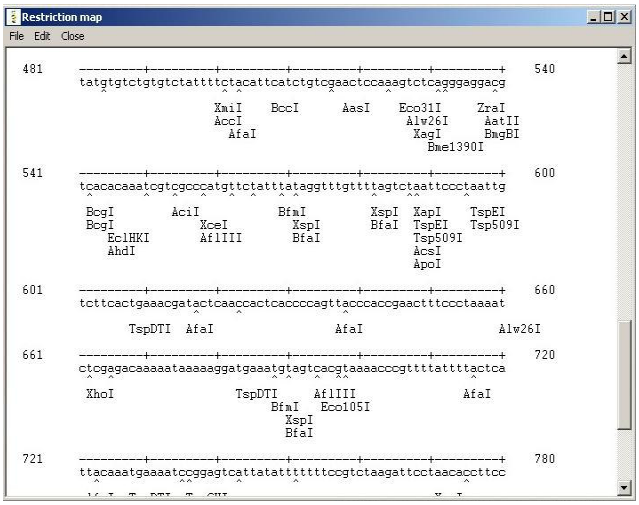
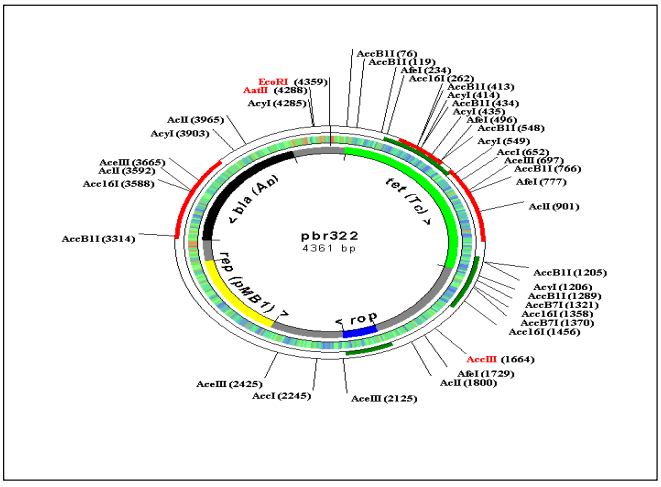
MB DNA Analysis Documentation (printable version)

For MB Version 6.80

(14th November 2005)





Help Topics:

This file was last reviewed on the 14th November 2005. We recommend you to print this document for a better reading.

1. About the program

MB is a FREE Windows program for DNA analysis.

Key features:

- restriction sites search and mapping
- plasmid/linear DNA draw, with a possibility of changing fonts of annotations and restriction sites on the DNA map and mark unique restriction sites with a specified colour
- plot GC percentage and ORFs for 1 selected frame on the DNA map
- mapping of enzymes' cuts positions on the map
- ability to save all restriction analysis reports into one HTM file.
- multiple sequence alignment (method of hierarchical clustering), various amino acids substitution matrices are included.
- tree building
- amino acids analysis (translation, chemical properties, prediction of the secondary structure of the protein using Chou-Fasman method)
- codon usage table calculation for selected ORFs
- primer design (self-hybridisation, combination of 2 primers and homology search within a given sequence)
- open reading frame search for all sense and antisense frames
- molecular weight calculation for single-stranded and double-stranded DNA
- dot plot (for DNA and amino acids sequences)
- ability to search for 4 promoter sequences (TATA, Pribnow, -35-Region, CAAT)

The program features amino acids library.

All results can be saved in different ways or sent to printer.
The package includes 687 restriction sites (including isoschyzomers) and some test DNA sequences. The database version: 502 from http://rebase.neb.com .

You can easily add new restriction sites and DNA to database. It is also possible to import DNA files from Internet (MB supports GenBank and FASTA formats). See p. 21 for more information.

New version features automatic update function.

MB runs under all WINDOWS systems (95, 98, NT, Me, 2000, XP). There *may* be a Linux or Mac support in the future, but I still do not know. Please write me an email, if you want these systems to be supported.

If you have some questions or critics, I'll be appreciated if you write me (see "Contact information").

Author and developer: Oleg Simakov

2. Features to be considered in the next version

- promoter analysis, gene prediction

You can see the progress of the development on the following page:
http://www.stud.uni-karlsruhe.de/~uzbar/downloads/todo.html

3. Program Files

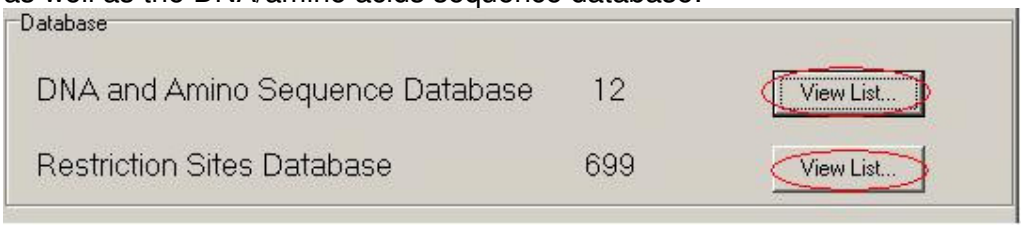
The following table represents the basic structure of program files.

