, all 3000+ off them. In programs where you have to specify what enzyme to use you can use any of the known enzyme names, AiO will recognize it and use it. In some programs AiO will make a selection, this is necessary, *Xho*I has more than 80 isoschizomers if all would be shown, the resulting graphic would be unusable.
  Several enzyme lists can be used, default is a list (called 'All first & comm') that is compiled according to the following rules: of all enzymes that recognize the same sequence the enzyme that was

described first will be used. If this enzyme is not commercially available then an isoschizomer is used instead. This will be the isoschizomer that is sold by the largest number of firms. If there is no commercial isoschizomer at all than there will be no entry for this recognition sequence.
Next to the default list three other lists can be used. One in which the neoschizomers are also included, and another where the non-commercial enzymes are included. The last list that can be selected is the 'Own list'. It can be defined by the user her/him self. For further info see: 'Programs under Data Bases'.

- *Authorization*: guided by past experience AiO has some DBs that can not be edited by just anyone. The group-oligo DB is such a DB, one or two individuals can have "group manager" privileges, only these people can edit/delete directly in this DB. Group managers also have a number of other privileges/tasks: they have access to the users DB, to the structural DNA DB and to the oligo manufacturer DB. Furthermore, they are allowed to change the group manager.
- *Formats*: AiO will recognize **files** in the following formats: Flat file (pure ASCII= just As, Cs, Gs and Ts), GCG, EMBL and GenBank. If sequence is pasted into AiO other rules apply, see under Editor.
- *Updates*: the restriction enzyme DB and the program itself are automatically updated. The frequency is once a month. For REs the program uses anonymous FTP to the New England Biolabs server to get the newest list of Rich Roberts. For updating itself it uses the HTTP protocol to contact the AiO download site, see also under 'Miscellaneous'.

- *Feedback*: integrated into AiO is a small Email program that can be used to mail a "bugreport" directly to the author. Use it! Use it every time something goes wrong or is behaving funny. **I can not change that I do not know**.

Running AiO

The start-up menu

When AiO is started the program is in full screen mode and the menu will have 8 items (see Figure 1).

***Figure 1**. The menu of AiO at start-up*

AiO makes a difference between programs that use just one DNA/protein file and those that use more then one file. I found out that most of the time you will use more then one program on a sequence. Most of the time you will look for single cutters and search for Open Reading Frames (ORF) and want to have a plasmid map. So the easiest way is to select the file first and then say what programs should run. So first thing a sequence file has to be opened. The opening will result in the loading of the sequence into an editor.

Open
New

The Open command will open an existing file from disk (*.DNA , *.seq or *.pep for formats see below), the new command will open an empty window for typing or copying sequences. Both these commands will open the Editor and the menu will change (see Figure 2).

Edit
Action

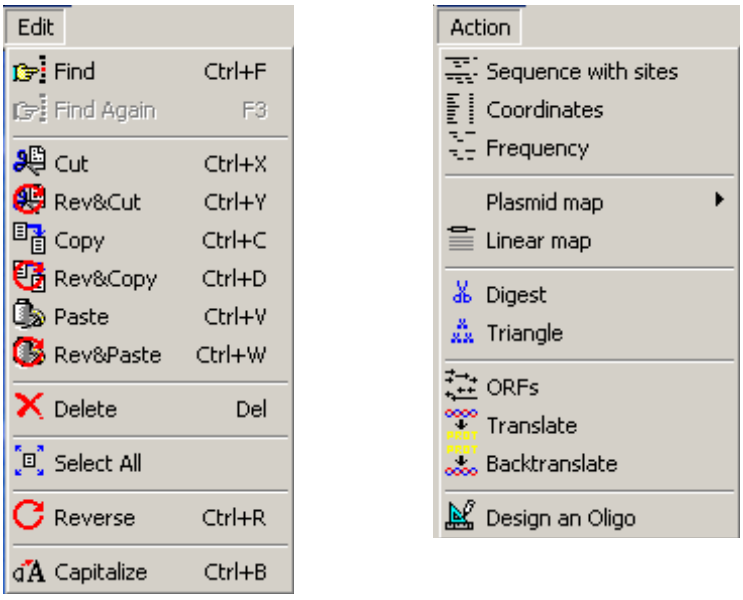


***Figure 2**. The Edit and Action menu items*

Print
Save

It will now sport things like Print and Save options. Two new pull down headers will appear: First, Edit, which covers Copy/Paste/SelectAll and Cut related commands, and secondly the Action item that will start the programs.

The edit options are not just normal text orientated cut/copy/paste

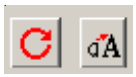
functions but are redefined for DNA. This means that the find function will look in two orientations: looking for ‚AAAA' will also search for ‚TTTT'. Restriction enzyme names that are prefixed with an "#" are also recognized by the search functions (e.g. '*#Eco*RI' is the same as 'GAATTC'). All functions that communicate with the clipboard have a companion that will allow you to use the reversed sequence instead. So if the sequence 'CCCCC' is copied and you put your cursor in another window, pressing Ctrl-W will result in 'GGGGG' being inserted. As can be seen in Figure 2 these functions all have a shortcut key combination. If you prefer you can also activate the various commands with the right mouse button (pop-up menu). The commands listed under Action will be discussed later, first the command buttons of the editor are presented.

***Figure 3a**. The three functional parts of the upper bar of the Editor; for a description see below*

The buttons are arranged into two bars (see Figure 3a and 3b), the topmost has all the buttons for the editorial functions, the bottom bar has the buttons that give the various programs their basic data.

On the Edit Bar all the functions that of the edit menu are also present. Again there are not only the normal functions but also those that work with the reversed sequence.

So there are Cut and CutRev, Copy and CopyRev and of course Paste and PasteRev.



Here is also the possibility to reverse the part of the sequence that is selected and to change the selected part into capitals.

Of course the Find and FindAgain functions are also on this bar

There are some extra functions. The 'undo' and 'redo' functions will reverse your last action.

The coordinates of the highlighted selection in the sequence are copied into the two buttons in the edit bar. By clicking these buttons the begin or end point of the selection can be changed.

The last button on the right has as icon a open book, when the sequence loaded is in a format (GCG, EMBL, GenBank) that has other information besides the sequence this can be viewed by pressing this button.

ASCII, GCG, EMBL, GenBank

This brings us to the different formats AiO can read. AiO will recognize files in the following formats: Flat file (pure ASCII= just As, Cs, Gs and Ts), GCG, EMBL and GenBank.
If you save these files there is a kind of conflict. Say you downloaded pBR322 from EMBL, you change the sequence and save it. Now you have a file with the identical name as the 'official' file from EMBL but with another sequence. For you to keep them apart, a line is added into the 'text' part stating that something was changed.
Checksums of GCG files are updated, so changed files will work with the original GCG software. Files that were created on mainframes use a different EOL code, so there is some problem of reading this kind of files for PC programs. AiO will recognize this and load them anyway.

The editor will show only the pure DNA/amino acid sequences and nothing else. All of the sequence will be in capital letters. Lowercase letters are not 'seen' by the program and can be used to comment the sequences. These comments will not be saved or used after a rebuild.
If you open a new editor window and paste in a sequence the following rules will apply:

Paste Sequences

- In case the text is all uppercase it will be treated as sequence information.
- In case the text is all lowercase it will be changed to uppercase and treated as sequence information.
- In case the text is of mixed case the program will check if it is in a format it supports if so it will load the sequence present.
- It will do this only if nothing was modified in the editor window before. When something has been modified the editor will behave like the normal editor.

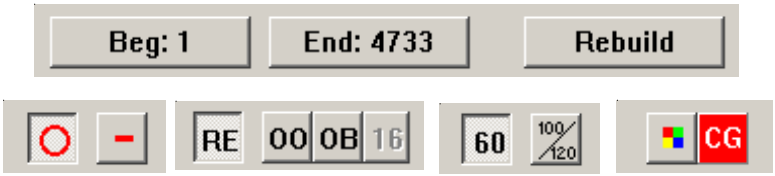


***Figure 3b**. The four functional parts of the under bar of the Editor, for a description see below.*

Program Bar

The lower bar, called Program Bar, **interactively** informs you of the settings that the functions of AiO will need (see Figure 3b). These settings contain the general informations all subprograms will need, some may require a few more.

When a new sequence file is loaded the programs shows you:

- Begin and End show the whole sequence. These can be changed by clicking on them. A window will pop up and ask for the new values. With the changing of these the subsequent programs will use just a part of the loaded sequence.

- Rebuild will rebuild and renumber the sequence shown in the editor after manual editing, it will also update all open windows that use the sequence in the edit window.

- Circular or linear, default is circular.

- With what should the program compare the DNA? Restriction Enzymes (RE), Own Oligos (OO) or the central Oligo Bank (OB). If you choose OB the minimal sequence homology can be set (default is 16 bases).

- Output: 60 or 100 characters. When proteins have to be displayed the choice is 60 or 120 characters.

- The last two buttons are important if you work a lot directly with the editor. Of course, there is the 'Virtual Cloning' subprogram and nothing has to be done directly in the editor but some people prefer it. You can visualize sequences in the editor in three ways.
  - The Find function will show you sequences, one at a time.
  - The Show Structural Sequences option will use the DB that is responsible for the drawing of plasmids and linear maps (see the chapter on programs under Action) and visualize the se quences by colouring the corresponding bases (see Figure 4).

  You can change the coloring by clicking on the correspond

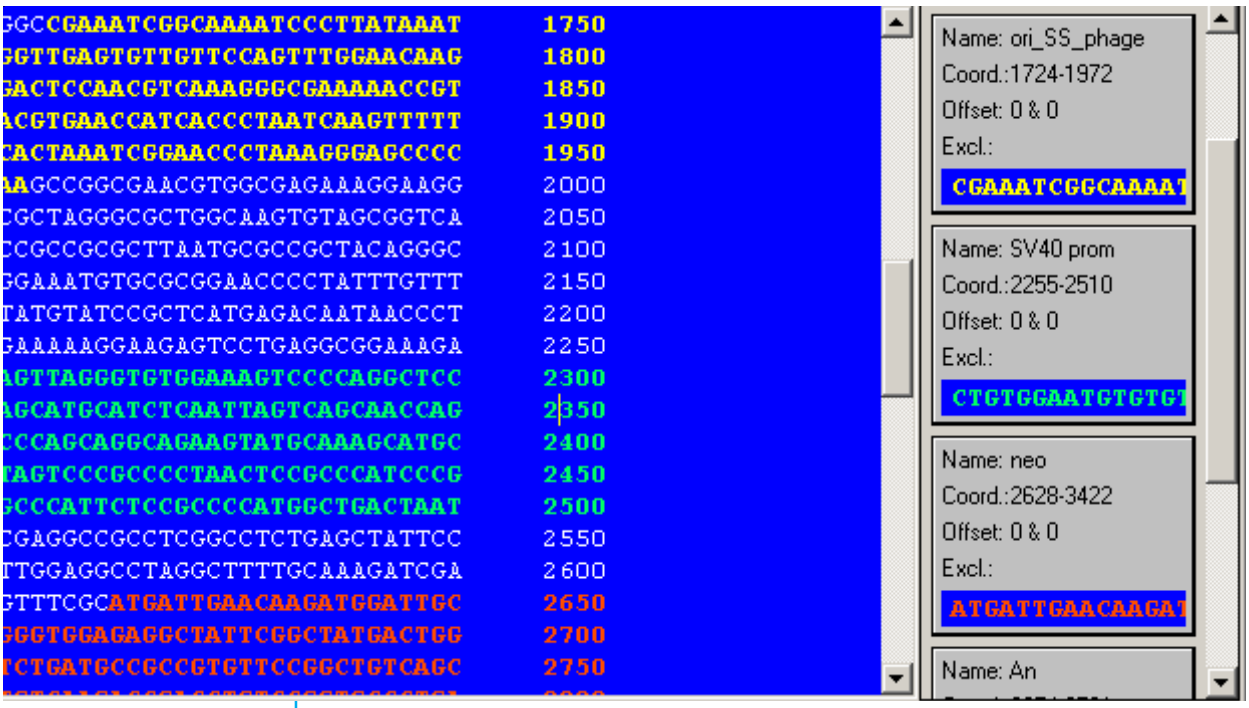


***Figure 4**. Part of the editor window is shown with three different 'structural DNAs in different colors.*

ing panel with the **right** mouse button. Clicking it with the left button will jump to part described in the panel.

- The Highlight function will show you sequences, all at the same time. It will light up all instances of the query sequence in red, or green if it is on the lower strand see Figure 5.

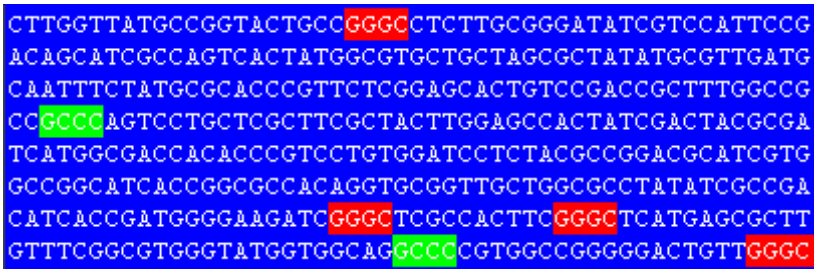
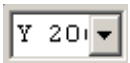
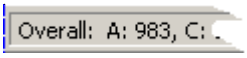


***Figure 5.** Sequence with highlighting, here the query sequence was 'GGGC'.*



Some features of the editor can only be seen under certain conditions like the warning shown here on the left. It tells you that there are some letters in your DNA file that denote degenerate code and how many of these. After the initial flashing warning a pulldowm is shown, listing all these bases. If you click on one of these you will 'jump' to it.



At the very bottom of the editor screen is a summary of your DNA, the file name, how many bases are in the whole sequence and, if applicable, in the selection.

As you can see there is a lot you can find out about your DNA without even leaving the editor.

Action

With the settings on the 'Program Bar' most programs that are under the menu-item 'Action' have enough data to run.

For these programs please read the next Chapter.

You can run each of the programs under 'Action' and they will all use the same sequence (that in the editor). So you can see a listing of all restriction enzymes that cut in one window and see the proteins that are encoded at the same time in another window. If you change the sequence in the editor and press the 'Rebuild' button all windows that use the same sequence as the editor will also be updated.
There is no limit on how many editors you can open other then limits of your computer (Windows has a limit on how many windows it can open).

The programs under Action

General

Here the programs that use just one sequence file are grouped.

By clicking on the menuitem under the ‘Action’ heading a subprogram is started and its output is shown in a new window.

This output has several nice (personal opinion) features. The most obvious one is probably; ‘direct-information’ see Figure 6.

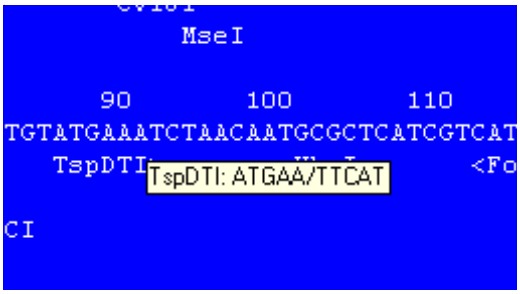
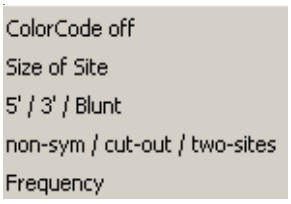


Figure 6. *Info at your ~~finger~~ mouse tip. As your pointer moves over the text information is shown for the restriction enzymes and oligos. The small colored box can be clicked. The left button will show information about the enzyme or oligo. In case the info is about a restriction enzyme you can also click it with the right mouse button. Then the digestion of the DNA with that enzyme is shown.*

Edit

For those programs where it is appropriate, the output-window has its own Menu-item called ‘Edit’. These are not really to edit these outputs. As a matter of fact they are read-only, but under this heading are functions like Cut/Copy/Paste, Find and ‘ColorCode’.



This latter allows you to overlay the output with another layer of info. You can select size of sites, for the ends generated (5'/3' or blunt), for the type of the enzyme and even for the total number of cuts, see Figure 7 for an example.

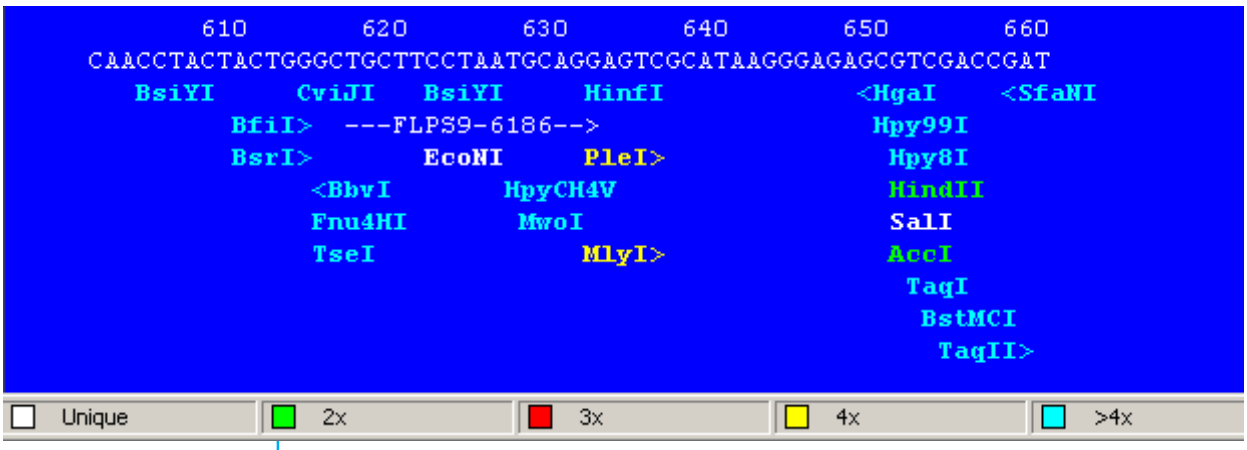


***Figure 7**. Shown is part of a output screen of the 'Sequence with Sites' function. The names of the various restriction enzymes are colored according to how often they will cut the DNA.*

Find

The 'find' of the output windows can have more options then the normal 'find' of the editor, see Figure 8 for an example.

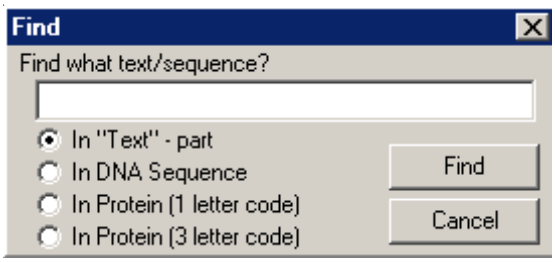


***Figure 8**. The 'Find' box of the program Backtranslate offers you four different possibilities to search. Text is literal, the other options are on sequence level and will find degenerated bases and sequences overspanning the linebreaks.*

The various Programs

Sequence with sites

Sequence with sites.

A typical example of the output can be seen in figure 7 (the default is without the coloring). As you can see, if one of the oligo DBs is selected the oligos are drawn in just like the restriction enzymes. Under the 'file' menu now a full series of Print/Save options for this output is shown. Just like for any of the other output windows under 'Action'.

Coordinates

Coordinates.

This function just gives you a list of all the RE/OO/OB that you selected and where they cut/hybridize.

