PHENOS GUIDE

v3.02

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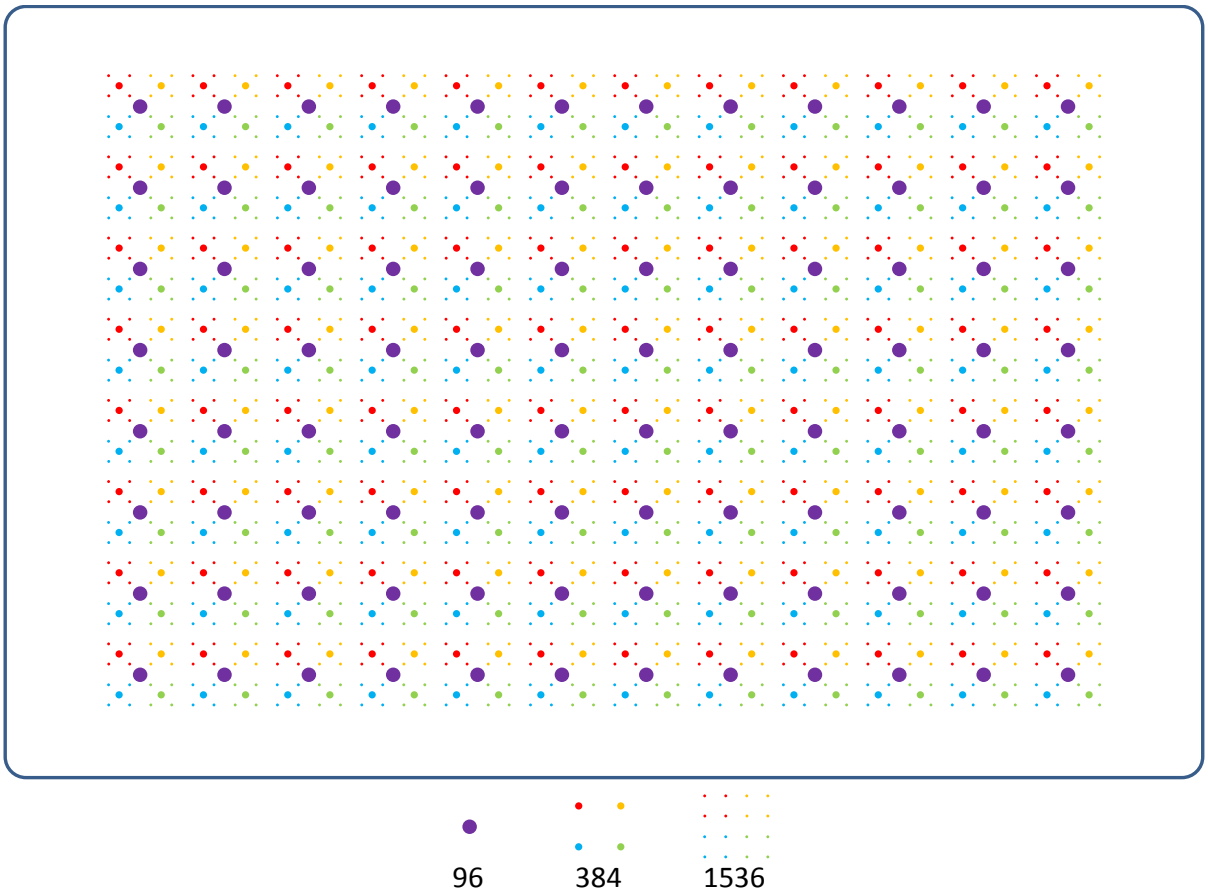
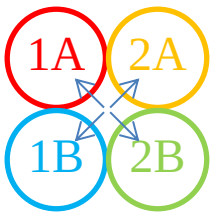
INTRODUCTION

PHENOS (Phenotyping On Solid media) is a pipeline for using a microplate reader to measure the growth of yeast colonies in arrays growing on solid agar. The data can be passed to QTL analysis software or used for other purposes.

Yeast strains can be grown in large arrays with 96, 384 or 1536 samples on a single rectangular plate containing agar media. These arrays are easily created, duplicated and maintained using the Singer Instruments ROTOR HDA colony manipulation robot, which uses disposable plastic printing pads called 'repads'. We've found that 384 arrays are the best for phenotyping.



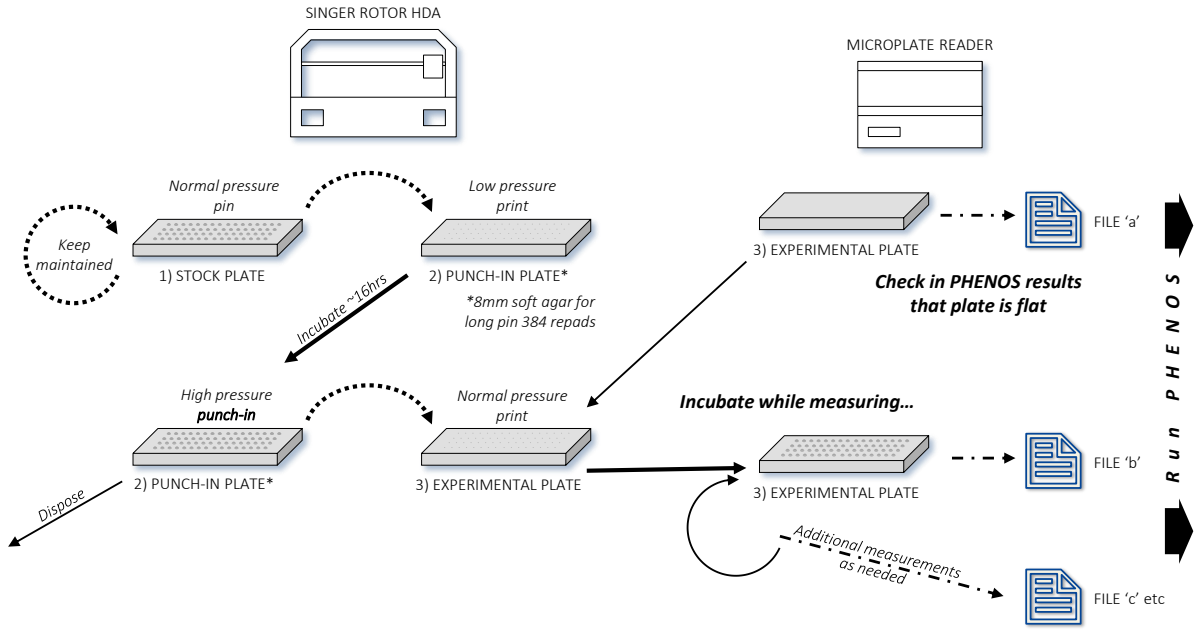


The ROTOR can easily combine four 96 arrays into a 384 array (or four 384 arrays into a 1536 array) by superimposing the source arrays, each with a different offset. It can reverse the process too, to split a larger array into four smaller ones. When using manual ROTOR controls, the four source or target plates are designated 1A, 2A, 1B and 2B according to their offset.

We developed a ‘punch-in’ technique to help equalize the amount of cells printed in each spot of the array. This requires creating a throw-away intermediate source plate called a ‘punch-in plate’.

We use the microplate reader to take readings of the empty plate (containing only solid agar media) immediately before it is printed to, and then take any number of sets of readings after printing, which the PHENOS software can join together, while subtracting any absorbance due to the agar.

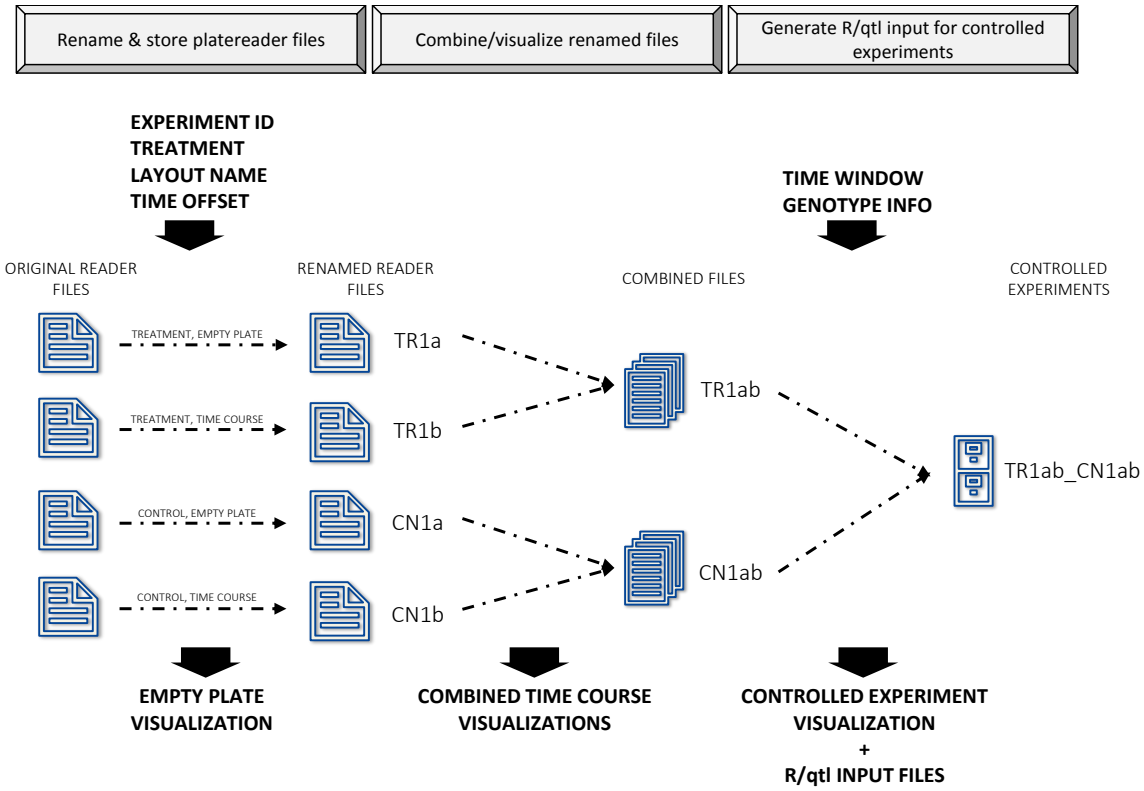
Absorbance readings, which measure colony thickness as a proxy for overall colony size, can be taken at 20 minute intervals to create high resolution growth curves.



PHENOS EXPERIMENTAL WORKFLOW.

The PHENOS software (for Windows, written in Python 2.7) collects vital information about the experiments, and then stitches together the different results files and generates growth curves and other useful visualizations.

The PHENOS program can also compare a treatment experiment with a control experiment and generate treatment ratios in a format that can be passed into the R/qtl package as phenotypes for QTL (Quantitative Trait Loci) analysis.



PHENOS ANALYSIS WORKFLOW.

As summarized in the figure above, the PHENOS program has three functional sections:

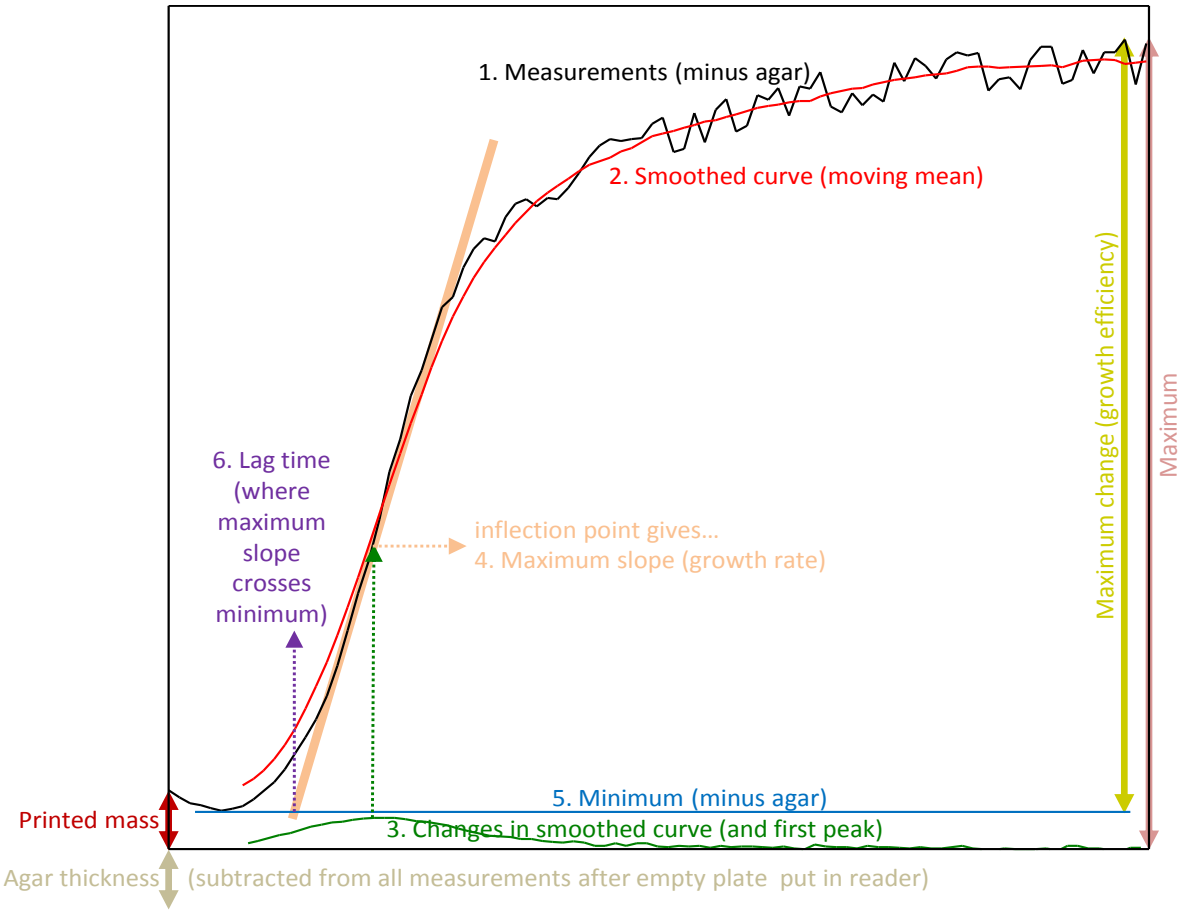
1) **Rename & store platereader files.** This will list all output files from the microplate reader and prompt the user to select one, then it will prompt the user for information about the experiment (including the treatment and layout) before copying the file and renaming that copy with the information in the new file name.
2) **Combine/visualize renamed files.** This joins together data from multiple ASCII data files (readings of the empty plate, plus any number of subsequent readings after printing) into a new combined file, which is stored in a database and visualization plots (see below) are generated.
3) **Generate R/qtl input for controlled experiments.** This compares a combined file from a treatment experiment with a matching file (with the same layout) from a control (no treatment) experiment, and, if genotype information has been put in the Genotypes folder in the correct format, PHENOS will generate R/qtl input files with phenotype and genotype data side by side.

DIRECTORIES

The PHENOS software works across three different locations on your PC:

1) **Installation directory**: e.g. C:\PHENOS. This is wherever the PHENOS installation files and scripts are placed.
2) **Source directory**: e.g. C:\Platereader output. This is where the microplate reader will store its output files, and the PHENOS software will look for them.
3) **Target directory**: e.g. C:\PHENOSdata. This is where the PHENOS software will place renamed copies of the microplate reader files, as well as all its databases, visualizations and R/qtl input files.

GROWTH CURVES

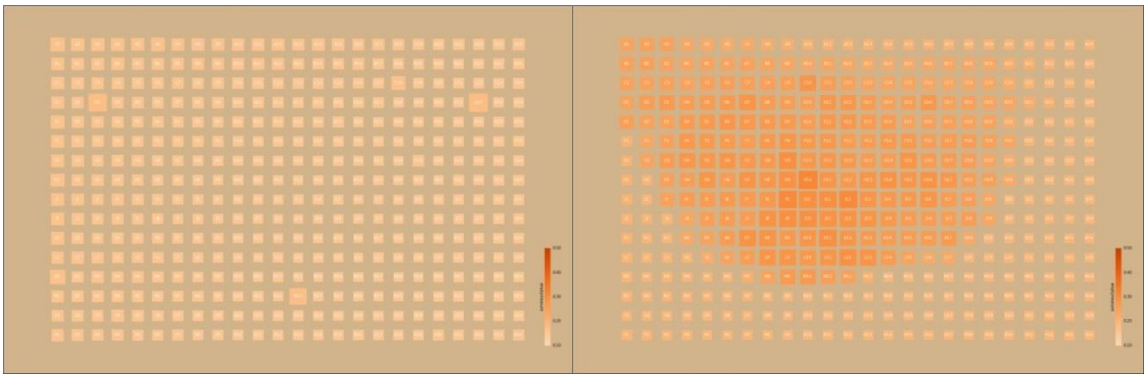


The measurements from the microplate reader (black curve, above), with absorbance from agar subtracted, are really measurements of the thickness of a colony, which is a serviceable proxy for actual colony size. PHENOS can extract measurements analogous to growth rate (maximum change/hour), growth lag (hours), and efficiency (maximum absorbance minus agar), and indicate through some of the visualizations below whether any of these correlate to the initial 'printed mass' measurement (lag often does) or to location on the plate.