Directories	**Files**
<MB>	"MB.EXE" - program file “README.TXT" - Readme file “MB Homepage” – a link to the homepage of the program
<MB\DOCS>	“mbhelp.pdf” – main help file “index.htm” – short info on the features of the program
<MB\AA>	amino acids pictures and data file ("aa.dat")
<MB\LIBRARY>	directory with DLL and *.wav files, needed to run the program
<MB\DATABASE>	- DNA files ("*.PRT") with 5'-3' sequences (nucleotides are written in small letters!) - Amino acids sequence file (*.AMI) containing only AA sequence in “one-letter-code” - Restriction sites database ("resenz.dat") with 5'-3' nucleotide sequences in small letters - Description file of DNA/amino sequences: "PROTEIN.0". You can edit it as you wish. - “PK.DAT”-file contains pK-values for charged amino acids and used for pI estimation - "SEARCH.0" – this file contains search list of restriction enzymes. It is used in restriction analysis.
<MB\OPTIONS>	- "CONFIG.INI", “PARAMETERS.INI” - configuration files - “plasmid_map.txt” – this file contains settings for the DNA map. The value in the first line represents the height of the map in pixels. The value in the second line is the width-value. If you cannot see all restriction sites on the map, then increase the numbers. This will enlarge the size of the map and it will become possible to see all annotations. - “performance.txt” – see Chapter “Configuration”
<MB\REPORTS>	- All reports have extension "REP" or “HTM” <ANY DIRECORY NAME> - exported restriction analysis report files
<MB\UPDATE>	This directory contains “plugins.installed” file. It is where the names of all the plugins, which you download through automatic update are stored. The program can then determine which plugins are already installed on your computer, so you will not download the same plugin for the second time. You may also discover some temporary files in this directory, they are used by the automatic update.
<MB\PLUGINS>	Directory for plugins (external programs, usually with *.dll extension)

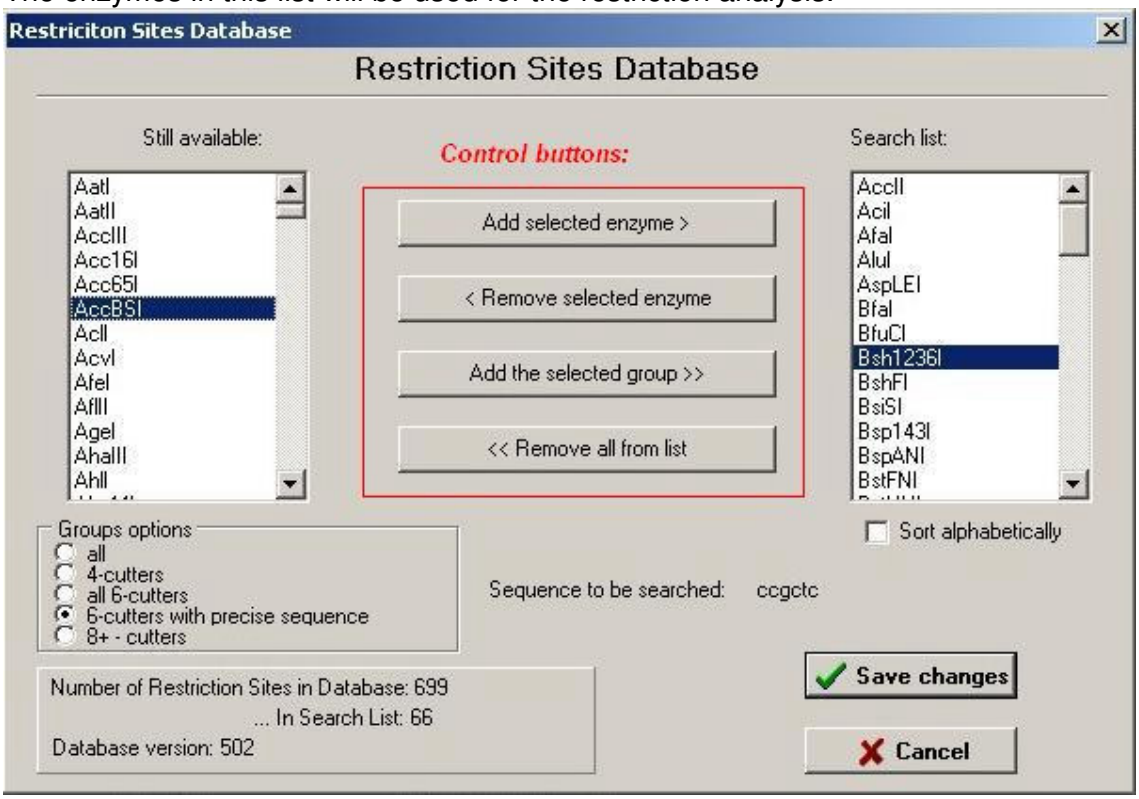
4. Recognition sequences, DNA and amino acids Databases

The explanation on the file format for the sequence can be found under “How to add new sequences to the database”-chapter.

Recognition sequences database is accessible from the main panel of the program, as well as the DNA/amino acids sequence database:



Restriction database contains all restriction enzymes’ recognition sequences. The enzymes can be sorted in 4 groups: 4 nt cutters, exact (no ambiguous nt) 6 nt cutters, all 6 nt cutters and cutters with more than 8 nts.
You can then add the enzymes to the search list by clicking the “Add to list buttons”. The enzymes in this list will be used for the restriction analysis.