PCR table

An extra feature is a table that lists all the PCR product with the oligos. The latter is only generated when OO or OB is selected without the RE (see Figure 8).

Table of all possible PCR products on circular molecule:

	<MPSVT7DEL	<ELSE-1605	<TET3-5645	<AMP1-3462	<DKL3-1023	<KASGEG	<R2NDE1
CLANHE1	481	1250	1647	2134	2248	2277	2288
TET5-5644>	489	1258	1655	2142	2256	2285	2296
FLPS9	4240	648	1045	1532	1646	1675	1686
GA0437>	3682	90	487	974	1088	1117	1128
DKL4-1025>	2592	3361	3758	4245	4359	27	38
KASSGEG	2592	3361	3758	4245	4359	0	38
NRU-NEW-53	2405	3174	3571	4058	4172	4201	4212
NRU-OLD-53	2358	3127	3524	4011	4125	4154	4165
TAN2-5235>	1950	2719	3116	3603	3717	3746	3757
NEOGAL1	1543	2312	2709	3196	3310	3339	3350
AMP2-3463>	1444	2213	2610	3097	3211	3240	3251
KB0155>	1382	2151	2548	3035	3149	3178	3189
AMPMS1	1258	2027	2424	2911	3025	3054	3065
SCMSEQ	576	1345	1742	2229	2343	2372	2383
SEQTKIF	529	1298	1695	2182	2296	2325	2336
WW0349>	545	1314	1711	2198	2312	2341	2352

Figure 8. *Next to a simple list of coordinates a table can be produced showing all possible PCR products and their length. This is only shown when either the Own oligos or Group oligos were selected* ***without*** *the restriction enzymes (see page 6: RE/OO/OB).*

Frequency

Frequency.

This program also lists all RE/OO/OB sites. However this time sorted on how often they cut/hybridize. Starting with non -cutters and listing up to the number of that enzymes that cut the most times.

Plasmid map

Plasmid map.

This is probably the most popular of all functions in AiO. This program will draw high quality maps of your plasmids. Since it uses a DB to get all its information you don't have to do anything more than just click the button. Only if you start to use some new gene you will

have to add this to the DB (only once). After that it will be recognized automatically and be drawn in all new Plasmidmaps. For how to edit the 'Structural DNA' DB see the chapter on 'Programs under Data Bases'

Plasmid map comes in two flavours: Standard and Custom.

Standard plasmidmap

Standard: This function will draw print quality plasmid maps. It uses the RE/OO/OB data that has been activated, and the structural DNA DataBase (DB). This DB contains information about how a gene or other DNA segment is to be drawn.
With this program you can draw high quality maps very easily, just one click. There is not much to choose from: print it or view it.
In the standard map only those REs that cut only once or twice will be drawn. An example with oligos and restriction enzymes is shown in Figure 9.

Custom plasmidmap

Custom: Allows you to select REs and oligos that you want to have on your plasmid map. In the resulting map REs that cut only once will be in **bold**, those that cut twice will have the normal font and those that cut more than two times will be in gray.

Linmap

Linmap.

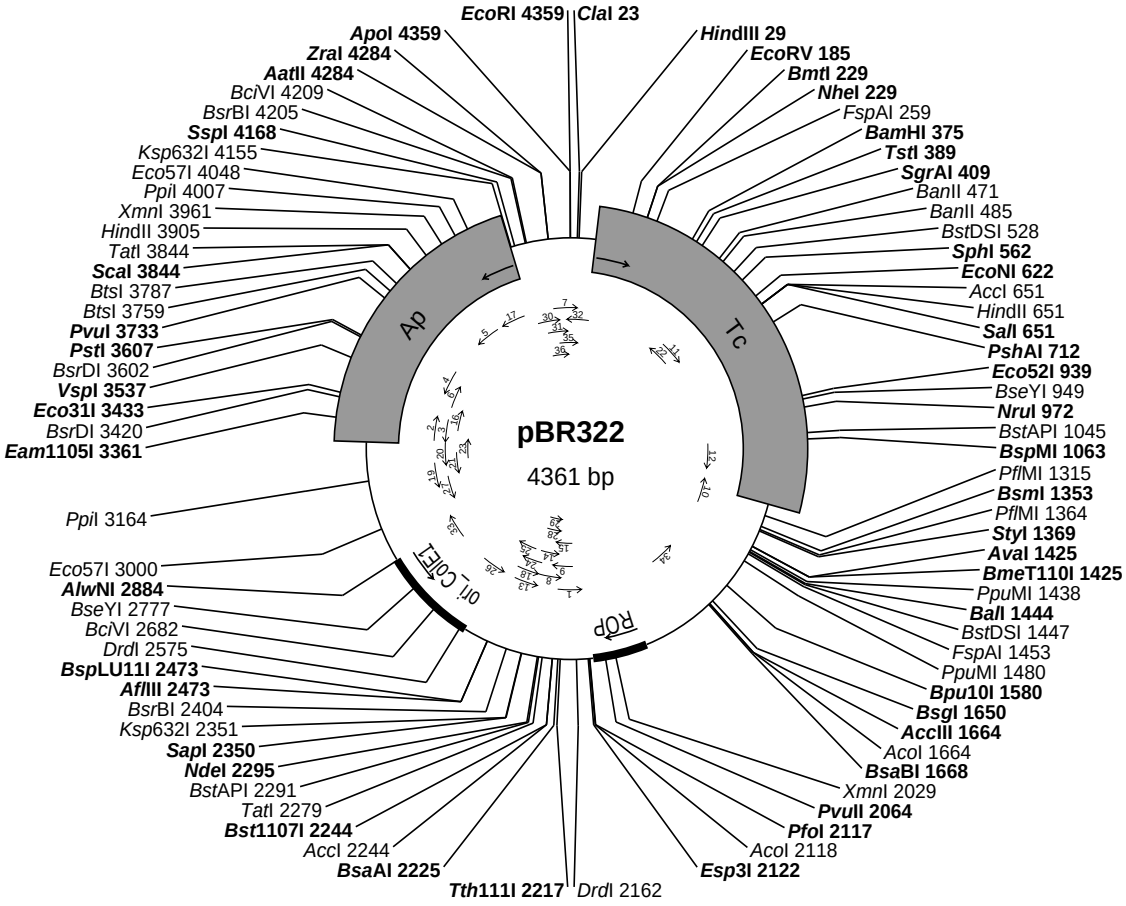
Uses the same DBs as plasmid map to draw linear maps.
If you selected for restriction enzymes this program will ask you for a selection based upon the times the enzymes should cut. So you can select enzymes that cut between 4 and 7 (including 4 , including 7) times. It generates multi pages output with the restriction enzymes first, than one page with those enzymes that do not fall within your selection and finally the pages for the oligos. The size of the restriction fragments is written above the corresponding stretches in the drawing. For the oligos the 5' to 3' direction is indicated by an arrow. On the right side the number of cuts/hybridizations is given.
In Figure 10 on the next page an example of the Linmap is shown.

***Figure 9**. PlasmidMap with restriction enzymes and oligos. Only those enzymes that cut once (**bold**) or twice are shown. Oligos are sorted alphabetically at the bottom of the picture.*

ALL

Program AiO; 06/04/2006 17:15:06 hr.

DNA file: C:\DNA\vectors\Pbr322.dna from 1 to 4361
Using: enzymes that cut 1 or 2 times
GenTher oligos, cut to 16 bases



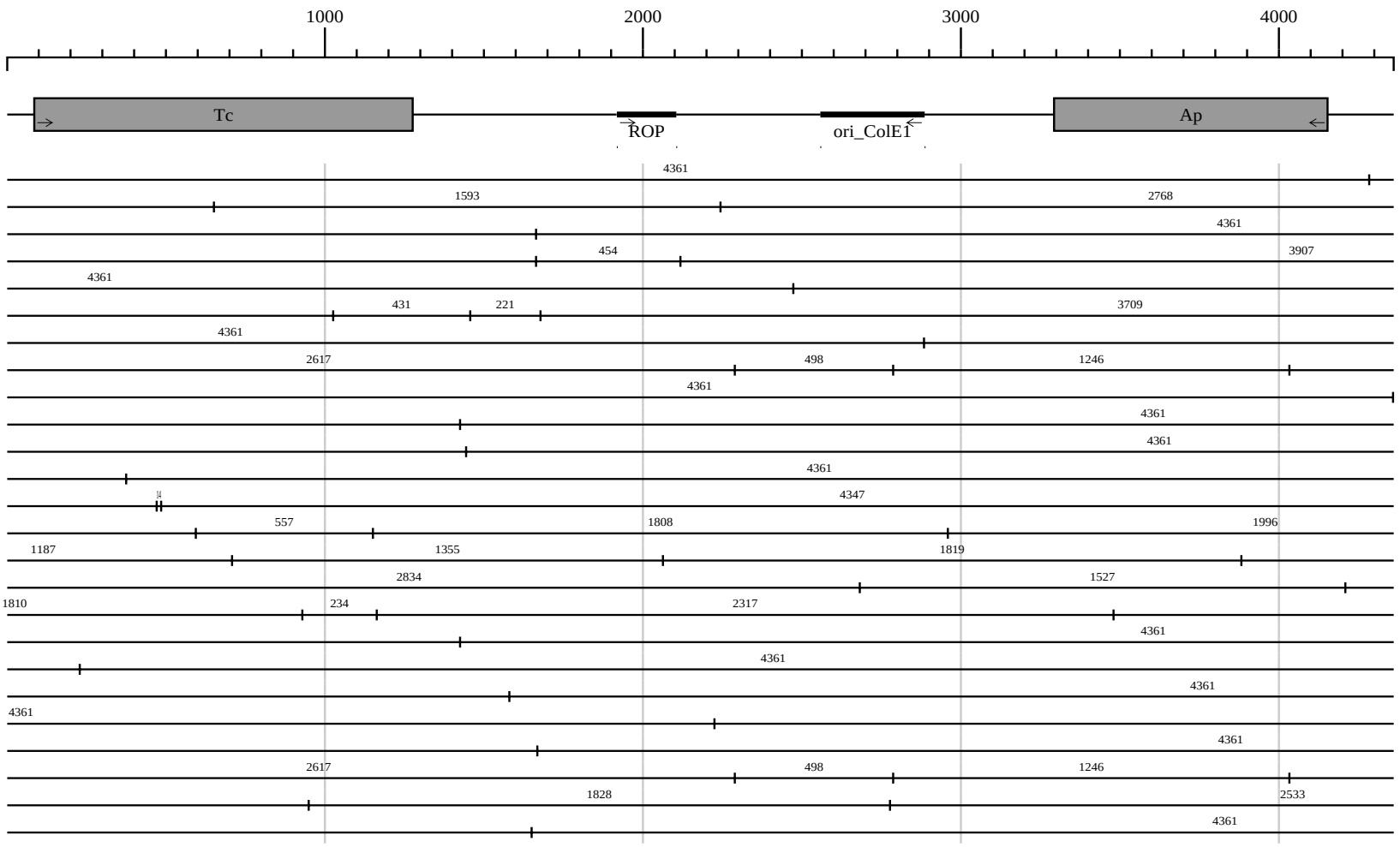
Codes of oligos:

1:	AMP1-3462	2:	AMP2-3463	3:	AMP3-5688	4:	AMP4-5689	5:	AMP5-5690	6:	AMPMS1-4243	7:	CLANHE1-6501	8:	DKL3-1023
9:	DKL4-1025	10:	ELSE-1605	11:	FLPS9-6186	12:	GA0437	13:	HYG2-3131	14:	KASGEG-6597	15:	KASSGEG-6561	16:	KB0155
17:	KB0156	18:	MCSSEQ-60597-001	19:	MFE-OLD-53748-003	20:	MFEPLC-53748-005	21:	MFEPLL-53748-004	22:	MPSVT7DELTACLAI-WW02	23:	NEOGAL1-5850	24:	NRU-NEW-53748-002
25:	NRU-OLD-53748-001	26:	ORI1-5686	27:	ORI2-5687	28:	R2NDE1-5209	29:	R2NOTI-5745	30:	SCMSEQ-267783	31:	SEQTKIF-1234	32:	TAN1-5234
33:	TAN2-5235	34:	TET3-5645	35:	TET5-5644	36:	WW0349								

Program AiO; 07/04/2006 08:18:17 hr.

DNA file: C:\DNA\vectors\Pbr322.dna from 1 to 4361
Using: enzymes that cut between 1 and 3 times

estriction enzymes



Digest

Digest.

This function calculates the position of RE cuts and the size of the generated fragments.
It is a 'command line' like program. This means you will have to type in the name of the restriction enzyme. I have tried it with mouse control, it's terrible. There are just too many enzymes. You are much, much faster if you just type in the name. Digest recognizes **all** enzyme names. So you can type in *Ava*I or *Sin*I, it doesn't matter all isoschizomers are recognized. As you just saw AiO uses the 'real names' *Sin*I and not *Sin*1.
Output is a listing of all cut positions and fragments generated. Underneath is a listing of the fragments sorted by size.

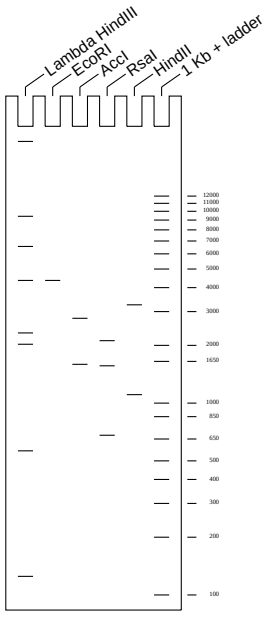
Multiple digests

Multiple enzymes digests are no problem. You can type in all the names separated by a space.

Partial digests

You can also calculate partial digests. Just put an "*" behind the RE name and all possible partial cuts will be calculated. If more than one enzyme is used you can have them all cut partially or just a subset.

Virtual Gel



There is another very handy feature in Digest: the virtual gel. This is a diagram of the gel as it would look with the selected digests (figure 11).
You can view this picture even without printing if you press the preview button that is located in the lower right hand corner.
The "hardcopy" of the output has this picture and the listing of all cut positions and fragments.

***Figure 11**. Virtual gel. Shown is the predicted gel of the plasmid pBR322 cut with various enzymes.*

***Figure 10**. LinMap with restriction enzymes that cut up to three times. Only the first page of the output is shown.*

Triangle

Triangle.

Triangle is an automatic variant of Digest; it will generate all double cuts. i.e. the input “EcoRI AccI RsaI HindII” will generate the following digests: EcoRI, EcoRI + AccI, AccI, AccI + RsaI, RsaI + HindII, HindII. Again, as in Digest, a predicted gel can be printed and viewed, see Figure 12.

This feature was programmed for plasmid mapping. The generated combination of enzyme digests allow easy determination of relative site positions.

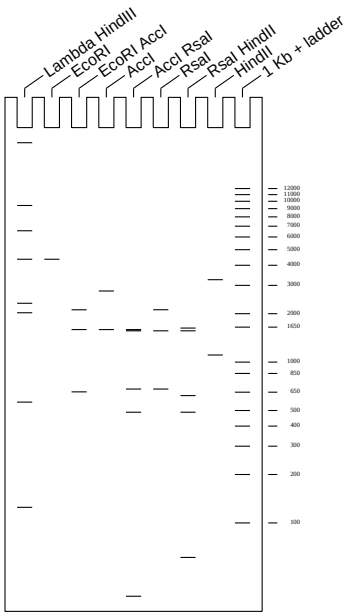


***Figure 12**. Virtual gel. Shown is the predicted gel of the plasmid pBR322 cut with various enzymes and their combinations.*

ORF

ORF.

The **O**pen **R**eading **F**rame finder (see figure 13). The default of this program shows all ORFs that are greater than 300 bases and it draws all ATGs.

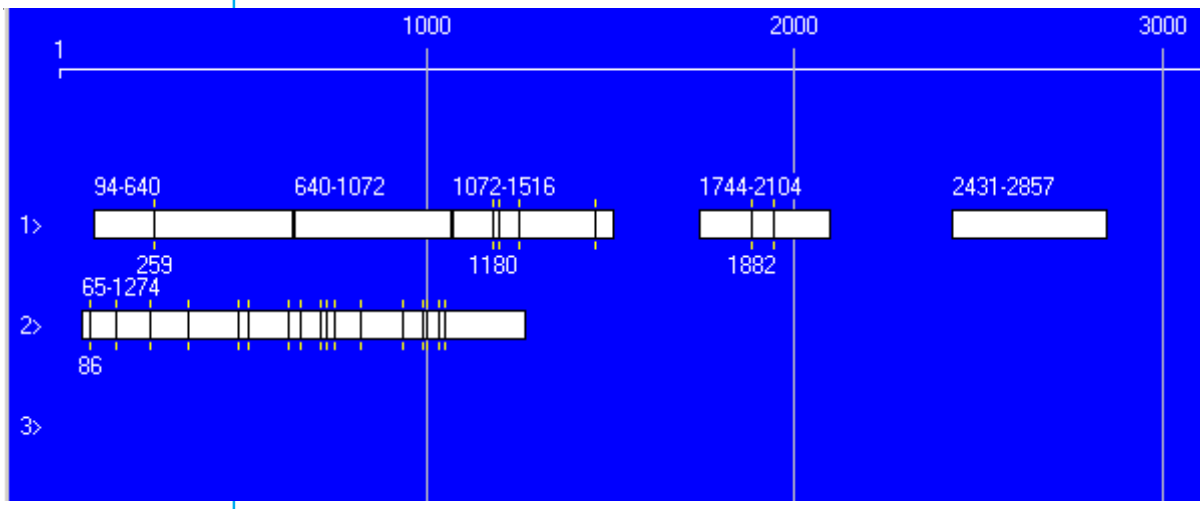
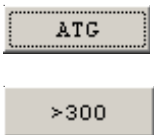


***Figure 13.** Part of the output of the ORF program. In this small part only the upper strands are shown. The coordinates of the ORFs are shown above the white boxes that represent them. All ATGs are drawn as black, vertical lines. The coordinate of the first ATG is given beneath.*

The ORFs are represented by open white boxes with the coordinates of the ORF above. The start codons are shown as black, vertical bars. The coordinate of the first start codon is printed below the ORF.



This program can use extra input. There will be two buttons visible on the right hand site of the window, if you click the button labelled "ATG" you can enter any three letter combination that should be treated as start codon. The minimum length of the ORFs is also changeable via the second button.

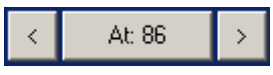
Translate

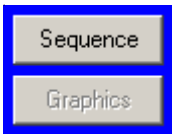
Translate.

The program that translates DNA into protein. The start window of translate is very similar to that of ORF. There are some visible differences; on the left side there six numbered boxes (representing one of the six possible frames of the DNA) and on the right there are two extra buttons.

However, the big difference in this window is the fact that the ORFs can be activated by clicking. When clicked, a ORF turns red. It is also possible to click upon one of the small, numbered boxes, these also turn red. When a ORF is clicked the statistical information of its translation is shown in the dark box on the bottom. When a second ORF is clicked the first ORF turns pink and the newly clicked ORF's information is put in the dark box. Here are those things like: composition of predicted protein, whether this ORF was translated from Stop to stop or from a ATG, the total number of amino acids and the calculated values of the pI and Molecular Weight.
The small numbered boxes just stay red when something else is clicked, Both can be changed back to white by clicking upon them with the **right** mouse button.