VISUALIZATIONS

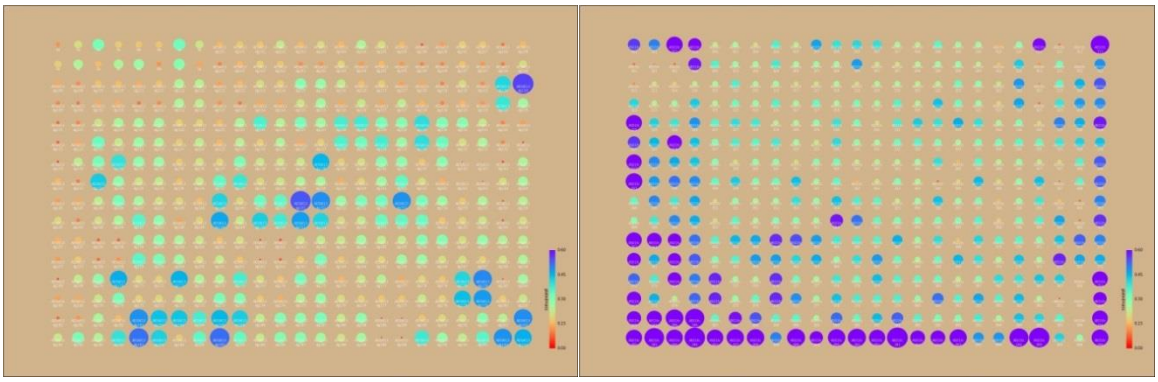
The visualizations and output produced by PHENOS (in the target directory Plots subfolder) can include the following (you can customize which are produced by default, or which are produced for a given set of user initials, by editing the config.txt file: see "CONFIGURING PHENOS BY EDITING CONFIG.TXT" on pg. 18):

AgarThickness



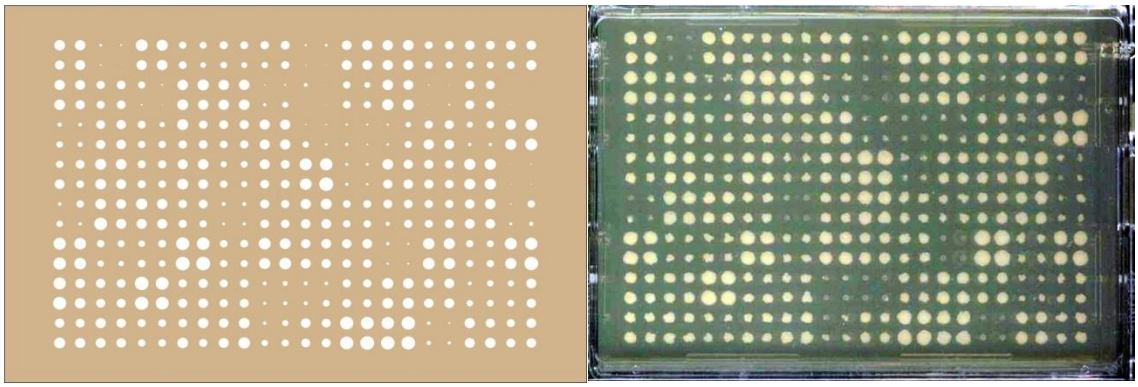
These plots are based purely on measurements of an empty plate (before printing cells to it), showing how flat and smooth the agar is. The colour of the squares should ideally be somewhere in the middle of the range, with no particular gradient from side to side (e.g. above left, not above right). Squares are scaled based on maximum and minimum values for the plate, the smallest square being the lowest reading and the largest being the highest. This illustrates any general slope to the agar, which may result in poor printing quality as the repads may never touch the thinner parts.

PrintingQuality



These plots show the 'printed masses' for each colony (the initial set of readings after printing, with the empty plate measurements subtracted). As in the EmptyPlate plots, the colour scale is fixed and comparable between plots, while the spot sizes are scaled between the local minimum and maximum. *The spots should ideally be mostly green with no discernible pattern.* Large purple spots indicate overly large printed masses while small red spots indicate overly small printed masses, which may take significantly longer than average to begin growing.

FinalGrowth

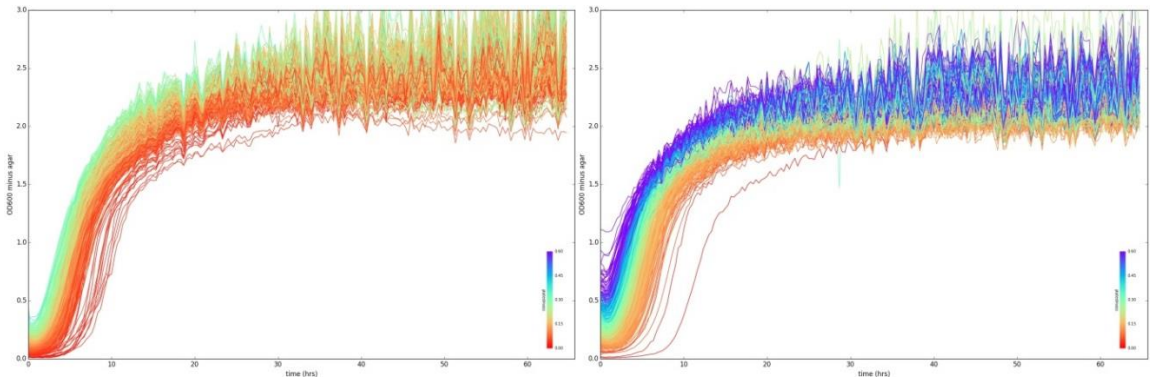


These plots (example above left) show the final readings, with agar absorbance subtracted, and so should resemble the appearance of the plates at the end of the experiment (see above right). Two variants of this plot can also be created: 'FinalGrowth_Lag' and 'FinalGrowth_MaxSlope', which color the spot for each curve according to the maximum slope measured and the lag

Animation

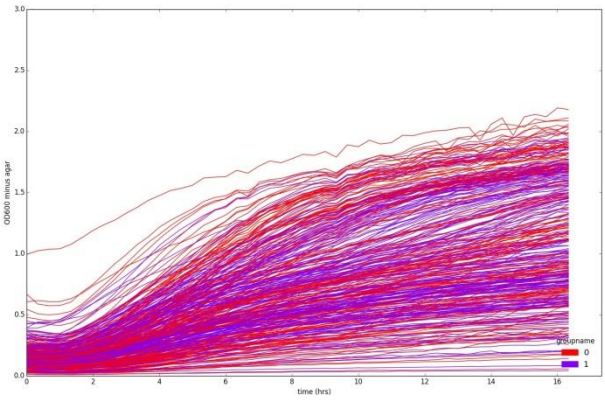
These mp4 animations present a time-lapse view of the plate throughout the course of the experiment, as measured by the microplate reader. If a wave of growth is seen moving across the plate, this may indicate a misalignment between the printed locations and the measured locations (a common problem if working with 1536 arrays printed using fine-pinned 1536 repads). In the 'Animation_Temp' visualization, the spots are colored by microplate reader temperature measurements, and this can draw attention to temperature irregularities.

CurvesWithoutAgar_PrintedMass



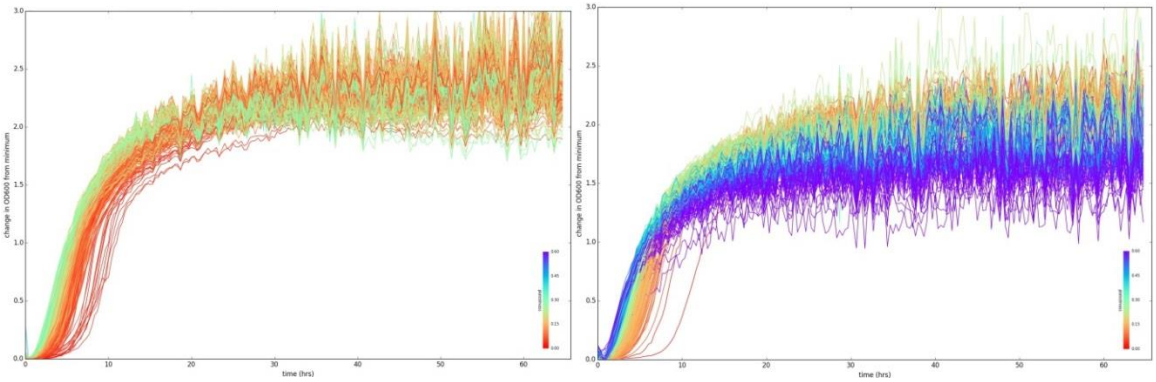
These plots show each growth curve (with agar absorbance subtracted), colored by the value of the printed mass at the start of that growth. It is easy to see that excessively small printed masses show longer lags before peak growth. The variants 'CurvesWithoutAgar_Lags' and 'CurvesWithoutAgar_Slopes' show the same curves colored by lag or slope value instead.

CurvesWithoutAgar_Groups

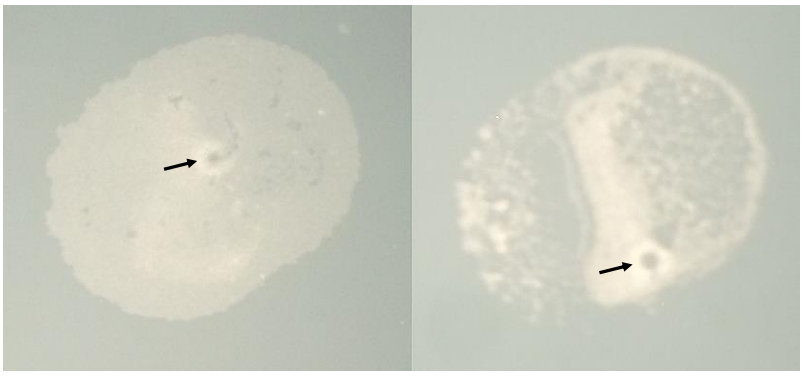


These plots show each growth curve (with agar absorbance subtracted), colored by the value of the groupname if ‘groupnames’ are defined in the layout file. This gives you a quick and flexible means of visually dissecting your data.

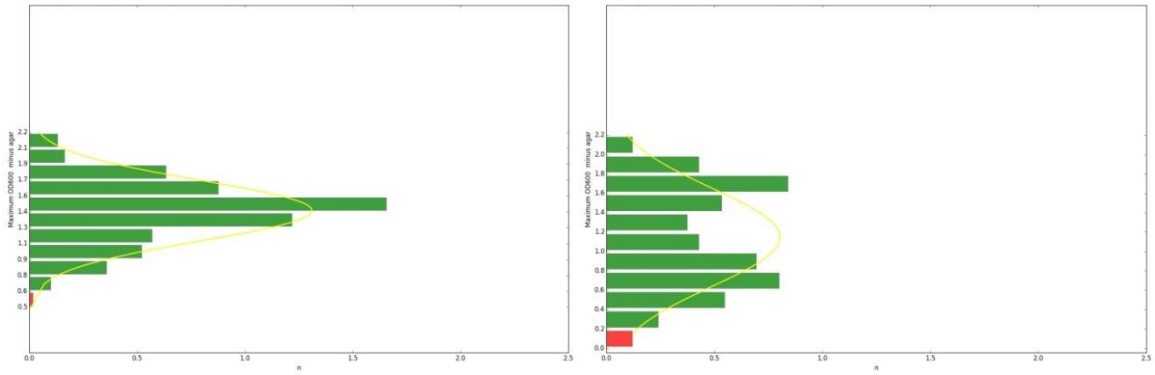
CurvesNormalized_PrintedMass



These plots are similar to the previous, but all curves are normalized to their minimum values, and thus show the overall change in colony thickness. Note that a slight dip at the start of the curves is quite common, thought to be due to the presence of microscopic bubbles after printing which diffract light until they evaporate (see below).

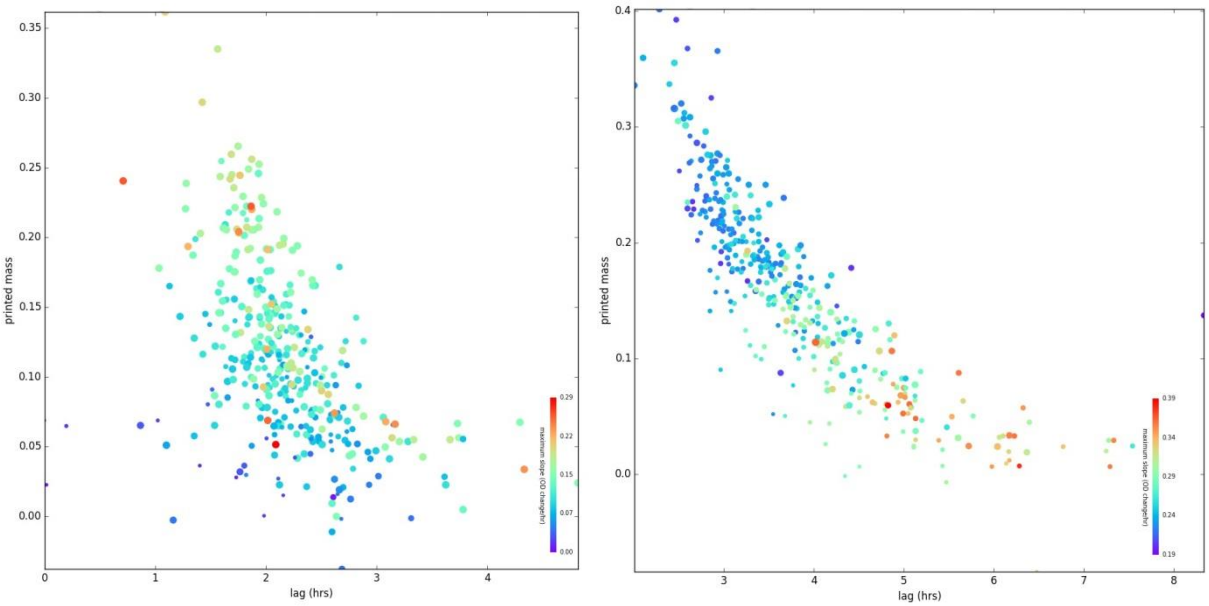


Histogram_MaxWithoutAgar



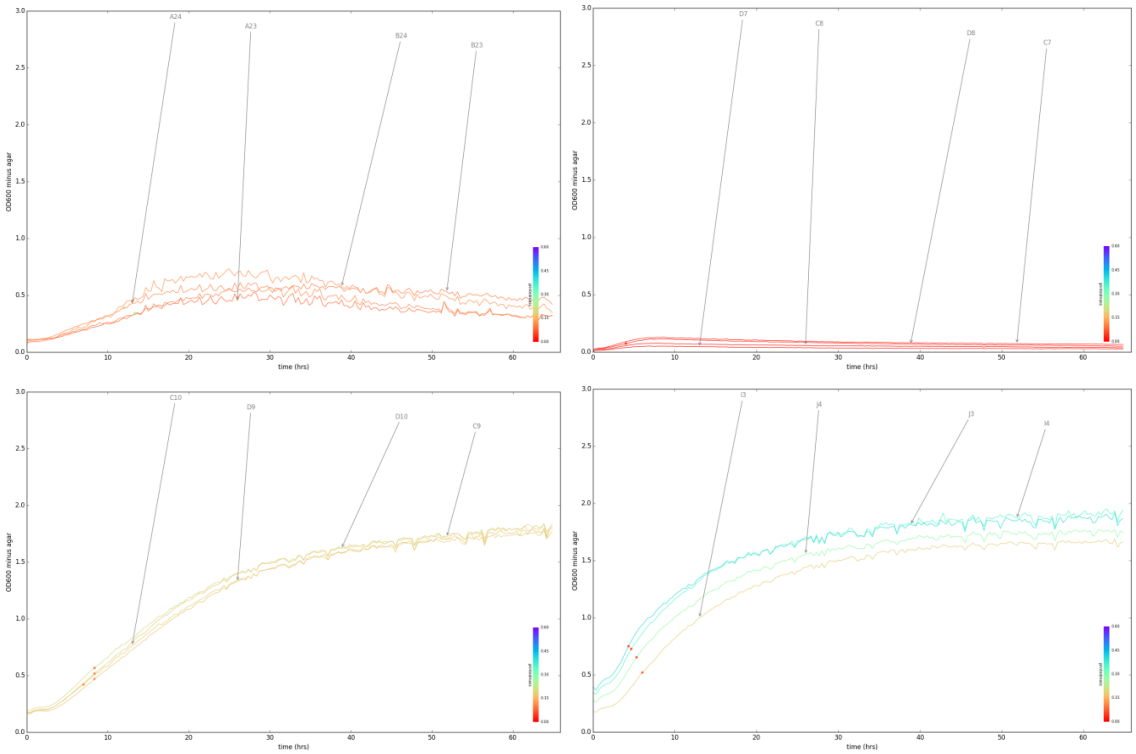
These histogram plots show the distribution of the maximum change values (the difference between the last measurements and the minimum measurements), and can help you notice patterns such as bimodal distributions of phenotypes (see above right). The plot attempts to fit a normalized distribution curve to the data (in yellow).