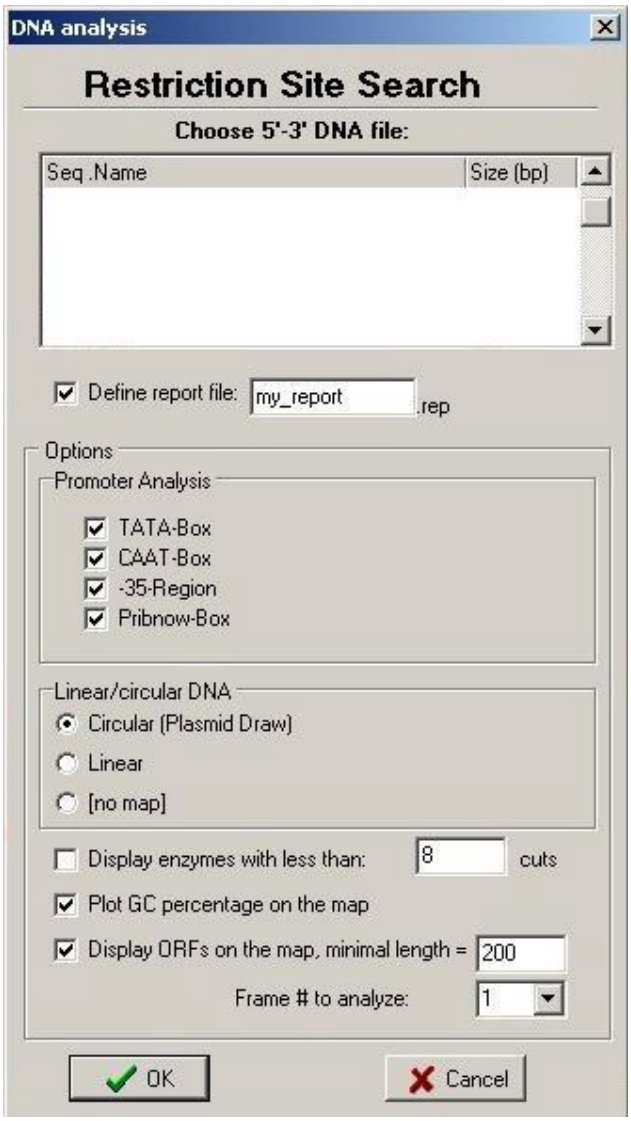


DNA/amino acids sequence database contains all DNA files (file extension .prt) and amino acids files (.ami). Other file types will not be detected. To view the sequence, please click on “Show Sequence” button. In the sequence window it is possible to edit the sequence.

5. Analyzing DNA in MB

5.1. Restriction Analysis

5.1.1. Basics



1. To launch this tool go to: Main Menu – Analysis – Restriction Analysis.

2. In appearing window choose a protein file to analyze.

3. Choose some promoter sequences to search for (TATA-box, Pribnow-box, -35-Region or CAAT-box).

If these sequences were found, the program calculates the approximate position of transcription regions.

4. Choose whether to create a plasmid or linear DNA map.

5. Check the box “Display Enzymes with less than … cuts” to limit the number of enzymes which will be displayed on the restriction and DNA maps.

6. Check the “Plot GC percentage on the map” to be able to see the GC content of every 10 bp block for the sequences under 7000 bp. If the sequence is longer, than the program will adjust the length of the read fragment. The program will assign a specific color to every percentage.

7. Check “Display ORFs on the map” box to be able to see the ORFs on the DNA map. You will have to define a minimal length (200 by default) and a frame, which is going to be analyzed (1, 2 or 3). The sense and antisense ORFs will be plotted on the map in different colors (by default: sense is green and antisense is red, but you can change the color in the options)

8. Click OK button

The program will count the bases and their percentage.
Melting temperature of the DNA will be calculated using the following formula:

Tm = (0.41*(nc+ng)/length)+59.9-600/length

“nc”, “ng” are the amounts of cytosine and guanine
“length” is the total *length* of DNA in nucleotides

The program will search for restriction sites, which you have added to the search list (for this purpose click on the button 'View list…' to see the restriction sites database on the main panel, there you can manage it). The “main report” (NOT restriction map) will be saved to the file you have previously defined from the analysis window.

If some restriction sites contain ambiguous bases (for example if the enzyme recognises either a A or a G, etc. at some position), they are being coded according to IUB-IUPAC standard code:

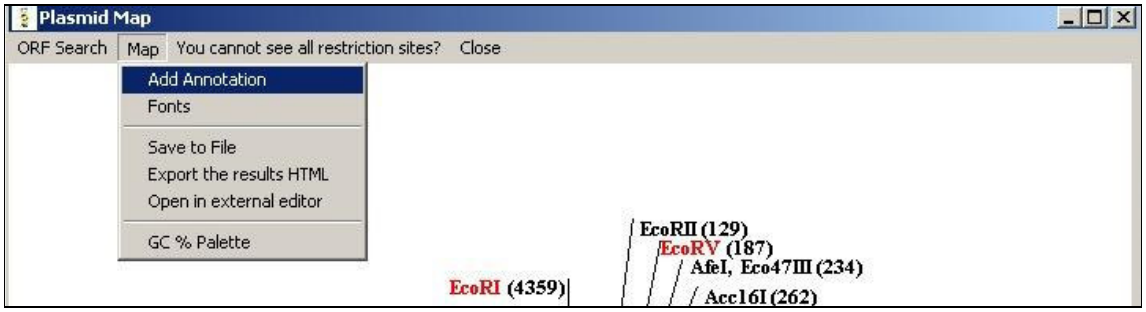
Code	Bases
a	A (Adenine)
c	C (Cytosine)
g	G (Guanine)
t	T (Thymine)
r	A or G
y	C or T
s	G or C
w	A or T
k	G or T
m	A or C
b	C, G or T
d	A, G or T
h	A, C or T
v	A, C or G
n	A, C, G or T

Please note that only restriction sites “positions” are given in your report, and not the cuts positions of enzymes.

5.1.2. Vector/linear DNA Map

If you have chosen to create a plasmid/linear DNA map, then a new window shall appear.

You can add annotations to the map by clicking “Add annotation”.