As you probably guessed (or tried) the translation is directed by the exact location of your click. You can translate just part of the ORF. The starting point is the next start codon upstream. A problem could be that two -or more- start codons are so close together that exact clicking is impossible, for that reason the control box was included, by clicking on the arrows you can the select the next/previous start codon. The option to save the protein is self-explaining.
Just something about the name giving of the proteins; default is the name of the DNA with the coordinates of the clicked ORF attached. You can change this at the saving stage.





When you click on the "Sequence" button, the sequence of the DNA is shown with all the information you expect; restriction enzymes and oligos - if these were activated in the editor, see Figure 14. The ORFs you activated, both the red and pink ORFs, will be translated and printed above the DNA sequence. If you activated one of the

Figure 14. *Output of the Tc-ORF of pBR322. The DNA, and Protein sequences are shown, as is the number of the translated frame (2) and the restriction enzymes that cut in this stretch of DNA. The REs are colored on their cut-property (5'/3'/blunt).*

small boxes on the left, that whole frame will be included. If you are not satisfied with the result, you can switch back to graphics mode by clicking the so-labelled button.

Backtranslate

Backtranslate.

This program answers the question: "What changes - i.e. introduction of restriction enzyme sites - can I make in my DNA without changing the encoded protein?"

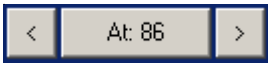
It can also answers questions like:
"What is the optimal codon usage?"

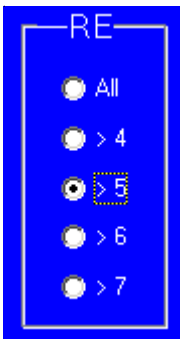
?

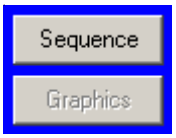
and

"What is the DNA sequence that encodes this protein?".

The latter only when a protein file is loaded :).







The introductionary window and the control elements are very much like those of Translate, a difference is the fact that only one feature (ORF or frame) is activatable. Again for difficult cases there is the possibility to use the controlbox with the two arrows.
When the RE option was chosen in the editor there is a group of radio buttons on the right. With this feature you can choose between RE's that recognize a minimum length of DNA. This might be necessary, the possible number of sites can be enormous and most of the time you don't want to introduce an enzyme site that is there 15 times already. So you just want to see the '6-base and up' enzyme that can be introduced.
To see the DNA with the protein and possible even REs and oligos aligned click on the 'Sequence' button.

For a part of a typical output see Figure 15.

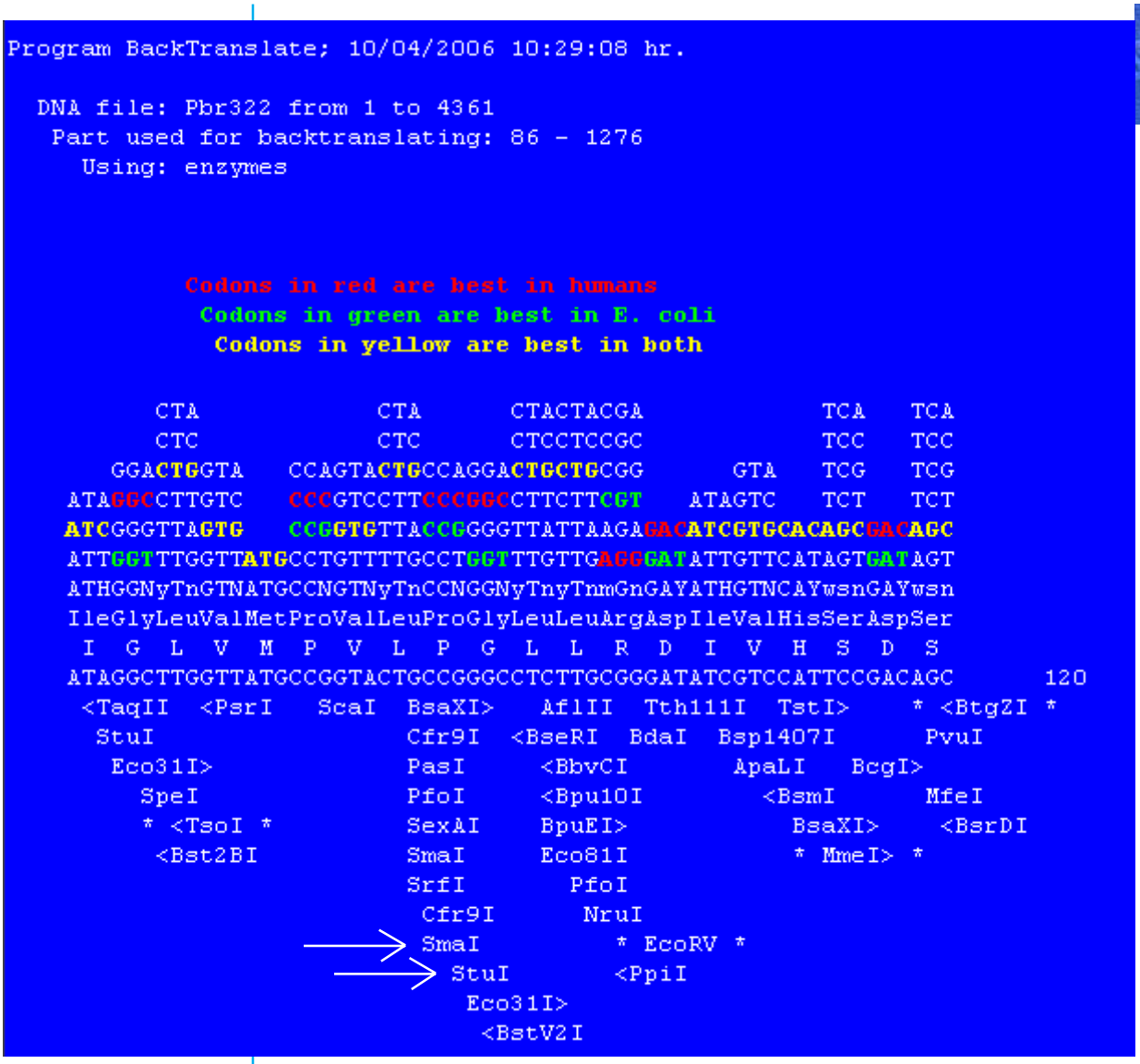


Figure 15. *Output of Backtranslate. All possible codons for the amino acids are given, and based on these the consensus DNA sequence is calculated. The codons that are optimal for humans or for E. coli is colour encoded. In the figure REs were selected (>5), since the original DNA sequences has a sites for Tsol, Mmel, BtgZI and EcoRV these sites are marked by '*'s. All the other sites can be created, albeit that a lot of these site are mutually exclusive: when the SmaI site at position 24 is created, the StuI site at position 26 can not be created and vice versa.*

Explanation of the various lines (third column of Figure 15).

```
CTA          ( 1)- First 6 lines give the possible codons
CTC          ( 2)-
CTG          ( 3)-
CTT          ( 4)-
TTA          ( 5)-
TTG          ( 6)-
yTn          ( 7)- Consensus DNA
Leu          ( 8)- Three letter Amino Acid code
 L           ( 9)- One letter Amino Acid code
TTG          (10)- Original DNA sequence
```

Oligo Design

There are two subprograms here to design oligos manually or fully automatic.

Manual

Manual

When the OligoDesign window opens it is in the “Manual” mode. All information is depicted in a very compact way so let’s see what is where.

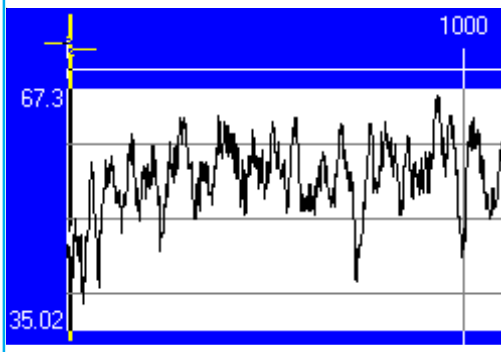


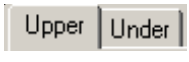
***Figure 16**. Meltingcurve graph.*

At the top is a graphic depicting the melting temperature (Tm) of all possible oligos of a specific length. When the program starts this length defaults to 20 bp. The maximum and minimum Tm is written on the left. The Tm is calculated for every oligo i.e. the oligo corresponding to bases 1-20 of the DNA then the oligo 2-21, 3-22 etc.
Notice the two arrow in the top left hand corner they symbolize the oligos you are designing.
The DNA sequence is right under the Tm graph and it shows both the upper and under strand. As you can see in Figure 17 it has no trouble with circular molecules.

***Figure 17**. The sequence.*

Directly under the sequence is a collection of controls. Here you can jump or step to another coordinate of the sequence, select the way the Tm’s are calculated, determine the endconcentration of salt and oligo(this influences the Tm) and the length of the oligos.

***Figure 18.** The controls*



Under these controls is the selector for the strand you want to design: Upper or Under and an editline for showing the selected sequence.

At the botton are a number of calculated graphs and values. On the left the predicted 2D structure of the best possible hairloop and next to it the best possible hybrid of two of the upper oligo, see Figure 19.

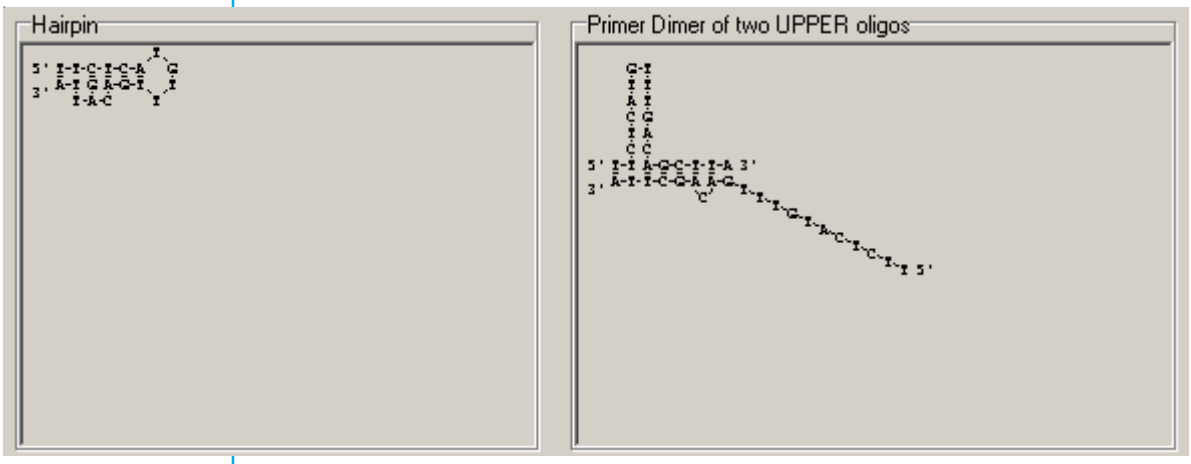


***Figure 19**. The predicted 2D structures.*

For most applications the oligos should have no stable 2D structure so AiO calculates the Tm of every structure and if it is close to the Tm of the oligo/DNA hybrid the pictures of Figure 19 will turn orange or even red.
The last of the info about the oligo you can find at the bottom on the right. The panel is called statistics and gives you all the data

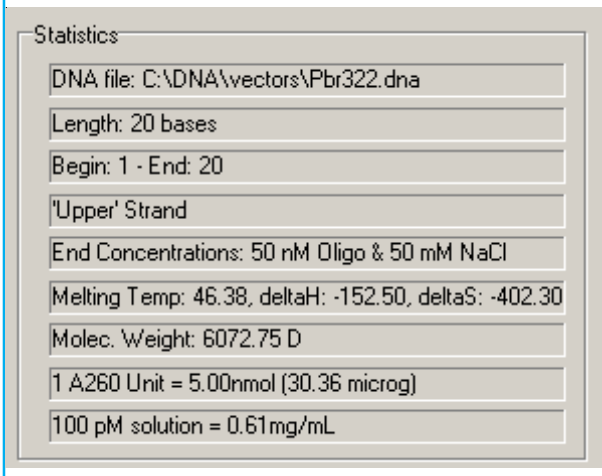


***Figure 20**. The statistics panel*

So after these panels there are only some buttons left in the window (Figure 21):

***Figure 21**. The buttons*

HowTo

How do you work with the manual mode?

The manual mode has been programmed so you can see what is happening and be master of the process of oligodesign. It is good if you want to design oligos at a certain position (say a start or stop codon), you want to modify your oligos or want to play with the conditions of the calculations of the Tm.

1 or 2 oligos

You can design just one or a pair (PCR) of oligos with manual mode. As there are so many option the easiest way to explain everything is just to describe how to design a pair of oligos. Consider that what is to come as a tutorial :)

The Oligo Design Tutorial

First some data to begin:

You want to:	design a pair of Oligos use them for PCR to get the ORF of Tc from pBR322 incorporate extra RE sites
You want to check:	the Tm's are not to far apart make sure they don't stick to anything else print all the data put them permanently in the DB of AiO order them on-line

Beginning

So:
- start AiO
- click on 'File' and 'Open' the file for the plasmid pBR322
- click on 'Action' and select 'Design an Oligo'.

You see the window as described previously. Now the ORF of Tc is from 86/88 for ATG to 1274/1276 for TGA (check it with the ORF program).

- jump to the beginning of the Tc gene (three times on the double arrow and you're almost there). Click a few times more on the single arrow and you have an oligo from 86 to 105. You can also type in the number 86 and you will be there in one go.

Now that is a nice oligo, Tm of 49^{O} C, length of 20. Don't worry we will get the Tm up a bit. But here is a nice change to get into how the Tm is determined. AiO knows three ways to calculate it:

- *Nearest Neighbour: the method of choice, a method that uses thermodynamic formulas to calculate the Tm. Part of these formulas are: the various end-concentra tions of salts and the oligo itself, the exact sequence and if it is hybridizing to other oligos, DNA or itself.*
- *A/T and C/G: classic rule of the thumb method. works OK for oligos around 20 bases. Simply takes 4^{O} C for*

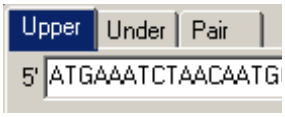
every C or G and 2^{O} C for every A or T.

- *Wetmur: takes the percentage of C/G bases of the oligos and calculates the TM with an emperic formula. There is a correction for the end-concentration of salts.*

- Make the length of the oligo 25 bases, use the arrow up next to the length or type in '25'.

So the Tm is now about 56^{O} C, that suits me fine.

- Select this oligo as 'Upper' by clicking the 'Make Upper' button.



The sequence of the oligo appears now in the editline, the tabsheet for 'Upper' turns blue and another tabsheet, 'Pair' becomes visible. Now an extra site.

- Click in the editline, in front of the ATG and type: 'GGAATTCC'.

The sequence appears in red, as this sequence is not directly from the DNA file. As you can see the Tm is now 63^{O} C (but remember not in the first round!). So the first, upper, oligo is now ready, now for the under oligo. You can do that by just clicking on the tabsheet that is labelled 'Under' and repeat the process, but we will do it differently.

- Click on the tabsheet labeled: 'Pair'.

Hey!, that 'Make'-button just changed. This is the combination tabsheet. On the bottom are infos that show how well the oligos go together. In the middle is a graphic display that shows the already familiar hairpin and intra-molecular hybridization of the 'Under' oligo. New is the third panel showing the best posible structure of the Upper and Under oligos. As the two overlap at the moment the last will be in red.

- Go to position 1257 and change the length to 20 bases. That should be no problem for you, if it is; see how we got to position 86, on the previous page.

OK, Tm = 59.3^{O} C and the rest looks good. If you want more info click on the 'Under' tabsheet, it will show you all about the oligo. Time for another extra site.

- Click on 'Make Under'
- Go to the 'Under' tabsheet, it will become the second blue one.
- Go to the editline and add (at the 5' end!, **all oligos are always depicted 5' - 3'**) 'AATTAAT'.

Good, now we have got two oligos with extra sites (EcoRI and VspI), they have Tm's not to far apart and exactly where

we wanted them. So let's check if they are only sticking to our DNA and not to anything else.

- Click on the 'Pair' tabsheet.
- Click on 'Blast them'

Blast

What you will see is somehing like Figure 22. The upper panel is info, under it you can select against what you want to runyour oligos against.

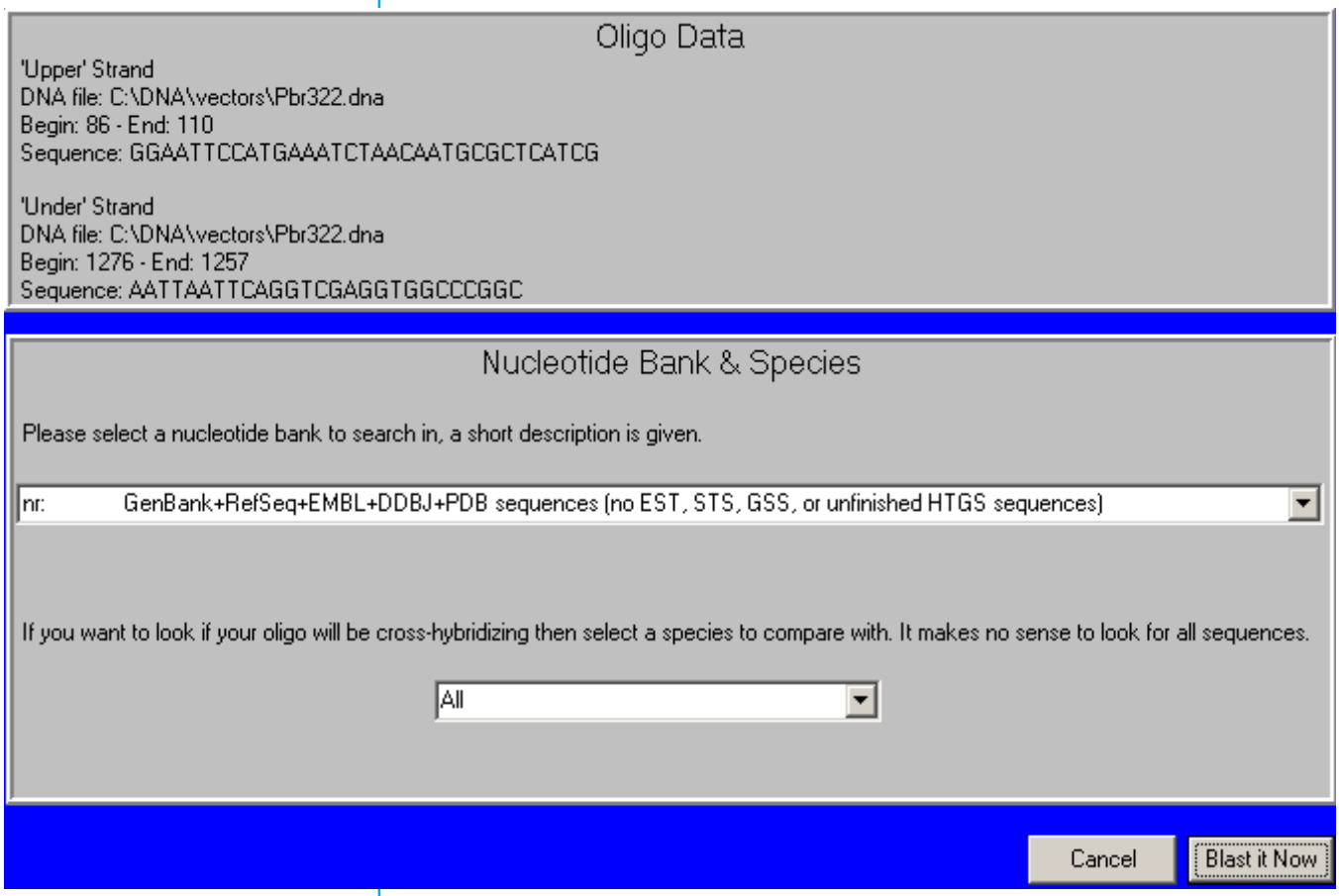


***Figure 22**. The Blast window.*

There are two dropdown boxes here; the upper allows you to select all the bank or exclude est's ect. The bottom dropdown box to select a species.