Scatterplot_PrintedMass_Lag



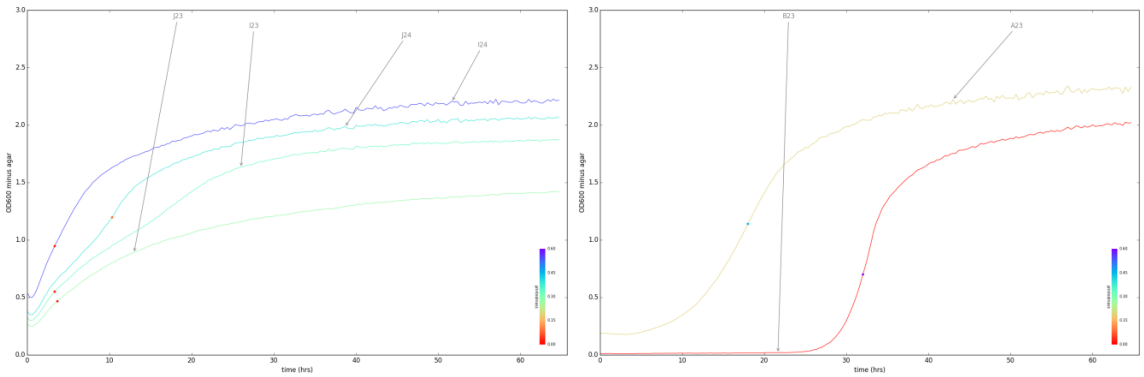
These scatter plots show the relationship between ‘printed mass’, ‘lag’, ‘maximum slope’ and ‘maximum without agar’ values (the scale of the spots) for each colony. This can reveal if there is any noticeable correlation between these values (e.g. on the right, lag is inversely proportional to the printed mass of the colony and therefore may not be a reliable measure of response to this treatment).

ReplicatePlots

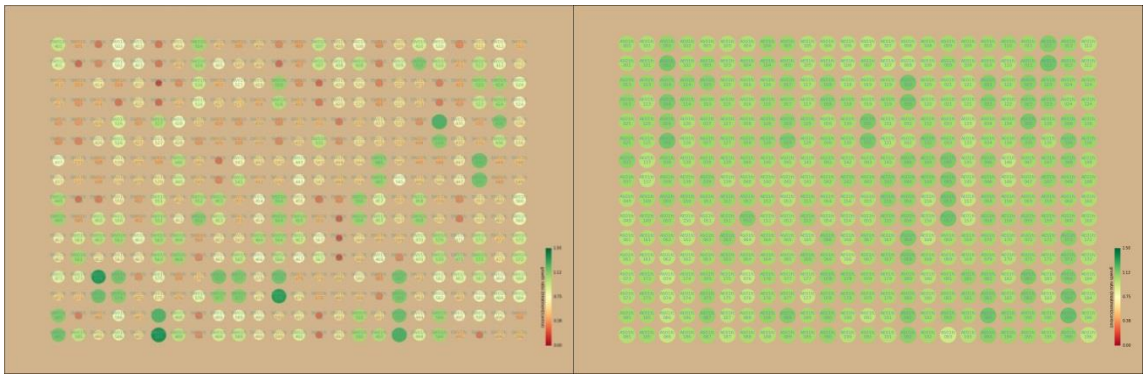


If there are multiple replicates of each strain on the plate, these plots, collected in a subfolder called 'ReplicatePlots', show each set of replicate curves on a single graph, which shows you how reproducible your experiment is. If the replicate curves are generally similar (as above, and contrast with below) then the experiment can be considered reliable. The plots also label the curves by plate position, to help you identify any abnormal colonies.

PHENOS also attempts to identify the inflection point for each curve, which is used to calculate the maximum slope and lag values. This inflection point is marked on each curve with a dot (the colour of which is scaled by the lag value). For some curves, the function that determines the inflection point will fail or give inaccurate and misleading results, so these plots are a useful indication of that.



ControlledRatios



If you compare a treatment experiment to a control, this plot is generated showing the 'treatment ratios' for each colony in the designated time window, which is the treatment:control ratio of the averaged readings (minus agar) within that time window. Green spots indicate high resistance to the treatment (with dark green showing a positive effect), while red indicates sensitivity. For effective QTL analysis, you want to see as much phenotypic diversity as possible (as above left, not as above right).

Tabular data file

PHENOS also outputs the data in each combined file as a tab-delimited text file (with the extension '.tab') which can be easily opened in Microsoft Excel. The timepoint measurements in this have had the agar absorbance measurements from the empty plate subtracted. Add the 'agar absorbance' to each reading to get the raw measurements recorded by the microplate reader.

R/qtl input file

If you have provided genotype data in the correct format, and linked to it in the 'strains.csv' file in the Genotypes folder (see "Strains.csv" on pg. 20), then PHENOS will generate a file suitable for inputting into the R/qtl program. The phenotypes included will be determined by the 'PhenotypeCalculators' section in the configuration file 'config.txt' (see "PhenotypeCalculators" on pg. 19).

ABOUT THE AUTHOR

David Barton is a Research Associate in the GACT lab of Professor Ed. J. Louis at the University of Leicester. He devised the PHENOS pipeline, the punch-in technique and the PHENOS software, and wrote this guide. Any problems with any of those things are his fault alone. Please report any problems or mistakes directly to him at dbh8@le.ac.uk.

SET UP

To start using PHENOS, first follow these instructions to set up your microplate reader and its PC with the PHENOS software and the correct settings. If this has already been done, you can skip ahead to the "EXPERIMENTAL WORKFLOW" chapter on pg. 31.

SETTING UP THE MICROPLATE READER FOR PHENOS

PHENOS has been created for and tested with BMG Labtech microplate readers in the FLUOstar Omega and FLUOstar Optima ranges. It may work with other machines but would require additional code to process different formats of output. The following instructions apply to the Omega software, but key differences in the Optima software are noted.

Setting up a new user

Upon starting the Omega or Optima control program, it may be useful to set up a new user (called 'PHENOS'). You will need the administrator password to do this. In the 'login' window, double-click on the password box next to ADMIN which should contain asterisks, and type the password, which BMG Labtech should have provided. The 'New' button below should become useable now, and with this you can create the new user and fill in the name (e.g. "PHENOS") and the User path to which protocols etc are stored (e.g. "C:\Program Files (x86)\BMG\Omega\PHENOS"). Make sure that, for now, there is no check mark in the Run Only column. Putting one there will lock the user so that its protocols cannot be accidentally altered by users. You may wish to do this later after you've created the protocols. When everything necessary has been completed, the Run button will be unlocked. Click this.

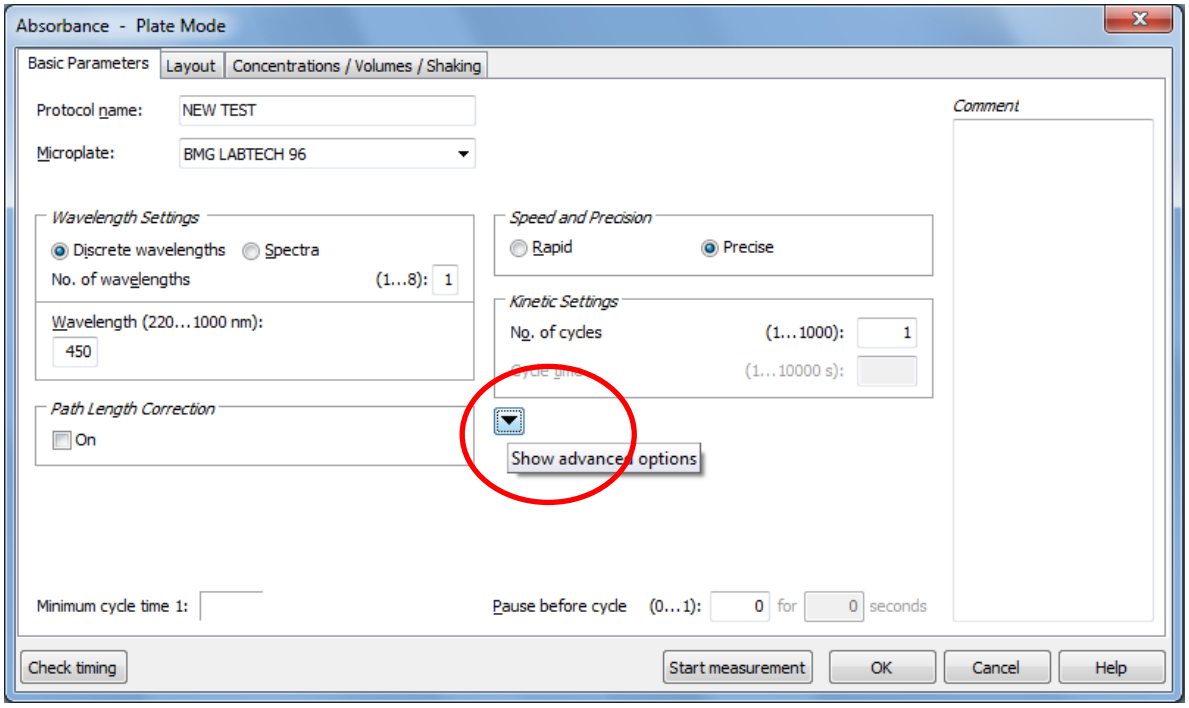
Setting up protocols

Find the Manage Protocols button on the toolbar and click it.

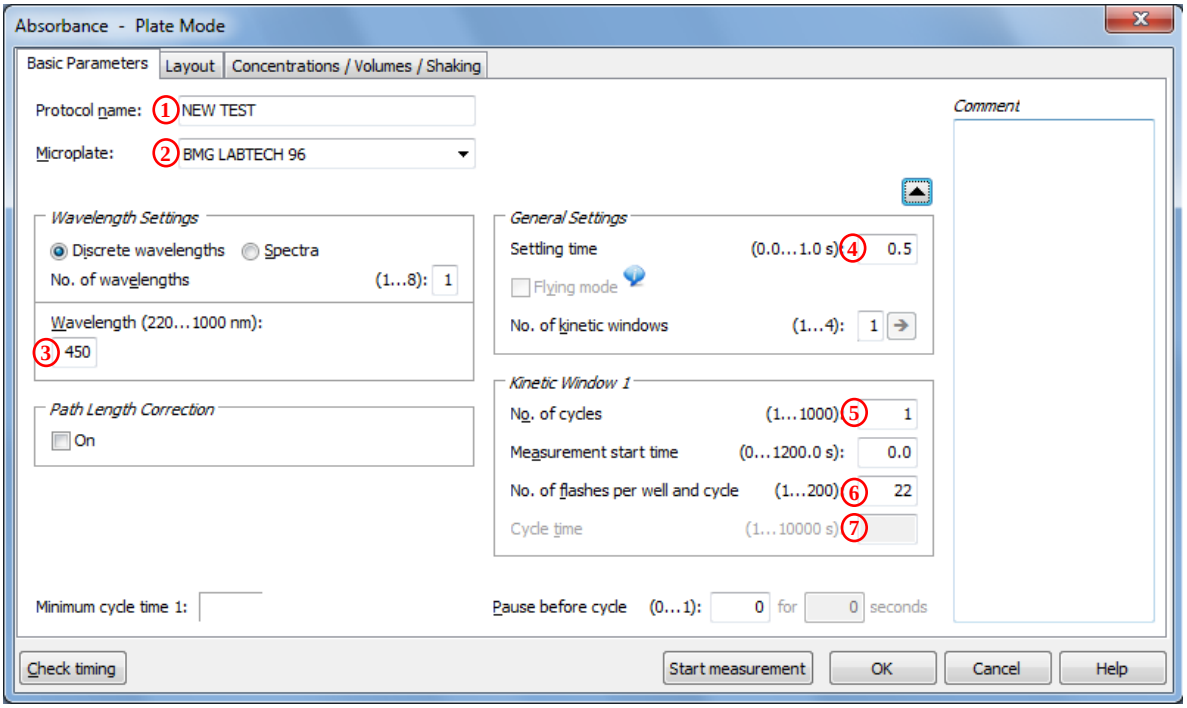
For the first protocol, find the New button and click it. For subsequent protocols you can save time by finding an already created PHENOS protocol and selecting Copy.

In the Measurement Mode window, select "Plate mode (slow kinetics)" and hit OK.

In the Basic Parameters tab that appears you need to find and click the “Show advanced options” button (shown below) to access all the parameters that need to be set.



…becomes…



For each protocol in the table below, set the parameters as listed (you will also have to visit the Layout tab before you can save each protocol; see below):

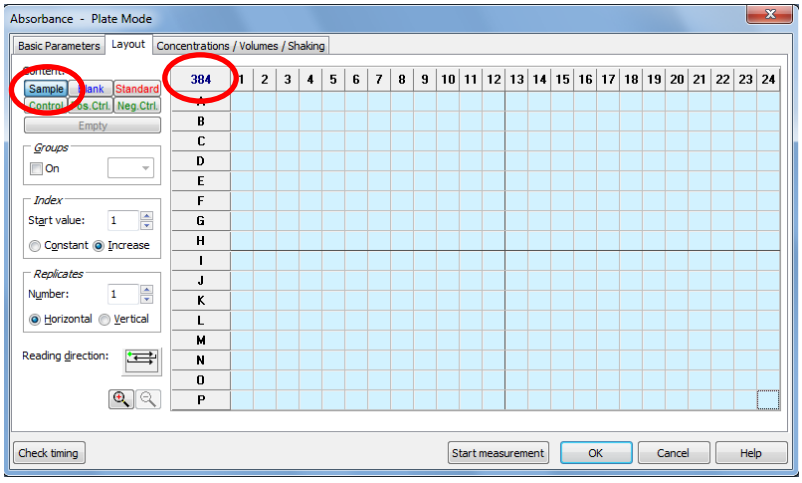
Protocol name	**Microplate**	**Wavelength** (nm)	**Settling time** (s)	**No. of cycles**	**No. of flashes…**	**Cycle time** (s)	(time to run)
1	**2**	**3**	**4**	**5**	**6**	**7**	
96-emptyplate	COSTAR 96	600	0.0	1	20		40 secs
96-snapshot				4		70	4 mins
96-17hrs				50		1200	16.3 hrs
96-65hrs				195		1200	64.6 hrs
384-emptyplate	CORNING 384 LOW VOL FL. BOTTOM			1			2 mins
384-snapshot				4		300	17 mins
384-17hrs				50		1200	16.3 hrs
384-65hrs				195		1200	64.6 hrs
1536-emptyplate	CORNING 1536			1	10		5 mins
1536-snapshot				4		300	20 mins
1536-17hrs				50		1200	16.3 hrs
1536-65hrs				195		1200	64.6 hrs

The emptyplate protocols are for measuring agar thickness prior to printing. 17hr protocols are intended to run overnight (e.g. 4.30pm to 9.00am) while 65hr protocols run over a weekend (4.30pm Friday > 9.15am Monday). Snapshot protocols are for briefly measuring colonies at irregular intervals, but take 4 readings in rapid succession in order to average out variance at high absorbances. You can of course create any custom protocol to suit your purposes.

In the Optima software, the above settings are slightly different, and instead of specifying a wavelength, you have to choose the correct filter from a dropdown list. The 595nm filter should be selected.

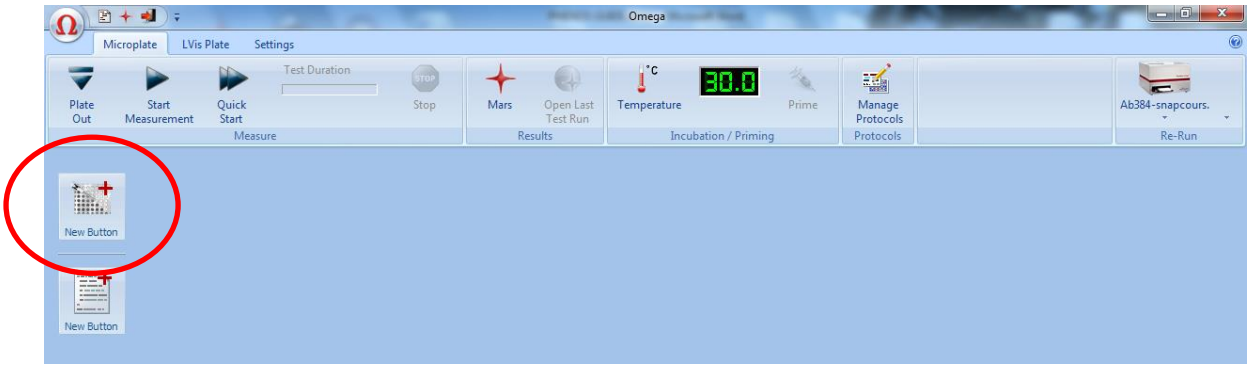
On the Layout tab for each protocol, click the sample button and then the top left corner of the grid to auto-fill the layout with numbers.