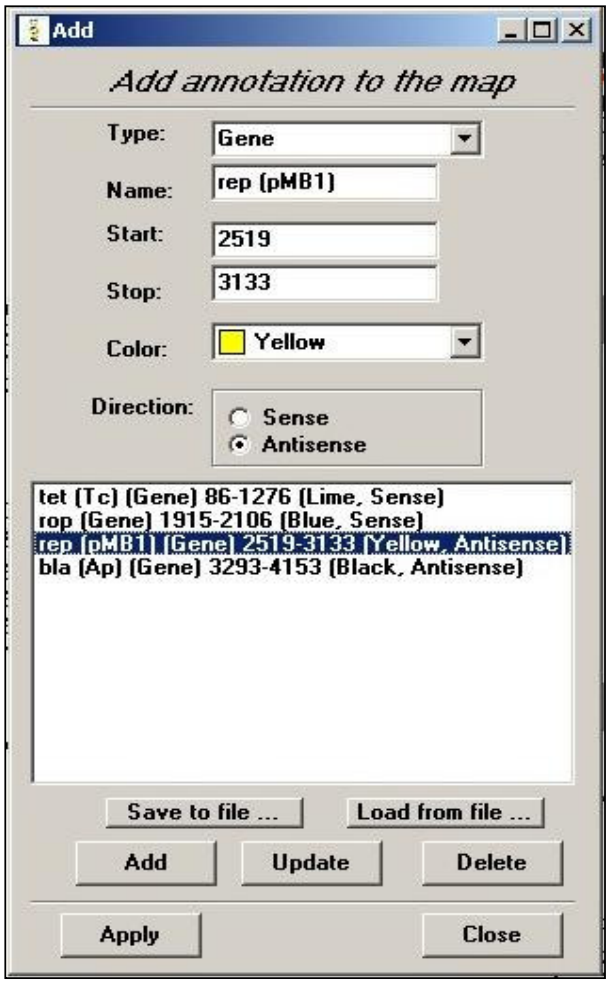
Then you shall specify whether to add a promoter/origin or a gene to the sequence.

Please note, that the start positions are always lower than the stop positions and maximal value of the end nucleotide is the number of the last nucleotide of the sequence.

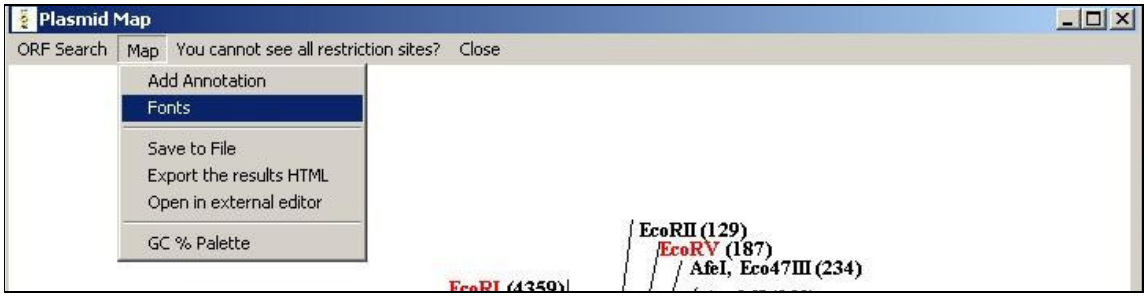
You can also save your annotation list to a file, by clicking “save to file”. Before saving please note, that you need to specify your file’s extension (like “annotations1.TXT”) to be able to load it again.

When loading a list from a file please be sure that none of the given positions extent the maximal length of DNA.

MB does not check the values, so you can get bad results.



To change fonts please click on the “Fonts”-Menu item from the “Plasmid Map” window



Please remember that it absolutely necessary to have some annotation on the map, before changing their fonts properties.

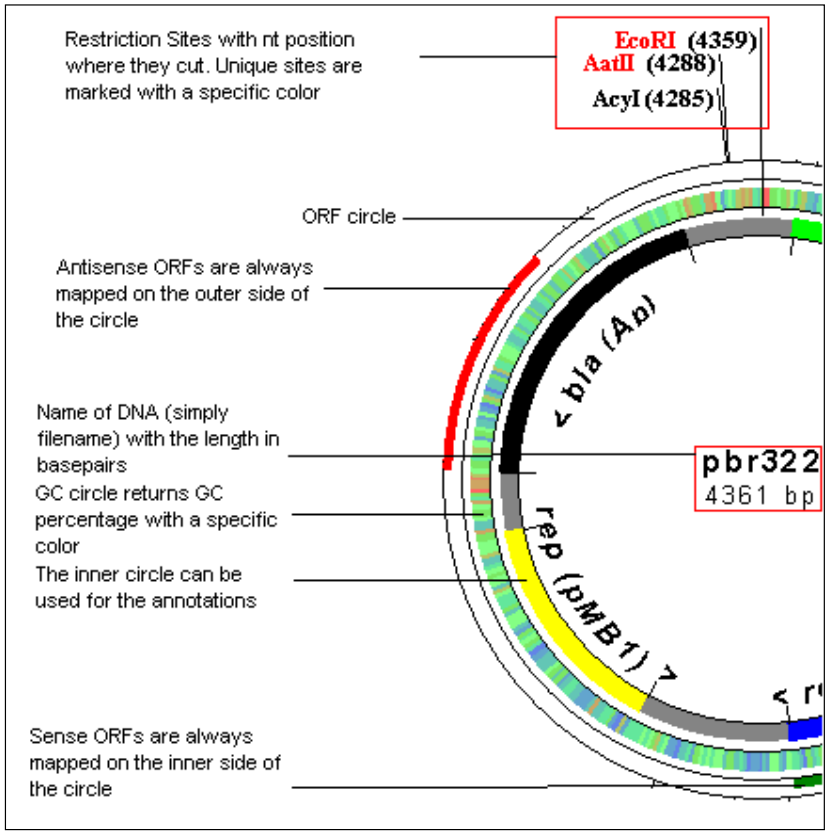
From the window which will appear, you will be able to change sizes, styles and spaces between the characters only of the annotations. To change parameters of the restriction sites fonts, please go to “Options” Menu – “DNA Drawing” Tab.