- Select *E. coli* in the bottom dropdown box
- Click on 'Blast it Now'

So now some small windows will appear in the taskbar (at the very bottom of your screen) that will count down untill the results are shown in new windows, see Figure 23.

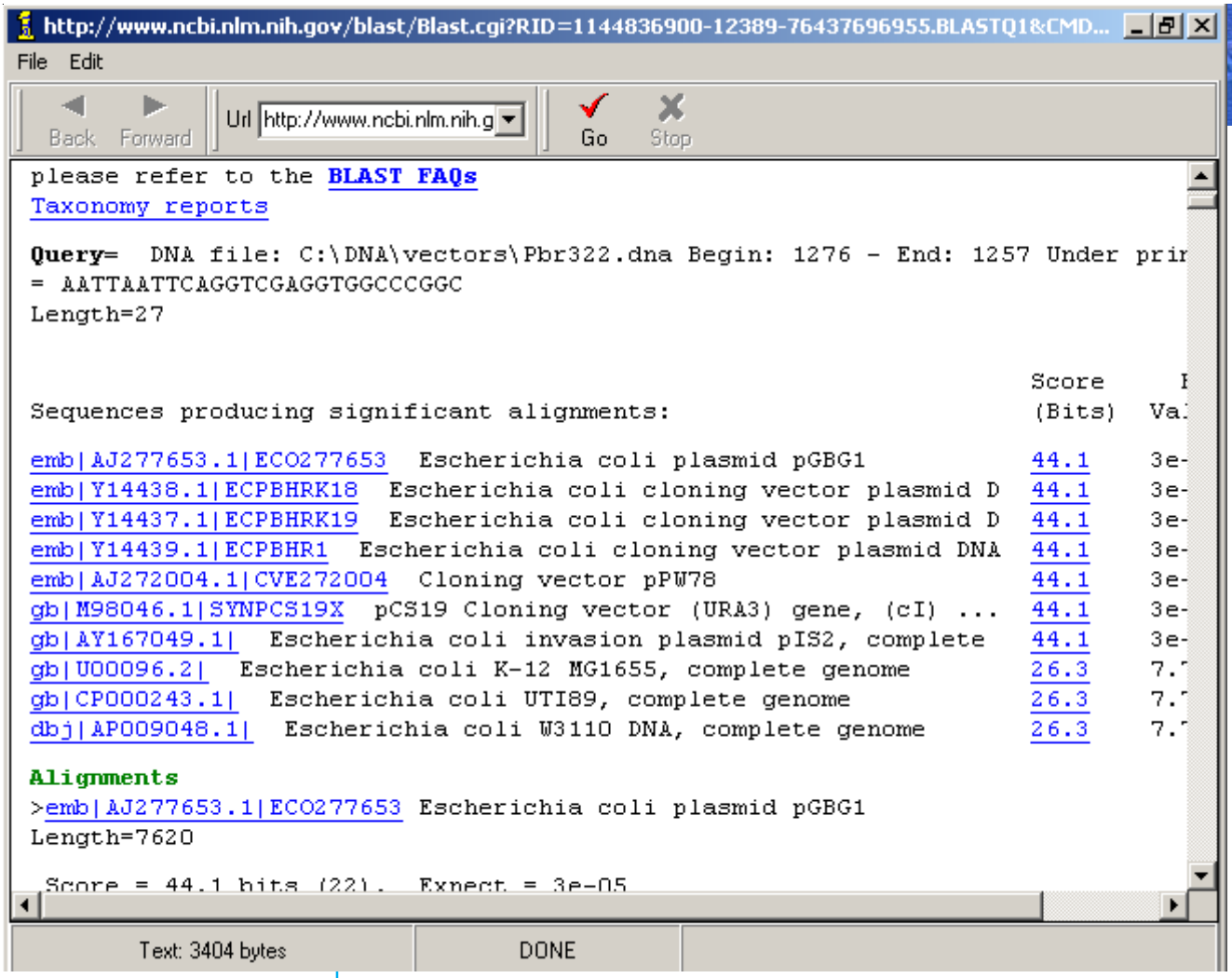


***Figure 23**. The blast results*

This looks good, only plasmids with the TC gene are found. So we can go on with our oligos.

- Click on 'Print all Data'

- Click on the 'Create Both' button

Now we are really almost there, everything we wanted has been done: the last click opens the 'New Oligo' window. This is described on page 48. After that you can also order them on-line with the 'Send Oligo Order to Manufactorer' function described on page 49.

There are only a few things that have not been described so far:

These two small buttons. You'll find them in the middle of both the the Upper and Under tabsheets. These allow you to type in/paste an oligo from another source and to clear the editline.

Automatic

Automatic

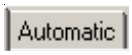
You can change from manual to automatic mode by clicking the corresponding tab.
When you do this there will be a lot of things that will now be rather familiar: the 2D panels and the dropdown box with the different method to calculate the Tm for instance, if not see pages 21 and 22.
To cut the whole process short: let's have another tutorial.

Tutorial

First some data to begin:

You want to:	design a pair of Oligos use them for RT-PCR to get the mRNA of the Tc gene generate a fragment of approx. 200 bp
You want to check:	the Tm's are not to far apart make sure they don't stick to anything else print all the data put them permanently in the DB of AiO order them on-line

So:
- start AiO
- click on 'File' and 'Open' the file for the plasmid pBR322
- click on 'Action' and select 'Design an Oligo'.



- Click on 'Automatic

Most of this looks very familiar, but let's concentrate on the Parameter panel, see Figure 24.

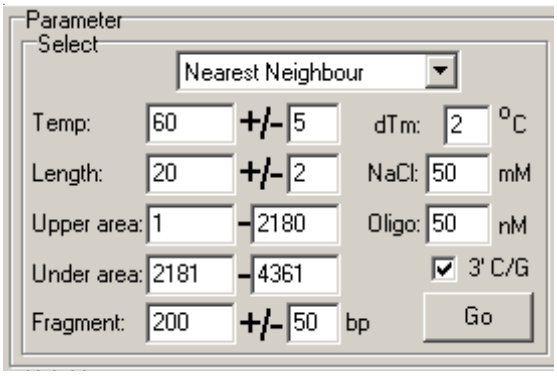
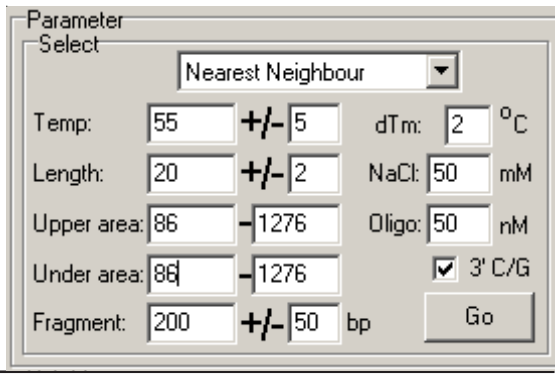


***Figure 24**. The parameter Panel*

- Change the settings to:



So what did you just do?

You asked AiO to select oligos that:

- *use the 'Nearest Neighbour' method to calculate the Tm*
- *have a Tm near 55^{O} C, and not higher than 60^{O} C and not lower than 50^{O} C.*
- *have an length between 18 and 22 with an optimum at 20 bases.*
- *that are both selected in the area of the ORF of the Tc gene (86-1276).*
- *generate a fragment with an optimal size of 200 bp and are not longer than 250 bp and not shorter than 150 bp*
- *the Tm's of the two oligos should not differ more than 2 ^{O} C*
- *the Tm is calculated with salt at an endconcentration of 50 mM and the oligo of 50nM*
- *the oligos should end (3') with a C or a G*

- Click on 'Go'

Well, that's about all. AiO will list the best 128 oligo pairs in the yellow listbox in the right hand bottom corner, see Figure 25. The actual valuwes of the pair are shown above it. The 2D structures are drawn. And again you can print them, compare them to the nucleotide bank (blast) and can enter them into your personal DB (Create). Nice detail; if you now go to manuel-mode all data from the selected oligos is also on the various panels.

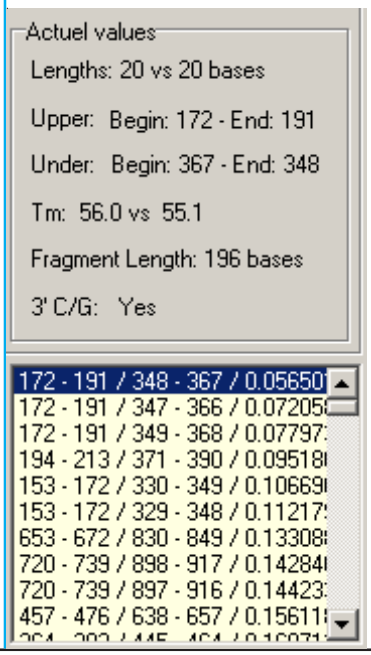


***Figure 25**. The best 128 oligo pairs and the data of the selected pair.*

The programs under FOF

General

General

Under “FOF” (file of files) are those programs that use more than one DNA/protein file. Most of them compare sequences with each other. It is not very handy to load all the sequences every time you want to compare them, say you have 5 sequences and want to compare these five with a sixth sequence. Tomorrow you will clone another gene and you will want to compare this new gene with the other six. So you would have to select all these files again. To prevent this AiO works with a file of files (FOF). The sequence information will not be duplicated from your normal DNA files into the FOF. The needed information is contained in a very compact manner, see figure 26.

How a fof file looks

```
0 U:\DNA\methylases\aqui.pep 0 1-387 0 387  @
0 U:\DNA\methylases\sini.pep 0 1-461 0 461  @
1 U:\DNA\fusions\nat1.pep 0 1-188 0 188  @
1 U:\DNA\fusions\sat.pep 0 1-174 0 174  @
```

Figure 26 *example of the contents of a “.fof” file. Only information about the sequence (total length, your selection if applicable etc.) is stored in here,* ***not*** *the actual sequence.*

The FOF-file just contains information to find the DNA/protein file and the coordinates of the part of the sequence to use. You can have as many different fof files as you like.

Control

All programs share the necessary controls to manage the FOF file architecture, see figure 27. So you can open fof files and subsequently add sequence files to the list, delete them or choose another part of the sequence. Of course you can save the so created fof files for later use under a name you can choose freely.

Figure 27. *The common control boxes of the programs grouped under the menuitem ‘FOF’ . These controls enable the user to change the DNA/protein files that are used in the currently active FOF file (the upper box) and to manage the different FOF files (lower box).*

How to create a new fof file?

When you start up AiO for the first time there will be no FOF-files for you to work with. To create a new FOF file go through the following steps:

- Click on the FOF menu and then on the program you want to use (Dotplot, Align, tAlign or Plasmid-maps - for a description of these programs see below).
- The program will open with an empty FOF file; there will be no names listed on the left.
- Use the 'Add' function on the right (see Figure 27) to add new files to your FOF-file.
- Use the 'Save As' function to save your FOF-file and to give it a name.
- That is all.

You don't have to use all entries

Another feature that all of the FOF programs share is a listing of the files contained in the FOF file and the information thereof. The listing is also a means to select which files to use, Not all files have to be used, only those which are checked will be used.

The various Programs

Dotplot

Dotplot

As this is a FOF program, first read the general introduction for these programs if you have any problems with the opening, creating or managing of fof files.

Dotplot is a program to compare two sequences, either DNA or protein. It gives graphic results and this is an enormous advantage over other comparison programs. Because there is no alignment necessary, the program will recognize conserved regions in two proteins even if these do not have the same relative order in both sequences.

Some Background

Principle of Dotplot.

Like all Dotplot programs, this one works with two parameters called the Window (W) and the Score (S). A block of homology is defined as that part of the sequences, with length W, where at least S residues are the same.

As an example, let us take two DNA files and leave everything to its default values. In this case only a line is drawn in the resulting picture if at least 14 out of 21 bases are identical. For a complete picture all possible stretches, with length W, of one sequence have to be compared with all possible stretches, with length W, of the

other sequence. For DNA this means that bases 1 to 21 of the horizontal sequence have to be compared with bases 1 to 21, 2 to 22, 3 to 23 etc. of the vertical sequence.
After this first round bases 2 to 22 of the horizontal sequence will again be compared to the vertical sequence: 1 to 21, 2 to 22, 3 to 23 etc. This is the general principle, but AiO's Dotplot uses an algorithm that has to calculate a lot less than this description suggests at first glance. However, it does give you an idea about the number of calculations needed for a run.

It also shows the quadratic nature of Dotplot: an increase in length by a factor two will increase the time necessary for a run with a factor of four.

Scoring tables

In the case of proteins the whole comparison is not necessary so straight forward. Of course you can run two proteins like any DNA comparison. Better is to use a scoring table.

To explain this option I will have to tell you something more about the various methods of comparing proteins and their amino acids.

AA vs. AA Chemically

Some amino acids are chemically more related than others; Glycine (R-H) is nearer to Alanine (R-CH3) than to Cysteine (R-CH2-SH).

This fact can be expressed as a fraction. As an example consider the following situations

Hor. Protein	Vert. protein	Score
Glycine	Glycine	1.00
Glycine	Alanine	0.80
Glycine	Cysteine	0.02

The same considerations can lead to another approach: just make groups. Here Glycine and Alanine would be considered to belong to the same group and have a score of 1. Cysteine would be in another group and it would score 0, both with Glycine and Alanine. In table form:

Hor. Protein	Vert. protein	Score
Glycine	Glycine	1
Glycine	Alanine	1
Glycine	Cysteine	0

AA vs. AA Evolutionary

A completely other approach is to score for evolutionary relatedness. This means two processes have to be considered and expressed in a number. First, the chance of a certain codon mutating into another has to be calculated, and secondly, the fitness of this mutation has to

be assessed. Both the chance and the fitness have to be expressed by a single number.

Both the chemical and the evolutionary method have been incorporated into Dotplot.

Jiminez

The chemical scoring table is called “JIMENEZ” after the man who described it first. It does not score for individual amino acids but divides them in groups.
The groups are:

PAGST	: neutral, weakly hydrophobic
QNEDBZ	: hydrophilic, acid amine
HKR	: hydrophilic, basic
VILM	: hydrophobic
FYW	: hydrophobic, aromatic
C	: cross-link forming

All amino acids within the group score equal (=1), between groups they score 0.

The evolutionary approach is represented by four other tables included in AiO.

Dayhoff

The oldest is the classical table called “DAYHOFF” again named for an important contributor of this work. It is a completely individual scoring table. The relatedness of every amino acid with every other amino acid is expressed as a number between 0 and 2.73.

PAM250
PAM500
VTML240

Newer are the tables called PAM250, PAM500 and VTML240. They are developed from the work of Dayhoff and are based either on the idea of the accumulation of mutations over the generations or on Hidden Markow Modells.

Own

There are three more tables available in Dotplot, the contents of which, as well as their names and defaults can all be changed. So if you feel you have developed an improved scoring system you can change one of these tables to fit, complete with an appropriate name and defaults.

Running Dotplot

Running Dotplot

When you start Dotplot you will see the empty field as described in the ‘General Introduction’. If you open a old fof file or create a new one the you can run Dotplot with the dotplot-specific controls that are left of the general fof controls, see Figure 28.

On the top the two names of the sequences that are to be compared are listed, which one is on the horizontal axis and which on the vertical one can be switched by clicking the ‘Flip’ button.

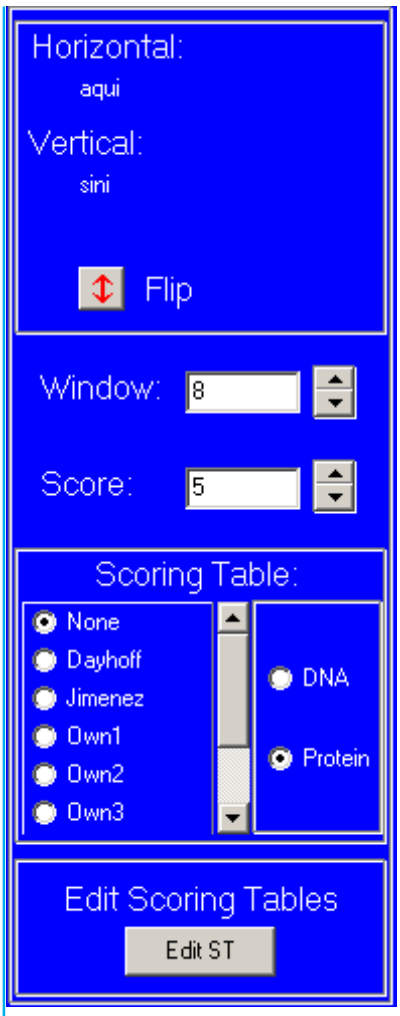


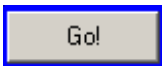
Figure 28. The dotplot-specific controls.

W & S

The next panel down shows the window and score parameters.

Select Scoring table

The third panel the different scoring tables. With the radio buttons you can select the table to use. The tables you can change are called Own1 to Own3. This name can be changed by you into any other, so if this program was installed by someone else, don't be alarmed if there are different names. This is also true for a lot of the numbers that are described as default values. If they are not the same as described, somebody probably has changed them to fit his/her needs. If you don't agree with these new values or if you don't agree with mine you can change them yourself. If you want to do this: the lowest panel gives you the possibility to edit the three 'Own' scoring tables.



If everything is as you like it then all you will have to do now is click the "Go" button. The picture will be drawn and the results of the comparison will be obvious, see Figure 29.

