MANUAL FOR THE PHP SOFTWARE (PhPwin)

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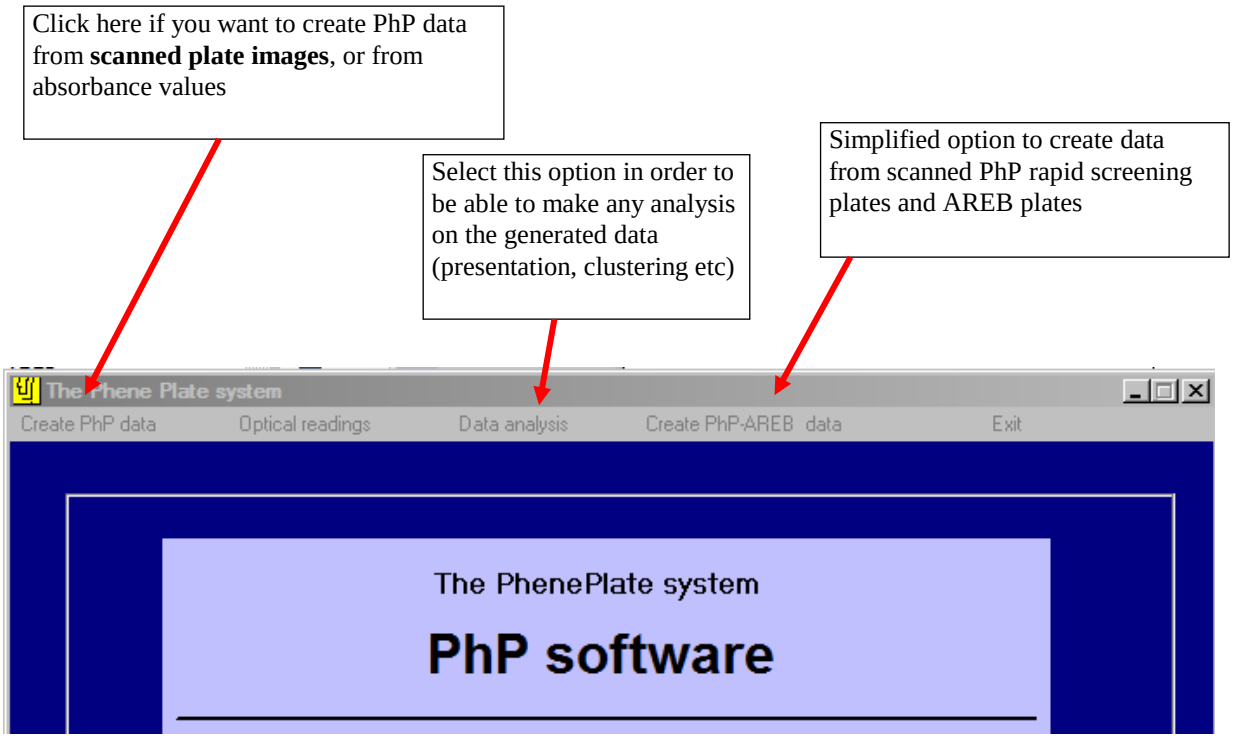
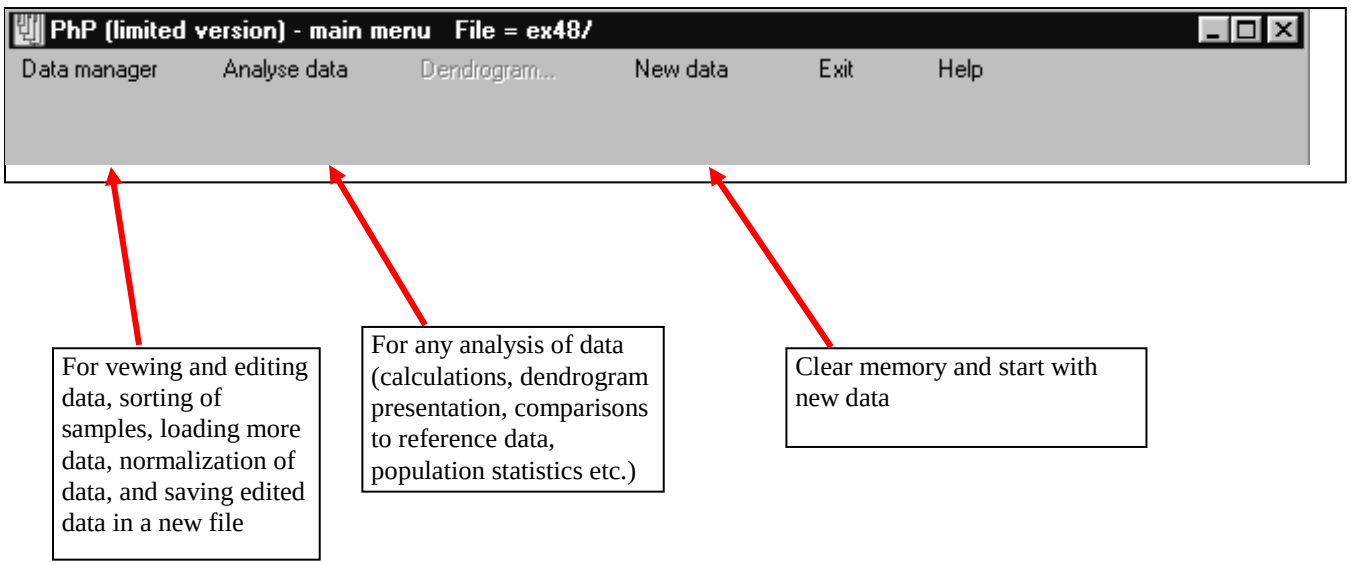
Additional information can be found on our home page www.phplate.se
Especially look at the download page for updates of manuals and software. Also try the power point presentation of the PhP software, PHPWIN.pps, which can be downloaded from the same web site

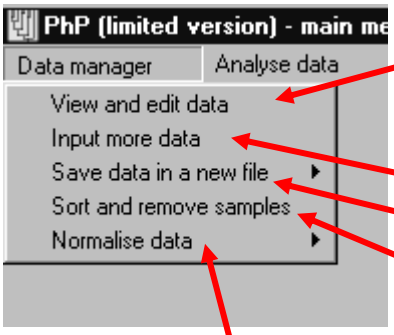
Startup menu



Main menu



Data manager



View and edit data – allows changing data and sample names, deting tests, printing, copying to clipboard and pasting into an Excel sheet etc

This option can also be used to create a reference data base

Input more data – allows input of data from another file

Save data in a new file – allows saving edited or combined data in a new PhP or Text file

Sort and remove samples – allows picking samples and sorting them in the picked order. Note that samples that are not selected will be removed from the computer memory, but will remain in the file

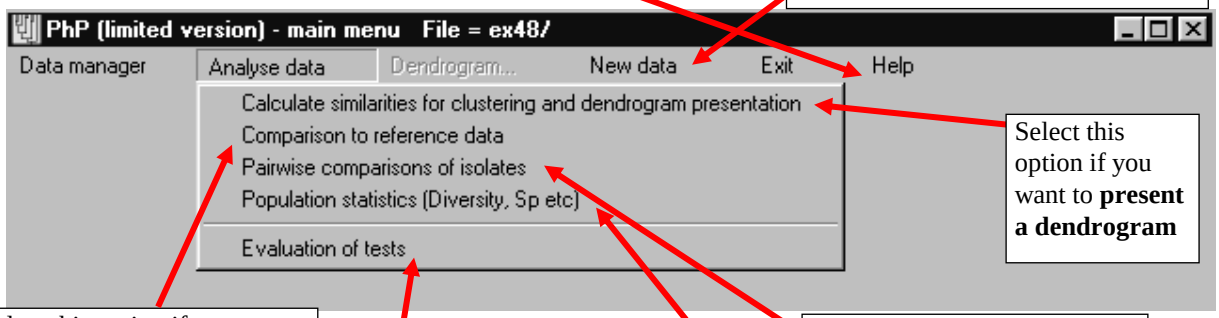
Substrate normalization: Normally negative wells should give OD values of 24-26 (if scanner was used) or 19 – 23 (if a microplate reader was used). If this was not the case, the values can be transformed here, provided that one of the samples in the actual file was a negative control, containing only substrate. If you want substrate normalization, give the number of the sample containing the negative control. The computer will suggest a value for the negative control in the actual file (e.g. 25 if absorbances are slightly high) and if this value is accepted, all data in the file will be multiplied by a factor (20/25 in this case).

Note that the normalisation procedures will not affect the raw data file. If you want to recalculate from the original raw data, you can always do so.

Note! If a name of a sample is printed with an exclamation marks (!) and 'NF' this means that that particular sample gave very weak reactions with the PhP plate, and that that sample can be regarded as non-typeable . If it is printed with !nf, it means weak reactions, but still valid data. However, weak reactions may result in a decreased reproducibility for that sample

Calculation menu

After data have been read into the computers memory, select 'Analyse data' for analysis of data. Click 'Help' in order to get some help with the items on the screen



Select this option if you want to start working with new data. Any previously loaded data will be removed from memory

Select this option if you want to **present a dendrogram**

Select this option if you want to compare new unknown data to known reference data (e.g. for species identification)

Population statistics – if you are analysing bacterial populations and have tested several isolates from each bacterial population. Calculates diversity indices and population similarities

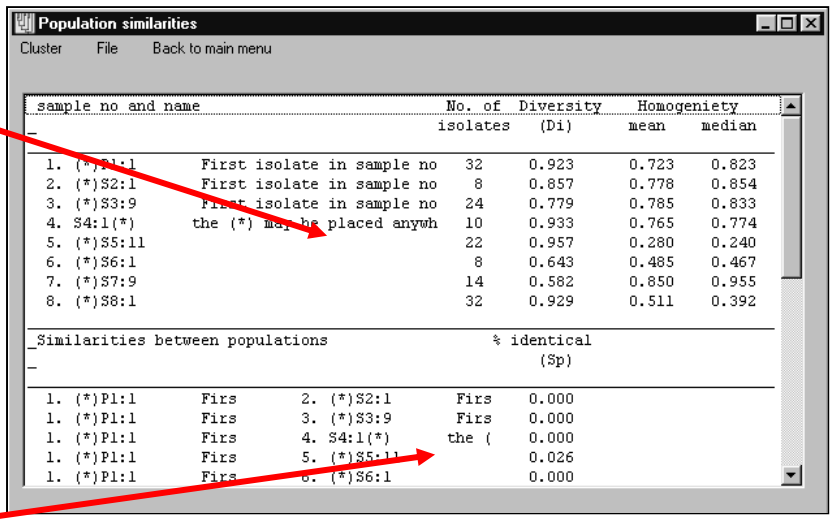
Select this option if you want to view data and similarities from pairwise comparisons, e.g. **for determine the reproducibility of duplicate assays**

Evaluation of tests – if you want to see the performance of individual tests

List of Population Similarities (Sp)