If you get a problem, that not all restriction sites can be displayed, then refer to “Troubleshooting”-chapter at the end of this manual.

To view the GC palette and the colors definitions of the ORFs please go to: “Map – GC Palette and ORF Colors”. In the window it is possible to copy the GC palette to the clipboard for further editing in an external editor.

5.1.3. Legend to the DNA map

People, who use MB for the first time, may be a little confused about the appearance of the DNA map. So here is an explanation on the information, which such a map can contain:



Linear DNA map has got the same structure.

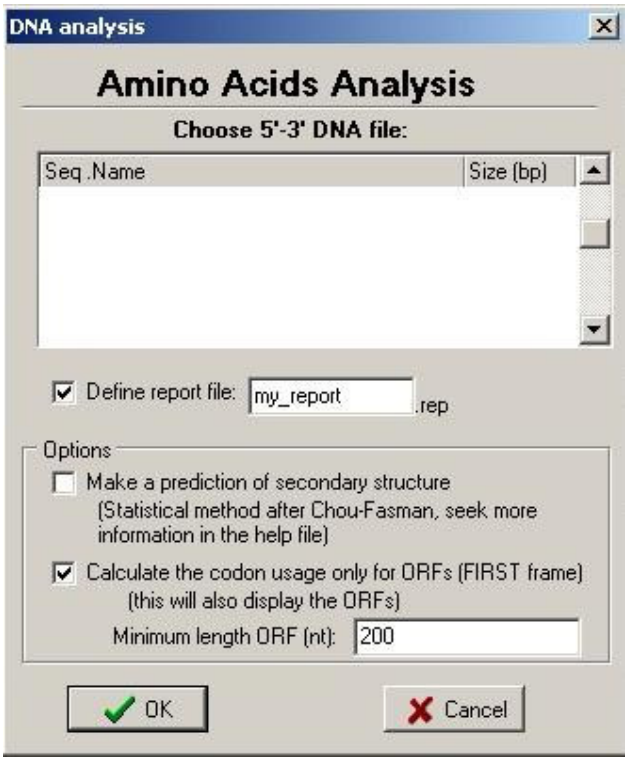
5.1.4. Exporting reports to Microsoft Excel

You can export the list of restriction sites and their cuts positions to Microsoft Excel. A file named “cuts.map” in the “DATABASE” directory contains all the information. You have to open this file in Excel, choose “space” for the column separation. The data will be then imported and you can sort it afterwards at your wish.
Please note: the cuts.map file is a temporary file. This means, every time you make a restriction analysis it will be overwritten. So please be aware of that.

5.2. Amino Acids Analysis

5.2.1. Basics

To launch this tool go: Main Menu – Analysis – Amino acids analysis.



In appearing window choose a DNA file to analyze.

Set the name of the report file.

Choose whether to make a prediction of the secondary structure (after Chou-Fasman, see below).

Check the "Calculate the codon usage only for ORFs of first frame)" to get the codon usage table only for the found ORFs. This will also display the translation of the ORFs in the "translation" results. Please set the minimal length of such an ORF.

Otherwise the translation window will contain the translation of the entire sequence.

The main report with the codon usage table and amino acids count is always saved to the file, which you have previously defined in the starting window. Other reports (like translation window, secondary structure prediction) should be saved separately.

5.2.2. Calculating the properties of a protein

The amino acids are dived into 4 groups:

- hydrophobic: alanine,valine, phenylalanine, proline, methionine, isoleucine, leucine
- charged: aspartic acid, glutamic acid, lysine, arginine
- polar: serine, threonine, tyrosine, histidine, cysteine, asparagine, glutamine, tryptophan
- glycine: is only glycine

The program counts all these residues. It also calculates the molar mass of the protein and the maximal possible number of the disulfide bridges.

5.2.3. Predicting the secondary structure

The graph, which will be displayed after the analysis process, shows the amino acids sequence with the “propensity” of being in one of the 3 known configurations.

A propensity of more than one means that the amino acid is more likely to be in a given configuration (in other words to be helix-, sheet- or loop-forming).

You can then apply the Chou-Fasman method to determine the configuration:

1.) A cluster of four helix-forming residues out of six contiguous residues will nucleate a helix. The helix segment propagates in both directions until the average value of propensity for the alpha structure falls below 1.00 for a tetrapeptide. The average values can be approximately determined from the graph. Proline however can only occur at the N-terminus of an alpha helix.
2.) A cluster of three beta sheet formers (propensity > 1) out of five contiguous residues nucleates a sheet. The sheet is propagated in both directions until the average value of propensity for the beta structure falls below 1.00 for a tetrapeptide.
3.) For regions containing both alpha and beta forming sequences, the overlapping region is predicted to be helical if its average value of propensity for the alpha structure is greater than the average value of propensity for the beta sheet.

You can use this method “manually” to determine the secondary structure from the propensity graph. The method itself is not implemented in the program due to its inaccuracy: its reliability is only 50%, in the best case 80%.

5.2.4. Translation window and ORFs

It is possible to search for sense first frame ORFs. All detected ORFs will be displayed in the translation window. The codon usage will be calculated only for the region of the DNA, which is occupied by the ORFs.