Then hit ‘OK’. The protocol will be saved. You may copy and edit previous protocols to avoid having to re-enter every single detail.

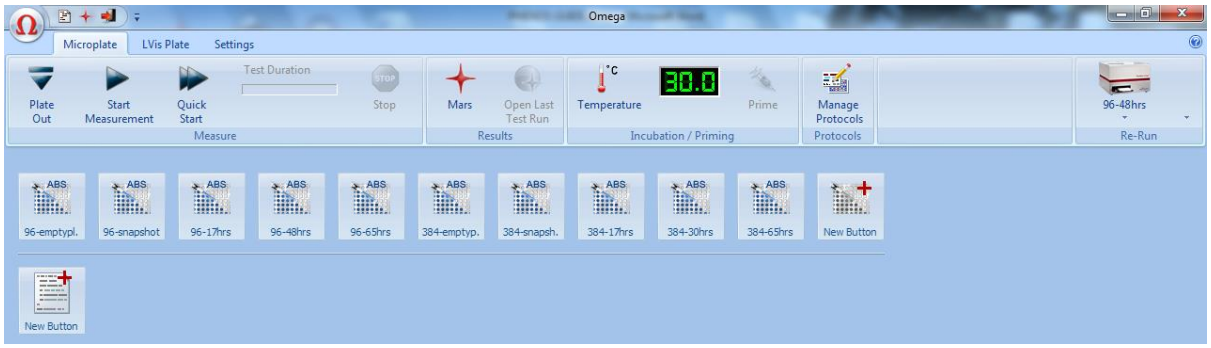


In the Omega software, after creating protocols, you may add them as buttons to the main window by clicking on the uppermost ‘New Button’ button (a tooltip shows ‘Create a new user button’ when you hover the cursor over the icon), then select the protocol from the list and hit ‘OK’.

Before:



After:



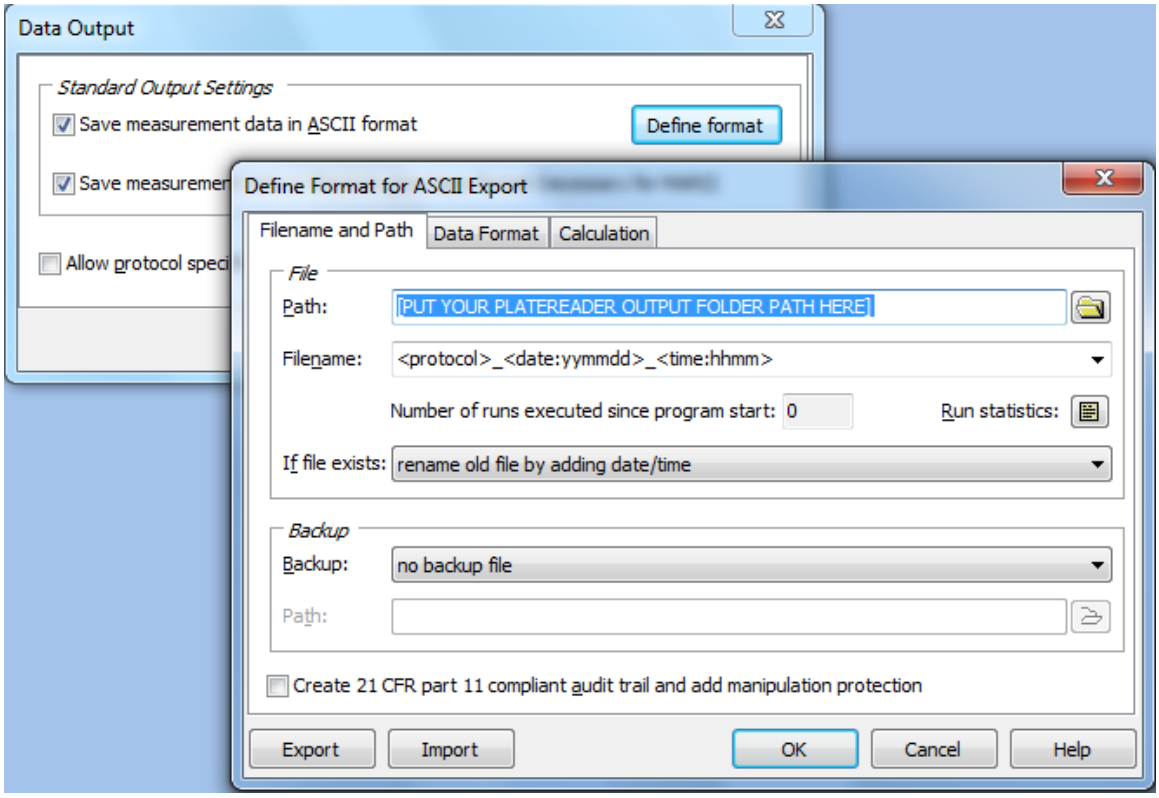
After creating all protocols, go back to the Login screen, put in the admin password, and put a check mark in the Run Only column to prevent anyone accidentally altering the protocols.

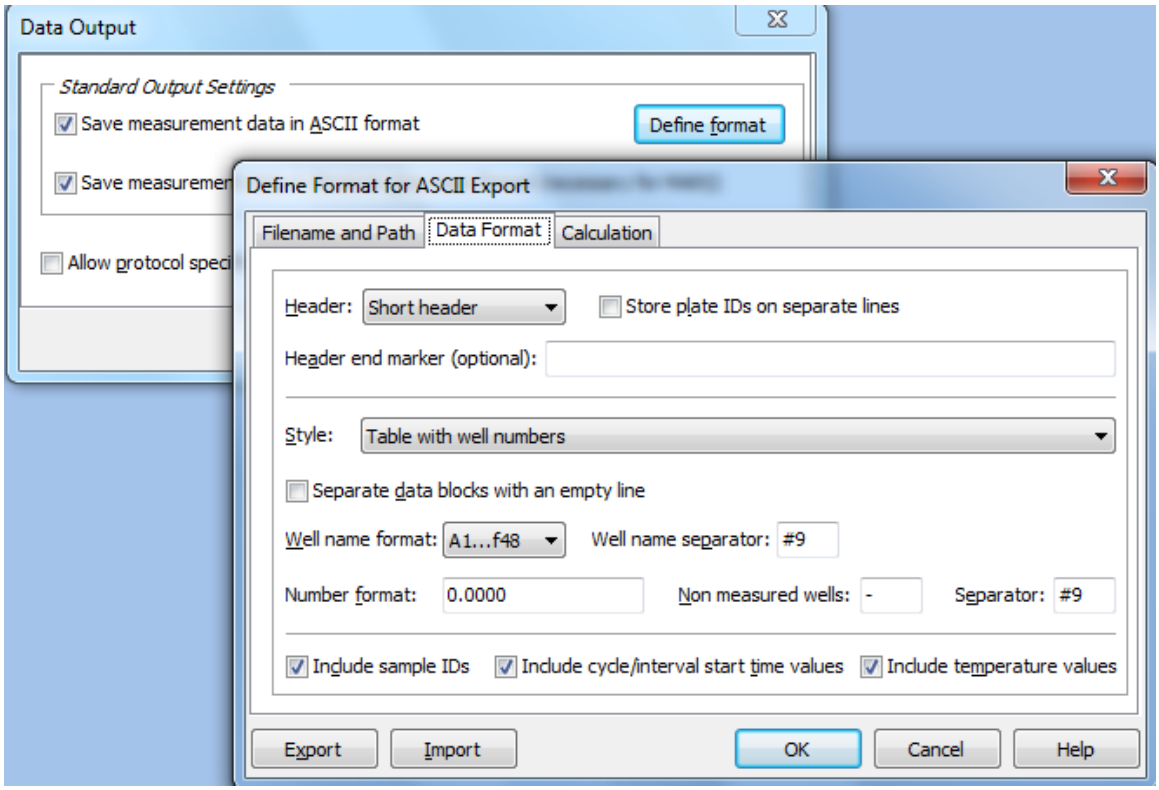
Setting up ASCII formatted data output

Before running the protocols, you need to change some user settings to tell the software to save the data (from all protocols) in a text format that PHENOS can then read.

In the Omega software, select the Settings tab along the top, and then select “Data output” and put a check mark in front of “Save measurement data in ASCII format”. In the Optima software there is an icon with a spanner on it to reach these options.

Then select “Define format” and alter the settings to match the screenshots below (apart from the ‘Path’ (highlighted), which should be the full path to whatever ‘source directory’ you wish to store microplate reader ASCII data files in (e.g. C:\Platereader output). Make a note of this as you will have to enter the same path into the config.txt file (see “Locations>source_directory” on pg. 19).





The output files should look as below, and will have either the .csv extension (newer Omega software) or the .DAT extension (older Omega or Optima software):

```
Testname: 96-48hrs
Date: 05/12/2016  Time: 11:35:19
ID1:    ID2:    ID3:
No. of Channels / Multichromatics: 1
No. of Cycles: 144

Chromatic: 1
Cycle: 1
Time [s]: 0
T[°C]: 30.1
A1   0.4727   A2   0.6564   A3   0.5507   A4   0.6867   A5   0.6656   A6   0.4914   A7   0.4939   A8   0.4906   A9   0.6307   A10  0.6309   A11  0.4830   A12  0.5340
B1   0.5156   B2   0.5368   B3   0.4165   B4   0.4940   B5   0.4539   B6   0.5209   B7   0.4999   B8   0.4963   B9   0.4822   B10  0.4657   B11  0.4460   B12  0.4211
C1   0.4676   C2   0.5998   C3   0.4927   C4   0.5057   C5   0.5240   C6   0.5478   C7   0.4957   C8   0.4912   C9   0.5101   C10  0.4268   C11  0.3854   C12  0.3086
D1   0.4872   D2   0.5294   D3   0.4969   D4   0.4613   D5   0.4833   D6   0.4986   D7   0.4735   D8   0.3790   D9   0.4076   D10  0.3975   D11  0.4291   D12  0.3561
E1   0.4577   E2   0.4066   E3   0.4359   E4   0.4418   E5   0.4529   E6   0.4731   E7   0.4516   E8   0.4251   E9   0.4119   E10  0.3917   E11  0.3157   E12  0.3011
F1   0.4541   F2   0.4636   F3   0.4647   F4   0.4718   F5   0.4696   F6   0.4007   F7   0.3824   F8   0.4590   F9   0.3717   F10  0.4025   F11  0.3346   F12  0.3264
G1   0.3870   G2   0.3723   G3   0.4465   G4   0.4588   G5   0.4554   G6   0.4458   G7   0.4209   G8   0.4066   G9   0.3536   G10  0.3169   G11  0.3218   G12  0.3412
H1   0.4762   H2   0.3705   H3   0.4494   H4   0.4635   H5   0.4306   H6   0.4004   H7   0.3647   H8   0.3831   H9   0.3131   H10  0.3088   H11  0.2708   H12  0.2743
Cycle: 2
Time [s]: 1200
T[°C]: 30.1
A1   0.4571   A2   0.6119   A3   0.4924   A4   0.6713   A5   0.6404   A6   0.4671   A7   0.4740   A8   0.4731   A9   0.6009   A10  0.6117   A11  0.4720   A12  0.5187
B1   0.5158   B2   0.5051   B3   0.4067   B4   0.4869   B5   0.4430   B6   0.4949   B7   0.4845   B8   0.4812   B9   0.4675   B10  0.4504   B11  0.4283   B12  0.4108
C1   0.4361   C2   0.5812   C3   0.4704   C4   0.4919   C5   0.5028   C6   0.5170   C7   0.4782   C8   0.4760   C9   0.4865   C10  0.4083   C11  0.3755   C12  0.3028
D1   0.4765   D2   0.5159   D3   0.4804   D4   0.4492   D5   0.4741   D6   0.4837   D7   0.4551   D8   0.3646   D9   0.3960   D10  0.3864   D11  0.4127   D12  0.3531
E1   0.4498   E2   0.3978   E3   0.4232   E4   0.4265   E5   0.4423   E6   0.4665   E7   0.4459   E8   0.4231   E9   0.4038   E10  0.3878   E11  0.3093   E12  0.3002
F1   0.4486   F2   0.4557   F3   0.4502   F4   0.4578   F5   0.4584   F6   0.4006   F7   0.3903   F8   0.4518   F9   0.3723   F10  0.3958   F11  0.3349   F12  0.3290
G1   0.3838   G2   0.4033   G3   0.4457   G4   0.4399   G5   0.4504   G6   0.4367   G7   0.4351   G8   0.4175   G9   0.3531   G10  0.3188   G11  0.3268   G12  0.3416
H1   0.4521   H2   0.3974   H3   0.4370   H4   0.4558   H5   0.4310   H6   0.4107   H7   0.3837   H8   0.3982   H9   0.3193   H10  0.3140   H11  0.2727   H12  0.2748
Cycle: 3
Time [s]: 2400
T[°C]: 30.0
```

INSTALLING THE PHENOS SOFTWARE

The PHENOS software is best set up on the same PC that operates the microplate reader and its associated software. This must be a Windows PC, and you must have administrative privileges to install software on it. It does not necessarily need to be networked as the installation files can be copied to the machine on a USB drive.

1. Copy the PHENOS files from https://github.com/gact/phenos/ to C:\PHENOS.
2. Install Python 2.7 using the installer included in "PHENOS/Installation files" (or downloaded from https://www.python.org/downloads/release/python-2712/) preferably using the default installation path "C:\Python27".
3. Add this installation path to the Windows PATH variable, by following the instructions at http://superuser.com/questions/143119/how-to-add-python-to-the-windows-path.
4. Install Microsoft Visual C++ from https://support.microsoft.com/en-gb/kb/2977003.
5. For animations of plate growth, install ImageMagick using the installer found in "PHENOS/Installation files/32bit" (or "PHENOS/Installation files/64bit" if using a 64 bit version of Windows), or downloaded from https://www.imagemagick.org/script/binary-releases.php.
6. You must now install a number of Python modules in the order given in the table on the next page. Run cmd.exe in administrator mode (google for instructions if needed). For each module in the list, if the PC is connected to the internet, you should be able to type "python -m pip install [module name]", but if not, or if this fails, copy the appropriate module wheel file from "PHENOS/Installation files/32bit" or "PHENOS/Installation files/64bit" to the directory you are working in in cmd.exe and then type "python -m pip install [module wheel file name]". The wheel files come from Chris Gohlke's site http://www.lfd.uci.edu/~gohlke/pythonlibs/.

[module name]	[module wheel file name]	
	32bit version	**64bit version**
Cython	Cython-0.24.1-cp27-cp27m-win32.whl	Cython-0.24.1-cp27-cp27m-win_amd64.whl
numpy	numpy-1.11.2+mkl-cp27-cp27m-win32.whl	numpy-1.11.2+mkl-cp27-cp27m-win_amd64.whl
numexpr	numexpr-2.6.1-cp27-cp27m-win32.whl	numexpr-2.6.1-cp27-cp27m-win_amd64.whl
urllib3	urllib3-1.18-py2.py3-none-any.whl	
six	six-1.10.0-py2.py3-none-any.whl	
h5py *	h5py-2.6.0-cp27-cp27m-win32.whl	h5py-2.6.0-cp27-cp27m-win_amd64.whl
*N.B. the h5py module installation will probably not work unless you use the wheel file, as outlined at http://docs.h5py.org/en/latest/build.html		
tables	tables-3.3.0-cp27-cp27m-win32.whl	tables-3.3.0-cp27-cp27m-win_amd64.whl
scipy	scipy-0.18.1-cp27-cp27m-win32.whl	scipy-0.18.1-cp27-cp27m-win_amd64.whl
pytz	pytz-2016.7-py2.py3-none-any.whl	
dateutil	python_dateutil-2.5.3-py2.py3-none-any.whl	
cycler	cycler-0.10.0-py2.py3-none-any.whl	
pyparsing	pyparsing-2.1.10-py2.py3-none-any.whl	
matplotlib	matplotlib-1.5.3-cp27-cp27m-win32.whl	matplotlib-1.5.3-cp27-cp27m-win_amd64.whl
biopython	biopython-1.68-cp27-cp27m-win32.whl	biopython-1.68-cp27-cp27m-win_amd64.whl
pypiwin32	pypiwin32-219-cp27-none-win32.whl	pypiwin32-219-cp27-none-win_amd64.whl
xlrd	xlrd-1.0.0-py2.py3-none-any.whl	
brewer2mpl	brewer2mpl-1.4.1-py2.py3-none-any.whl	

CONFIGURING PHENOS BY EDITING CONFIG.TXT

When run, PHENOS checks a file called config.txt for certain configuration options:

```
[Locations]
source_directory = C:\Platereader output
target_directory = C:\PHENOSdata
user_folder = Test

[Graphics]
type = png
!available = png, jpg, eps

[GUI]
position = 1100,800,50,50
!low-res = 750,550,25,25

[Controls]
controls = YPD, YPD 30C, COM, COM 30C, Control

[PhenotypeCalculators]
!default = TreatmentRatioCalc, LagCalc, MaxSlopeCalc
!available = MaximumChangeCalc, AverageWithoutAgarCalc, TreatmentRatioCalc,
LagCalc, MaxSlopeCalc, MaxSlopeTimeCalc, DifferentialTimeCalc, PrintedMassCalc,
PrintedMassControlledCalc, ShrinkageCalc, HalfPeakTimeCalc
MMS.* = TreatmentRatioCalc, DifferentialTimeCalc

[CombiFileVisualizations]
!default = AgarThickness, PrintingQuality, FinalGrowth, FinalGrowth_Lag,
FinalGrowth_MaxSlope, CurvesWithoutAgar_PrintedMass, CurvesWithoutAgar_Groups,
CurvesWithoutAgar_Slopes, CurvesWithoutAgar_Lags, CurvesNormalized_PrintedMass,
Histogram_MaxWithoutAgar, Scatterplot_PrintedMass_Lag, Animation_Temp,
ReplicatePlots
!available = AgarThickness, PrintingQuality, FinalGrowth, Animation_Temp,
CurvesWithoutAgar_PrintedMass, CurvesWithoutAgar_Groups,
CurvesWithoutAgar_Slopes, CurvesWithoutAgar_Lags, CurvesNormalized_PrintedMass,
Histogram_MaxWithoutAgar, Scatterplot_PrintedMass_Lag, ReplicatePlots,
LayoutView, Animation, Histogram_MaxChange, FinalGrowth_Lag,
FinalGrowth_MaxSlope
```

When you first run PHENOS, the config.txt file from the PHENOS folder will automatically be copied to "C:\Users\[your windows user name here]\AppData\Roaming\PHENOS", and it is this copy that must be edited in the future. Before this copying happens you should any of the following fields that you need to:

Locations>source_directory

If necessary, edit this entry to match the path where you have told the microplate reader to store ASCII data output (by default, it is "C:\Platereader output").