A list of true diversities and homogeneieties (as mean and median similarity coefficients) for each sample (population) containing the selected isolates

A list of similarities between the population, measured as population similarity coefficients

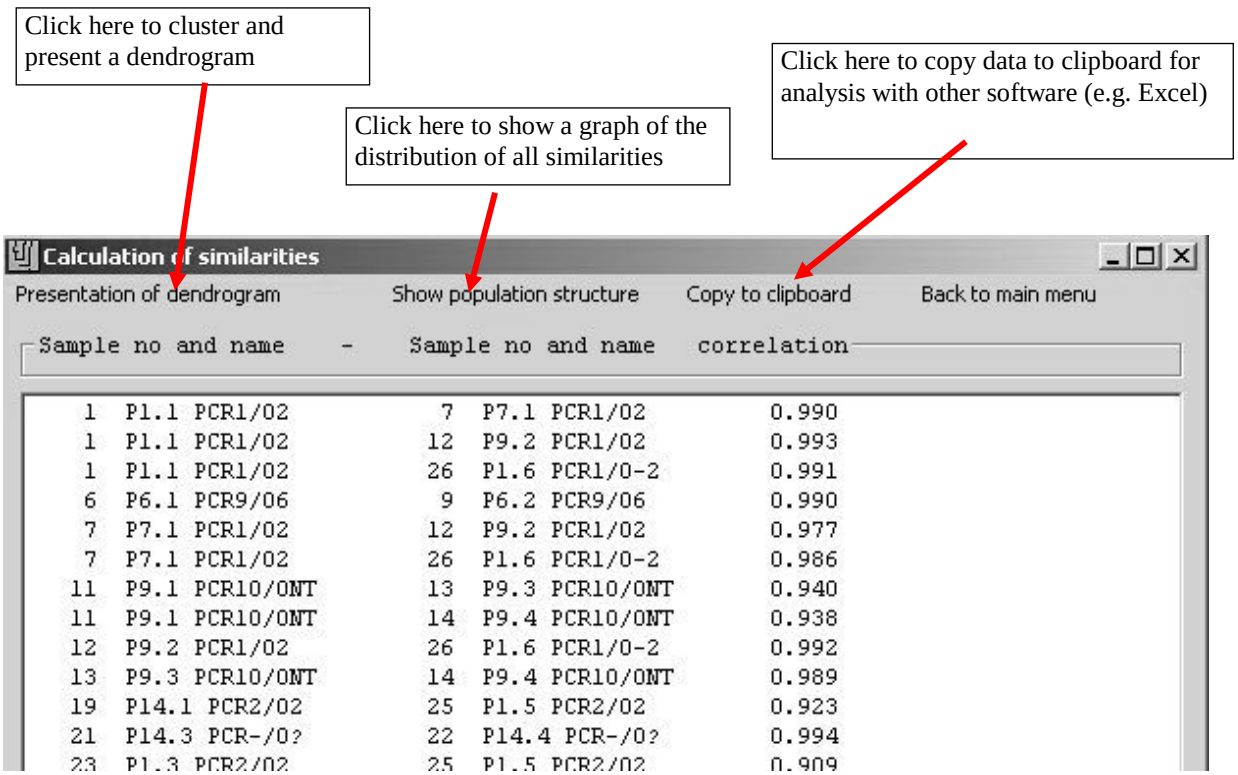


Population similarity coefficients are the proportion of identical strains in two different compared samples (where each sample consists of several isolates).
Example: If 10 isolates have been assayed from sample A and 10 isolates from sample B, and 5 isolates in sample B are identical to isolates in sample A, then the population similarity between the samples is 0.50 (it is not really that simple, but you can check the reference Kühn et al. 1991: Biochemical fingerprinting of water coliform bacteria - a new method for measuring the phenotypic diversity and for comparing different bacterial populations. Appl Environ Microbiol 57(11): 3171-3177 for more info)

Diversity index: A measure on how the isolates are distributed into types.
A value of 1 means that all isolates are unique (single types)
A value of 0 means that all isolates are identical.

The true diversity index is calculated as 1 - (the number of pairwise comparisons yielding similarities above the identity level divided by the total number of pairwise comparisons)

Calculation of similarities



If the list contains too many values, only the first values will be displayed. The text 'Too many values to display' will appear at the end of the list.

Clustering options and Dendrogram

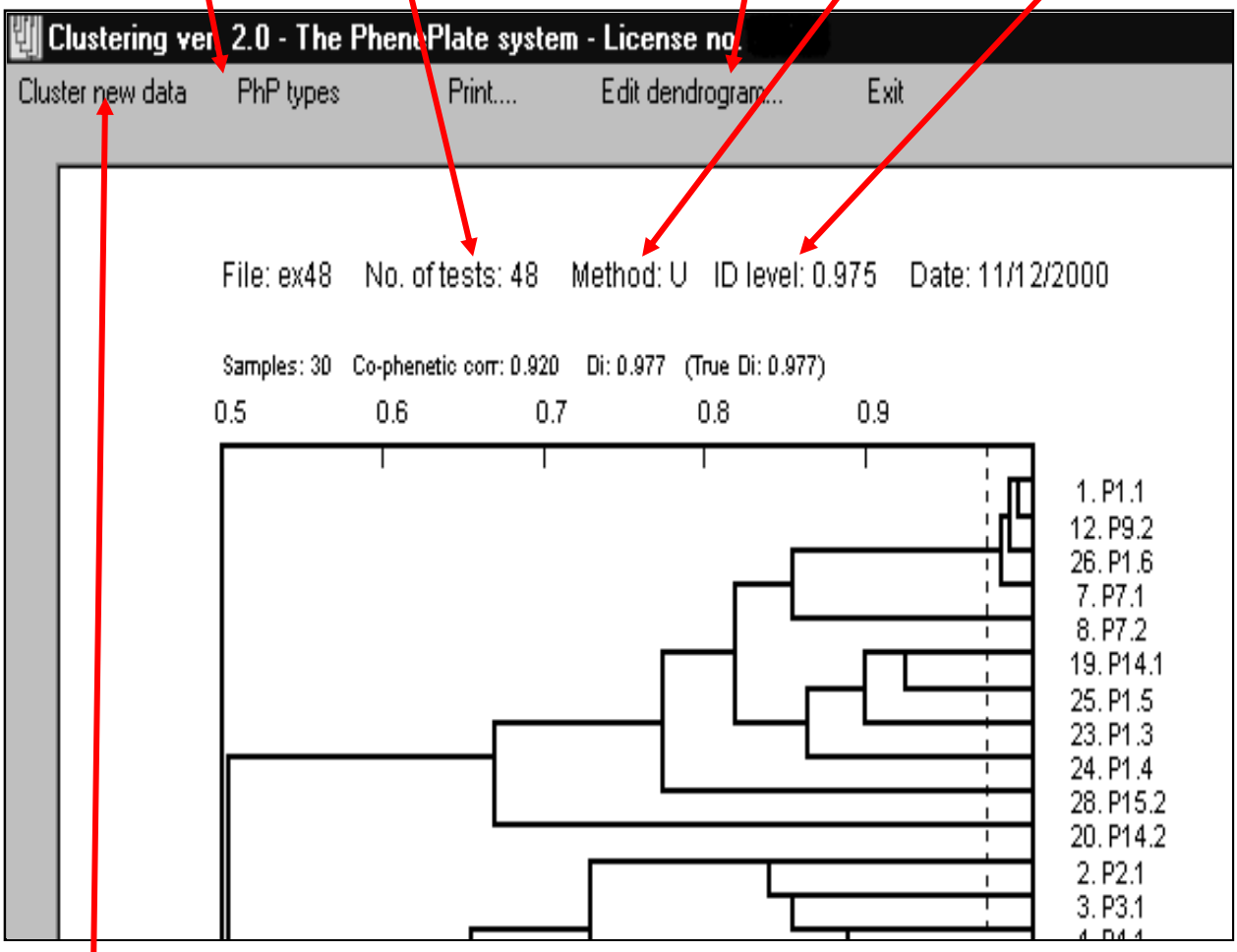
To obtain help with the clustering options,
click on the frame of the option you need help with

This option can be used to set color marks on the samples in the dendrogram, and is very useful for getting an overview on how samples of different origin are distributed. It can also be used to add text to the dendrogram, or to remove items

Gives a list of the types obtained from the clustering procedure

The **Co-phenetic correlation** is a measure on how well the dendrogram corresponds to the data it was created from (see exercises 1 and 9)

The **calculated Di** (diversity index) is calculated from the clustered data. The **true Di** is calculated from the raw data, and is thus a more true value (see above)



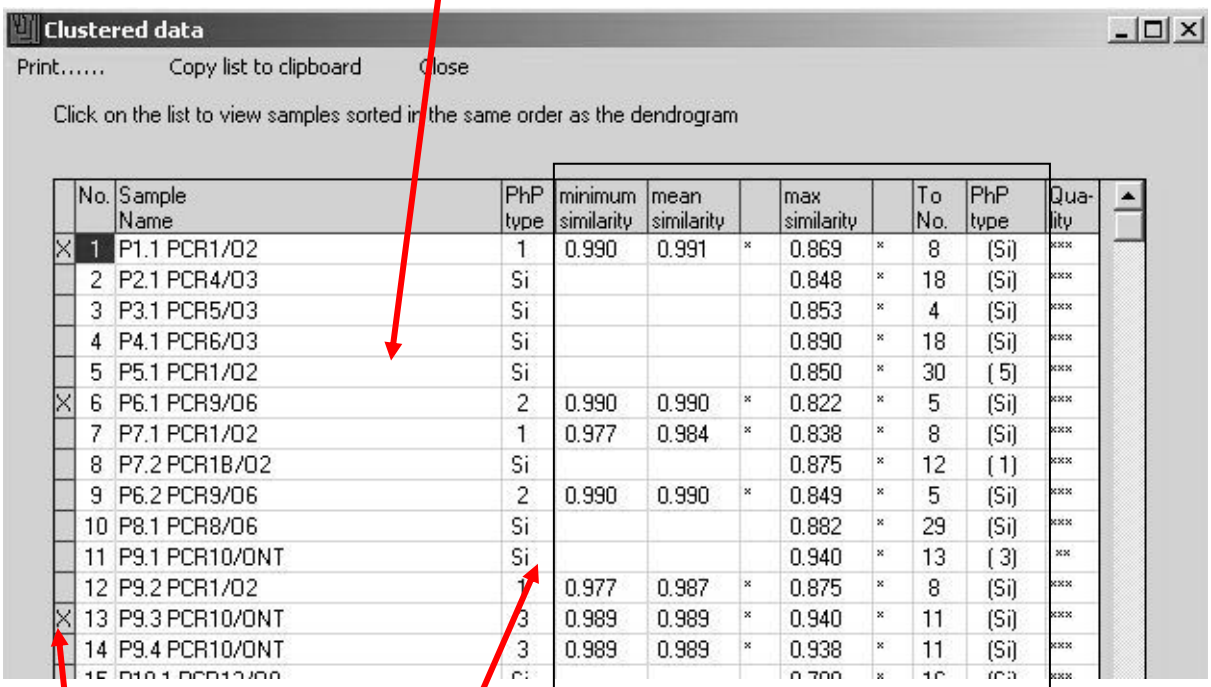
You can cluster different subsets of your data several times and present several dendrograms on the same page. To do this, you first have to calculate similarities of all isolates you want to present on the same page. Alternatively, cluster your data, using two or more dendrograms per page, then minimize the dendrogram page (click - in the upper right corner), go back to PHPWIN software, select a new datafile, and calculate these data for clustering. Instead of selecting Clustering from the menu bar, click on the icon for the last clustering (bottom menu bar in Windows95)