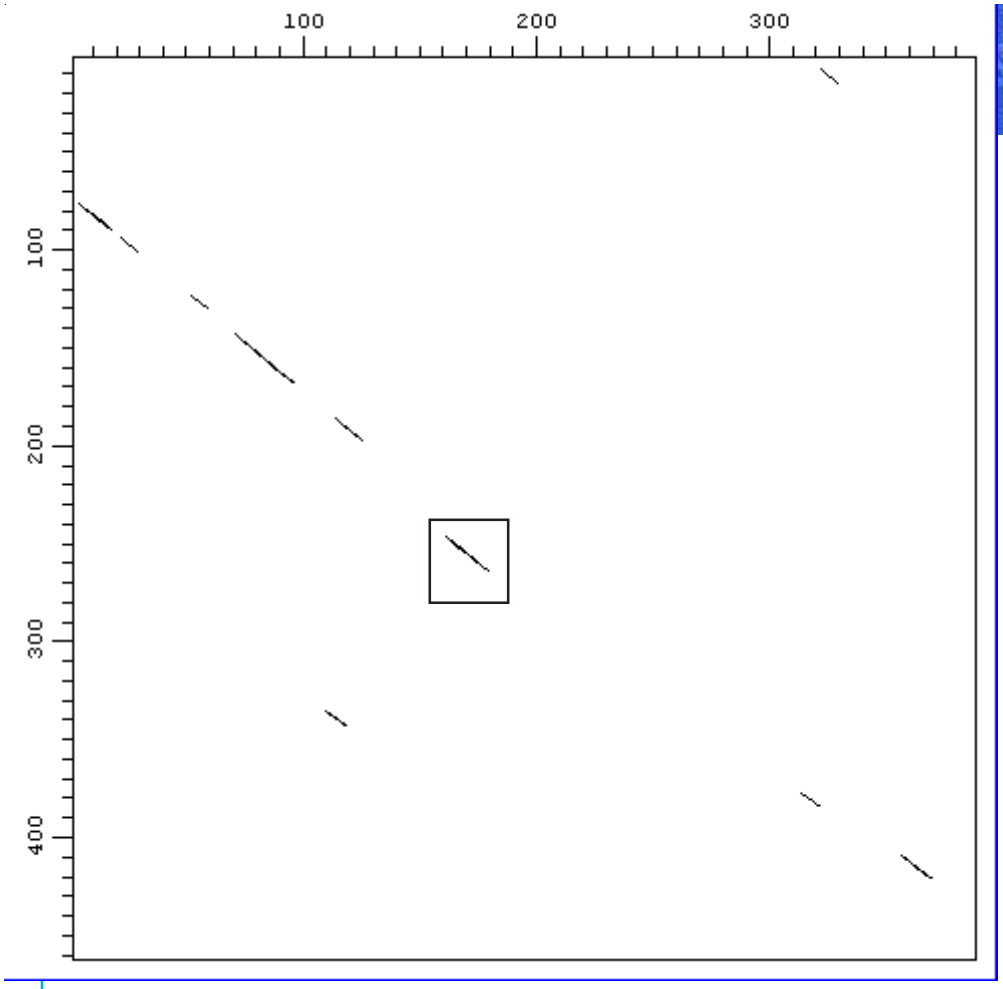


***Figure 29**. Dotplot with selected part.*

Zoom in

The mouse is now a very important tool, by clicking the **left** mouse button, keeping it pressed and moving it across the picture you can select a part of the picture that will subsequently be zoomed in. To get the full picture again use the “Full Length” button.

Sequences responsible for line

To display the sequence alignment that is responsible for the lines in the picture click on any of these lines with the **right** mouse button, see Figure 30.

```
Horbeg: 70  Horend: 97
Verbeg: 142  Verend: 169

aqui      PLEIDLVIGGPPCQSFSLAGKRMGMDDP          97
            ||||  |||||| || |||| |  |
sini      GNEIDLIMGGPPCQAFSTAGKRLGLEDE         169
```

***Figure 30**. The sequences ‘behind’ a line in a dotplot.*

Scoring tables again

So and now some final words on the scoring tables.

When using scoring tables, the definition of the Score has also to be revised, now it better be defined as the sum of the various, individual, scores.
If you run without any score tables this is the same as for DNA, but with other standard values for the Window and Score. With the use of score tables the new definition comes in really handy; it will explain the Dayhoff defaults where Score is ten out of a Window of only 8. The very high values can be explained by the use of numbers larger than one for some combinations. If you click the button labelled “Edit Scoring Tables” you will see these values and you can even change them, see Figure 31. As you might have noticed the table is a 26 by 26 matrix. This means that not only the standard amino acids are represented, but also B(Asx) and Z(Glx). There are four letters that do not stand for any amino acid (J,O,U,X), although the X is sometimes used for “any amino acid”; however, this allows you to use the extra letters for such eccentrics as selene-coupled amino acids and their likes. It is advisory to give these extremely high auto-score values to highlight their rarity.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A	1.18	0.00	0.80	1.03	1.03	0.65	1.13	0.86	0.95	0.00	0.88	0.81	0.89	1.02
B	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.80	0.00	2.19	0.49	0.47	0.57	0.66	0.66	0.77	0.00	0.46	0.40	0.48	0.64
D	1.03	0.00	0.49	1.39	1.34	0.44	1.06	1.07	0.76	0.00	1.01	0.60	0.74	1.21
E	1.03	0.00	0.47	1.34	1.38	0.46	1.02	1.07	0.80	0.00	0.99	0.66	0.79	1.14
F	0.65	0.00	0.57	0.44	0.46	1.91	0.52	0.82	1.10	0.00	0.47	0.75	1.02	0.65
G	1.13	0.00	0.66	1.06	1.02	0.52	1.48	0.79	0.74	0.00	0.83	0.59	0.72	1.03
H	0.86	0.00	0.66	1.07	1.07	0.82	0.79	1.65	0.76	0.00	1.00	0.79	0.79	1.16

***Figure 31**. Part of a scoring table.*

Align

Align

As this is a FOF program, first read the general introduction for these programs if you have any problems with the opening, creating or managing of fof files.

This program can take the checked DNA or protein sequences of a FOF file and aligns them. It takes the selected sequences and cuts them according to the begin and end coordinate specified.

Parameters

There are just two parameters, the ‘smallest area’ and ‘limit’. The first describes the smallest number of bases/amino acids that constitute a homology domain. The second the limit of what is still considered a significant number.

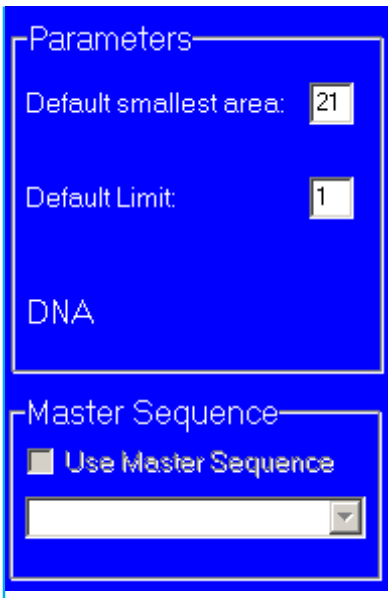


***Figure 32**. The Align-specific controls.*

The defaults are 21/1 for DNA and 8/1 for protein sequences. These work in almost all cases I encountered sofar - that is why they are the defaults!! ;).

Master Sequence

Under the parameter panel is the Master Sequence panel. Normally all sequences are compared to all other sequences and a tree is produced according their relative homology.
In some cases this is *not* what you want. Take for instance the case that you cloned a number of PCR products and you want to check if their were any errors incorporated. In this case you know what the sequence should look like. You don't want to compare your PCR fragments among themselves, just with the original sequence. So load your original sequence and make it the Master Sequence. All the others will be compared only to this one. It will be quicker and the generated alignments are probably more like what you wanted.

For the program to run just press the 'Go' button. As the program is progressing you will see the various comparisons printed on your screen.
The end is a screen filled with the various sequences aligned. This is a pure ASCII alignment, it is thought for the export to other programs or for further editing. For a better view of the alignment see the 'graphics' below.

```
..FSLFSGAGGLDIGA
..ISLFSGCGGLDLG.
..LSLFSGAGGLDLG.
..ISLFSGAGGLDLG.
..ISLFSGAGGLDCAI
NFVDLFCGAGGITQG.
..ISLFSGAGGMDIG.
..IDLFSGAGGFSLG.
..LELFAGAGGMALG.
```

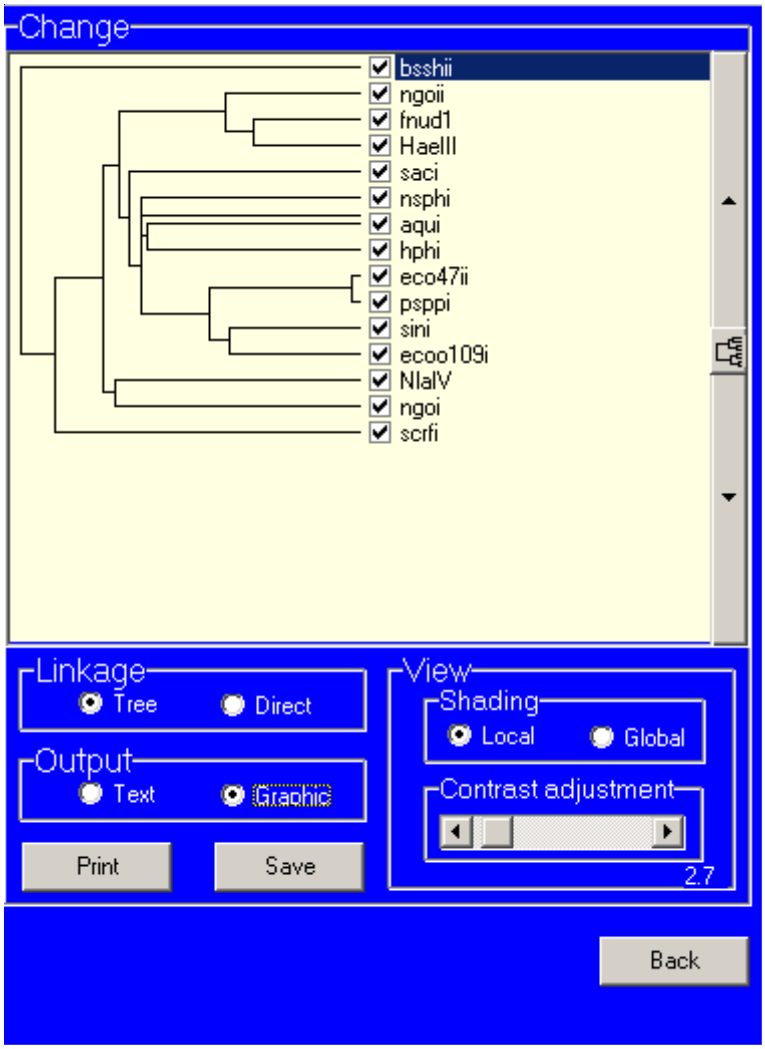


***Figure 33**. The various controls of the Alignment.At this stage it is possible to make sequences 'invisible' (uncheck them), move their order, influence the shading.*

The Tree

On the right hand site of your window is a tree figure displaying the *overall* (!) homology between the various sequences, see Figure 33. Directly under this part of the window is the 'Linkage' box, with the radio buttons you can switch between the classical 'tree' view and the direct-linkage view. The best fitting pairs can be seen directly in the latter mode.

As an example: the sequence of the file called HaeIII is very homologous to that of the file called fnud1, a third sequence -ngoii- is the next best fit. In the right hand part of Figure 33 you can see that it matches more closely with Haeii than with fnud1. As you can see ngoii is directly adjacent to Haeii, the program sorts the sequences in such a way to minimize the vertical distance. You can move to files by hand, use the arrows up and down on the right. You can always go back to the best possible ordering by clicking on the tree-button in the middle. By the way all these sequences really exist, they are all type II methylases.

Graphics

Global vs local

Now to the next set of radio buttons labelled :'Output'. Here you can switch between the pure ASCII mode and the graphic mode. The graphic mode comes in two flavours: global and local, if you click on the graphic radio button you will see the sequences highlighted as a measurement for the homology. Furthermore, a box labelled View/ Shading can be seen with the global/local options. To understand the difference you will have to keep in mind that the program makes the best pairs nearest neighbours. Global just calculates how good the homology is, at a certain position, for all sequences. The better this overall homology is, the darker the corresponding base/amino acid will be. Local looks at each sequence and its nearest neighbour(s) and calculates how good it fits in.

As a rule: with global you can see the domains that are conserved in all sequences very well and with the local setting you can also see the domains that are conserved in just a subset of your sequences. For an example see Figure 34.

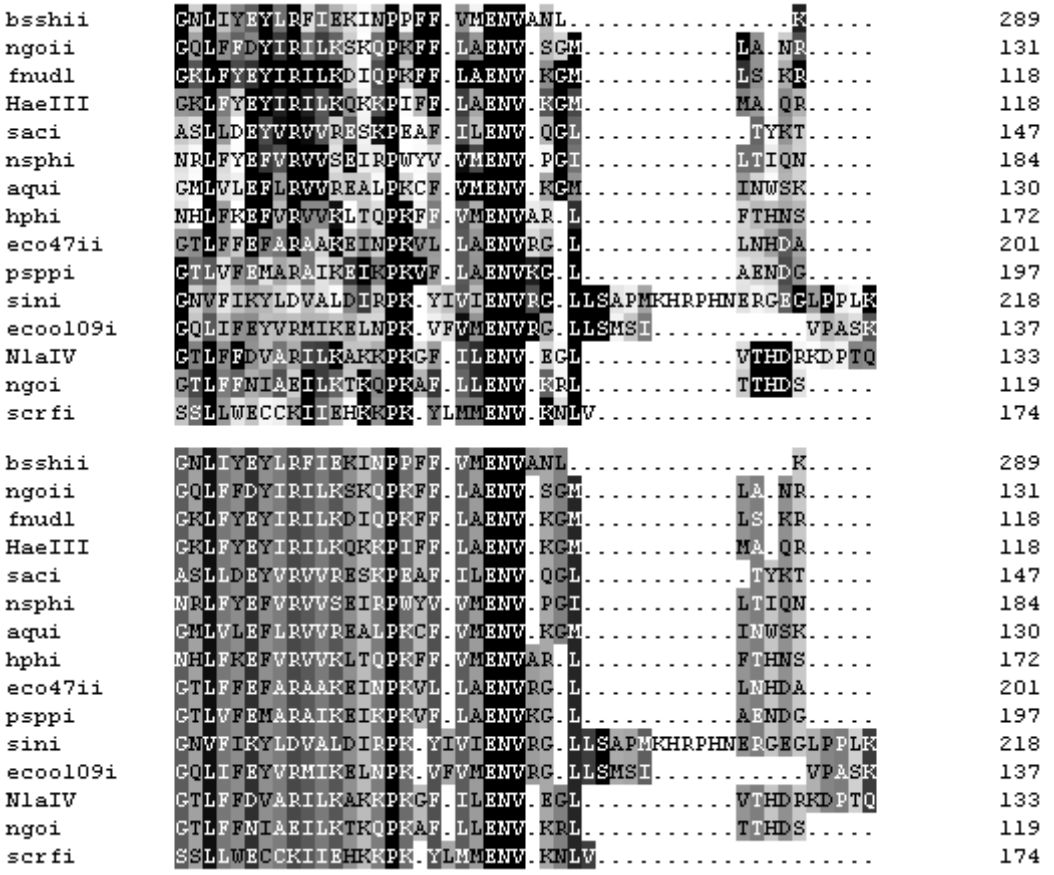
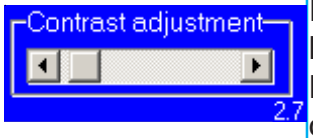


***Figure 34**. The same area of an alignment, with local and global shading.*



In case you compared protein files there is another small box labelled: 'Contrast adjustment' in the 'View' box. The alignments of DNA sequences are in black and white, those of proteins are in hues of gray. The difference between a good match (black) and a not so

very good match (white) can be represented by a linear or a number of other functions. In fact the shading is logarithmic (that is the reason for the factor 2.7 in the box). You can change this for a better contrast between domains by using the slider provided.

If you used DNA fies there is another box instead of the 'Contrast Adjustment'. It is called 'Splice' and I programmed it for the comparison of chromosomal DNA with cDNA. It works very well with the chromosomal DNA as Master Sequence and various splice isoforms. For a simple example see Figure 35.

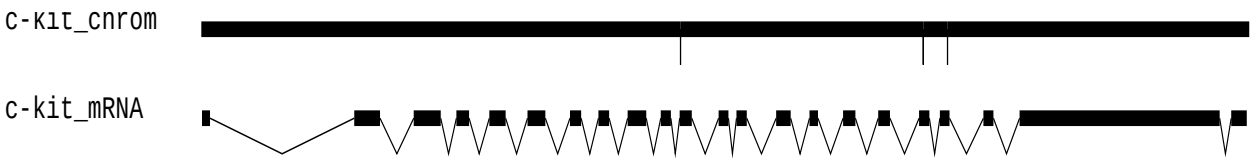


Figure 35. *An example of the 'Splice' drawing subroutine. It shows the alignment of the chromosomal DNA and cDNA of the c-kit oncogene.*

Limits

Limits

There is a limit of 250 files, but this will not be the main bottleneck. Time will be. With small numbers this is no problem: 15 proteins like the methylases in the example take 4 seconds (350 MHZ, 64 Mb RAM). But the program compares all sequences to all others, for n sequences that is (n2-n)/2 comparisons. This goes up very rapidly.

tAlign

tAlign

As this is a FOF program, first read the general introduction for these programs if you have any problems with the opening, creating or managing of fof files.

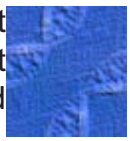
This is a variant of Align. This program will take DNA sequences in a FOF file, translate the part specified and align the resulting proteins. For all further information see under Align.

If the files are already protein files it will run them straight away.

Plasmid Maps

Plasmid Maps

As this is a FOF program, first read the general introduction for these programs if you have any problems with the opening, creating or managing of fof files.



This program is intended to print a large number of plasmid maps. It takes a FOF file and prints all activated entries. The only things that can be changed are the DBs used; own oligos, group oligos and restriction enzymes.

The programs under Line Commands

General

General

Dig and Triangle

There are just two programs under this heading, Dig and Triangle. They give the same kinds of result as the programs with the same name under ‘Action’, see page 15 and page 16 respectively.

Why?

The difference is that in this instance the name of the plasmid should also be typed in. These commands were included for speed, as it was found out that it is very inconvenient to open a DNA file in the editor, choose Dig and type in the REs for every DNA.

Now this can be cut short as follows:

- activate Dig under Line Commands and type: ‘ pBR322 HindII BamHI’ followed by a RETURN.

The program will now look in the entire directory for a DNA file named pBR322 and when found will cut it with the indicated enzymes.

‘doskey’

If you want to cut another plasmid with the same enzymes use the ‘arrow up’ key. This will bring back the previous command (this ‘doskey’ feature has a memory 64 command deep). Now edit pBR322 into the new name and press RETURN, voila....

All other features like partial cuts, are the same as in the case of the normal Dig. This program will, of course, also generate virtual gels.

For this version of Triangle everything, but the name-entry, is also the same as in its namesake under ‘Action’.

The programs under Data Bases

Programs with an '*'

!

As mentioned in the 'some things you should know' part of the introduction the management of some of the DBs is handled by the group manager (GM). Some of the programs under Data Bases are just for the use of the GM, other users will not see them displayed. When AiO is run by a GM these programs are labelled with an '*' at the end of their names.

General

All the DB-editors that are under this heading are programed extremely conservative: they do not change the DB unless this is explicitly asked for and confirmed.
In reality the user is editing a copy of the actual DB, all changes are made in this copy not in the DB itself. So at the end of a session the "Done" button must be clicked for the edited copy to replace the old DB.

If this is omitted the copy is deleted and nothing is done with the original DB, discarding all changes.

Programs

Search in 'group name' Oligo DB

Search in 'group name' Oligo DB

With this program it is possible to search the group's oligo DB on various criteria and mark them for printing. The term 'Group name' will be the name that was entered at the time of installation.
Search criteria are:

- Synthesis Number, the number given by the manufacturer.
- Name of Oligo, given by whoever ordered the oligo.
- Owner, the login-name of the person who did the ordering.
- Free Text, all lines of the oligo entry in the DB.
- Sequence, the sequence is searched. Degenerated and reverse sequences are also searched for.
- Site for RE, type in a RE name and all oligos that have the site for it are displayed.
- Browse, not really a search but sometimes very handy.