Locations>target_directory

If necessary, edit this to match the location of the folder where you want your renamed data files and visualization plots to be stored (by default it is "C:\PHENOSdata"). Don't forget to create this folder if necessary and you will also need to copy the Layouts and Genotypes folders from the PHENOS installation files into this target directory, as described below in "COPYING LAYOUTS AND GENOTYPES FOLDERS" on pg. 20.

Locations>user_folder

This entry will be constantly updated by PHENOS to whichever user folder you created or selected last, so there is no need to edit this. It defaults to 'Test'.

Graphics>type

By default, graphics are generated in the 'png' raster format, but you can change this to any of the other extensions listed under Graphics>!available, e.g. 'jpg' or 'eps' (a vector format suitable for importing into PowerPoint).

GUI>position

If using a lower resolution monitor, change this to the settings listed under 'GUI>!low-res'. These numbers control the window width, window height, top left x coordinate and top left y coordinate of the PHENOS window respectively.

Controls>controls

This comma-delimited list contains all treatment names that PHENOS will treat as controls (these experiments will then be available to compare against non-control experiments). You may add to this list if you need custom control treatments for any reason.

PhenotypeCalculators

This section controls how phenotype values are generated for R/qtl input files (see "R/qtl input files & phenotype calculators" on pg. 52). Each phenotype calculator produces one phenotype column in the R/qtl input file. The possible calculators should be listed under '!available'. You can change the default calculators listed under '!default', or make special arrangements for particular treatments, e.g. adding the

line ‘Ethanol 10% = TreatmentRatioCalc’ means that for any experiments with the treatment ‘Ethanol 10%’, only the TreatmentRatioCalc column will appear in the R/qtl input file. If you end the treatment name with ‘.*’, it will match any treatment that begins with the previous characters, e.g. ‘MMS.*’ will match ‘MMS 0.05%’ as well as ‘MMS 0.01%’.

CombiFileVisualizations

This section controls which visualizations will be generated. All the possible visualization types should be listed under ‘!available’ (they are described in “VISUALIZATIONS” on pg. 5). You can change the default visualizations listed under ‘!default’, or make special arrangements for particular experiment user initials. E.g. if you use the initials AB for your experiment, and this section contains the paragraph ‘AB = LayoutView”, then for any experiment given those initials, only the LayoutView visualization will be generated. All visualizations will be placed into the target directory, inside a subfolder called ‘Plots’, inside another subfolder matching the name of the user folder. PHENOS will open this folder as an explorer window when it creates them. You might choose to create a desktop shortcut to this folder for your convenience, as well as a desktop shortcut to the PHENOS script.

COPYING LAYOUTS AND GENOTYPES FOLDERS

Copy the Layouts and Genotypes folders from the installation folder (C:/PHENOS) to the target directory (e,g, C:/PHENOSdata). You will need to create a layout file (‘.xlsx’ or ‘.csv’ format) for each yeast array you use in PHENOS (see “PREPARING LAYOUT FILES” on pg. 33), and you should also keep track of all your strains in a single file called “strains.csv” in the Genotypes folder. To create R/qtl input, you must also have genotype information in a separate file or files that are referred to from within “strains.csv”. These files are explained below:

Strains.csv

A ‘comma-separated values’ text file in the Genotypes folder called ‘strains.csv’ should be used to store additional information about your strains that might not be included in the layout file. A basic version of this file is included in the installation Genotypes folder. You can add additional auxotrophy columns to the right side of it if you want to. Most importantly, if generating input files for R/qtl, you need genotype information for each strain, and PHENOS needs to know where to find it. It looks up the strain in ‘strains.csv’ (N.B. the strain name must be exactly as it was in the layout file, including the same capitalization, punctuation, spaces etc). Then it checks the ‘GenotypeFile’ column for that strain. If it finds a file name, it looks for that file name in the Genotypes folder and tries to open it as a genotype file (see below) and extract genotype information from it.

Genotype files

You can have as many different genotype files as you need. They should be formatted like the 'Example genotypes.csv' in the installation Genotypes folder, a portion of which is shown below (but columns are separated by commas in the actual file). Missing or unknown parental genotypes can be denoted with a "-". At present R/qtl can only handle two different parental genotypes (these can be single letters or single numbers).

strain names

marker names
chromosomes
positions
parental genotypes

id	c01:0040000	c01:0119000	c02:0472000	c02:0517000	c02:0522000	c04:045400
	1	1	2	2	2	4
	40000	119000	472000	517000	522000	454000
AW12fc001	W	W	A	A	A	W
AW12fc002	-	-	-	-	-	-
AW12fc003	W	W	A	A	A	W
AW12fc004	W	A	A	A	A	W
AW12fc005	W	W	A	A	A	W
AW12fc006	W	W	A	W	W	A
AW12fc007	W	A	A	A	A	A
AW12fc008	W	A	A	W	W	W
AW12fc009	W	W	A	A	A	A
AW12fc010	W	A	A	A	A	A
AW12fc011	W	W	A	A	A	W
AW12fc012	W	A	A	A	A	W
AW12fc013	W	A	A	A	A	A
AW12fc014	W	W	A	A	A	W
AW12fc015	W	A	A	W	W	A
AW12fc016	W	A	A	W	W	W
AW12fc017	W	W	A	W	W	A

You should now be ready to begin using the PHENOS pipeline.

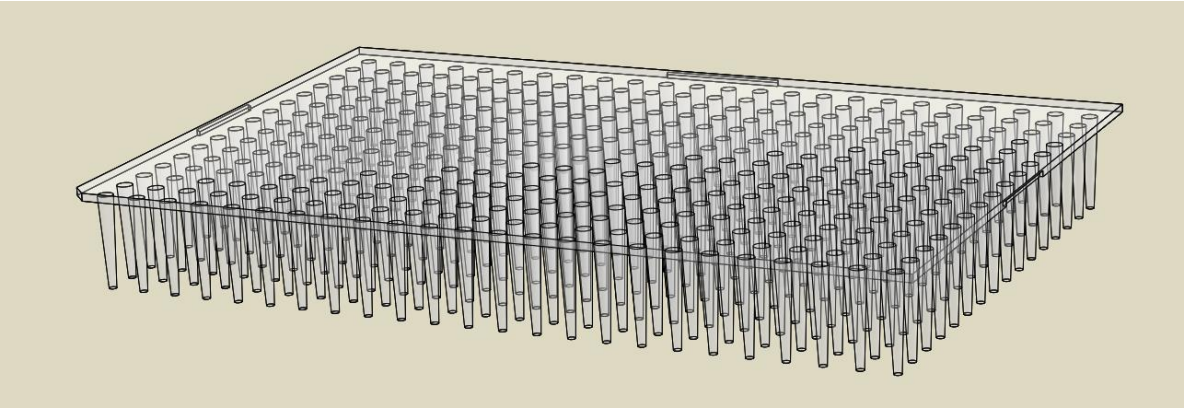
OPERATING THE MACHINES

The PHENOS pipeline relies on two pieces of equipment, the Singer Instruments ROTOR colony manipulation robot and a microplate reader (we use the BMG Labtech FLUOstar Omega model, though it should be possible with other machines too, although PHENOS would need to be updated to read different styles of ASCII data input).

Please refer to the documentation for these machines for full instructions on their use, however a limited overview is provided below.

USING THE SINGER INSTRUMENTS ROTOR ROBOT

The Singer Instruments ROTOR HDA colony arraying robot is essentially just a little block printer which uses disposable plastic 'repads' (see below) to copy cells between solid agar plates or microtiter plates (MTPs). The most common arrays are "96" (12x8), "384" (24x16), and "1536" (48x32) though the latter can be difficult to work with. The repads come in long-pin (like below) and short-pin varieties. The long-pin ones are really meant for working with liquid-filled MTPs but because these repads can be recycled more easily, and they can be used for the 'punch-in' method described below, we tend to use them for most purposes.



The robot is very user-friendly and the software generally tells you what to do in a step-by-step manner. When you have a choice, presented on the ROTOR software as a green tick or red cross icon, or the ROTOR wants you to click a tick to proceed, you can click the icon within the software, or you can use the 1/0 button on the front of the ROTOR (1 means yes/tick, 0 means no/cross).

Use gloves when touching any part of the machine itself, or when handling repads, in order to minimize the risk of contamination.

There are two gaps in the front of the machine, below the level of the main deck. The gap on the left should have a metal drawer in it which collects used repads. Don't use the machine without this drawer or you'll have a mess to clean up. The gap on the right should contain a blue metal hopper that can be pulled out and filled with clean repads.

The main printing head of the ROTOR is a nondescript grey colour with a metal underside full of little suction holes. There is a special alternate head called the Stinger which is gold coloured with a black bumper surrounding a little robotic arm. The Stinger can be used to rearrange colonies and if you want to use it seek direct instruction because changing the heads must be done with care. Make sure that the normal grey default head, not the gold Stinger, is in the machine.

The ROTOR will need to be told, in advance, what plates are being put into it and where: which bay each goes in to (colour-coded black, red, blue, green and yellow), what array type (96, 384, or 1536), what media type (solid, MTP, etc), and whether each plate is a source plate or a target plate. Only once you've entered this information does it present further options.

When you put plates in the bays, make sure that they are oriented as they should be so that your target plates will have the same orientation as the source plates. The rectangular PlusPlates have little nicks in the edges so that they should only fit into the bays one way round (in this orientation, when you hold the plate up you should see the word Singer on the lid the right way up, and position A1 should be at the top left). Always check that plate lids are loose and will come off without difficulty. Some PlusPlates arrive defective, with reinforced struts inside the lids having dug little grooves in the edge of the plate, and these can make it hard for the ROTOR to remove the lid which causes problems.

Turning the ROTOR on

Turn on the ROTOR using the red switch near the bottom right edge of the machine, and the accompanying computer. When prompted (or before), turn on the compressor using the square green button on the end of it, which will light up when it is on. You should hear the pump working immediately. Click on the green tick or hit the 1 button on the front of the ROTOR to skip to the next screen, which asks you to remove obstructions from the stage. If it's clear, hit the tick / 1 again. Let the "Initializing the ROTOR" screen pass and the main menu should appear, which says "Select an operation mode" across the top.

UV-sterilizing the ROTOR

Before using the Singer ROTOR each time, it is wise to UV sterilize it for at least 20 minutes. Find the purple "UV" icon on the bar down the right hand side and click it. Set the minutes to 20. Put a tick in the checkbox labelled 'Cycle lid lifter arms…', hit "Start", then go and do something else.

Loading repads

There is a hole at the bottom right corner of the front of the robot under the deck. Reach in (with gloves on) and feel for a handle. Pull this towards you to pull the blue metal hopper out. This is where the repads go. If there is any paper at the bottom of it (from an earlier run), discard it. Keep this hopper clean.

If you are using new repads, they will be sealed in stacks of 4 with a paper sheet covering the bottom pins and a loop of paper around the stack. When asked to load repads, wearing gloves, pull out a whole stack of 4 and insert it in the hopper with the pins pointing downwards, making sure to get the orientation of the cut-off corner correct (as illustrated by the software; it should be oriented towards the back right corner of the robot when loaded. After that, remove the strip surrounding the stack.

If you are using recycled repads, then it's wise to give the base of the hopper a quick spray with IMS and wipe it clean with a sterile wipe. You can then load up to 5 repads in at the time, oriented as described just above.

Standard programs

The ROTOR comes with a variety of built-in programs under 3 main categories: "Replicate" simply copies one plate to one target. "Replicate Many" copies one plate to multiple targets. "Mate" takes multiple source plates and combines them on a single target plate. For more complex arrangements it's easiest to use 'manual control' (see pg. 26).

The standard programs are straightforward and the software should be easy to follow. One thing that can catch you out, however, is that the pinning settings (or program options) are not stored with each program, but carry over from whoever used the machine last. Therefore you must always check these settings before proceeding with any program or manual control (see below).

Pinning settings / program options

The pinning settings or program options control precisely where, how fast, how hard, how deep and how often the repads are pushed into the source or target plates (there are different settings for each), and the program options menu also allows you to customize repad recycling so that you can reuse repads (if sensible to do so).

When a program is running, a series of icons down the left side of the screen control which of these settings are applied. Make sure that, if you want dry mixing, for example, that you not only set the pinning settings sliders how you want them, but also select the dry mixing icon for whichever plate type (source or target) you want to do dry mixing on.

A brief overview of the main options here:

Offset: by default the pins go down at a very precise location, but you might want to vary that, perhaps because you are printing multiple targets from a single source and you want to pick up as much of the

source colony as you can. You can control the offset manually (for each pinning, you get a little target screen and select the offset location by eye), or set it to a fixed value, or selecting automatic offset will make the robot remember each pinning from a source plate and make sure it pins from a slightly different location each time.

Pinning: you can control, for source plates and target plates separately, the Pin Pressure, Speed, Overshoot and Repeat Pin number. Pin Pressure should usually be 100%, but if printing to a soft agar punch-in plate (see "Printing to the punch-in plate" on pg. 36) you might want to use 50% so as to not dig into the agar. Speed can usually stay at the default of 19 mm/sec. Overshoot is normally 1.5mm, but you can push this up to 2.5 for the 'punch-in' method. Repeat Pin is normally 1 time, but for 'punch-in' can be set much higher.

Dry mixing (for agar plates) or **Mixing** (for MTPs): for solid agar plates, if you want to be sure of picking up cells when colonies are small or irregular, you can instruct the robot to rapidly print (from source or to target) in a little cross pattern to cover more area. If 'step-in' is selected, each cross will be a little smaller than the last. You can control the size and number of repeats of this dry mixing. A similar setting is available for liquid MTPs, and this can be used to thoroughly stir plates of glycerol stock that have just come out of -80°C storage.

Manual control

This mode allows you to control every individual move the machine makes and then record your series of moves as a new program which can be repeated exactly. As with standard programs, you first need to tell the robot what plates you are putting where. You then need to check your pinning settings. Then you get a screen of controls which should be simple to follow. Options will be greyed out for logical reasons. For example you won't be allowed to pin to a plate if you have not first removed the lids. The 'get fresh' option combines dumping an existing repad with picking up a new one. If you are using, for example, a 96 repad to pin to a 384 array, after you hit pin you will be presented with a screen allowing you to select which quadrant to pin to. By default this shows the A1, A2, B1 and B2 positions (at the top left corner of an array), but it will show them upside down as you will be viewing the array upside down. However you can deselect the 'Display as plate in ROTOR?' checkbox to invert this view and get a 'machine's eye view' of these four positions. Click on the one that you want the first pin of the repad to visit. Confusingly, the ROTOR software refers to the position that would be called A1 on a 384-well MTP as '1A', A2 as '2A', B1 as '1B', and B2 as '2B'.

When you've finished, you can click on the green tick to end manual control. If you have not already dumped the repad or replaced the lids, the software will do that for you, and it will then tell you when to remove plates.

You then get a screen headed 'Manual mode complete' with 5 options on it. If you want to save what you have done to repeat it exactly on a later occasion, the best option to choose is 'Automatically evaluate and save as a new program (recommended)', which just performs a few common-sense checks on things and weeds out unnecessary moves. You will be prompted to select a suitable icon for your program and then

name and describe it (the name can only be quite short and special characters aren't allowed, so keep it simple and put more info into the description). Your program will then be available from the 'Select and run stored programs' option of the main menu, once you have specified the appropriate source and target plates and repad type. Note that precise pinning settings (see below) are not saved with the program and must be set manually each time you use the program.