The dotted line indicate the identity level (ID level)

List of phenotypes

The list of phenotypes is obtained from the dendrogram, by clicking ‘PhP types’ on the menu bar. You can view a short list, with only types, or a list containing additional information on the types (see below). You can also display the types directly in the dendrogram by selecting option ‘Print types’ in the clustering options

First the list contains the isolates sorted in the same order as they were input. Click on the list and isolates will be sorted in the same order as they were clustered in the dendrogram



X denotes the isolate that forms the center of each type, i.e. that is most representative for the particular type

Si denotes single (unique) type that is not identical to any other isolate

These quality of each identification is based on these data. They are shown only if you have selected ‘Show a list of PhP types with all information’

Quality of the identification. A question mark (?) indicate that the phenotype of the isolate may be questioned, either due to a too low similarity to isolates belonging to the same type, or a too high similarity to isolates belonging to other types.

*** indicate a good identification, ** and * indicate acceptable identification

Note that this list can also be partly displayed in the dendrogram, by clicking ‘Show types’ before clustering

The first digits denote the number of tests

Data file format

A. Raw data (.dta files)

```
(12) 4*testfile
***  16 TIMMAR
RS15A KLEB
NDDDDFODE[[I
SAMPLE NAME     INFO1/INFO2
WDDDDVVWD\[Q
RS15A DS17
VWVUVVDCVKHU
RS15A PS
QMNHNKMMNY\H
RS15A AERV
UEDUUUSUUHGJ
RS15A SINT
VUDUVVTUUHGJ
RS15A BD
VUVUCUCCUHGW
RS15A NEG
UUTTUTQTTFEH
*** NEXT
***  40 TIMMAR
 1
FEEDEEFDF[\G
 2
VEDDDUUUE\ZN
 3
VUUDVUDCOKIU
 4
OJKDJHIHH[\G
 5
TEEUUVSUJIHJ
 6
VUEUVUSUUHGJ
 7
UUUUBUCBTHGU
 8
UUTTUTQSTFEH
*** NEXT
***  64 TIMMAR
 1
EEEEFEEDF[\F
 2
VEDDDVUUE\\L
 3
VUUEVUCDJJJV
 4
NHHCIIGGG\]G
 5
UFFUUVSUDIIJ
 6
WVFVVVSUUHGK
 7
VWVVBVCBUHGW
```

A header that is selected at the first reading occasion

Text written with bold letters can not be changed, since it steers the data output format

The first 25 digits of each line contains name of samples, and the rest are the data. If option 'Print info' is selected in the dendrogram, the format should be: first 15 digits = sample name; digits 16-25 = two information fields separated by / (See file EX48 [illegible] example)

The 4* here means that plate type no. 4 (PhP-RS) was used.

Here the next reading occasion starts (40 hours)

B. Added data (Biochemical Fingerprints)

```
*12*4*testfile
RS15A KLEB      8  5  5  4  5  5  9  4  6 27 28  7
SAMPLE NAME    22  5  4  4  4 22 21 22  5 28 27 14
RS15A DS17     22 22 21 10 22 21  4  3 16 11  9 21
RS15A PS       15 10 11  5 11  9 10  9 10 27 28  7
RS15A AERV     21  5  5 21 21 22 19 21 12  9  8 10
RS15A SINT     22 21  5 21 22 22 19 21 21  8  7 10
RS15A BD       22 22 22 21  2 21  3  2 21  8  7 22
!NF RS15A NEG 22 21 21 21 21 20 17 19 20  6  5  8
```

Here the next reading occasion starts (64 hours)

If a name of a sample is printed with !NF this means that that particular sample gave very weak reactions with the PhP plate, and that those results are not valid. If it is printed with !nf, it means weak reactions, but still valid data.

If you need to **edit raw data** (because of mistakes when reading data, a plate was read twice, or different readings in different files etc) make sure that there is the same number of samples in each reading, and that the two lines starting with *** between each reading are intact!

Each letter or digit in the dta file represents an OD value. A means OD 0.1, B means OD 0.2, C 0.3 etc. Values above 2.6 are also represented by the corresponding ASCII charachters (e.g. [is OD 2.7)

The raw data file, named with the extension 'DTA', is created when data are read with a microplate reader. When using the old DOS reading software, this file can not be used for any analysis, but must first be converted to a biochemical fingerprint file, named with the extension 'ADD'.

File format - other data than PhP data

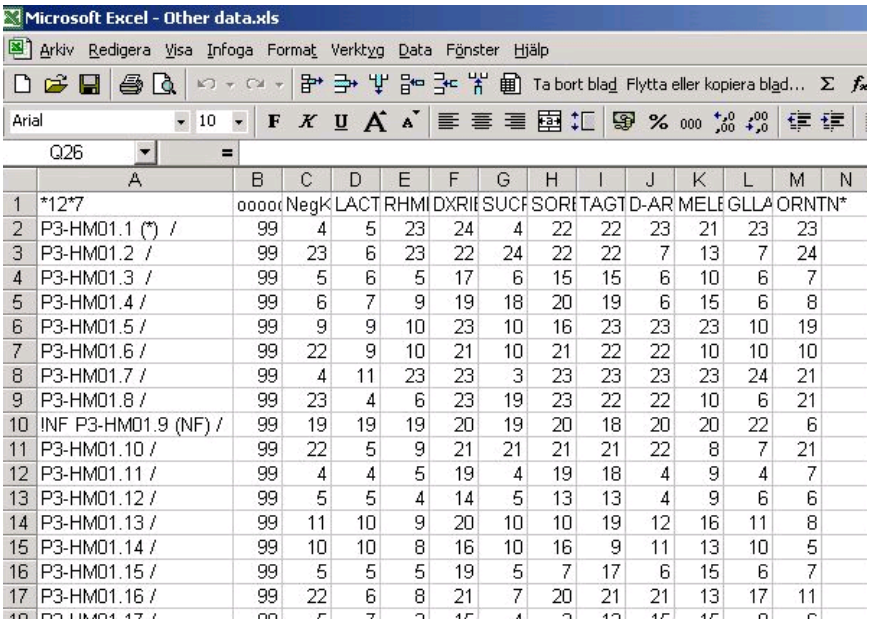
You can calculate on data generated in other ways than from PhP plates, if you follow the instructions below (it is a printout of the file 'EXOTHER.TXT' included in software diskett no. 3).

```
*8  The first digit must be *. Next two digits denote the number of tests
*** any line starting with *** is ignored and can be used for comments
*** spaces separate the test values
*** not more than 48 tests allowed
*** the test values must be integers between 0 and 98
*** 99 denotes missing data
*** Sample no. 5 is not a valid sample name (no spaces allowed in names)
*** Sample.no.5 is thus a valid sample name
*** The sample names can be up to 20 digits long
Sample1    1 2 3 4 5 6 7 8
Sample2    9 10 11 12 13 14 15 16
Sample3    11  2  13  4  15  6  17  8
Sample4    1 2 3 4 55 66 77 88
Sample.no.5    99  1 11  1 21  2 33  1
Sample6  1   2   3   4   5   6   7   9
Sample_6_contains_27_digits 0 10 20 30 0 10 20 30
```

How to load data from EXCEL files

The PhP software can interact with Excel (or other databases) via the clipboard. Data from an Excel sheet can be copied to clipboard and then pasted into PhP-WIN. Similarily, data from PhPWIN can be copied to clipboard and then pasted into an Excel sheet. We recommend that you use Excel sheets for storing all data

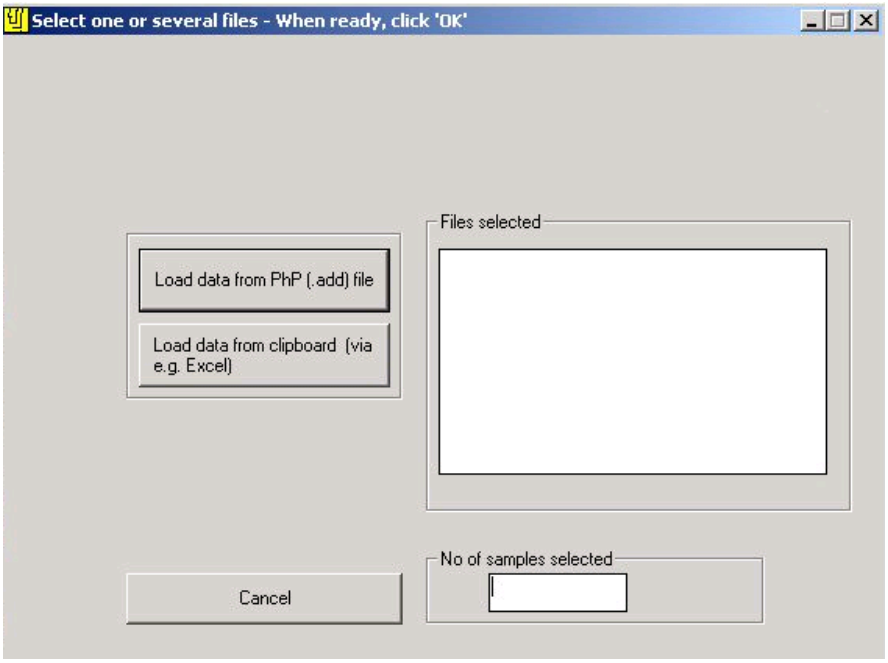
First, make sure that both the Excel file and the PhPWIN software are open. In the Excel sheet, make sure that the first cell on the first line contains *N (N is the number of tests, e.g. *12 for 12 tests). Always use 2 digits (e.g. *08 for 8 tests)