Search in Restriction Enzyme DB

Search in Restriction Enzyme DB

Find out how RE cuts, who sells it, what are the isoschizomers. You can look for all existing REs, the program knows them all. You can also type in: '#GAATTC' followed by a RETURN. This will display the information for *Eco*RI, the hashmark will make the letters that follow

into a sequence instead of a name. The information to generate the DB is automatically updated once a month. All other restriction lists that AiO uses are created from this DB. So all other lists are always up to date. The only exception is when you use an 'Own Selection' RE list. This list will be static. For all possible lists see below under: 'Change Restriction Enzyme DB'.

Search NCBI DB

Search NCBI DB with either text or sequence.

Just two links that will open your default web browser and point you to websites where you can search the whole nucleotide bank. Used to be EMBL DB but as the NCBI is faster, in my experience I changed it. The default for searches with text is the 'Nucleotide' DB, sometimes it is better to change this to 'Gene'.

Change Restriction Enzyme DB

Change Restriction Enzyme DB.

All AiO programs run with the RE DB you select here. This selection is personal; if 10 people use the same copy of AiO, all 10 can make their own selection and AiO will remember this and use it every time.

There are four different selections of restriction enzymes.

All first & comm

- All first & comm. This is the default selection. 'All' enzymes are in this list. For an exact description of how it is compiled see 'General Introduction', under 'Restriction enzymes'.

Neoschizomers added

- Neoschizomers added This list contains all the enzymes of 'All first & comm' and all the neoschizomers of those enzymes. So next to SmaI (CCC/GGG) there will also be an entry for XmaI (C/CCGGG). This list shows enzymes that generate alternative ends.

Non-Comm. added

- Non-Comm. added The list for masochistic users. It also shows the enzymes that are not commercially available. In my experience this means that there always be at least one perfect enzyme to cut exactly where you want. Only problem is how to get it.

Own Selection

- Own Selection In the under-menu, you can either select your personal list or edit/make one. This list will be personal, every user can make his/her own. It will be saved along with the personal oligos.

The selection you make has no influence on other users. AiO looks who is logged on and will activate this person's preferences. This means that this choice is permanent; until you change it AiO will always use the list you selected.

Compatible Enzymes

Compatible Enzymes

I just liked this kind of table. You can find it in catalogues of RE sellers. But of course it is never up to date. That's why I included it in here. AiO will make it with the data it gets from the internet, for a typical output see Figure 36 overleaf.
You can only print it or have a preview shown. The latter uses the Ghostview program. I tried various ways to have it shown directly on the screen. Without a very high resolution it was impossible to show the whole page on any normal screen. However, the lettering became unreadable.....
So that is why only the printed version has a good overview.

Edit own Oligo DB

Edit own Oligo DB

Rather straight forward little program, you can edit the sequence or the name of an existing entry. It is also possible to add a new oligo or delete one. As only the owner of these oligos can access this DB, (s)he has absolute control over it.
If AiO is used for designing and ordering of Oligos (see page 47 for an overview) this DB will be kept up to date automatically. Not only new entries will be incorporated they will also be linked to their sysnthesis number when profided.

Edit 'group name' Oligo DB

Edit 'group name' Oligo DB*

Program only for GMs, other users will not see it displayed.
With this program the Group oligo DB can be edited. This includes deletion of entries, adding completely new entries and changing existing oligos. All information about the oligo is editable.
Do not forget to use Save and Done or all changes will be void.
This DB contains all the data that AiO has of any of the oligos. Not all this data are necessary for AiO to run. So there is a second file that contains just the name and the sequence of every oligo. The latter is used to map oligos. If you edit anything in the DB and go through the right procedure (Save and Done) the working-file will be generated for AiO to use. Do not edit this file manually, all these changes will be lost the moment the 'Edit group name Oligo DB' program is used and it generates a new version.

Edit Structural Sequence Data

Edit Structural Sequence Data (Plasmap + Linmap) *

Program only for GMs, other users will not see it displayed.
This one is a beauty (I think). With this editor you can change how various parts of a sequence should look in the plasmid map and linmap programs. Most of the controls are self-explanatory (I hope). However, something has to be explained: the hierarchy of the data

Program AiO; 21/04/2006 09:20:52 hr.

Restriction Enzyme Compatibilities

	AATT	ACGT	AGCT	ATAT	CATG	CCGG	CGCG	CTAG	GATC	GCGC	GGCC	GTAC	TATA	TCGA	TGCA	TTAA
^~~~	**TspEI**				**FatI**				**MboI**							
~^~~		**MaeII**			**CviAII**	**HpaII**		**MaeI**		**Hin6I**		**Csp6I**		**TaqI**		**MseI**
~~^~			**AluI** CviJI				**AccII**		**DpnI**		**HaeIII** CviJI	**RsaI**			**HpyCH4V**	
~~^									**BstKTI**	**HhaI**						
~~^		**TaiI**			**NlaIII**											
A^~~~T	ApoI		**HindIII**		**BspLU11I** AflIII	**AgeI** Cfr10I BsaWI	**MluI** AflIII	**SpeI**	**BglII** XhoII			TatI				
A~^~~T		**AclI**												**ClaI**		**VspI**
A~~^~T				**SspI**						**Eco47III**	**StuI**	**ScaI**				
A~~~^T																
A~~~~^T					NspI					HaeII					**NsiI**	
C^~~~G	**MfeI**				**NcoI** StyI BstDSI BsaJI	**Cfr9I** BsaJI AvaI AcoI	BstDSI BsaJI	**AvrII** StyI BsaJI			**Eco52I** CfrI	**BsiWI**	BfmI	**XhoI** SmlI AvaI	BfmI	**AflII** SmlI
C~^~~G				**NdeI**		BmeT110I								BmeT110I		
C~~^~G		**PmaCI** BsaAI	**PvuII** MspA1I			**SmaI**	MspA1I									
C~~~^~G							**SacII**		**PvuI** BstMCI		BstMCI					
C~~~~^G															**PstI**	
G^~~~C	**EcoRI** ApoI					**MroNI** Cfr10I	**BsePI**	**NheI**	**BamHI** XhoII	**KasI** BanI	**Bsp120I**	**Acc65I** BanI		**SalI**	**ApaLI**	
G~^~~C		AcyI								**NarI** AcyI			AccI	AccI		
G~~^~C		**ZraI**	**Ecl136II**	**EcoRV**	Cac8I	**NaeI** Cac8I	Cac8I	Cac8I	NlaIV	**DinI** NlaIV	NlaIV	NlaIV	**Bst1107I** Hpy8I	Hpy8I HindII	Hpy8I	**HpaI** Hpy8I HindII
G~~~^C																
G~~~~^C		**AatII**	**SacI** SduI Bbv12I BanII		**SphI** NspI			**BmtI**		**BbeI** HaeII	**ApaI** SduI BseSI BanII	**KpnI**			SduI BseSI Bbv12I	
T^~~~A					**BspHI**	**AccIII** BsaWI AcoI		**XbaI**	**BclI**		CfrI	**Bsp1407I** TatI				
T~^~~A					Hpy188III	Hpy188III	Hpy188III	Hpy188III						**AsuII**		
T~~^~A		**SnaBI** BsaAI					**NruI**			**FspI**	**BalI**		**PsiI**			**DraI**
T~~~^A																
T~~~~^A																

files. Consider the case of a fusion gene as depicted in Figure 37.

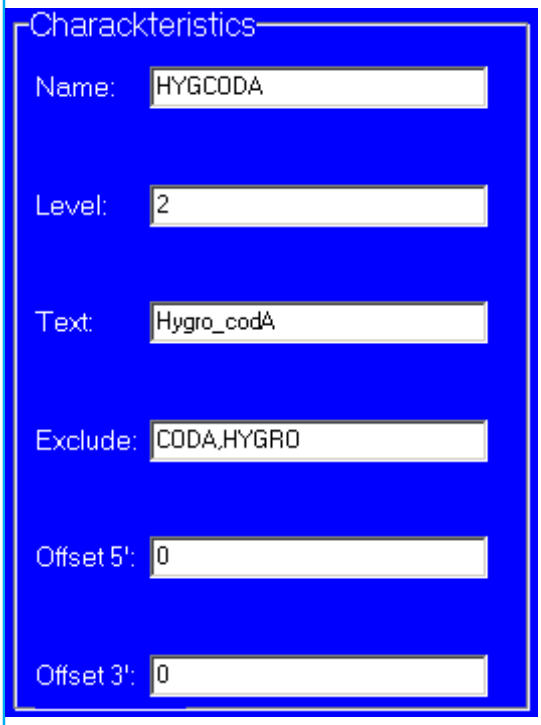


***Figure 37**. The central control box of the structural DNA editor.*

If the program would just use all the data it can access, it would draw the HygCodA fusion gene in a plasmid map, but also the Hygro and the CodA gene. These three would be overlapping and it would not be a nice picture. That is why the fusion HygCodA is level 2, it is checked before any level 1 genes. If a level 2 gene is drawn in a picture **this part** of the drawing is not overlapped with any of the genes mentioned in the exclude box. There are 9 possible levels and any with a higher number will be considered before those with lower numbers.

Offsets

As can be seen in Figure 37 there is also the possibility to offset the DNA. This is a way to recognize a large part of DNA and draw just a little part.
What uses that has? Well a good example is the Cmp gene of *E.coli,*

***Figure 36**. The table showing all compatible enzymes.*

in its natural host it causes chloramphenicol resistance. This is a prokaryotic gene and is drawn in gray (if you want you can change that).
However, it can also be used as the CAT gene to measure promoter activity in eukaryotes (white).

How to solve this?

Making the drawing of a stretch of DNA dependent of neighbouring sequences

Answer:

-make two files one with level 2 priority call it CAMP and in the exclude box write CAT. As sequence load the ORF of the Camp gene **with 300 bases of prokaryotic promoter at its 5' end**. In the offset5' box fill in 300. The graphics are: gray box with arrow and text. The actual drawing is just the ORF, the beginning of the box is set off 300 bases, exactly the length of the promotor.
-Now make the second file, call it CAT make it white and with 'CAT' instead of Cmp etc. But most important make it level 1 and let the DNA consist of **only** the ORF.

The program now makes a very intelligent choice: if there is a procaryotic promotor in front of the gene it is Cmp, if not it is CAT.

With the 9 levels and the offset option a number of gene systems can be determined automatically and be drawn without any further manipulation.

Offsets can also be negative, which comes in handy if the symbol in the drawing should be larger than the recognized DNA. This is the answer to the request: everything that starts with this sequence should be labeled with the same text and have an overall length of ... bases.

Edit User DB

Edit User DB*

Program only for GMs, other users will not see it displayed.
Here the GM can edit the information of the users. There is no need for the addition of new users as AiO does this automatically. The deletion of users is hardly ever needed, the only exception is when one of the members of your group switches to another group that uses the same copy of AiO.

Edit Oligo Manufacturer DB

Edit Oligo Manufacturer DB*

Program only for GMs, other users will not see it displayed.
This is a simple editor to edit your list of firms that make oligos, all AiO needs is the Email address to send its orderforms (see below).

The programs under Oligos

General

General

Under this heading are the programs to **order new** oligos.

Background information
There are two oligo DBs. There is the central DB of the group, where all the oligos are stored with all relevant information about them. This DB can be accessed be all members of the group. Every member of the group has it own 'working set' of oligos in his/her own oligo DB. The administration of these two DBs is mostly fully automatic.

There are several possibilities how to design, check, order and manage oligos in AiO see Figure 38 for an overview.

What does what

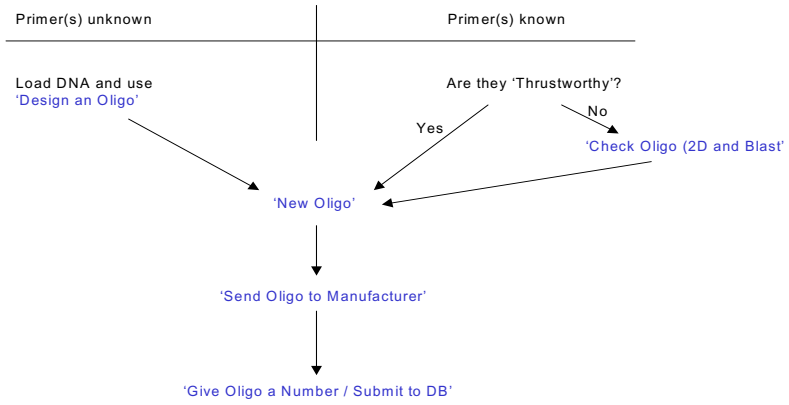


***Figure 38**. The use of the various programs. Program names are in blue.*

As you can see in Figure 38 the central program of the whole process is 'New Oligo'. This program is integrated in both 'Design an Oligo' and in 'Check Oligo (2D and Blast)'. Both will open this program when you press the 'Create' buttons of these programs (see page 25 and page 49 respectively). You can even start with this program if you already know the sequence of the oligos you want to order and thrust them.
For the use of 'Design an Oligo' and in 'Check Oligo (2D and Blast)' see the entriesunder 'Programs under Action' and on the next page. The general flow of these programs will be explaind before the programs are discussed in detail.

Flow explained

The ideal method of working with the programs is as follows:

- Create a new virtual oligo (subprogram ‘New Oligo’)

  This oligo will then be automatically in the ‘Own Oligo’ DB.An ordering-form can be printed, either to use as such or for your personal administration.

- Virtually test the oligo by running the programs with option ‘use Own Oligos’.

- If the oligo is OK then order it (subprogram ‘Send Oligo Order to Manufacturer’)

  AiO has its own built in Email functions. The list of Manufacturers is freely editable. (See subprogram under ‘Data Bases’).

- When the physical oligo arrives add the synthesis number (‘Give Oligo a Number’)

  Only now the oligo will be submitted to the oligo DB of the group. The number will be added to the name of the oligo in the ‘Own Oligo’ DB.

In this way you can be sure that the oligo you typed will be exactly the same as the oligo in the DBs. The other nice thing is that all oligos in the DB of the group will really physically exist in the lab.

Programs

Programs

New Oligo

New Oligo

Here the information of the new oligo can be supplied. Most of these things will give no problems, the ‘copy from base’ box might. For this I will have to explain what happens if you use this program.

The oligo you type in (or paste in) will automatically be incorporated into your own oligo DB. It will not (yet) be in the group oligo DB. This gives you the change to run the other programs with the new oligo in a kind of test phase. The own oligo DB is very much your working subset of the groups oligo DB. In the group oligoDB is all the information about the oligos that really exist. The own oligo DB is free editable. If your oligo has a 5' tail of say 12 bases consisting of two RE sites, there is a large possibility that a lot of your plasmids will not have the exact 5' sequence of your oligo because you cloned with the second site. The programs would not find such an oligo. That is the reason why the group oligo DB can be cut to various lengths (default 16 bases). Your own DB should be ready to use, and here is the possi-

bility to cut it to size. The cutting will not affect the later submission to the group DB, there the whole oligo is saved.

If you use the 'Done' button the oligo with all information is saved and a FAX form can be printed (default is: Print, if you do not want this un-check the option in the lower left corner). You can use the FAX form to order and/or for your own administration as hardcopy.

Check Oligo

Check Oligo (2D and Blast)

This program allows you to check known oligos. It gives you the opportunity to type / paste in an oligo or two oligos. All controls are the same as in 'Oligo Design', see page 20.
If you type in you will notice that only values for oligos longer than 8 bases are calculated.

Give Oligo a Number / Submit to DB

Give Oligo a Number / Submit to DB

Once your oligo was ordered you will have the physical oligo and a synthesis number. These numbers are very handy as they are different for every oligo, say something who synthesized it and when. With this information all the program needs to know is finally together. Now you can start this little program and it will ask you the synthesis number and the concentration of the stock. If the oligo was ordered by Email the name of the synthesizing firm will be automatically added. After this step your oligo will be **in the group oligo DB.**

Transfer Oligo from Group DB to Own DB

Transfer Oligo from Group DB to Own DB

This program allows you to transfer a oligo from the group oligo DB to your own DB. This program is under this heading for historical reasons, maybe it should be shifted to the Data Bases heading.
With this program you can search oligos in the group DB mark them for transfer and when your done, your own DB will contain the new oligos.

Send Oligo Order to Manufacturer

Send Oligo Order to Manufacturer

with this program you can order your oligo by Email. All information about you, your institute and the oligo should be here. Just select an oligo, fill in the scale and purification needed, select a manufacturer and click 'Send'. The letter that will be send is shown, if everything is OK say so, otherwise edit it (it is a text-editor). AiO has its own built-in Email program and will not use any external program.

The programs under Family

Origin of plasmids

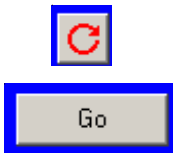
Under this heading are programs that work with the relationships of the origin of plasmids, **not** for evolutionary origin but how they were made.

Find and Replace

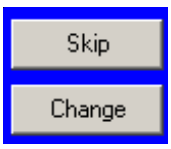
Find and Replace

This will solve a problem I had sometimes:
I had this plasmid I was working with for quite some time, the sequence was deduced from published parts. All of sudden some digests were not conform with the sequence. I started sequencing and found the errors. By that time I had at least 15 plasmids that were based upon the original plasmid. I had to change all those sequences as well. I do not like this kind of editing; it is boring and error prone.

So that is what this program is for. On the opening window of this program you give the old sequence and the new sequence. You can type in sequences, paste in from another window and load from disk. To reverse the sequence there is the button with the red circular arrow, all shortcuts e.g. Cntrl-D, Cntrl-W, Cntrl-Y, Cntrl-R and Cntrl-B will also work. If you have filled in both sequences then press the 'Go' button



The window you then get is something like in Figure 39. AiO will now search the whole DNA subdirectories for files sequence files. These will be listed on the left. These files will then be searched for the old sequence (the one to be replaced).
In the gray area (top right hand corner) you can monitor the progress. Hits will be displayed in the yellow area under the gray area. You can change this instance by clicking on the 'Change'-button or skip the file with the other button. If there is more then one hit in a file these will be listed in the afore mentioned yellow area. You can select which of this hits in a single file you want replaced by (un)checking the checkboxes in front of the hits. Only checked instances will be changed when you press the 'Change'-button.



You do not have to wait until all files are screened before starting to Change/Skip, the searching was programmed as a separate thread and will not be interrupted by your typing. As a matter of fact you can even do something else in AiO (e.g. calculating digests) without stopping the search thread. So you don't get bored if you will have to wait a long time for all files to be screened.

Plasmid maps

In case some files were changed, AiO will ask you if you want to draw new maps of the changed plasmids.