Shutting down the ROTOR

Remove all plates, then go back to the main menu (the home button on the right should take you there) and hit the off button on the right. The software will guide you through the shutdown procedure, so turn off the compressor and the ROTOR when instructed to. Take the metal dump drawer out and deal with used repads appropriately (see below). Spray the inside of the empty metal dump drawer with IMS and wipe it clean before putting it back for the next user.

Recycling Singer repads to save money

Repads are expensive but the long-pin ones can be reused several times if they are properly cleaned and sterilized first: they can be autoclaved at 110°C for 10 minutes without deforming, and recycled repads can reliably be used for ordinary stock plate maintenance. However it is best to use brand new repads for printing experimental plates for the PHENOS pipeline because the recycling process may leave pins slightly misaligned or alter their adherence properties.

USING THE FLUOSTAR OMEGA MICROPLATE READER

Turning the FLUOstar Omega microplate reader on

If there is no green light at the left end of the red band on the front of the plate-reader, feel around the back bottom left corner for the power switch and turn it on. Ensure the connected PC is turned on and the Omega software has been started.

If you are using a FLUOstar machine that has been equipped to take fluorescence or luminescence readings, then ensure that the correct leads are properly installed, with sufficient spacer blocks to keep the heads clear of the lids on your plates.

Next to the light on the front is a small round black button which opens and shuts the tray below, which is where you put your plate. Always put plates in this tray the right way up (lid on top) and the right way around, so that the A1 (top left) position of the array is at the back on the left. If using Singer PlusPlates™, the word Singer on the lid should be the right way up as you look down on your plate.

Using the FLUOstar Omega software

Log in on the PC that controls the microplate reader, and on the desktop look for a black and red icon named “Omega” (see right) and double-click this to run the application.

The first window that should appear says “Login” at the top. From the list of users, find “PHENOS”, click to highlight it, and click the Run button.

In the main program window there is a row of large icons across the top. Notice the one labelled “Temperature”. If you wish to measure long-term growth curves, you need to click on this to set the temperature appropriately in the Temperature Control window that appears. 30°C is standard, unless you’re measuring something like heat or cold resistance. Then click the “Incubator On” button and then “Close”. The temperature meter to the right of the Temperature button should light up. It only takes a minute to warm up to 30°C.

Ensure the platereader is shaded from direct sunlight at all times, as this can raise the internal temperature.

Assuming that the microplate reader has been set up properly (see “Setting up protocols” on pg. 11), you can find the PHENOS protocols laid out as square buttons in the main window, or else listed when you clock on the “Manage Protocols” button on the tool bar.

You need to pick the appropriate program for your array size and for your purposes:

Protocol name	Purpose	No. of cycles	Cycle time (s)	Time to run
96-emptyplate	For measuring agar absorbance before you've printed any cells to the plate. It just takes one reading for each point on the plate, which is fine for this purpose	1	-	40 secs
384-emptyplate		1	-	2 mins
1536-emptyplate		1	-	5 mins
96-snapshot	For taking intermittent measurements at arbitrary timepoints. Taking 4 measurements in a row accounts for sampling errors (especially with larger colonies)	4	70	4 mins
384-snapshot		4	300	17 mins
1536-snapshot		4	300	20 mins
96-17hrs	For generating growth curves overnight, from about 4pm until about 8.30am. Sufficient for some purposes.	50	1200	16.3 hrs
384-17hrs		50	1200	16.3 hrs
1536-17hrs		50	1200	16.3 hrs
96-65hrs	For generating full growth curves over a weekend, from about 4pm Friday until about 8.45am Monday. Useful for late-emerging phenotypes and controls for all experiments	195	1200	64.6 hrs
384-65hrs		195	1200	64.6 hrs
1536-65hrs		195	1200	64.6 hrs

You can create your own protocols if you have the Administrator password for the Omega software, and copy the programs enough and then make changes to the copy, but make sure you don't accidentally alter the existing protocols above.

When you have clicked on the correct protocol, a window labelled "Start Measurement" should open with a "Start Measurement" button at the bottom right. Click this to begin the protocol.

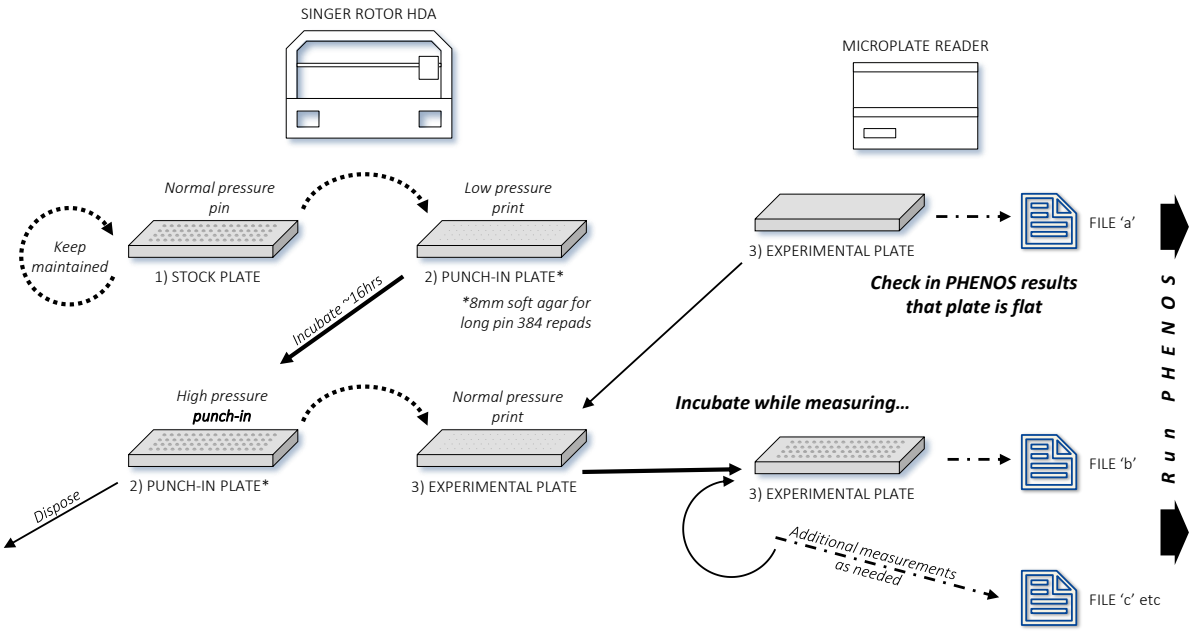
You should hear the plate-reader making a few noises that correspond with moving the plate around and checking the optical gear, and after a few seconds the actual measurements should begin: a regularly spaced series of buzzes, with the sound of the plate being repositioned at the end of each row (by default the reader moves left to right on the first row, then down to the next row and right to left, then down again and left to right, and so on in a slalom pattern). Once you hear the plate-reader shifting down a row, you can be confident that everything is OK and you can leave the machine to run.

Take precautions to ensure that other users know that the reader is in use and should not be interfered with.

Scanning or photographing plates

It's a good idea to take a picture of the plate after you take it out of the plate-reader, so that you can compare it to the PHENOS output and check that everything looks like it should. You can take pictures with a smart phone, or else use a flatbed scanner.

EXPERIMENTAL WORKFLOW



PHENOS experiments follow the flow illustrated above. You keep your arrays on **stock plates**, copy them on to temporary **punch-in plates** which you then use to create normalized **experimental plates**, which are normally then incubated in the microplate reader while readings are taken.

DESIGNING YEAST ARRAYS

Before beginning, you need to design and create your yeast arrays, and create layout files which describe them.

Array sizes

While 96 arrays may sometimes be better (e.g. to allow colonies to grow big enough to see different colony morphologies), for most purposes a 384 array containing 2-4 replicates of every strain provides much more reliable phenotype data, and is simple to create from one or two 96 source arrays. 96 arrays can last longer however, and there is a reduced risk of cross-contamination, so it is worth maintaining both 96 stock plates and their 384 derivatives. Stock plates can be refrigerated for many weeks but need to be backed-up, checked regularly, and duplicated before agar dries up or gets contaminated.

Fingerprint your arrays

Early on, these arrays should be characterized for any phenotypes that may discriminate between individuals such as auxotrophies, mating types, low and high temperature growth, and colony morphologies. This information can be used to check that later copies haven't been mislabelled, misoriented, or suffered from obvious cross-contamination. Consequently, it's good practise to have a mixture of these discriminating phenotypes in a given array, although it may be wise to separate mating types to avoid accidental mating and takeover of colonies by faster growing diploids.

Maintain and normalize stock plates

Slow-growing colonies (e.g. petite mutants) can be lost from arrays through repeated duplication, as small colonies produce even smaller copies. If this becomes problematic, normalize the stock arrays using the punch-in method described below. Allowing stock plates to grow at room temperature can help.

Note on working with 1536 arrays

The BMG Labtech FLUOstar Omega is capable, with a quick free vendor adjustment to the base settings, of reading 1536 arrays. 1536 yeast arrays are challenging to maintain because slower growing colonies can easily be lost over subsequent reprintings, although PHENOS could itself be adapted to check arrays for missing spots and generate instruction files for the ROTOR Stinger attachment to replace them. Another problem is that Singer Instruments 1536 repads come only in a short-pin variety with very fine pin cross-sections. The precision of spot placement and of microplate reader measurement coordinates is insufficient to produce reliable alignment, so the reader measurements tend to generate an illusory sweep of growth across the plate as colonies on one side aren't picked up at all until they grow large enough. These problems might be overcome by using four long-pin 384 repads to print the 1536 array from four soft agar 384 punch-in plates. However there are other concerns. The greater proximity of colonies in 1536 arrays reduces peak growth efficiency across the plate and brings forward the timepoint at which edge effects become prominent (peripheral colonies continuing to grow for longer due to greater availability of nutrients). Also, mating pheromone inhibition may complicate growth in mixed mating type arrays.

PREPARING LAYOUT FILES

The “Layouts” subfolder (which should have been copied to the target directory e.g. C:/PHENOSdata/Layouts) contains three Excel template files you can copy and edit to create your own layout files: “Basic96.xlsx”, “Basic384.xlsx”, and “Basic1536.xlsx”. These each contain four worksheets named ‘Samplenames”, “Groupnames”, “Backgrounds” and “Matingtypes”, each of which has rows and columns matching the layout you see when looking down on the plate.

Samplenames

These cells should contain the ID of the strain in each location of the layout (each name must be <= 30 chars). If multiple locations contain the same strain, then they should have the same strain ID, and PHENOS will use this information to create ‘replicate plots’ showing all the growth curves of all these replicates on the same graph. If the location is empty, it should be kept empty or contain a ‘b’ for ‘blank’. Therefore do not use a ‘b’ as a strain ID. Capitalisation and spaces matter, so ‘MY234’ is treated as a different strain from ‘my 234’, and the exact same strain name should be used in ‘strains.csv’ file in the Genotypes folder. If you wish to perform QTL analysis with a strain, it must have genotype information supplied in a file that is named in the strains.csv file (see “Strains.csv” on pg. 20).

Groupnames

You can also split locations on the plate into groups of your choice, giving each group a distinct ID (<= 30 chars). The “CurvesWithoutAgar_Groups” visualization (pg. 7) produced by PHENOS plots growth curves from these different groups in different colours.

Backgrounds

This information isn’t stored, but can be used for your own reference to record relevant genotype information instead of storing it in strains.csv.

Matingtypes

This is also for your information: ‘a’ is used for MATa; ‘b’ for MATα; ‘ab’ for diploid; and blank where not known.

Entering your new layout

Once you run PHENOS, you will be asked to specify the layout for a particular experiment, and if your new layout isn’t in the list, there will be a ‘*browse*’ option that will let you select it from the files in the Layouts folder (see “RENAME & STORE PLATEREADER FILES” on pg. 41).

MAKING PLATES

We define three different types of plates as follows:

1) **Stock plates**: for the maintenance of your yeast arrays, these are rectangular plates normally containing YPD and agar, copied every few weeks with backups.
2) **Punch-in plates**: these are disposable intermediary plates used in the 'punch-in' technique to normalize the size of spots being printed. They are made with a double thickness layer of media, and if working with 384 arrays, half the usual amount of agar must be used. More detail on the punch-in technique is given in "Overview of the punch-in method of print normalization" on pg. 36.
3) **Experimental plates**: these are the plates that are measured in the microplate reader. They must be flat, smooth, even, clean and the agar must not be too dark or cloudy or it will block the measurement beam.

Making media

Our standard YPD agar medium is made with 10g/l yeast extract, 20g/l Bacto peptone, 20g/l D-glucose, 1% adenine solution (0.5% adenine in 0.05M HCl), adjusted to pH 6.3 using 1M HCl (this is important as agar will not set properly in particularly acidic or alkaline media), then autoclaved with 20g/l Bacto agar.

We commonly make up batches of 400ml (10 plates), by adding 400mL ddH_2O to 4g yeast extract, 8g Bacto peptone, and 8g D-glucose in a 500mL Duran flask, mixing thoroughly with a magnetic stirrer then adding 4ml adenine solution, 10 drops of HCl, and then 8g agar, and autoclaving at 120°C for 20 minutes. After that the agar media is kept at >60°C, with extra stirring if needed to break up agar clumps and eliminate bubbles, until we are ready to pour.

For some applications, complete synthetic (COM) medium may be preferred, but colonies will grow more slowly on this.

Adding treatment compounds

In treatment experiments for R/qtl analysis, we generally add the compound to a fixed volume of this same agar media after autoclaving and mix gently to avoid introducing more bubbles. We use stripes of coloured marker on the plate edge as a code to identify what's in the medium of our plates.

Pouring plates

Plates should be level and sterile, e.g. poured in a flow hood on a levelled platform, normally 40mL per plate giving a 4mm thick layer. They are best left to dry without the lids on to minimize condensation. Thereafter they should be wrapped in clingfilm to prevent drying out, although leaving them on a benchtop only partly wrapped (not closing the edges) for one night can help reduce condensation problems further.

Special punch-in plates

For soft agar punch-in plates (see “Overview of the punch-in method of print normalization” on pg. 36) we add ¼ the normal amount of agar (5g/L or 2g in 400mL) and for all punch-in plates, soft agar or not, they should be double the usual thickness (80mL giving an 8mm layer). Different brands of agar produce different results, so experiment to find the optimum amount of agar to prevent punching-in at low pressures (when creating the punch-in plate) while allowing punching-in at high pressure (when printing from the punch-in plate).

Use soft agar punch-in plates within a few days of pouring them. They do not keep well.

Checking evenness

After pouring and setting, plate evenness should be checked using the microplate reader and the PHENOS software (see “USING THE PHENOS SOFTWARE” on pg. 39). Sloped plates (caused by bad levelling) or bumpy plates (caused by prematurely cooling agar media) tend to produce uneven printing quality and less reliable growth curves if used as punch-in or experimental plates.

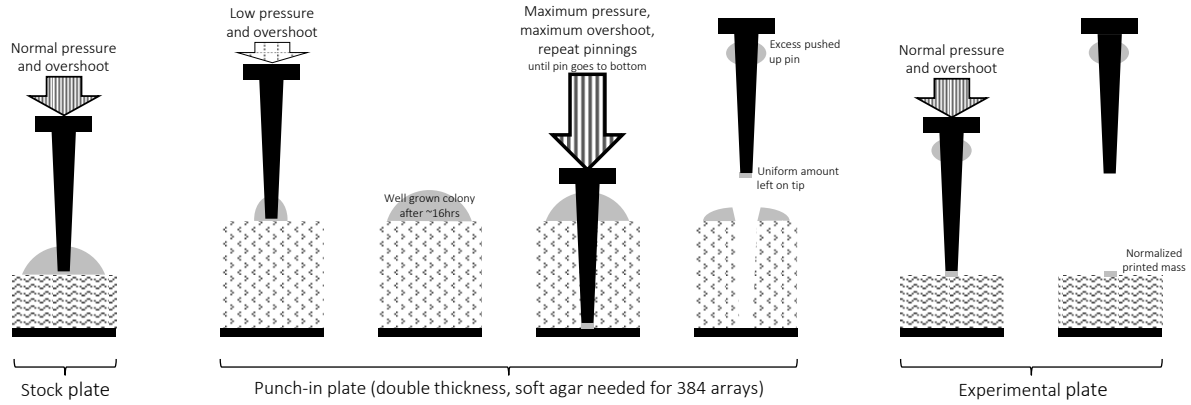
Caution about plate orientation

Be consistent and careful about plate orientation. Rectangular plates, such as Singer PlusPlates™ or Nunc OmniTrays™, have a distinct orientation. With PlusPlates, the notches and lid ridges are always at the top/back of the plate (where row A lies) while the word ‘Singer’ on the lid faces you at the bottom/front of the plate. Consistently label your plates on this bottom/front edge to minimize orientation errors, and always put plates into the microplate reader with this bottom/front edge towards you.