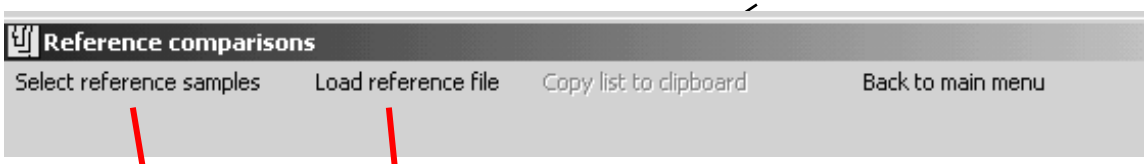
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	*12*7	ooooo	NegK	LACT	RHMI	DXRI	SUCI	SORI	TAGT	D-AR	MELI	GLLA	ORNTN*	
2	P3-HM01.1 (*) /	99	4	5	23	24	4	22	22	23	21	23	23	
3	P3-HM01.2 /	99	23	6	23	22	24	22	22	7	13	7	24	
4	P3-HM01.3 /	99	5	6	5	17	6	15	15	6	10	6	7	
5	P3-HM01.4 /	99	6	7	9	19	18	20	19	6	15	6	8	
6	P3-HM01.5 /	99	9	9	10	23	10	16	23	23	23	10	19	
7	P3-HM01.6 /	99	22	9	10	21	10	21	22	22	10	10	10	
8	P3-HM01.7 /	99	4	11	23	23	3	23	23	23	23	24	21	
9	P3-HM01.8 /	99	23	4	6	23	19	23	22	22	10	6	21	
10	INF P3-HM01.9 (NF) /	99	19	19	19	20	19	20	18	20	20	22	6	
11	P3-HM01.10 /	99	22	5	9	21	21	21	21	22	8	7	21	
12	P3-HM01.11 /	99	4	4	5	19	4	19	18	4	9	4	7	
13	P3-HM01.12 /	99	5	5	4	14	5	13	13	4	9	6	6	
14	P3-HM01.13 /	99	11	10	9	20	10	10	19	12	16	11	8	
15	P3-HM01.14 /	99	10	10	8	16	10	16	9	11	13	10	5	
16	P3-HM01.15 /	99	5	5	5	19	5	7	17	6	15	6	7	
17	P3-HM01.16 /	99	22	6	8	21	7	20	21	21	13	17	11	

If PhP plates have been used, also indicate the plate type, preceeded by an * in the first cell. Example *48*1 means that the data will be treated as 48 tests, and that the PhP-48 plates were used
Then make sure that the first column of all other lines contain the names of the samples, and that the other cells contain the test data (must be numerical integer values, e.g. between 0 and 30 for PhP data)

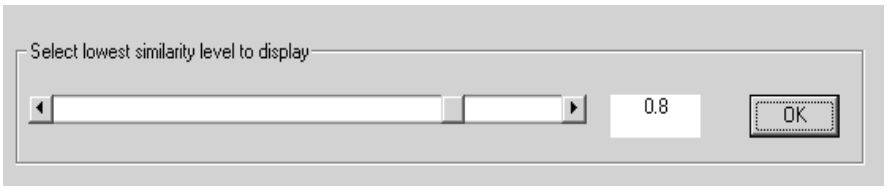
In Excel, copy the data to clipboard
In PhPWIN, select ‘Data from clipboard’
and follow the procedures in the software



Comparisons to reference data



Either select the reference samples that all other samples are to be compared to, or load a previously prepared database file that contains reference data (see Data manager above).



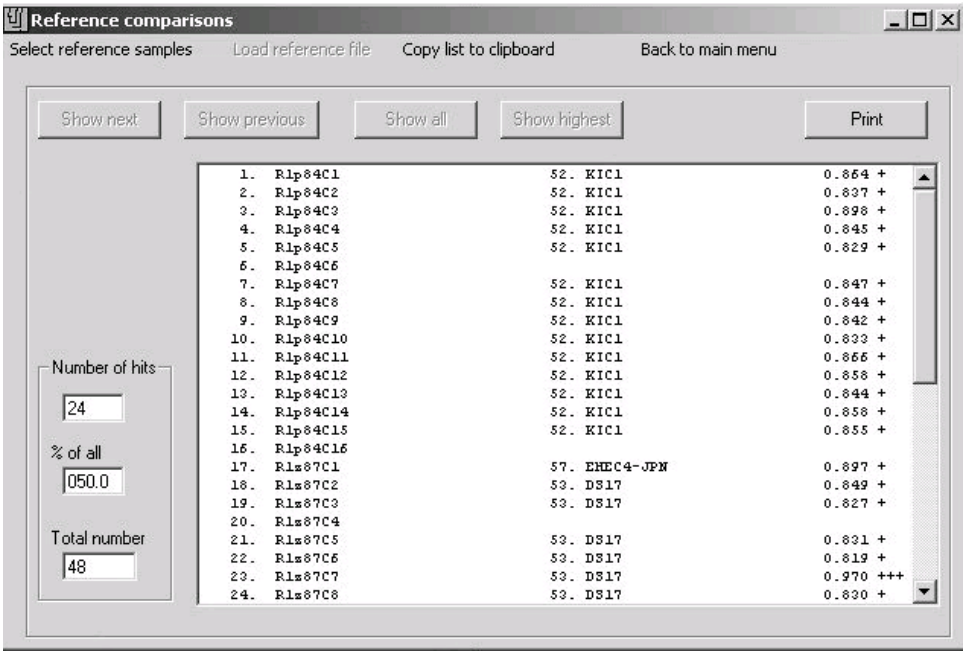
Click ‘Show all’ for a list of similarities above the selected level. The unknown isolates are displayed to the left, and the reference isolates to the right.

This option is particulary useful if you compare to only one reference isolate at a time.

The number of hits (i.e. the number of comparisons yielding a similarity above the selected level) is displayed

The first unknown (not selected) isolate is printed to the left

A list of similarities to the (selected) reference isolates is printed. Only similarities above the lowest selected similarity level is printed. If the unknown isolate has lower similarity to all selected isolates, it is skipped and the next isolate is displayed



App. A. Installation of the PhP software

A1. To install PhP-software the first time

(If you already have an existing version of the PhP software in your computer and want to install a more recent version, proceed to A2 below)
If you already have an old reading version installed, keep that in a separate directory if you should have problems with the new version.

Make sure that all other windows programs are closed except for program manager (press Alt + tab keys to see and close programs)
Select the setup program

The software contains som examples and reference files (in the directory namned 'EXAMPLES'). To wiew the files, any text editor can be used, such as Windows Notepad, Word for Windows (remember to use text format and not Word format) etc.
Click 'Help' on the menu bar if you want more help on the items on the screen.

App. C. Creation of PhP data from flatbed scanners and from absorbance data generated by other microplate reader softwares

Part I describes how to read PhP plates with a transmission flatbed scanner, and how to convert the generated plate images to PhP data

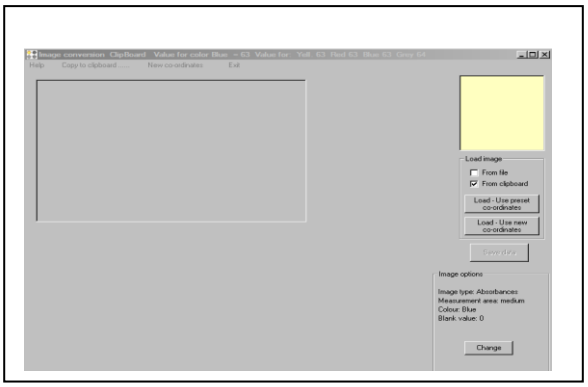
Part II describes how to convert absorbance data generated by other reader softwares to PhP data

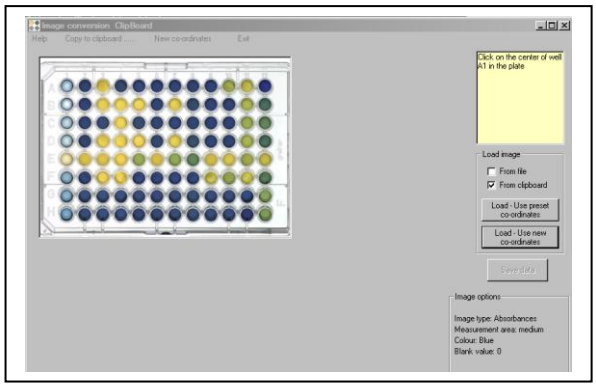
C I. Use of a transmission flatbed scanner to generate PhP data

(See also file HP-scanner.ppt)
Use the scanning software supplied with your scanner.

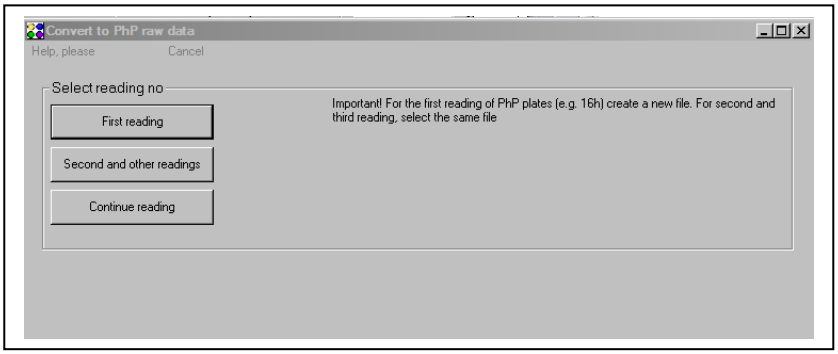
Scanning options:
Read transmissible originals. Scanner resolution: 75 - 100 dpi.
Preferably scan the plate images to clipboard and paste them into an Excel sheet. If this is not possible, save plate images as .jpg or .bmp files
Scan all PhP plates using the same options, and position all plates at the same location on the scanner. Note that it is very useful to write a clear plate identification on the top corner of the plate - the identification will then be visible in the scanned image
From software PhPWIN, select 'Create PhP data' – 'Create PhP data from absorbance data and from scanned images'
Click 'Convert plate image to PhP data' – Click OK

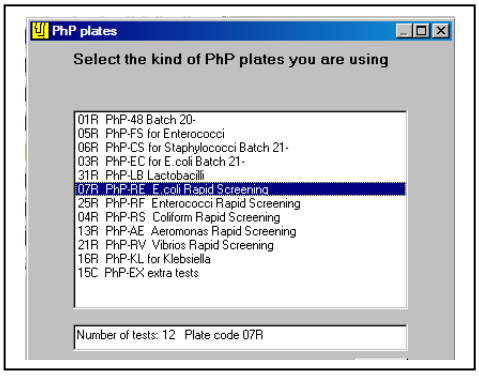
I.1.0. Click 'Load' to load scanned image of plate no 1 (from file or from clipboard) and load a plate image





I.1.1. Click on the center of well A1 in the plate (it does not have to be in the upper left corner) (the size of the measurment area can be changed by selecting 'Normalisation' on the menu bar)
I.1.2. Click on the center of the last well (H12) of the plate





A new frame appears, and you must now give information on where to save the PhP data that will be generated

I.1.3 Select whether you reading the plates for the first time or whether it is later readings
I.1.4. Type a new file name for storage of PhP data (if it is the first reading occasion) or select an existing PhP data file (.DTA) if it is reading occasion 2 or later
I.1.5. Select the kind of PhP plates you are using