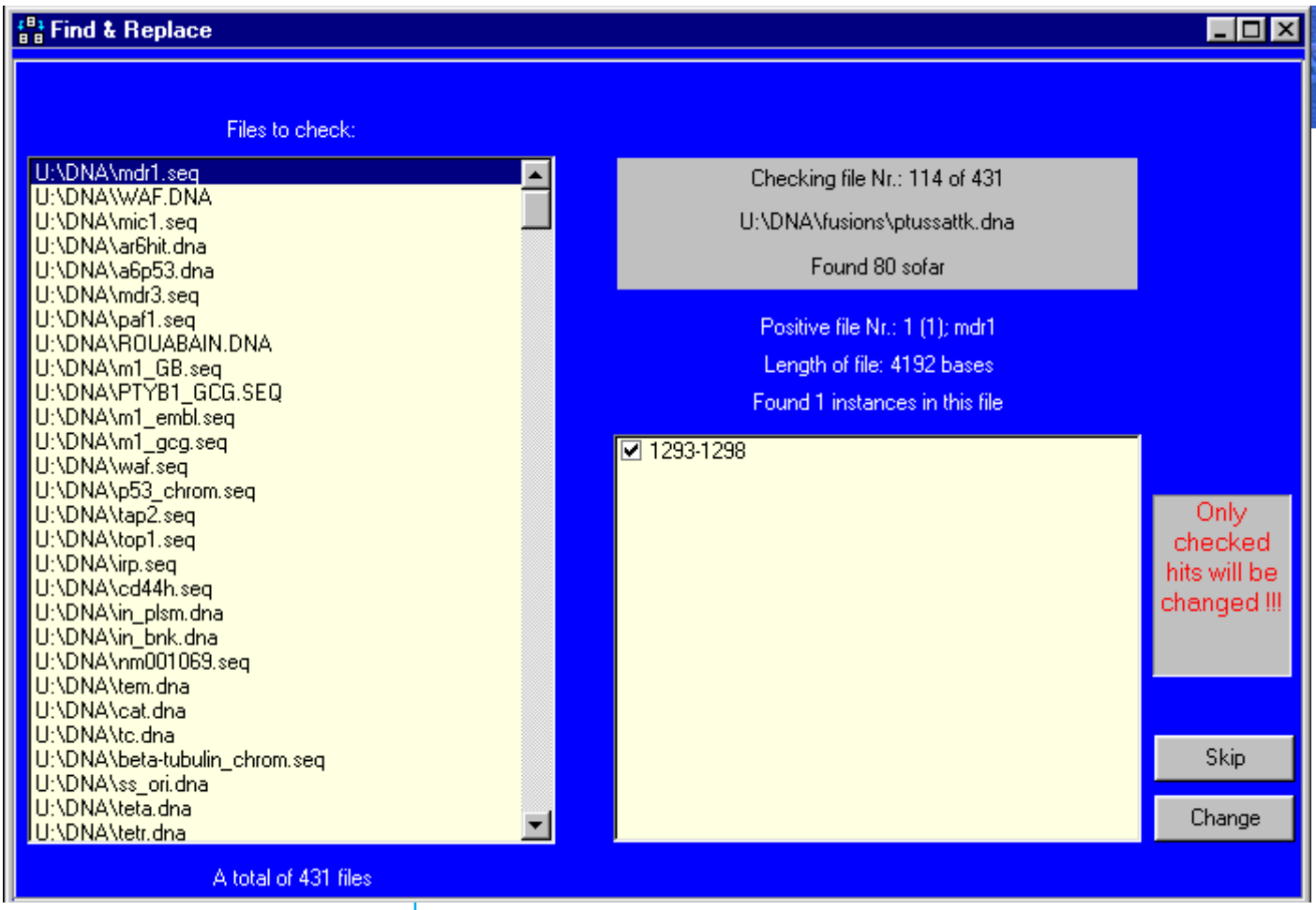


***Figure 39**. The interactive window of 'Find and Replace'.*

Flow

This program will let you **draw** flow maps of cloning strategies. If you start it the right part of the new window will look like Figure 40.

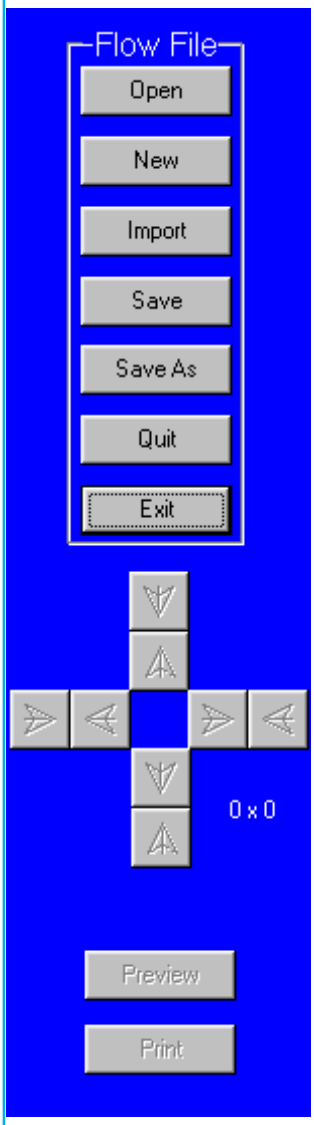


***Figure 40**. Control elements of the Flow program.*

This program uses Flow-files (.flow), you can save, load and edit flowfiles. So a drawing you make today can always be changed later. The top buttons are all clearly linked to opening and saving Flow-files. I do not think these function will give you any problems. Just remember 'Quit' is leaving this part of AiO without saving the Flow-file, 'Exit' will save it first.

The import function is rather nice: it allows you to 'fuse' two (or more) flow files. You can combine all the flows you created into one big flow, showing the complete cloning strategy. This function was written to use in combination with the 'Virtual Cloning' function. The latter can be used to generate flow files of all virtual created plasmids. These will normally feature only 3 or 4 plasmids. To generate the overview use the import function (see the example under virtual cloning, page 61).

If you press 'New' or load a old Flow-file the gray area on the left will be filled with Draw-Units (DUs) see Figure 41.

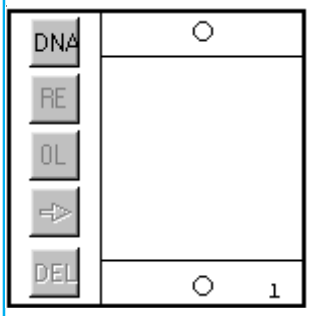
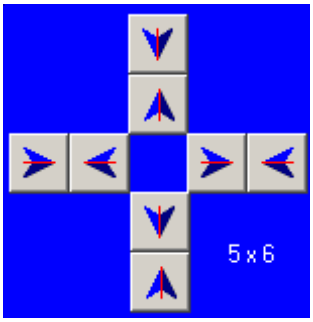


Figure 41. An empty DU.

In every DU there are 5 buttons, a central white area and two small circles. Every DU has a number in the right hand lower corner. When you make a new drawing it will start out with a 4x4 field of DUs.



You can add row and columns, both on the right and the left, top and bottom by using the fancy arrows on the right. You can always get rid of too many rows/columns by using the arrows 'turned in'.

The buttons DNA is there to load new DNA or change to another DNA. RE means restriction enzyme names to draw into the maps. OL = oligo names to draw into the maps. '->' is there to edit the arrows that connect the various plasmid in a flow map.
And the last, 'Del' is to get rid of everything in the DU.

The two circles Top Circle (TC) and Bottom Circle (BC) are for easy mouse directed drawing of arrows.

Colour coding
Most elements in a DU come in two colours: red and green. Red means: not done yet, and green means done. So if you loaded a plasmid and have not yet giving any REs to be drawn the RE-button will have red lettering. After you have given in some REs it will be changed to green. Arrows will be green if you have given them comments, red if they are without comments.

Example / Tutorial

The easiest way to show you the use of Flow is to tell you the fastest way to make a flow. In this example I will use three common plasmids: pBR322, pBR328 and pACYC184. You can find the sequences in any DNA-DB. This example is boloney, pBR328 was **not** cloned this way. The picture looks nice even though the lengths do not match.

Example

- Open flow and press the ‘New’ button.
- Click on the ‘DNA’ button of DU with the number 2 (DU2). A win dow will open like in Figure 42.

***Figure 42**. DNA input of the Flow program.*

- Click on the small black triangle to load pBR322 (I hope you have it somewhere on your disk). You can see that Flow also offers you a change to draw symbolic plasmids. This in case you want to incorporate a plasmid of which you do not have the se quence only a name and approx. length. These two can be put into the white edit boxes labelled ‘Text1’ and ‘Text2’.

  In the lower left hand corner you can see that it is also possible to draw linear molecules. Again this can be done ‘real’ or ‘symbolic’.

- If you successfully selected pBR322 the name and length will be displayed.
- Press ‘Done’.
- Now the DU2 will look like Figure 43.

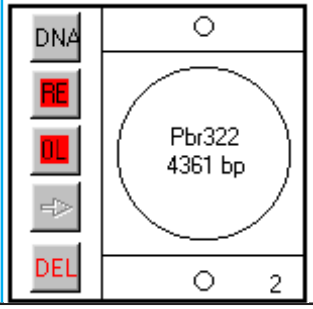


***Figure 43**. The new DU2.*

- Repeat the above procedure with DU4 and the plasmid pACYC184.
- Repeat the above procedure with DU11 and the plasmid pBR328.
- Now click on the BC of DU2 (it will turn red) and then on the TC of DU11. Another window will open like in Figure 44.

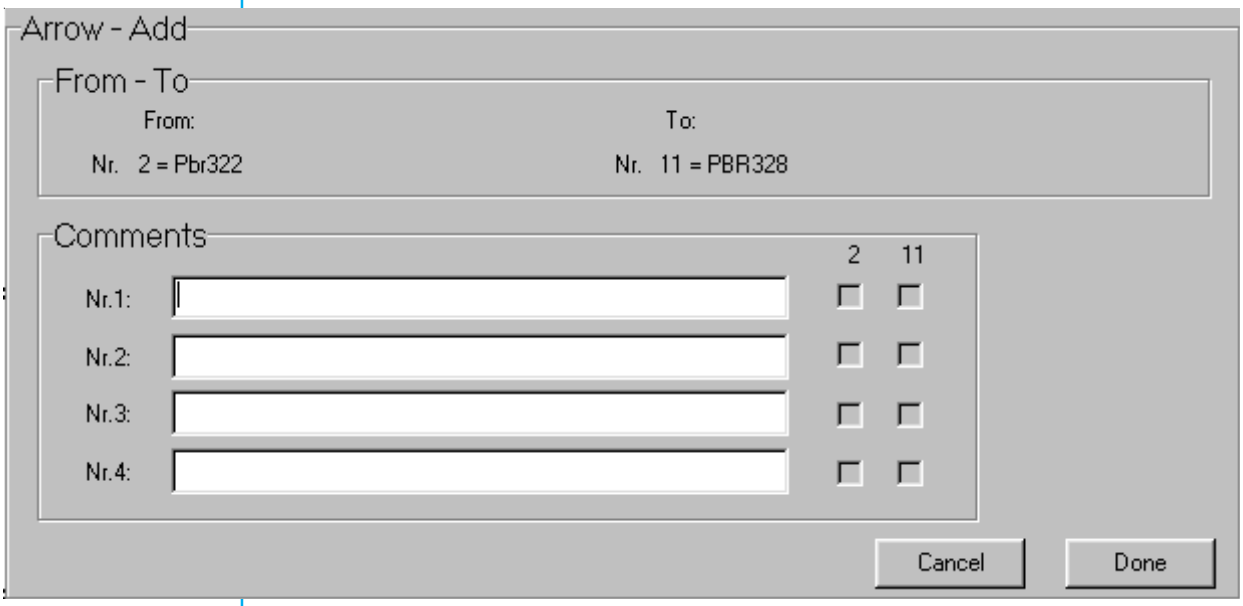


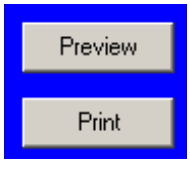
Figure 44. *The Arrow-Add function of Flow*

- Now type in (Comments Nr 1) 'x ecori'. The program will change this to: 'x EcoRI', indicating it recognized the RE. It scans for an 'x' followed by a space followed by an RE name. Notice the two check boxes behind Comments Nr 1.

  When they are checked the RE will also be used to draw the map of the plasmids.

- Give a tab or click in the editbox of comment Nr 2. Type in 'x Klenow + dNTPs'.
- Give a Return.

  *The window will close and a green line (there are comments!!) will connect DU2 with DU11. Both the RE buttons of pBR322 and pBR328 will be green: both will feature Eco*RI *sites.*



- Repeat this between DU4 and DU11, type in comment Nr 1:' x xmni', comment Nr 2:'x bcli' and comment Nr 3:'x Klenow + dNTPs'.
- You will not believe this but you are finished!!
- Use the 'Preview' or 'Print' buttons to see the results.

I'd like to move it

Wait a minute, I think pBR328 is too far away it should be in DU7!

That is easy:

- Click with right mouse key on pBR328, fill in '7' and voila. PBR328 has moved.

I think the other functions like adding oligos or editing arrow should give you any problems. So enjoy!

Virtual Cloning

Virtual Cloning:

with this program you can create new DNA files using those tools you are most familiar with: restriction enzymes, ligase, PCR and a number of other modifying enzymes. Of course you can use the editor for these things but here you can do everything with a graphical interface.
The principle is easy: you create a number of fragments and ligate them together. The various fragments can be modified in a number of ways. AiO uses 4 basic fragments:

Basic fragments

- those generated by restriction digest
- Topo TA vectors
- PCR fragments
- Adaptors (oligos)

With the exception of the Topo vector fragment all these fragments can be modified further. The ends of the adaptors and of the fragments generated with REs can be modified with enzymes like exonucleases, phosphatases, polymerases or nucleotide transferases (the program automatically detects which of the enzymes can use the generated ends as a substrate). The ends of the PCR fragments can also be further modified by applying REs, the ends that are so created can be modified further.

Tutorial

AiO checks if the ends of your fragments are compatible, if so you can ligate them. The best way to demonstrate the functions is by showing them. So what follows is a kind of tutorial. It uses very common plasmids and is only intended for showing the possibilities of AiO; the end products of the presented strategies do not make any sense.

Example I

Cloning two restriction fragments

This is the most common and the easiest way to generate new plasmids.

- Click on the menu item 'Virtual Cloning'

A new window is created it has -apart from a large gray area- only a few controls, see Figure 45.

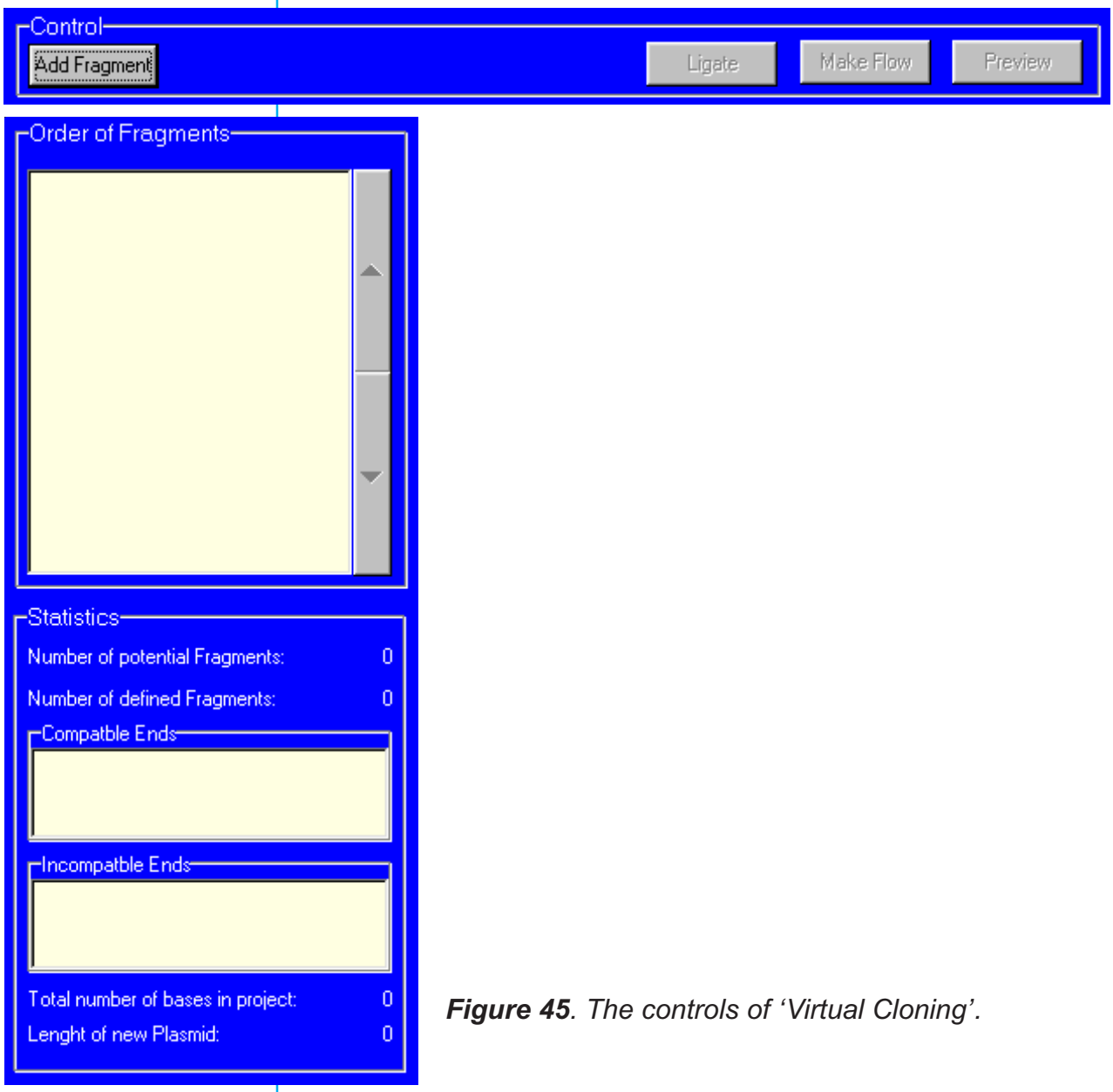


***Figure 45**. The controls of 'Virtual Cloning'.*

The most important is of course the button labelled 'Add Fragment'. The other buttons; Ligate, Make Flow and Preview can be used later when it is possible to create a new plasmid. As you can see in the info-panel, you can always change the order of fragments later, there is no need to create them in the order they should be in the final plasmid.
To check if the ends are compatible just watch the two yellow boxes in the 'Statistics' panel. If everything is in the top most. it is OK. Those ends that are incompatible are in red in the lower box.

- Click on the 'Add Fragment' button

A small window opens in the middle of the screen, it looks like Figure 46.

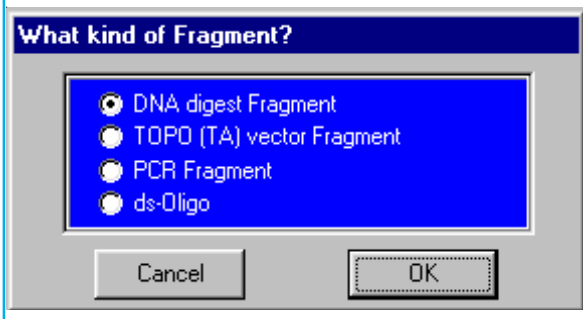


***Figure 46**. Selection of fragment type.*

- As the default is on 'Digest Fragment', just click on the OK button.

The gray area is now filled with a tabsheet, see Figure 47. This one is created for 'digest fragments', but those for the other kinds look very similar. There are four panels on it. From top to bottom: the graphic panel (GP), the end panel (EP), the modifying enzyme panel (MEP) and the main panel (MP).