PREPARING EXPERIMENTAL PLATES

Overview of the punch-in method of print normalization

Good quality PHENOS phenotyping depends on all the colonies starting with as uniform an amount of cells as possible. To achieve this, we developed the 'punch-in technique' in which long-pin repads are pushed down through the source colonies of a special throwaway source plate, and into the agar, which pushes excess cell mass up the side of the pins leaving a small relatively uniform amount on the tip. For more detailed instructions on using the ROTOR, see pg. 23.



Pouring the punch-in plate

The ROTOR usually has enough power to punch in to normal solid media (20g agar/L) when using 96 repads, but **not** when using 384 repads (for the same reason that the famous bed-of-nails trick works: the available pressure is divided out among the total number of pins). Therefore – as previously described – when working with 384 arrays, we make special soft agar punch-in plates using only 5g agar/L, which the ROTOR can more easily punch into.

Additionally, whether soft agar plates are required or not, this method seems to work better when the plates are *double the usual thickness* (8mm, requiring 80ml media).

Printing to the punch-in plate

First, the array must be transferred to the punch-in plate. The stock plate is copied to the punch-in plate using a 384 repad and low pressure settings (e.g. 50% pressure, 1mm overshoot, 1 pinning) because it is important not to punch into the agar until later. Once printed, this source array is allowed to *grow for ~16 hours*, and should be used for the punch-in method immediately after incubation as colonies tend to harden if refrigerated.

N.B. You need to create the 384 array on a normal agar plate first, and use a 384 repads to transfer it to the soft agar plate. If you try and use a 96 repad on the soft agar, it will probably punch in even at the lowest pressure and overshoot settings.

All colonies on the punch-in plate should have grown to a diameter of >2mm immediately prior to use. Refrigerating the colonies will alter their adherence and make the punch-in method less reliable.

Pouring the experimental plate and taking 'empty plate' readings.

Experimental plates should be poured as described above in "MAKING PLATES" on pg. 34. Immediately before printing, the empty plate should be put in the microplate reader to measure the base absorbance of the agar. Run the appropriate 'emptyplate' program for your array size, e.g. '96-emptyplate' for a 96 array or '384-emptyplate' for a 384 array (see pg. 39 for step-by-step instructions). This should only take 40 seconds (for 96 arrays), 2 minutes (for 384 arrays) or 5 minutes (for 1536 arrays).

After you have done this, we recommend you immediately run the PHENOS software to rename the results file, and PHENOS should automatically generate a visualization of the agar thickness at each location of the plate (see "AgarThickness" on pg. 5), and then automatically open the folder where this visualization has been placed (in the target directory at e.g. C:\PHENOSdata\Plots_Empty plate views\[XX] where [XX] is the user folder you have chosen). Once you have added this file to a combined file, the same visualization will be generated in the Plots folder for that particular combined file.

It's a good idea to check this visualization to confirm that the experimental plate is flat and even before proceeding further.

Printing to the experimental plate using the punch-in method

Use a standard Replicate program with the incubated punch-in plate as the **source** and the experimental plate as the **target**, but edit the pinning settings to ensure that the settings for the source plate are set to maximum (100% pressure, maximum 2.5mm overshoot, maximum number of pinnings) while the pinning settings for the target plate are normal (100% pressure, the standard 1.5mm overshoot, and 1 pinning).

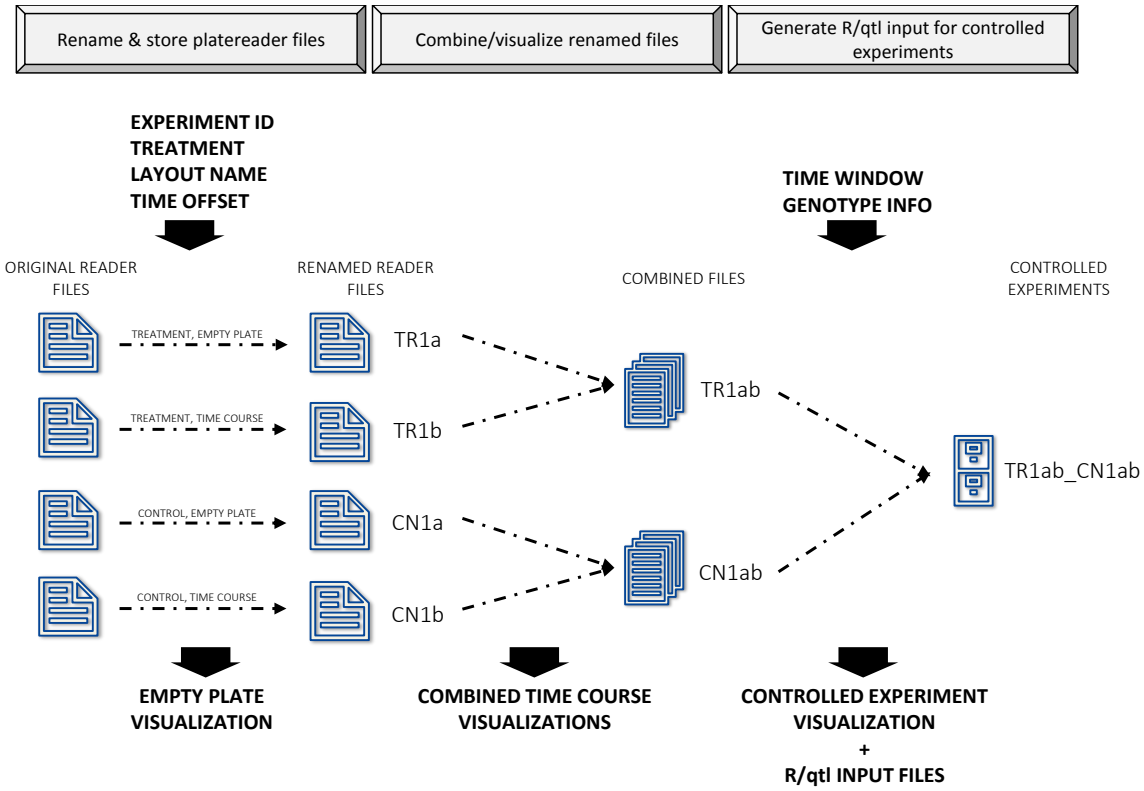
Ensure that the Repeat Source Pinnings icon is coloured in on the sidebar at the left. Keep your finger or the cursor ready over this icon, ready to turn it off *as soon as* the repad pins have pushed right down to the bottom of the punch-in plate agar. Repeat pinnings are necessary because it usually takes a few goes before the repad pins actually punch down to the bottom of the agar, which is critical to the normalization process. However, the pins must do this *only once*, so turning off repeat pinnings as soon as that happens ensures the ROTOR moves on to printing to the target plate.

After the punch-in plate has been used, it can be kept as a back-up stock plate, but shouldn't be used again for normalization.

Intermittent time courses

For some purposes, it may not be necessary or possible to generate continuous readings. PHENOS can stitch together multiple ‘snapshot’ readings, and will ask about the time offset of each in order to join them together.

USING THE PHENOS SOFTWARE



When the microplate reader has run, it leaves a file of the results in the source directory (e.g. C:\Platereader output). You can use the PHENOS software to fill in extra information about the experiment, enter these results into a database (it creates a copy of the file and renames that copy with the extra information) and also generate visualizations of the data and other output files.

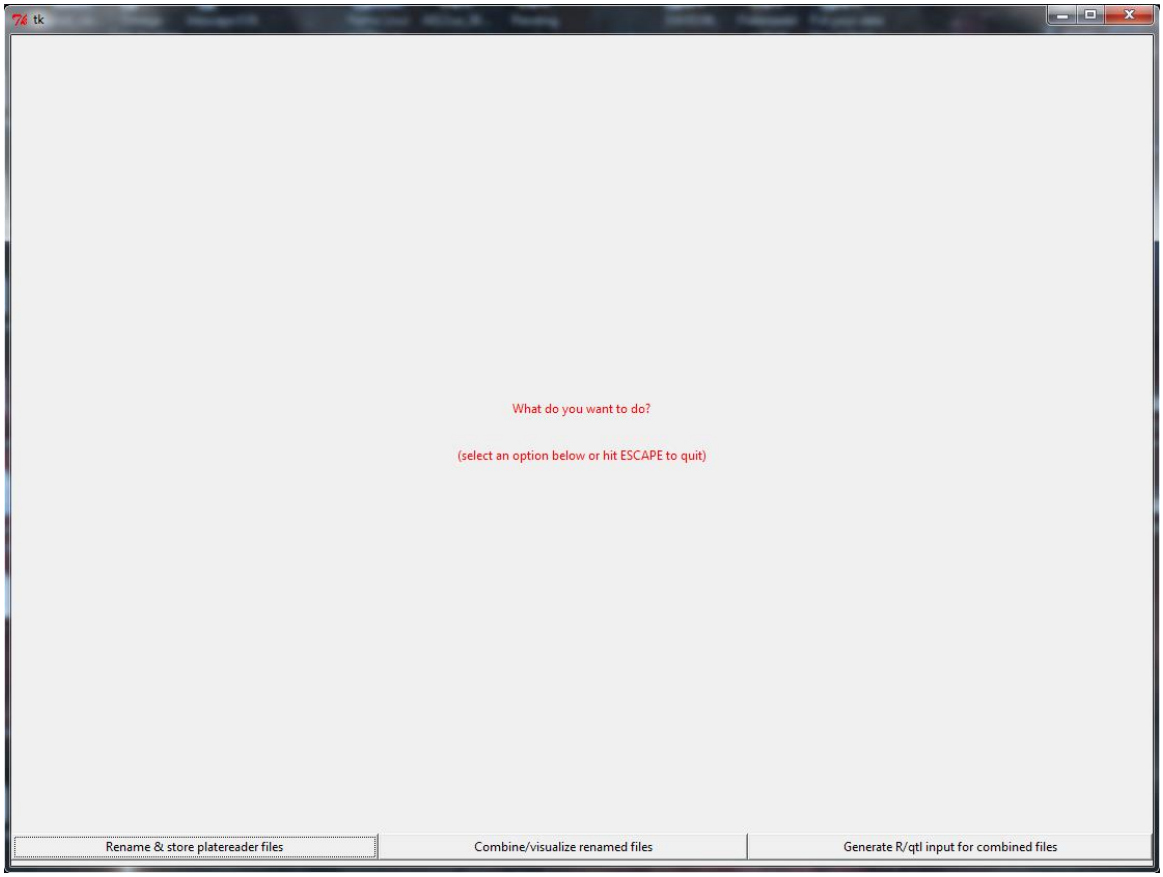
PHENOS can combine an ‘emptyplate’ reading with any number of subsequent measurement files to create a ‘combined file’, allowing you to subtract the absorbance due to agar alone, and create time courses even without continuous measurements.

PHENOS can also compare timepoints from two different combined files if one of them is designated a control experiment. Experiments will automatically be classified as control experiments if the treatment that you enter for it is listed as a control treatment in the configuration file (see “Controls>controls” on pg. 19).

By default, “Control”, “YPD”, “YPD 30C”, “COM” and “COM 30C” are all treated as control treatments, but you can change this or add others by editing that configuration file.

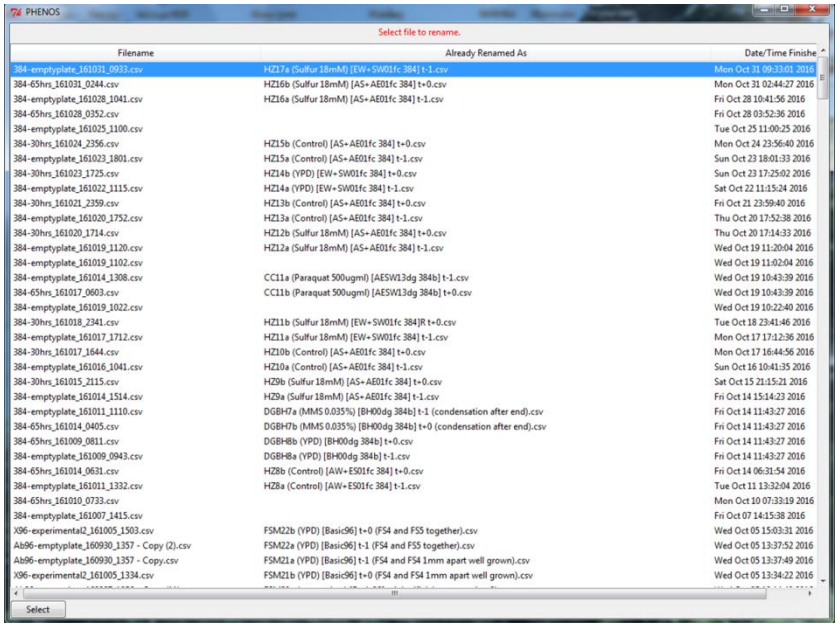
To run PHENOS, double-click on the PHENOS icon (you can create a shortcut to this on your desktop or in your toolbar for your convenience). A command line window (like the cmd.exe window) should appear in the background while PHENOS is running, while a GUI window appears in the foreground with the three buttons seen at the top of the figure above. If the command line window shuts down immediately then something has gone wrong. Log files are stored in Logs subfolder of the target directory (e.g. C:/PHENOSdata/Logs) which may help you work out what went wrong. When you first run PHENOS it may seem to hang for a short while as it creates databases and imports layouts and strain information. Be patient. **Do not attempt to run multiple versions of PHENOS at the same time on the same computer.**

The opening screen should look like this:



RENAME & STORE PLATEREADER FILES

This section of the program will list all output files from the microplate reader (stored in the source directory), with their original filenames in the left column. They are listed in reverse chronological order, with the most recent files at the top.



In the middle column you can see which of these files have already been renamed (and entered into the database).

You can select a file that hasn't been renamed, and double-click or hit <Enter> to proceed to the next step.

Alternatively you can select a file that has already been renamed, and hit <Delete> to remove it from the database, which makes the 'Already Renamed As' column blank but leaves the microplate reader output file in place so that you can rename it again.

Deleting a file will also delete any combined files or controlled experiments created from that file (see below).

If you have already renamed a file and placed it in the source directory (perhaps because you have already renamed it on a different PHENOS machine), PHENOS will recognize that it appears to be formatted correctly already and ask you if you wish to keep the same name.

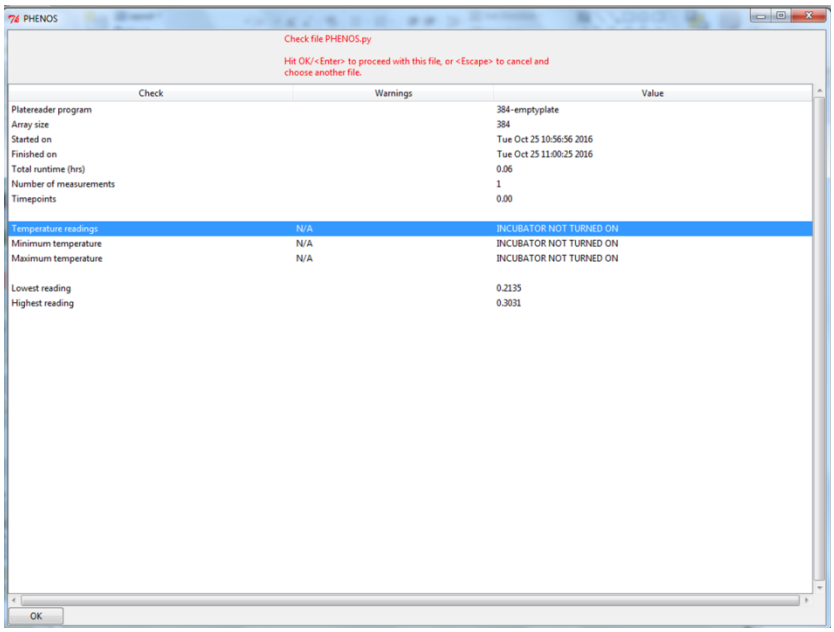
Check file contents

After you select a new file to rename, a new window opens containing a **summary** of the file contents:

You can examine this window to check that the correct program was used, with the correct timepoints, and that the temperature readings and measurements are within the expected range.

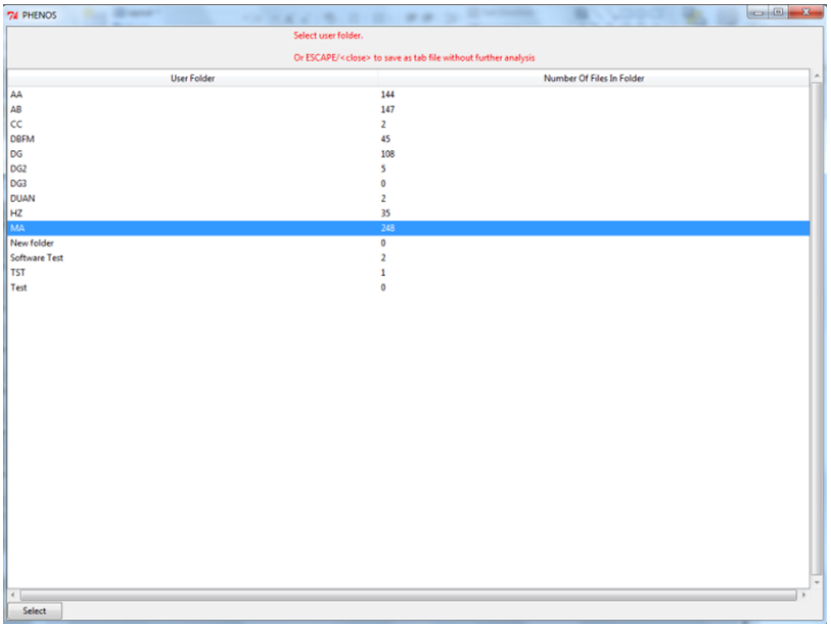
If everything looks OK, you can click ‘OK’ or hit <Enter> to proceed.