It is also possible to make a separate codon usage count for only one selected ORF. For this purpose, seek the “Extract ORF sequence” window, choose an ORF and click on the “Codon usage for the selected ORF” button. The extra window with a codon usage table has to be saved separately.

5.3. Dot Plot

To launch this tool go: Main Menu – Analysis – Dot Plot.

Select 2 DNA or amino acids sequences, you want to analyze. We recommend you to analyse DNA sequences of up to 10000 bp, because huge sequences take too much more time analyze.

Click on the map or hold the cursor to display the current position.

5.4. Molecular Weight Calculator

To launch this tool go: Main Menu – Analysis – Molecular Weight Calculator.

In appearing window choose the DNA sequence to analyze, set limits (start position, stop position), and choose option whether to calculate molar weight for double-strand or for single-strand DNA.

As a result you will get a molar mass of DNA in gram/mol.

The results will be saved to "[date].rep" file.

5.5. Open Reading Frames Search (Find ORF)

This is a new feature from version 6.40.

To launch this tool go to: Main Menu – Analysis – Find ORF.

Select the DNA file first, then choose whether you want to search for 6 (antisense and sense) or just 3 (sense) frames and the minimal length of frame.

The program will list you all ORFs, please do not forget to save the results, if you wish to.

5.6. Isoelectric Point Calculation (pI)

This is a new feature from version 6.50.

To launch this tool go to: Main Menu – Analysis – Calculate Isoelectric Point

Please select the amino acids sequence first, then click “OK”. The amino acids sequence should contain only one-letter-codes, no spaces. For “stop-codons” you may use “-“-sign.

Example:
File “test.ami” (name and extension may vary):
LTYILQNTERSSF-KRVKRVNCVSHGECLRVGSSGYVGWR-LGQHQLSDVDQ-C-QNAVCQINLIQIMCIYNSKCNMNKTNMNIFTGKGEGE-LEDVMF

If you have only nucleotide sequence of peptide and you want to determine its pI, simply translate the sequence to amino-acids-sequence using “Sequence Translator”-tool in Main Menu – Extras. The file will be created with extension .ami, It will contain you AA-sequence.

Results will be shown in form of graph, by wish, it can be saved to Bitmap-file (*.bmp).

Please note: if the pI-value is not being displayed in the results, you are to change some settings (s. 5. “Options/Configuration – Isoelectric” for more information).

The algorithm of isoelectric point estimation is following (taken from David L. Tabb):

1.) MB counts the amino acids residues, which possibly affect the pI value: lysine, arginine, histidine (basic side chains), aspartic and glutamic acids, cysteine, tryrosine (acidic side chains). N- and C-termini affect also the total charge of the molecule.
2.) Charge determination.

$$Z \text{ (total charge)} = Nterm + Cterm + K + R + H + D + E + C + Y$$

The letters with Nterm and Cterm represent the charges of every residue or terminus. The program assumes, that every residue is independent from another (approximation).

3.) pI determination. To calculate whether the group takes positive or negative charge is determined by its pK value. MB uses following data in “PK.DAT” data file in DATABASE directory:

Positive charge		Negative charge	
Group	pK-Value	Group	pK-Value
amino	9.6	carb	2.4
lys	10.5	asp	3.9
arg	12.5	glu	4.3
his	6.0	cys	8.3
		tyr	10.1

The next step is the determination of concentration ratio (CR). For positive groups this is:

$$CR = 10^{pK-pH}$$

For negative groups:

$$CR = 10^{pH-pK}$$

Once the CR is generated, the partial charge (PC) is calculated using:

$$PC = CR/(CR+1)$$

The summation of the partial charges is done using the formula for Z (total charge, see above). For C-terminus, aspartic acid, glutamine, cysteine and tyrosine the PC is defined as negative, while charges from N-terminus, lysine, arginine and histidine are assumed to be positive.

The pI value is then the pH where the total charge of the molecule is zero.

You can edit “pk.dat” file as you wish, but please keep the table structure. We recommend to backup the file.

5.7. Primer Design

It is possible to calculate the melting point of primer using the following formula:

$$Tm = 81.5 + Na + 0.41*(GC) - 675/length - 0.65*[FORMAMIDE]$$

Where $Na = 16.6* \log_{10}([Na])$

GC = GC Percentage in the sequence
Length = length of the primer in nt
[FORMAMIDE] = formamide concentration

The calculated primers’ combinations contain the highest number of complementary bases in a row.

You can also search for homology within a given sequence (template). This means, that the program will try to add the primer to specific positions of the template and check whether the hybridisation is possible. You can adjust the percent of complementary bases (80 by default).

6. Additional Features

6.1. Sequence Translator

Sequence translator tool can be used for translation of mRNA to 5'-3' DNA and 3'-5' DNA to 5'-3' DNA. You can also use it as “protein encoder” (translates the sequence of amino acids residues to 5'-3' DNA sequence). The last feature is not very clever to add, because you get just one of millions possible DNA sequences from protein, but in some cases it can of some use.
You can also translate DNA sequence to amino acids sequence. This will create an output file with AA-sequence in database directory with extension “*.ami”.

In each case you have to enter the name of the file in which the sequence will be written.

6.2. Amino acids library

This is just a library of amino acids. It does not do anything with analysis results.

You can choose amino acid by typing in DNA sequence or by clicking on the list.

The program will display the properties of the substance.

The data file for amino acids 'aa.dat' is found in your 'aa' directory. The jpg files of amino acids are also found in this directory.

6.3. Read aloud

Choose any DNA sequence from DATABASE directory and you can make the program read it for you.

6.4. MB Browser

Use it to explore the Internet!