I.1.9. Click Ok – continue to start generating PhP data from the scanned images

I.2.4. A new frame with plate data appears. If it was the first reading of the plate a list where sample names can be input is also shown (it is more easy to save data in Excel and add names there).
I.2.5. Click save data
I.2.6. Load next plate by clicking “Use previous co-ordinates”
I.2.7. The measuring points from the first plate will be used, and a new list of plate data appears.
If the points are not centered over the plate wells, click “use new co-ordinates” and “load”
When ready with all plates from one reading occasion click ‘Exit’
You can get help by clicking ‘Help’ on the menu bar
Note that when the plates have been converted to PhP data, the images can be removed from the computer, in order to save hard disk space
Note that if you scan the plates to clipboard, the images can be stored e.g. in an excel file (see file Example-of-results.xls) together with PhPdata and dendrograms

C II. Conversion of absorbance data to PhP data

The software can be used to transform absorbance values that have been read by other reader softwares into the PhP data format.
There are two different ways to use this software:

1. Either the microplates are measured by the existing software and the data are stored in a file using a text format (i.e. a file that can be edited using e.g. Notepad) or in an Excel file (see CIII below)
2. Or the plates are read by the existing software, copied to the clipboard and then converted to PhP format

In the first case, preferably read all plates from a PhP-assay (can be up to 100 plates) and save the absorbance data from all plates in one single file, see example below

Example of a text file containing absorbance data from 2 microplates

```
Plate 1
 0.042   2.407    0.64   1.746   2.535   2.453   2.313   2.164   2.439   2.599   1.802   1.075
 0.037   2.634   0.429   0.665   2.082   2.535   2.361   1.835   2.521    2.56   1.224   1.695
 0.162    2.42   0.456   0.706   2.481   0.465   2.214   1.876   2.409   2.486   2.398   1.796
 0.053   2.643   0.627   2.086   2.492    2.69   2.291   2.223   2.422   2.556     2.6   1.039
 0.117   2.635   0.487   0.668   2.651   2.523   2.179   2.213   2.456   1.713    2.64   2.058
 0.043   2.651   0.455    1.83   2.056   2.395   2.328   2.218   2.469   2.657   1.632   2.366
 0.327   2.449   2.318   2.387   2.499   2.376   2.193   2.097   2.284   2.344   2.396   0.794
 0.327   2.449   2.318   2.387   2.499   2.376   2.193   2.097   2.284   2.344   2.396   0.794
Plate 2
 0.03   2.538    0.73   1.261   2.192   2.523   2.303     2.1   2.425    2.48   1.422   2.261
 0.032   2.484   0.829   1.884   2.393   2.539   2.473   2.143   2.412   2.424    1.85   1.079
 0.065   2.595   0.559   0.736   2.464   0.635   2.431   2.254   2.556   2.061   0.928   1.874
 0.125   2.633   0.646   0.786   2.405   2.706   2.283   2.172   2.416   1.848   1.023   1.967
 0.073   2.663   1.372   1.549   2.402   2.616   2.435    2.13   2.625   2.236   1.838   1.408
 0.168   2.645   0.703   0.885   2.489   2.694    2.38   2.346    2.41   2.622   2.039   0.855
 0.231   2.591   1.134   1.302   2.511   1.197   2.487   2.129    2.38   2.529   2.036   1.741
 0.195   2.678   0.671   1.075   2.562   2.655   2.267   1.995   2.369   2.491   1.822   1.019
```

In the second case, the conversion software can be run in background while reading the microplates with an existing software, and after each plate has been measured the absorbance data are copied to clipboard and pasted into PFORMAT

How to convert data

There are several ways to convert the data. Which method to use depends on your existing reader software. Below is an example:

First read all plates and save all plates into the same text file.

II.1.1. From software PhPWIN4, select ‘Create PhP data’ – ‘Create PhP data from absorbance data and from scanned images’
II.1.2. Click ‘Convert absorbance data to PhP data’
II.1.3. Select whether to convert data from a file or from clipboard
If from a file, select the file the absorbance data was stored in
II.1.4. Select whether you reading the plates for the first time or whether it is later readings
II.1.5. Type a new file name for storage of PhP data (if it is the first reading occasion) or select an existing PhP data file (.DTA) if it is reading occasion 2 or later
II.1.6. Select the kind of PhP plates you are using
II.1.7. Type a header for the file (optional)
II.1.8. Type incubation time (optional)
II.1.9. Click Ok – continue to start generating PhP data from the absorbance file
Data from the selected absorbance file appears in the top list

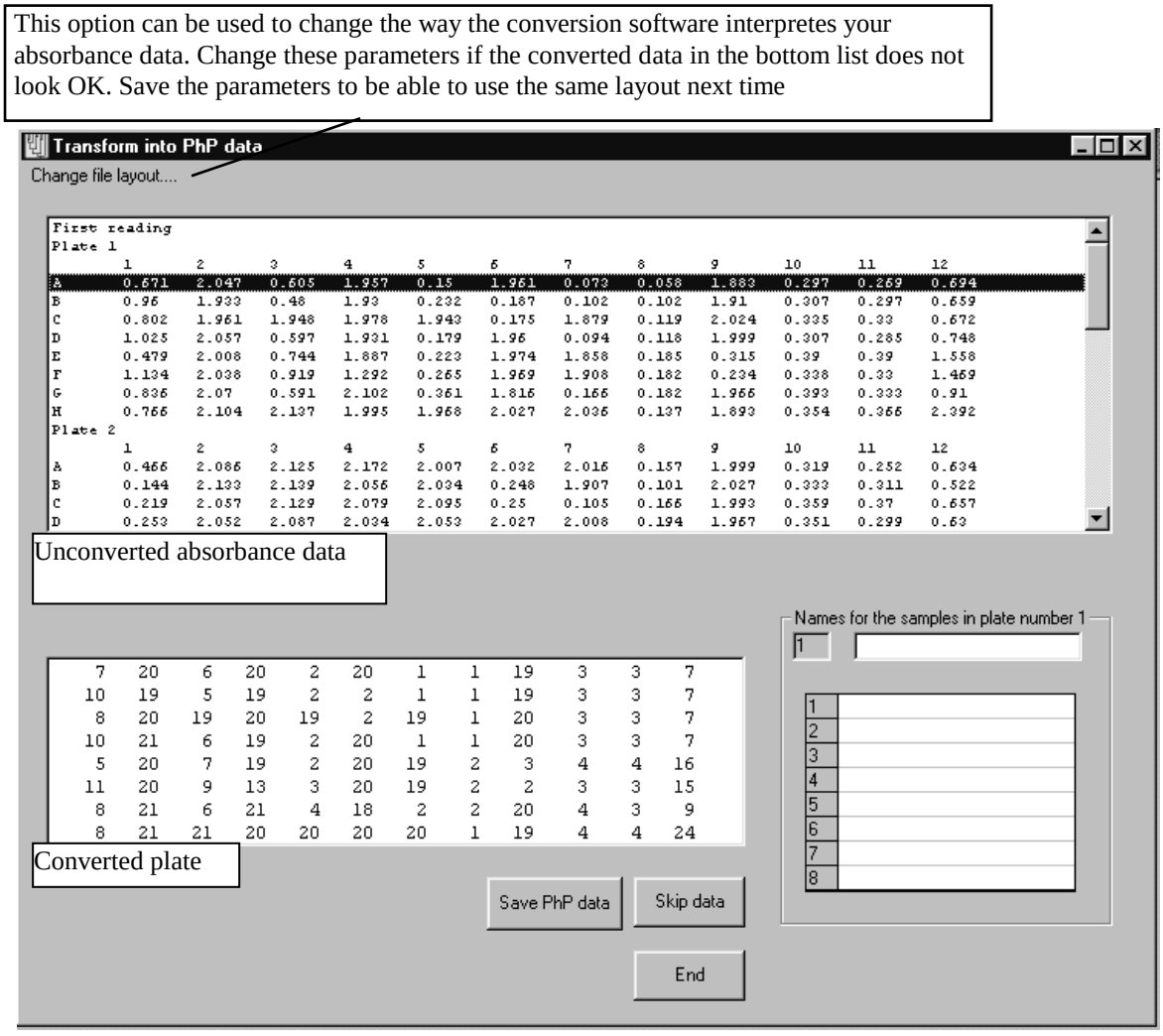
II.2.1 A new frame with plate data appears. If it was the first reading a list where sample names can be input is also shown. **First reading occasion only**: Give names of the samples (optional)

II.2.2. Click ‘Save PhP data’ when the data in the bottom frame corresponds to the plate data – if not, go to II.3.1

II.2.3. Click the first line of the next plate

All reading occasions: Continue until you get the message ‘End of data in this file’. Then click ‘Exit’ **For the second and othe readings** note that if you get the message ‘No more data available’ it means that you are trying to include more plates in the second and other reading than was included in the first reading

Transform the data from the plates in the same order as on the first reading occasion!



II.3.1. Changing of conversion parameters

Click ‘Change file layout’. The following options can be changed:

Separator sign between OD values: Spaces are used in the example above

Decimal sign: point or comma. Some software use integers of absorbance values x 1000

Number of lines per plate: Normally 8 (the file in the example above consists of 8 lines with 12 OD values in each line)

Max allowed OD value

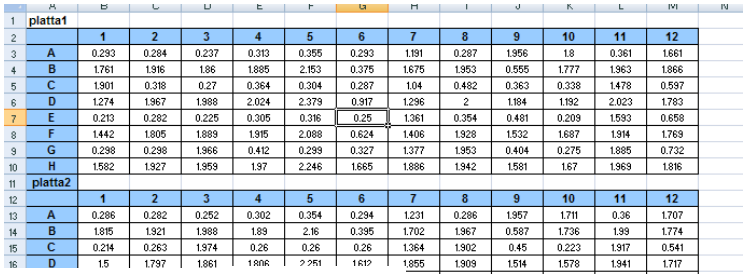
Number of lines with non OD values separating the plates: 1 in the example above

Click ‘Back to main menu’ and save parameters if you want to save them for future use

Note! If you obtain wrong data, E-mail us a unconverted data file (to info@phplate.se) and we will try to create the formatting file for you

CIII. How to convert absorbance data in an Excel file to PhP data

First, read all plates in the assay and save the data in the same Excel sheet



platta1

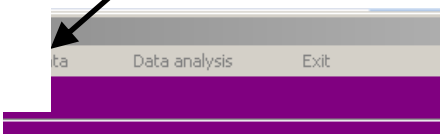
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.293	0.284	0.237	0.313	0.355	0.293	1.191	0.287	1.956	1.8	0.361	1.661
B	1.761	1.916	1.86	1.885	2.153	0.375	1.675	1.953	0.555	1.777	1.963	1.866
C	1.901	0.318	0.27	0.364	0.304	0.287	1.04	0.482	0.363	0.338	1.478	0.597
D	1.274	1.967	1.988	2.024	2.379	0.917	1.296	2	1.184	1.192	2.023	1.783
E	0.213	0.282	0.225	0.305	0.316	0.25	1.361	0.354	0.481	0.209	1.593	0.658
F	1.442	1.805	1.889	1.915	2.088	0.624	1.406	1.928	1.532	1.687	1.914	1.769
G	0.298	0.298	1.966	0.412	0.299	0.327	1.377	1.953	0.404	0.275	1.885	0.732
H	1.582	1.927	1.959	1.97	2.246	1.665	1.886	1.942	1.581	1.67	1.969	1.816