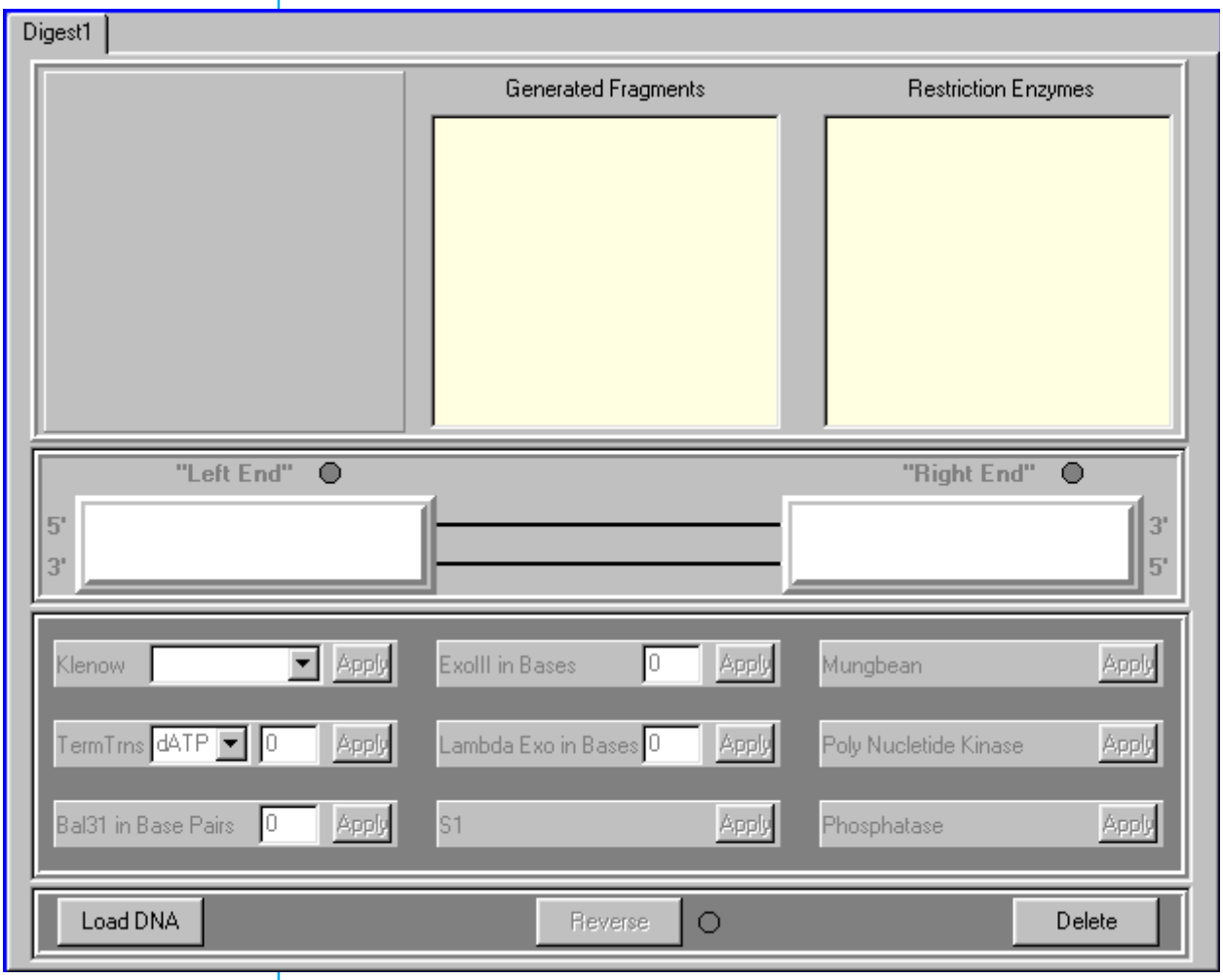


Figure 47. The tabsheet of a digest fragment

- Click on ‘Load DNA’ in the MP

The standard input will not give you any trouble, I hope.

- Load the plasmid pBR322

All restriction enzymes that have at least one site are listed in the right-most part of the GP. A (very) schematic picture of the plasmid is drawn on the left. You will notice that there also have been some changes in the ‘Statistics’ (Figure 45). Now it is time to cut the DNA.

- Scroll down the list of REs and activate *Bam*HI

The RE is drawn into the plasmid picture. The fragment (just one in this case) is listed in the middle of the GP.

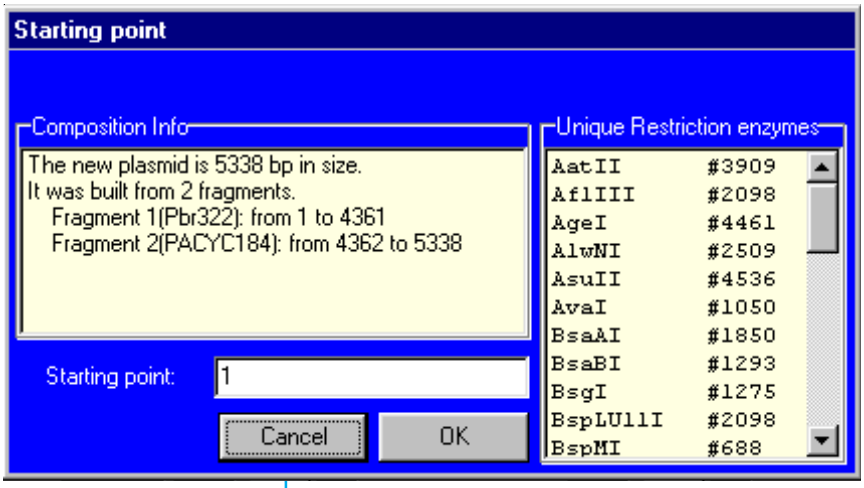
- Click on the fragment in the middle (BamHI/376 - BamHI/376)

The ends are now depicted in the EP. The statistics are updated. This fragment is ready. You could modify the ends but that is not necessary for this example. Let’s load the second fragment!

- Click on ‘Add Fragment’
- Click ‘OK’
- Click on ‘Load DNA’
- Load pACYC184
- Select the RE *Mbo*I

That’s better! That is a nice number of fragments. I don’t feel like looking up which fragment is which. Let’s do it the easier way: click on the fragment that is on 11 o’clock in the drawing. That works: it is selected (MboI/ 3543 - MboI/275). That is all, we have two fragments and their ends are compatible. Now for the ligation.

- Click on ‘Ligate’ (Top most part of the window).



Another small window opens: this one wants to know where the numbering in the newly created plasmid should start. You can use direct numbering or refer to a RE

***Figure 48**. The start of the new plasmid.*

- Click on 'OK'
- Save it under 'p_a_.dna' (yes, exactly like that: small 'a' between two '_'s)

αβχδε.....

Another little known feature of AiO: how to use Greek lettering. You can look at your new creation when you click on 'preview'.

Now comes a very important part: creating a flow file.

- Click on the button marked 'Make Flow'
- Click on 'OK', p_a_.flow is a nice name!

That was all. You have made a new plasmid and you have documented how it was made. The Flow-file can be loaded into the flow function and subsequently can be edited or printed.

Example II

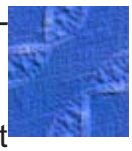
Cloning two restriction fragments with modification of their ends

For this example we will have to load two fragments with ends that are normally not compatible.

- Load pBR322 and cut it with *Sal*I. If you encounter any problems then please read the previous section. Select the only fragment.
- Load pACYC184 and cut it with *Mbo*I and select the same fragment as in the previous example.

The ends are now incompatible. You can not ligate them or watch a preview. These ends have to be made compatible. The easiest way would be to 'blunt' them with the klenow fragment of polI or mungbean exonuclease. But let's try something more elegant. Yes, this works even in the real world.

- Go to the tabsheet of pBR322 and click on both ends in the EP.



The small LEDs are red when the end is activated. The enzymes turn from gray to black in the MEP.

- Click on the dropdown item of the small klenow panel and select the combination 'dCTP+dTTP'. When this selection is in the editfield, click on 'apply', right next to it.

Look the ends have changed!

- Go to the tabsheet of pACYC184 and activate both ends.
- Use the klenow buttons again. This time use the 'dATP+dGTP' combination.

If you look in the Statistics section you will see that the ends fit.

- Ligate it and call it 'p_b_.dna'.

Fuse the flows

And now let's do something really convenient: let's fuse those flows into one, new big flow.

- Start the flow program, it is under 'Family' too.
- Open the p_a_.flow file.

For our purposes the plasmids are the wrong way around. That can be changed.

- Click with the RIGHT mouse button on pACYC184 and fill in '2'.
- Click with the RIGHT mouse button on pBR322 and fill in '3'.
- Click with the RIGHT mouse button on pACYC184 and fill in '1'.

They have been flipped!

- Click on 'Import' and load p_b_.flow.

You will see one flow in blue, in the centre and the other flow in green in the top left hand corner. To get the best picture you must optimize the overlap of the two flows. You can 'grab' the green flow or use the arrows on the screen or those on your keyboard to move the green flow.

- Put either the pBR322s or the pACYC184s exactly on top of each other

Thick green border.

- Click on 'OK'

The flows are now fused. Don't forget to save the new flow or all changes are void!

Example III

Cloning an adapter (two oligo hybrid) into a vector

- First Fragment: load pBR322, cut with *Bam*HI, select the only fragment available.
- Click on 'Add Fragment' and select the 'ds-Oligo' option. Click on 'OK'.

The tabsheet you see now is a bit different from the previous tabsheet. But you will recognize most of the functions. This time you don't have to select a fragment, but rather two oligos. Since I don't know what kind of oligos you have in your DB I will have to use the 'On the Spot' function. The button is where the 'Load DNA' button is on the digest-tabsheet.

- Click on 'On the Spot'
- Type: GATCGGGCTCGAGCCC
- Click 'OK'
- Click on 'On the Spot'
- Type in GATCGGGCTCGAGCCC once again.
- Click on 'OK'

So that was a *Bam*HI ->*Xho*I adaptor.

- Click on 'Ligate' and give it a nice name like 'p_c_.dna'

Example IV

Cloning a PCR fragment into a TopoTA vector

OK, the first part is really easy; getting the vector

- Click on 'Add Fragment'
- Activate 'Topo(TA) vector fragment' and click on 'OK'.
- Click on 'Load DNA'
- Select a Topo vector, say pCR2.1

That is it. You can not even change anything (apart from reversing the DNA). Now the insert.

- Click on 'Add Fragment'
- Activate 'PCR fragment' and click on 'OK'.
- Click on 'Load DNA' and load another classic plasmid: pUC19

'g-' and 'o-' oligos

I may not know what kind of oligos you have in your DBs,but I know that every AiO program comes with the M13 and M13-reverse primers. That is all I need because these two can be used in a PCR on pUC19. As a matter of fact they will be in the list twice: once in the groups oligo DB (starts with a small 'g'), and once in your own DB (those with a 'o').

- Select one of the M13 primers and one of the REV primers
- Click on the fragment in the listbox in the middle or on the frag-

ment in the drawing.

That is it. If you look at the top buttons you will see that you can ligate the fragments. AiO assumes you use taq polymerase and will add the 'As' at the 3' ends automatically. That is OK for Topo cloning, but it might be a problem for other projects. You can always apply the virtual Mung Bean Exonuclease on these fragments.

Example V

Cloning a PCR fragment into a digested vector

OK, now for the last example. Digest the vector, dephosphoralete it. Take a plasmid, PCR a fragment, cut it with REs and ligate it all together. The vector first.

- Load pBR322 and cut it with *Cla*I, select the only fragment. I think you can do this by now.
- Select both the ends by clicking on them (both the LEDs should be red).
- Apply Phosphatase.

See how you can not religate this fragment? No phosphates!

- Click on 'Add Fragment'
- Select 'PCR Fragment' and click on 'OK'.
- Click on 'Load DNA' and load pUC19
- Select one of the M13 primers and one of the REV primers
- Click on the fragment in the listbox in the middle or on the frag ment in the drawing.
- Click on the 'Digest' button at the bottom.

Yes, you are right: this is a button only visible on the tabsheet for PCR-fragments.When you click on it a window opens, giving you the opurtunity to digest the PCR fragment. It must look familiar; it is very much like the upper part of the tabsheet for a digest-fragment. However, the fragment is now linear.

- Select the RE *Taq*I and select the small *Taq*I - *Taq*I fragment in the middle (39-69).
- Click on 'OK'

You did it! Another fine clone. I do not think any other normal cloning scemes will give you any trouble if you managed to come this far. So, enjoy.

The programs under Windows

There are no real programs under windows. Here you can order your open windows in AiO or hop to a certain window by clicking on its name. The cascade command was rewritten, it is not the usual command as it is implemented in most text-editors. The open windows are sorted on the sequence they contain. This means that all windows that contain pBR322 albeit the editor, the plasmid map or a digest are grouped together as are all the pUC18 windows ect, ect...

The programs under Info

Here are programs that give you information about the program. Most give just that and nothing more, others are more demanding.

Help

Help

Opens this handbook in your favourite PDF-viewer.

Group Manager

Who is group manager

Just the info, who is the GM for your group.

System Manager

Who is system Manager

Again just info. The SM is the guy who is to be blamed for things going wrong with AiO that have nothing to do with the program but with protection of drives, firewalls and things like that.

Change GM

Change Group manager *

Only a GM can change the GM (and will thereby loose her/his own privileges).

Download

Download Info

Just info, when was what downloaded.

Bug Report

Bug Report

This is another nice one. Type in anything you ever wanted to say to somebody who programs things like AiO and click on 'Send'. Seriously, please, please give me some response, if there is something going wrong I want to know.

Homepage

Homepage

Just a link to the AiO homepage.

Miscellaneous

There are some screens that show up only at certain times or if certain events take place.

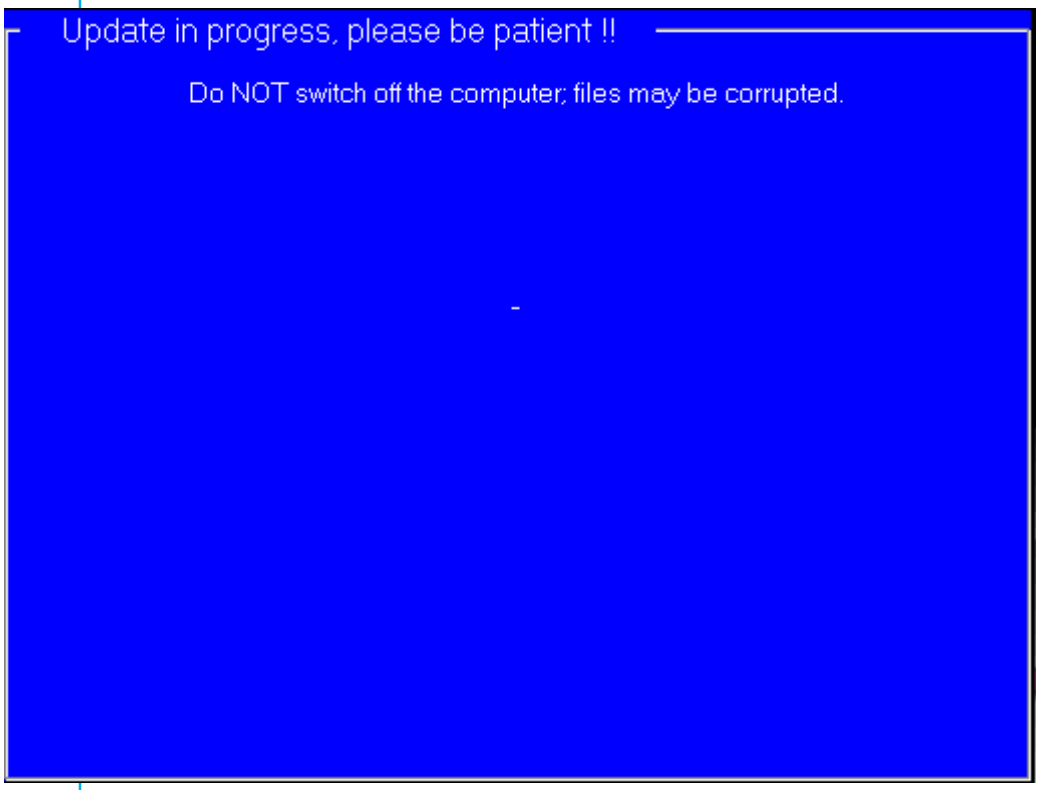


Figure 49. The update window.

In Figure 49 you can see the window that is displayed when AiO updates itself. If you can see this window something has probably gone wrong. Normally it will be shown for far less than a second.

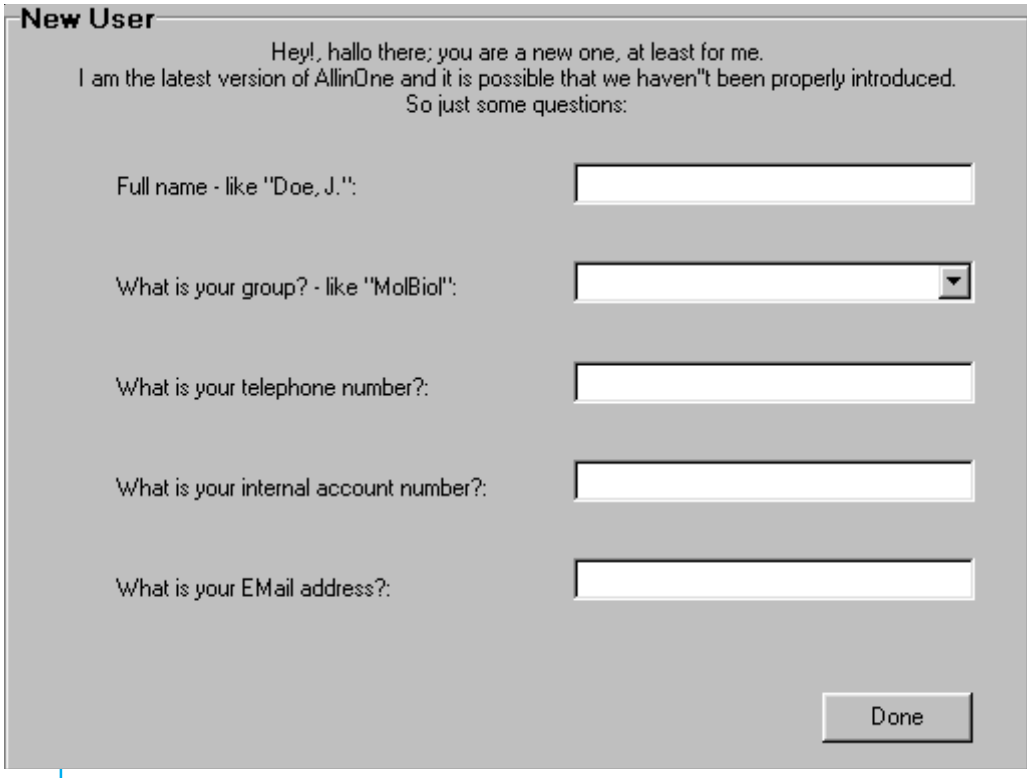


***Figure 50**. The 'New User' window.*

The latter window is only shown when somebody uses AiO that has never used it before. The information is rather straightforward and should not give you any problems. The telephone number asked is only the extension, as the general part of the telephone number is already in the group's description. The internal account number is handy if different members of the same group have different sources of money (grants). The oligos will than be charged to the right account.

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