6.5. Plugins (External programs)

External programs (plugins) are placed in a special folder called “PLUGINS”. They all have a *.DLL file extension and are automatically recondized by the program at the startup. If you are experiencing some problems starting the plugins, then please write me (see contact information).

Plugins have their own help files, which can be updated separately.

7. Configuration

7.1. Protein

Use it to configure the display-form of the amino acids sequence (if yes in which form: full name (Alanine) or 3-letter name (ala) or, may be, 1-letter name (A).

7.2. Restriction

[Count bases before restriction sites search]. By clicking this option you will enable the base count in "Restriction Site Analysis" feature.

[Open report files with Windows Notepad]. If you wish, the program will open the reports with NOTEPAD-program (if installed on your computer).

[Computer performance:]. Here you can choose between “high”, “middle” and “low”. You need to choose high level of performance, when you are analysing the sequence with more than 200 restriction sites in search list. The process will go a little bit slower, but the program wont crash. If you choose “low” instead, then the program wont be able to handle such a huge amount of sequences and you will get “Access violation error”.
Analysing sequences wit only 50 or so recognition sequences does not require huge arrays, so you can choose middle or low levels. If you still get “access violation”, then choose “high”.

[Restriction mapping]
By default the restriction sites and enzyme cuts mapping is enabled.
Maximal number of sites pro line of map means just the same thing as in “computer performance”. If you are analysing sequence with 200 enzymes, then the program needs to plot them somehow on the map. So it needs more memory to store the information. Enter the number of about 200. Note: huge numbers can cause loss of some seconds in analysis.
Maximal number of sites pro line map is 300. By default it is 50.

[Update]
The url mentioned is the page for MB update.

7.3. Isoelectric point

[Set pH changes value (max 1):]. Advanced 2 options consider pI-calculations. The way, MB calculates pI-value is quite simple: The program calculates the total charge of peptide at given pH. Afterwards, pH-value is being enlarged by the number, which was set in advanced 2 options. The process lasts till pH reaches 13. If you want get a (relative) precise result, please enter the number of about 0.0001.

Please note: By entering larger numbers it may happen, that the pI would not be calculated, so its recommended to leave the default setting unchanged.

[Mark pI-point with [X] on the graph]. You can also choose, whether you want to display the green X-marker on the graph

7.4. Update

[Update URL]
The URL mentioned is the page for MB update.

[Update Reminder]
It is possible to switch on the automatic update reminder, which will remind you of possible updates in X-given days.

7.5. DNA Drawing

[Mark unique restriction sites with color]
Define the color of the unique restriction sites on the DNA map.

[Restriction Font Size]
The size of restriction sites' font

[Style]
You can choose between normal, **bold**, ***bold and italic***.

[Plasmid Map Sizes]
You can change the size of the plasmid map in order to see all of the restriction sites

[ORF color]
Specify the color for the ORFs on the DNA map. By default the sense ORFs are green, the antisense ORFs are red.

7.6. Printer Setup

Here you can manage your default windows printers.

8. How to add new DNA, restriction site, amino acids sequence to database?

To add new DNA sequence...

From the program:
... go to Main Menu - Database - Add new... - ... Protein.
Enter the nucleotide sequence (all characters in file **MUST** BE IN LOWER CASE, NO SPACES, ONLY NUCLEOTIDES: ‘a’, ’c’, ’g’, ‘t’**!**). Then click “save sequence”, specify that the sequence is DNA and click “add”-button. You can also add a description of the sequence. It will be then saved to “protein.0” file in the database directory.

From World Wide Web:
To add new DNA sequence from WWW (file should be in FASTA or GenBank format):

Main Menu - Database - Import … - DNA from Web-Site. In appearing window enter path to import file with sequence. The name of sequence is the name of file under which it will appear in the database. The program will automatically find in resource file the description of sequence (i.e. in case of GenBank this will be the sentence after the word "DEFINITION").

To add new restriction site...

From program:
... go to Main Menu - Database - Add new... - ... Restriction Site.

Fill the fields specifying the name of a restriction site and its sequence. Press 'Add'.

Manually:
Open the “RESENZ.DAT” file in DATABASE directory. Add the restriction site at the bottom of the file. **Please do not change the existing “table” structure.** The number at the end of the line specifies the cut position of the endonuclease.

If recognition sequence of enzyme contains ambiguous bases then they are declared according to the IUB-IUPAC standard code (see 5.1.1. Basics).

To add new amino acids sequence...

1. Possibility. Go to “Main menu – Database – Import – DNA/amino sequence”. Then enter your sequence or load it from file. Click “Save sequence”. Please select “amino acids sequence” on the panel. Your sequence will be saved to “*.ami” file. Therefore in the right MB format: UPPER CASE, all one-letter-characters for amino acids residues, “-“ for “stop”-codon.

2. Possibility. You can add DNA sequence first and then use “Sequence Translator” from “Extras” Menu to get amino acids sequence in MB format (*.ami file)
3. Possibility. Add sequence file manually (we recommend to supply it with *.ami extension, because in that case it can be easily found by the program). The file should contain **only** amino acids sequence and no spaces. All amino acids residues should be written in UPPER CASE. Stop codons should be marked with “-“.
4. Possibility. Some amino acids sequence, which you download from the databases are actually in the right format. So just copy/paste them into the file. But please check, that there are no spaces (“ “) in the sequence, no numbers and all characters are in the UPPER CASE.