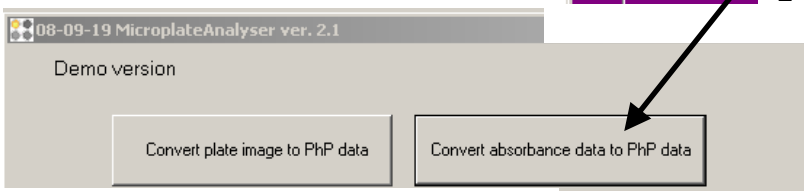
platta2

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.286	0.282	0.252	0.302	0.354	0.294	1.231	0.286	1.957	1.711	0.36	1.707
B	1.815	1.921	1.988	1.89	2.16	0.395	1.702	1.967	0.587	1.736	1.99	1.774
C	0.214	0.263	1.974	0.26	0.26	0.26	1.364	1.902	0.45	0.223	1.917	0.541
D	1.5	1.797	1.861	1.806	2.251	1.612	1.855	1.909	1.514	1.578	1.941	1.717
							1.279	0.314	0.489	0.238	1.38	0.606
							1.1	1.987	1.502	1.744	1.953	1.783
							1.25	1.95	1.911	1.935	1.973	0.431
							0.886	1.943	0.937	1.725	1.951	1.814

7	8	9	10	11	12

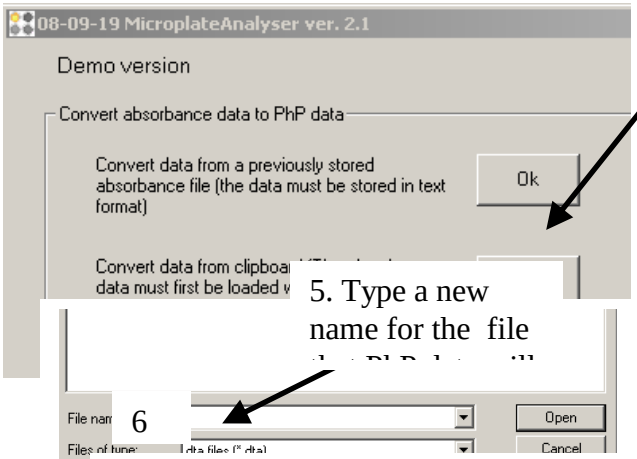
Load PhP software and select Create

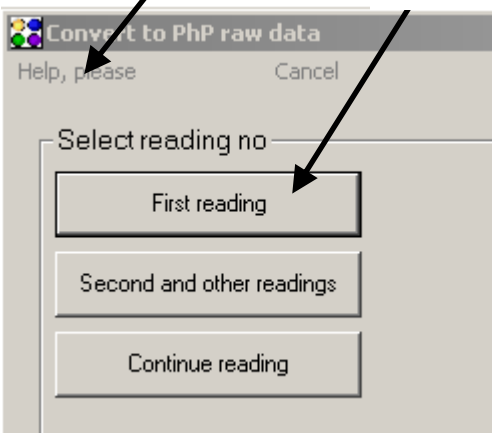




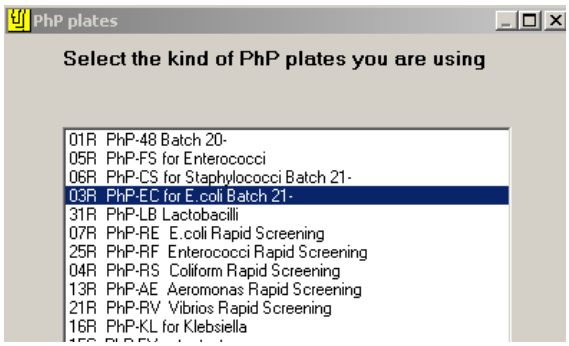
Click Help for

4. First reading to convert data from a new

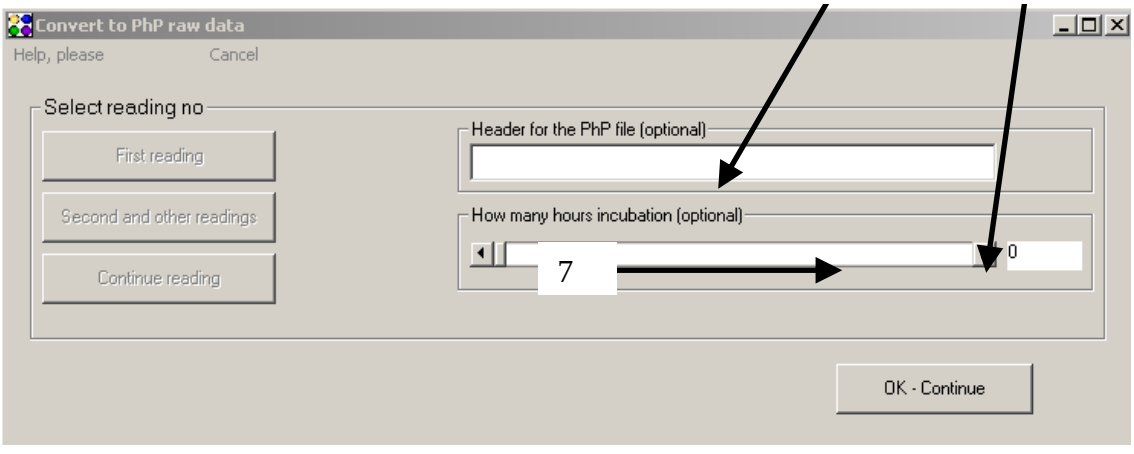


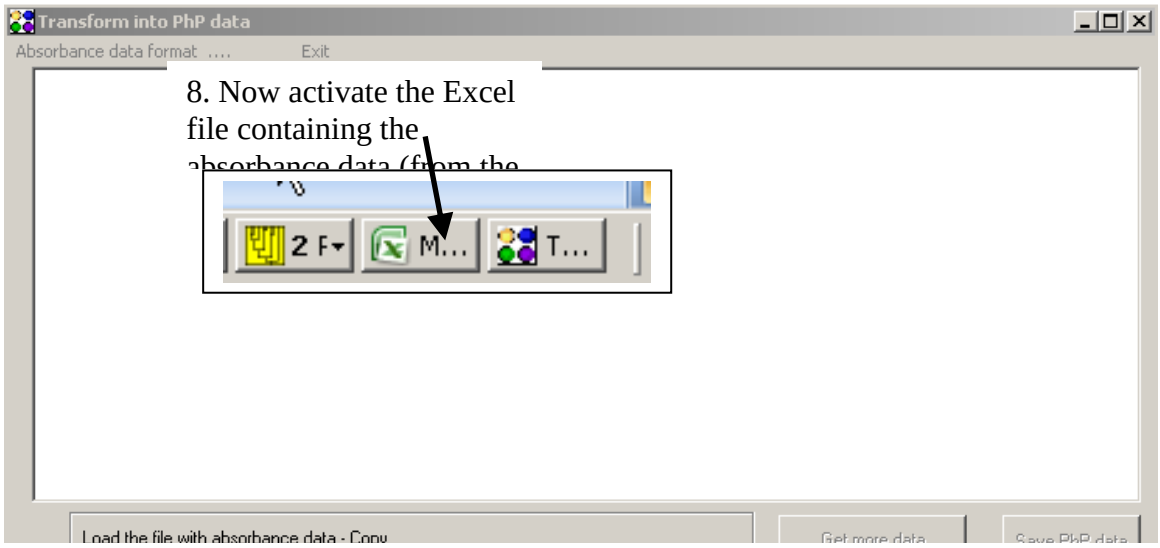


5. Type a new name for the file



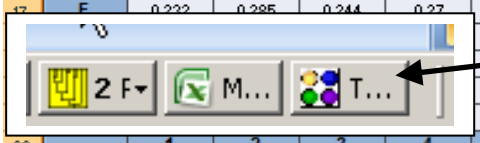
You do not need to give this information





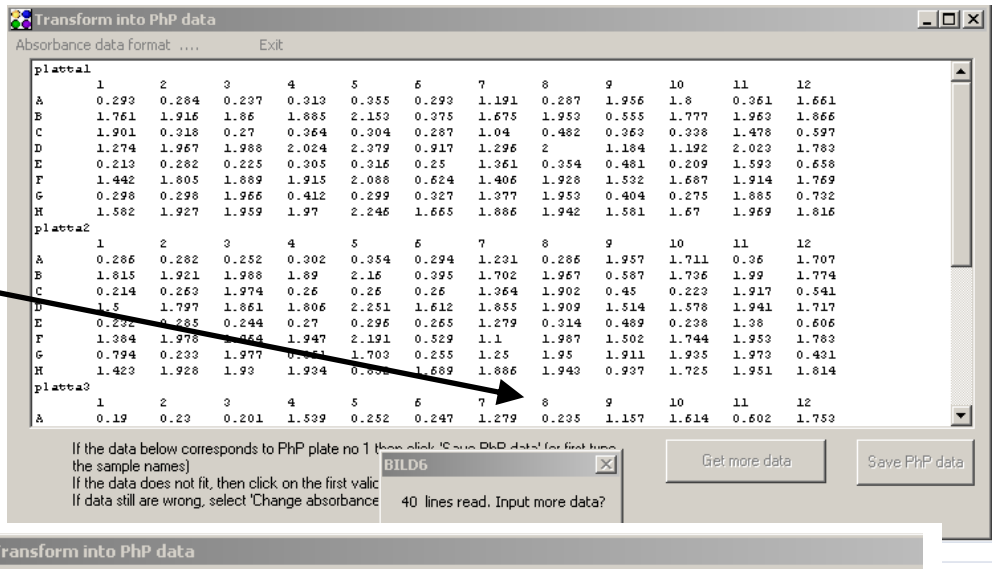
8. Now activate the Excel file containing the absorbance data (from the