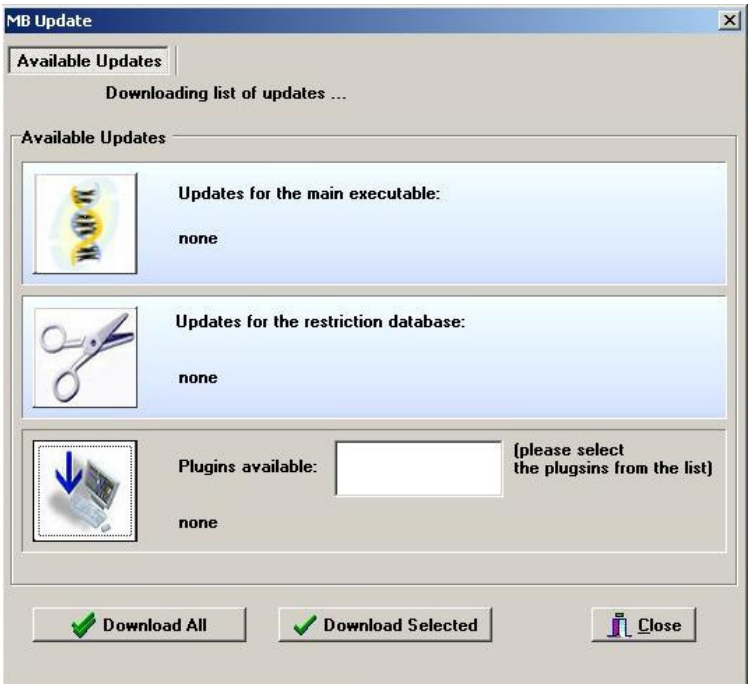
9. How to update the program

To update the program:
go to : Main Menu - Help - Check for updates.

Please note: automatic update within the program is only available in the registered copy of the program (see 8. Registration of the program).

The program will then connect to the web-site and search for possible updates.

You will see following window from which you can choose which of the updates you want to download:



Choose which of the updates you want to download, click on the download button and follow the instructions.

The program can actually install the updates automatically. You can try this out, but sometimes it may not function properly. So here is the manual way of the installation:

After the download is finished, close the program and remember the following:

If you have chosen to download an update for the main program executables, then execute the .exe file from the UPDATE directory and enter the path to the MB folder (like C:\programs)

If you have downloaded a newer version of ResEnz.DAT (restriction database), then copy this file from the UPDATE directory to the DATBASE directory overwriting the existing file.

If you have downloaded a plugin, then you can find it in the UPDATE directory. You can then install it to any other folder. The name of the plugin is saved in “plugins.installed” file, so the next time you start the automatic update it will not inform you that the already installed plugin is available for download.

In any case, please RESTART the program for the changes to take effect!

You can also download the updates from our web-site: http://www.stud.uni-karlsruhe.de/~uzbar following “Updates”-Link. But this will only list all possible downloads without providing you with the information, whether your version is up-to-date.

10. Registration of the program

- Why?

MB is a free program and it will remain to be so. By registering your copy you help us in the further development of this software. Registration also keeps us in contact with our users.

Registration of the program is absolutely free of charge.

Your advantages:

- By registering the program you will be automatically added to our mailing list.
- You can receive technical support
- You will be able to search for updates from the program ("Check for updates" feature) and automatically install them
- You can contact the authors and ask any question about the program.

- Entering the registration key

After sending the registration form via our homepage, you will get an email with a key (within a few seconds). Simply enter that key in right order in "Enter Key"-tab of "About"–box. Click ok. A conformation message should appear.

Please do not share this key with other people, it is for your personal use only.

If it happens, that an error messages will be displayed after clicking "OK"-button, please contact me at mb-dna@gmx.de.

11. Known problems and troubleshooting

Problems found:

- “Access violation error” during restriction analysis: refer to 5. Configuration (“advanced options”) for detailed explanation
- “Wrong character, process will be terminated” and I/O error 103 during restriction sites search: wrong format of *.prt file you are analysing. Prt-files are DNA files, containing only nucleotide sequence. FORMAT: no spaces, all letters in lowercase, only letters in file are ‘a’,’g’,’c’,’t’.
- Not enough place for all annotations on a plasmid map: You may try to reduce the number of recognition sequences or the font size (see “options”) OR enlarge the map size parameters in the “plasmid_map.txt” file, which can be found in “OPTIONS” directory.

Please report any bugs to mb-dna@gmx.de

You need to supply following information: your name, version number of the application (MB), text of the error message.

12. Contact information

You can write me a letter (with your critics or ask questions about the program) to
ileecoli@hotmail.com

Please note, that MB is a “spare-time” project, so please be nice in your criticism.

For updates please visit my web-site: http://www.stud.uni-karlsruhe.de/~uzbar.

If you want to know, when new updates are available, please subscribe to our mailing list at: http://www.stud.uni-karlsruhe.de/~uzbar/mailing.htm

13. Recent changes

MB Version 6.80 changes:

- hierarchical multiple sequence alignment, tree building
- removed "sequence alignment" from the main application
- stabilized performance: it is possible to cancel a long working process
- fixed bug: automatic update incompatible on some systems
- fixed bug: duplicated first amino acids residue in the structure prediction results
- fixed bug: sequence translator
- fixed bug: sequence editing disabled
- fixed bug: protein analysis feature
- text properties of the DNA map are saved on exit and loaded at the startup automatically
- fixed problems with text overlapping in DNA map
- dot plot graph for reverse sequence
- much faster dot plot algorithm
- possibility to select a specific sequence fragement for the dot plot
- "sense" and "antisense" ORFs are renamed to "forward" and "reverse" ORFs respectively
- plugin support
- improved structure of the configuration files
- window settings can be saved and loaded at the start

View the whole program history at
http://www.stud.uni-karlsruhe.de/~uzbar/downloads/changes.txt