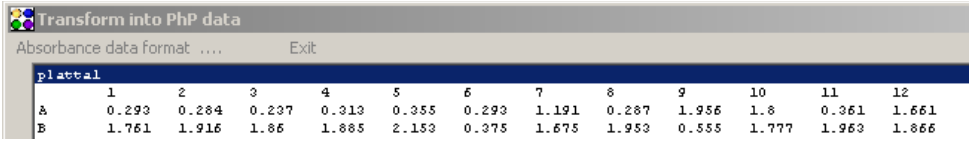
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	platta1												
2		1	2	3	4	5	6	7	8	9	10	11	12
3	A	0.293	0.284	0.237	0.313	0.355	0.293	1.191	0.287	1.956	1.8	0.361	1.661
4	B	1.761	1.916	1.86	1.885	2.153	0.375	1.675	1.953	0.555	1.777	1.963	1.866
5	C	1.901	0.318	0.27	0.364	0.304	0.287	1.04	0.482	0.363	0.338	1.478	0.597
6	D	1.274	1.967	1.988	2.024	2.379	0.917	1.296	2	1.184	1.192	2.023	1.783
7	E	0.213	0.282	0.225	0.305	0.316	0.25	1.361	0.354	0.481	0.209	1.593	0.658
8	F	1.442	1.805	1.889	1.915	2.088	0.624	1.406	1.928	1.532	1.687	1.914	1.769
9	G	0.298	0.298	1.966	0.412	0.299	0.327	1.377	1.953	0.404	0.275	1.885	0.732
10	H	1.582	1.927	1.959	1.97	2.246	1.665	1.886	1.942	1.581	1.67	1.969	1.816
11	platta2												
12		1	2	3	4	5	6	7	8	9	10	11	12
13	A	0.286	0.282	0.252	0.302	0.354	0.294	1.231	0.286	1.957	1.711	0.36	1.707
14	B	1.815	1.921	1.988	1.89	2.16	0.395	1.702	1.967	0.587	1.736	1.99	1.774
15	C	0.214	0.263	1.974	0.26	0.26	0.26	1.364	1.902	0.45	0.223	1.917	0.541
16	D	1.5	1.797	1.861	1.806	2.251	1.612	1.855	1.909	1.514	1.578	1.941	1.717
17	E	0.232	0.285	0.244	0.27	0.296	0.265	1.279	0.314	0.489	0.238	1.38	0.606
18	F	1.384	1.978	1.954	1.947	2.191	0.529	1.1	1.987	1.502	1.744	1.953	1.783
19	G	0.794	0.233	1.977	0.351	1.703	0.255	1.25	1.95	1.911	1.935	1.973	0.431
20	H	1.423	1.928	1.93	1.934	0.852	1.689	1.886	1.943	0.937	1.725	1.951	1.814
21	platta3												
22		1	2	3	4	5	6	7	8	9	10	11	12
23	A	0.19	0.23	0.201	1.539	0.252	0.247	1.279	0.235	1.157	1.614	0.602	1.753



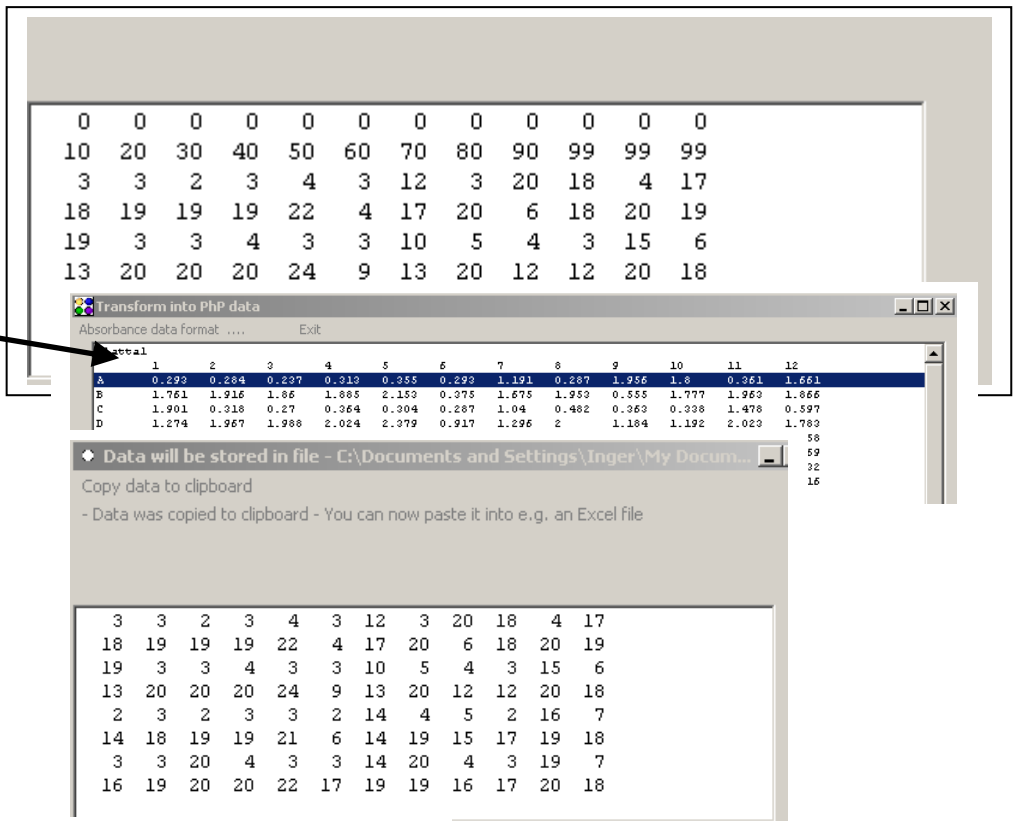
10. From the bottom menu bar activate the PhP data conversion again
Click Ok

1

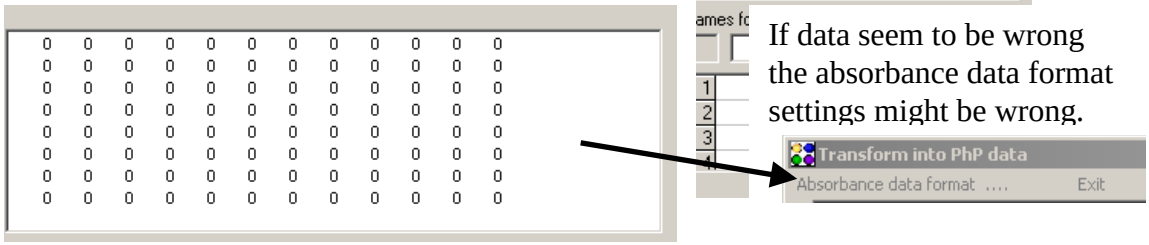




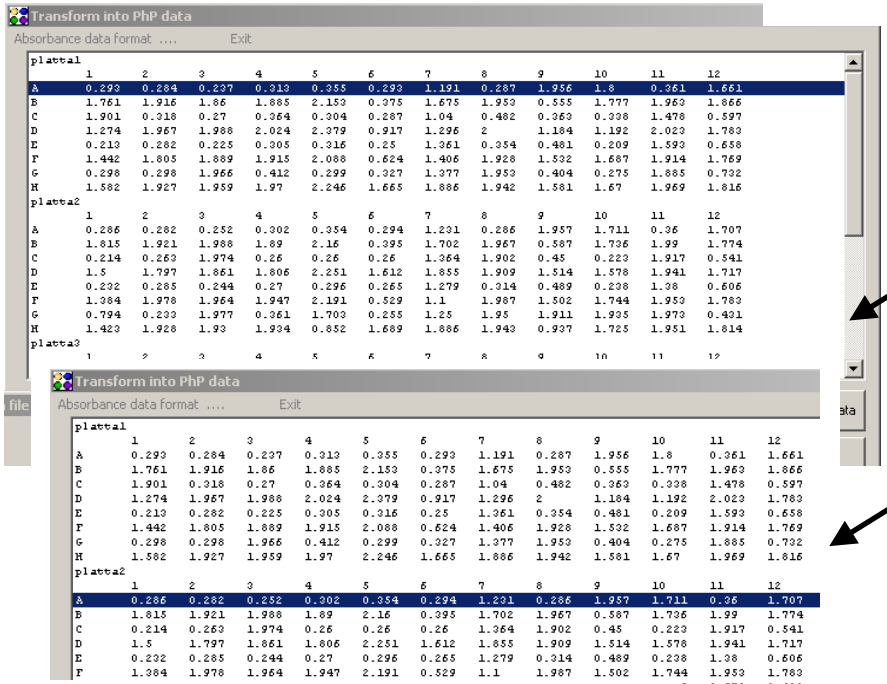
The software will try to convert data from the line activated in the upper frame. Check data carefully! In this example the first two lines do not contain PhP data.



12. Click on the
These data are ok. Sample names can be given here (not nessecary if data are going to be stored in Excel format, se later information)

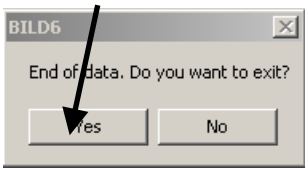


If data seem to be wrong the absorbance data format settings might be wrong.

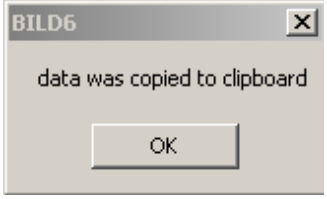


13. Click Save PhP data to save data from the first plate

14. Click on the first line of the next plate. Repeat step 12-13 for all plates

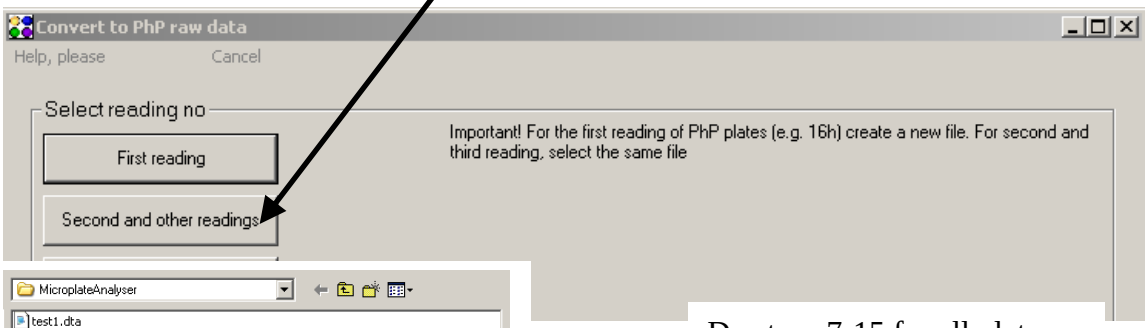


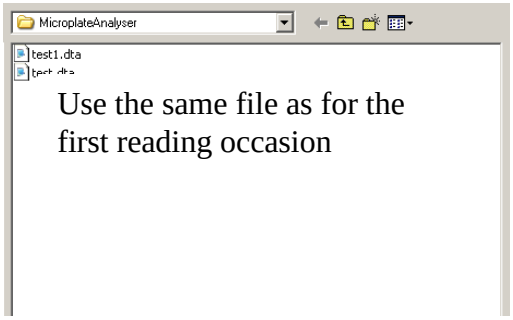
The PhP data were copied to the clipboard and can be pasted into an Excel file (you do not need to do this



Excel file with PhP data from the first reading

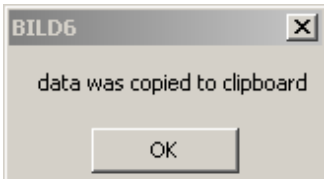
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA
1	*24*03R*																										
2	1	3	3	2	3	4	3	12	3	20	18	4	17	18	19	19	19	22	4	17	20	6	18	20	19		
3	2	19	3	3	4	3	3	10	5	4	3	15	6	13	20	20	20	24	9	13	20	12	12	20	18		
4	3	2	3	2	3	3	2	14	4	5	2	16	7	14	18	19	19	21	6	14	19	15	17	19	18		
5	4	3	3	20	4	3	3	14	20	4	3	19	7	16	19	20	20	22	17	19	19	16	17	20	18		
6	5	3	3	3	3	4	3	12	3	20	17	4	17	18	19	20	19	22	4	17	20	6	17	20	18		
7	6	2	3	20	3	3	3	14	19	4	2	19	5	15	18	19	18	23	16	19	19	15	16	19	17		
8	7	2	3	2	3	3	3	13	3	5	2	14	6	14	20	20	19	22	5	11	20	15	17	20	18		
9	8	8	2	20	4	17	3	12	20	19	19	20	4	14	19	19	19	9	17	19	19	9	17	20	18		
10	9	2	2	2	15	3	2	13	2	12	16	6	18	16	18	18	19	20	3	16	17	4	15	18	17		
11	10	3	3	3	3	3	3	10	3	3	3	12	4	9	19	19	20	24	6	8	19	12	13	19	18		
12	11	4	7	4	7	8	3	15	17	15	16	18	16	15	17	18	17	14	16	16	18	8	16	17	16		
13	12	3	4	19	5	3	4	13	20	4	3	19	5	15	19	19	19	23	15	19	19	14	14	19	18		
14	13	2	2	2	16	2	2	13	3	11	17	7	18	17	19	19	18	22	3	16	19	4	14	18	18		





Use the same file as for the first reading occasion

Do steps 7-15 for all plates

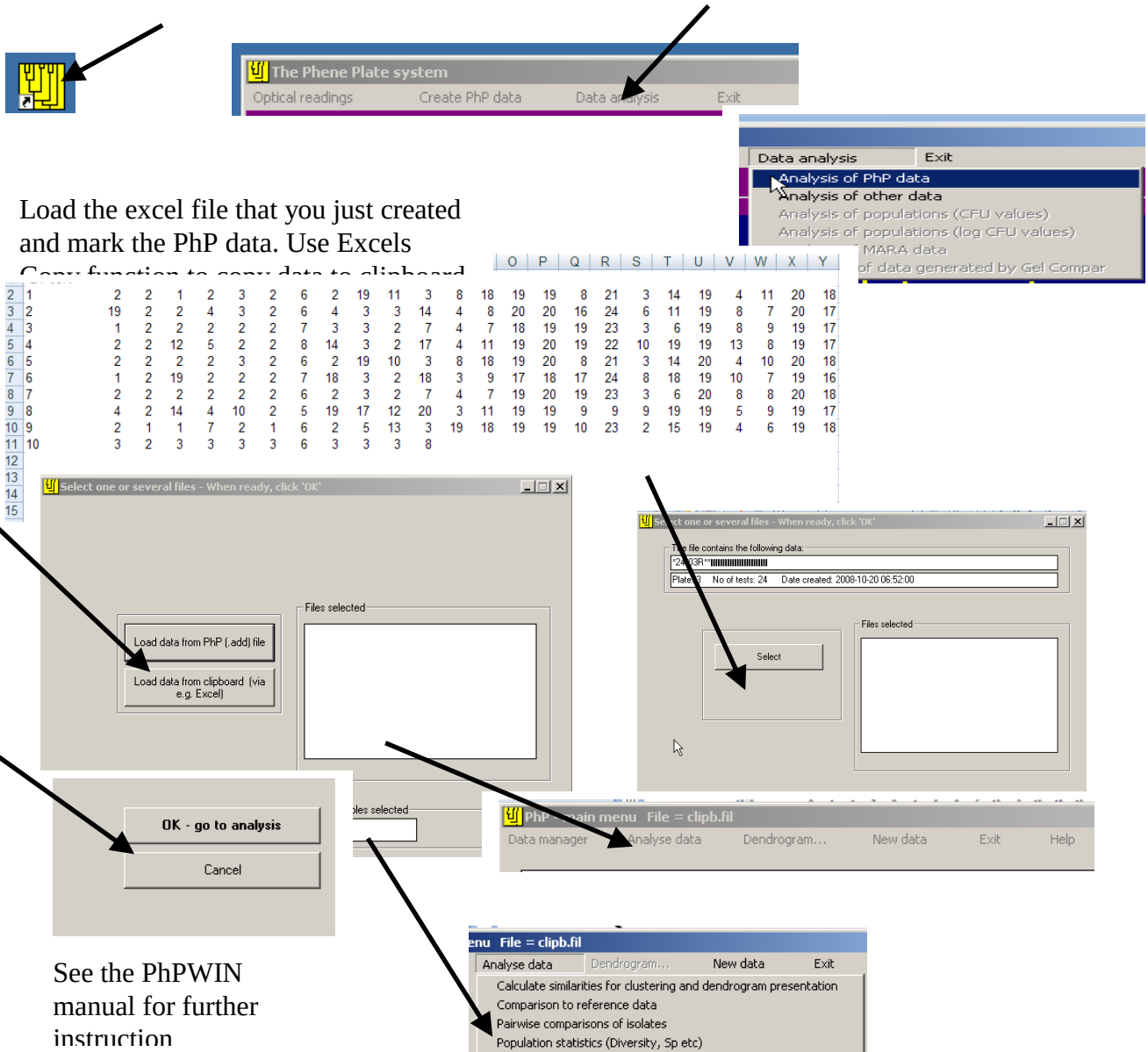


After the last reading, paste data into an

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	*24*03R**																								
2	1	2	2	1	2	3	2	6	2	19	11	3	8	18	19	19	8	21	3	14	19	4	11	20	18
3	2	19	2	2	4	3	2	6	4	3	3	14	4	8	20	20	16	24	6	11	19	8	7	20	17
4	3	1	2	2	2	2	2	7	3	3	2	7	4	7	18	19	19	23	3	6	19	8	9	19	17
5	4	2	2	12	5	2	2	8	14	3	2	17	4	11	19	20	19	22	10	19	19	13	8	19	17
6	5	2	2	2	2	3	2	6	2	19	10	3	8	18	19	20	8	21	3	14	20	4	10	20	18
7	6	1	2	19	2	2	2	7	18	3	2	18	3	9	17	18	17	24	8	18	19	10	7	19	16
8	7	2	2	2	2	2	2	6	2	3	2	7	4	7	19	20	19	23	3	6	20	8	8	20	18
9	8	4	2	14	4	10	2	5	19	17	12	20	3	11	19	19	9	9	9	19	19	5	9	19	17
10	9	2	1	1	7	2	1	6	2	5	13	3	19	18	19	19	10	23	2	15	19	4	6	19	18
11	10	3	2	3	3	3	3	6	3	3	3	8	3	5	19	20	19	24	4	7	19	7	7	19	17
12	11																12	20	8	15	19	4	8	18	18
13	12																18	23	9	19	18	11	7	18	16
14	13																11	24	3	13	20	4	6	19	19
15	14																19	24	9	18	14	3	8	19	17
16	15																14	21	8	13	20	4	9	21	19
17	16																15	19	12	17	19	9	11	17	15

Excel file with average PhP data from all three readings. You can add sample names in the left column

A. To analyse this data

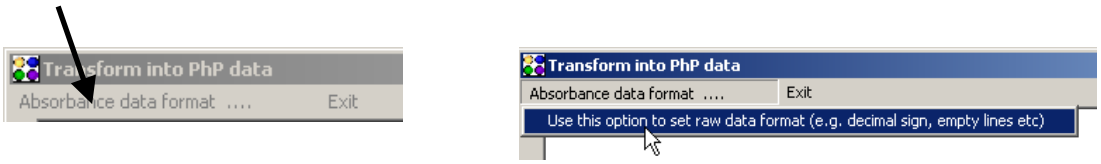


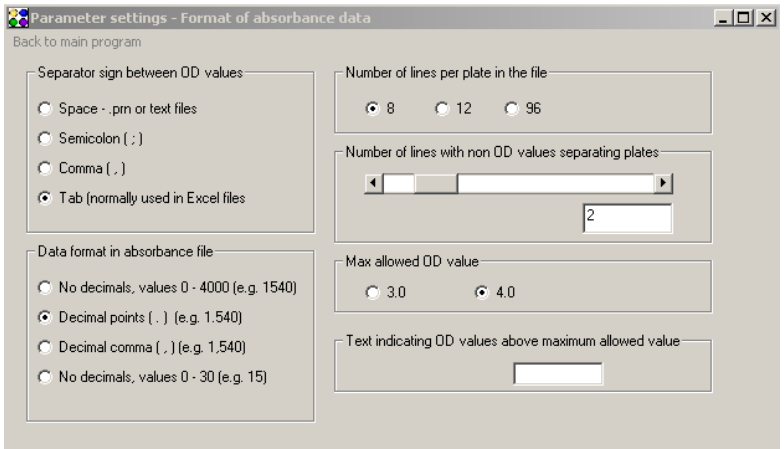
Load the excel file that you just created and mark the PhP data. Use Excels Copy function to copy data to clipboard

1		2	2	1	2	3	2	6	2	19	11	3	8	18	19	19	8	21	3	14	19	4	11	20	18
2	19	2	2	4	3	2	6	4	3	3	14	4	8	20	20	16	24	6	11	19	8	7	20	17
3	1	2	2	2	2	2	7	3	3	2	7	4	7	18	19	19	23	3	6	19	8	9	19	17
4	2	2	12	5	2	2	8	14	3	2	17	4	11	19	20	19	22	10	19	19	13	8	19	17
5	2	2	2	2	3	2	6	2	19	10	3	8	18	19	20	8	21	3	14	20	4	10	20	18
6	1	2	19	2	2	2	7	18	3	2	18	3	9	17	18	17	24	8	18	19	10	7	19	16
7	2	2	2	2	2	2	6	2	3	2	7	4	7	19	20	19	23	3	6	20	8	8	20	18
8	4	2	14	4	10	2	5	19	17	12	20	3	11	19	19	9	9	9	19	19	5	9	19	17
9	2	1	1	7	2	1	6	2	5	13	3	19	18	19	19	10	23	2	15	19	4	6	19	18
10	3	2	3	3	3	3	6	3	3	3	8													

See the PhPWIN manual for further instruction

B. How to change settings f





Change parameters that are wrong (e.g. digit used to denote decimal points). The parameters in this example are the ones to be used for the Excel file used in the conversions. Click Back to main program and Save parameters when ready

This step only has to be

Still have problems with the data? Mail a copy of your absorbance data file to info@phplate.se and explain the problem, and we will help you