If not, hitting <Escape> will return you to the previous screen.



Select user folder

If you have chosen to proceed, you will be presented with a list of available **user folders**. If none yet exist, just ‘New folder’ will be available. The window will also tell you how many files have already been added to each user folder.



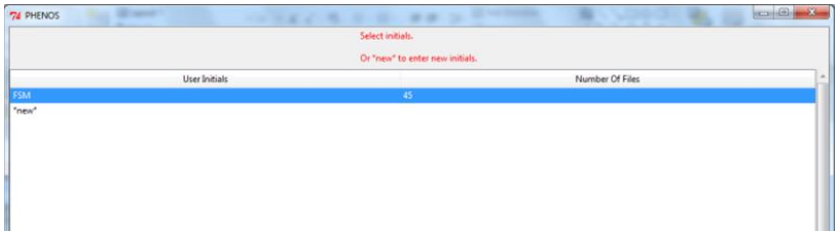
User folders allow you to keep your work separate from other users, or divide your own work into separate projects of manageable size. Each user folder gets its own database, so you can’t combine files from different user folders. However, later on, once you have created a combined file (see below) you will be asked if you wish to copy the entries to a special combined database, depending on whether it is a control experiment or not. If the former, you’ll be asked if you want to add it to the ‘Controls’ database, which will allow it to be used as a control experiment by any other user. Otherwise you’ll be asked if you want to add it to the ‘All’ database.

If you select ‘New folder’, you will then be asked to give a name for the new folder.

Select user initials

After selecting the correct user folder you will be asked to select the **user initials** for your experiment. You can use any number of different initials in a particular user folder, and any number of different experiments can share the same initials. The window shows how many files already bear each set of initials. These initials are primarily for your benefit to help you distinguish different groups of experiments. When renamed copies of the results files are created, these will be the first letters of their filenames.

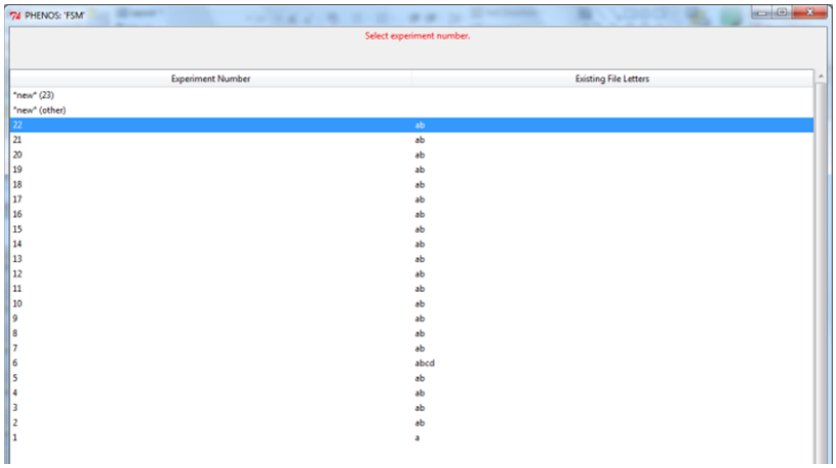
If you select *new* you will then be asked to enter new initials (1-5 letters)



Select experiment number

After entering user initials, you will be asked to select an **experiment number**, which – with the user initials – will be shared by all readings of a particular plate.

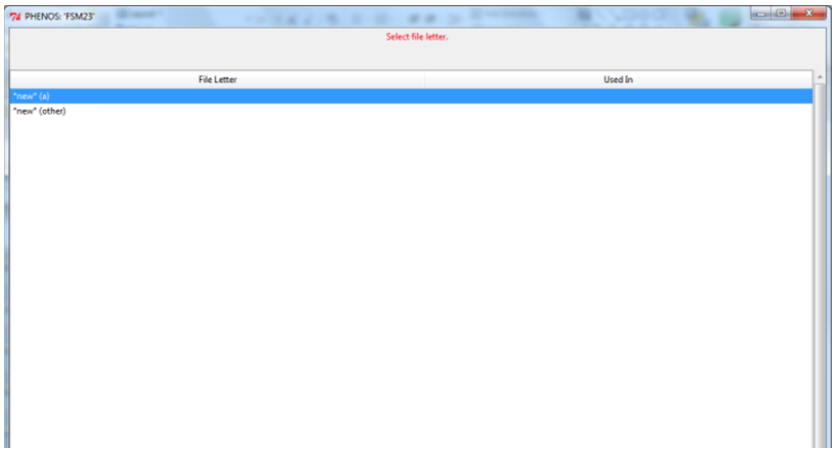
At the top, PHENOS offers the highest unused experiment number for new experiments, or you can select *new* (other) and will be prompted to enter a number of your choice. The default selection will be the last experiment number you used.



Select file letter

Next you will be asked to specify the **file letter**. The first file of readings (the 'emptyplate' readings) should be 'a'. The second set of readings, immediately after printing, should be 'b', and so on in alphabetical order.

If you have specified an experiment number that is already in use, PHENOS will default to the next letter after that. If for some reason you need to specify a different letter, you can select *new* (other) and will be able to put any letter in after that, so you need not enter files in order.



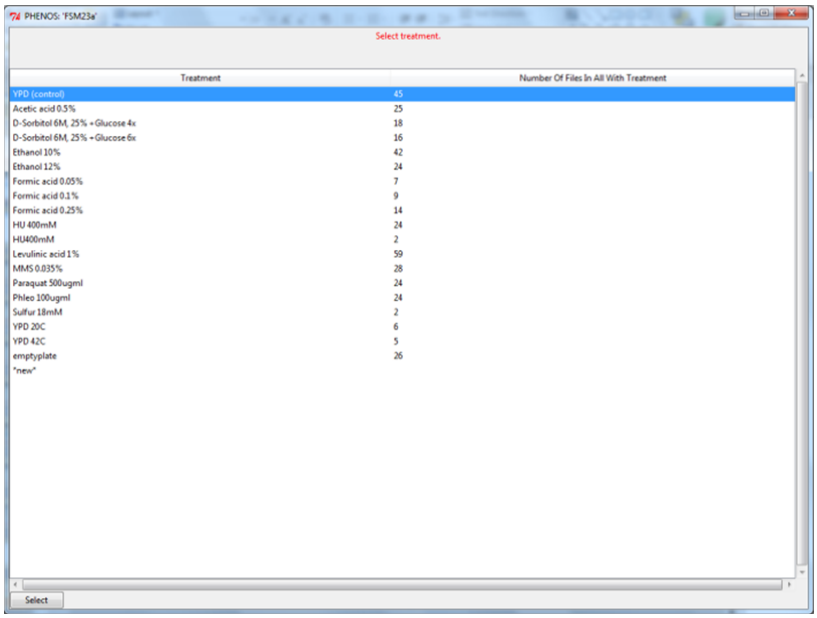
Select treatment

After this you will be asked to name the **treatment** for the file. The standard control treatment is 'YPD', although you can specify which other treatments will be treated as controls by modifying the config.txt configuration file (see "Controls>controls" on pg. 19).

PHENOS displays all the previous treatment names that have been used (including those copied to the combined 'All' database) so you can easily co-ordinate with other users.

If you need to enter a new treatment name, select *new*.

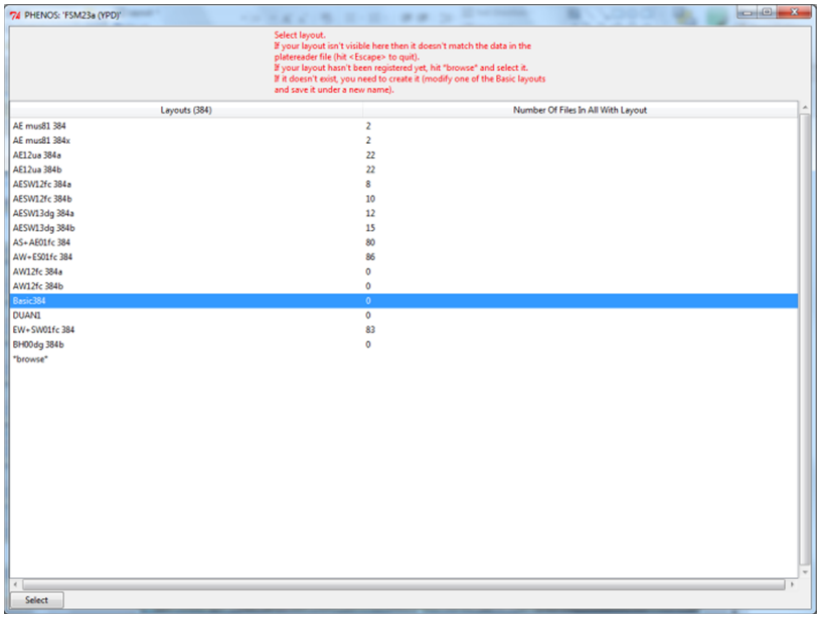
Note that treatment names must be exact- different capitalization or different numbers of spaces will produce different treatment names (see HU 400mM and HU400mM to the right)



Select layout

Next you must select the name of the **layout** for this experiment: this will be the name of the file (minus the extension) stored in the target directory Layouts folder, which specifies what strain is stored in each location of the array (see “PREPARING LAYOUT FILES” on pg. 33).

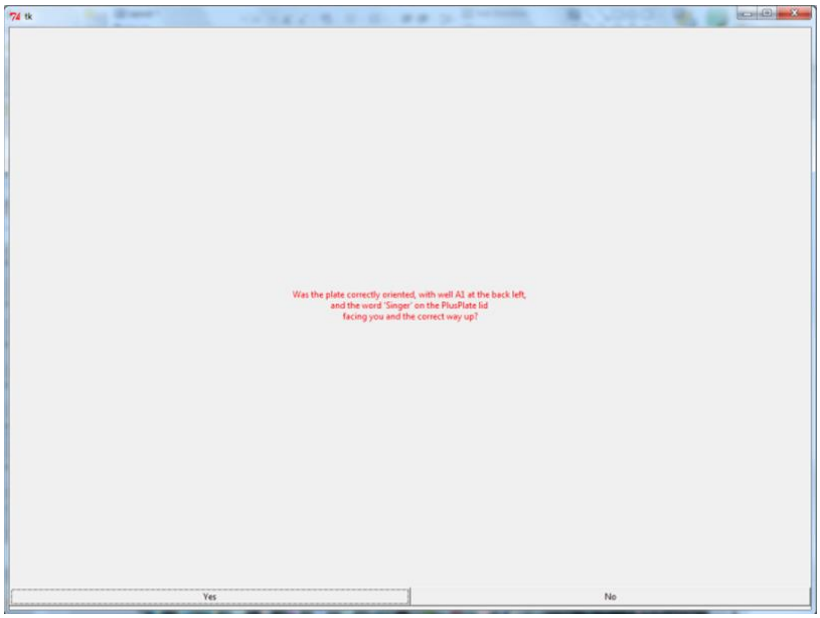
If your layout file is not in the list, select *Browse* and find the file in the Layouts folder. If it isn’t there, you’ll need to create it.



Confirm orientation

After this you will be asked to confirm that the plate was put into the microplate reader the correct way round. If it was put in back to front, PHENOS can change how it reads the file to account for this.

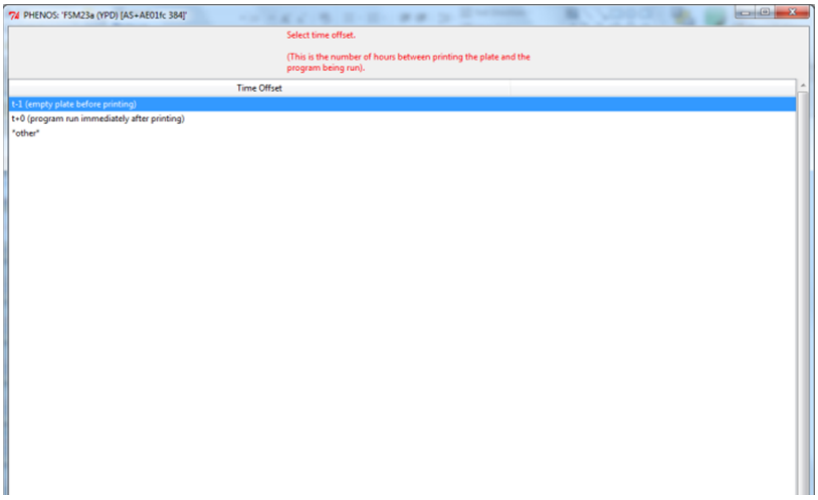
Respond with ‘Yes’ (the default option) or ‘No’.



Select time offset

Now you must enter the time offset of the file. If this appears to be an ‘emptyplate’ file (i.e. the file letter has been set as ‘a’), then ‘t-1’ will be selected as the default, which is used exclusively to denote pre-printing emptyplate readings. If it has been given a file letter of ‘c’ or higher, PHENOS will intelligently attempt to approximate the time offset by comparing the file’s timestamp to that of the one that came before it, and this result will be offered as the default.

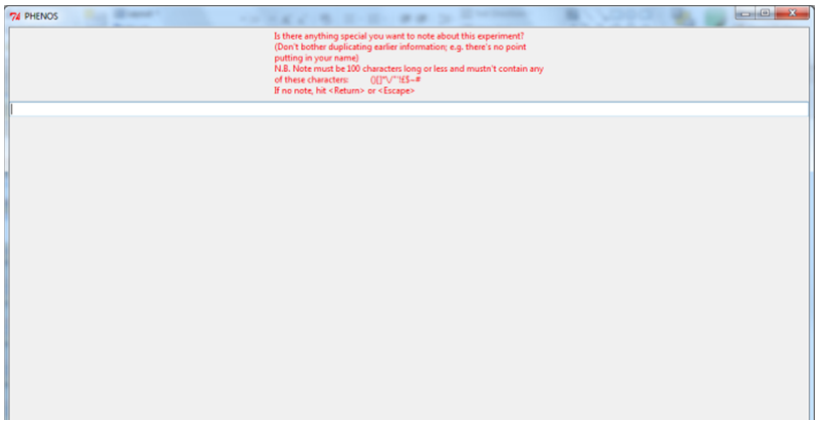
Otherwise, you can select *other* and put in any time offset you like. The offset, which should be a whole number, is the number of hours that should be added to each timepoint in the file, i.e. the number of hours after the initial printing that the readings were taken. ‘t+0’ indicates no delay between printing and the start of measurements.



Enter note

Finally, you may add a **note** of up to 100 characters containing any extra information you wish to remember about the experiment. There’s no need to duplicate any information you’ve already entered (such as the user), but you might wish to remember, for example, that you observed contamination on the edge of the plate.

You don’t need to enter a note: hit <Enter> to proceed without one. If a note existed for the previous file in this experiment, the same note will be offered as a default option.

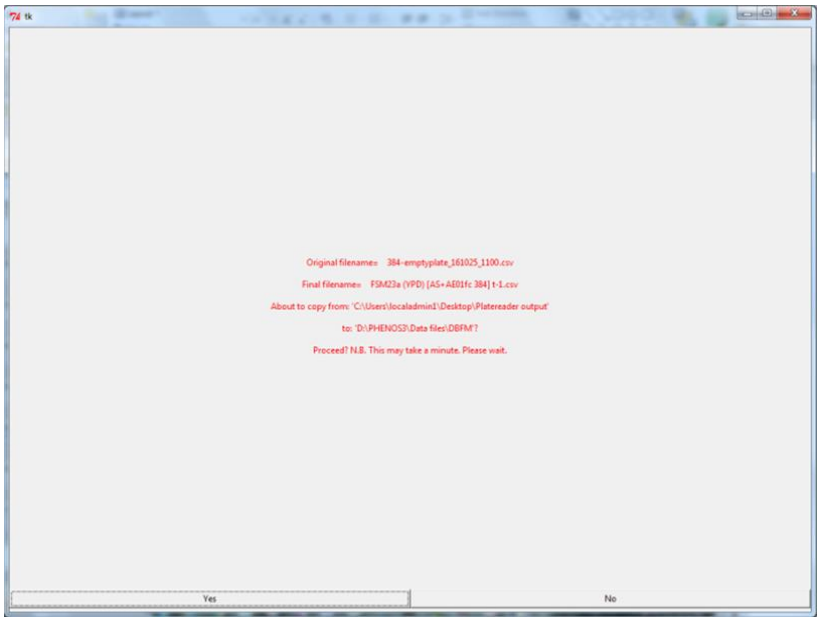


Confirm

When you've done this, you'll be presented with a confirmation window like this:

Click 'yes' or hit <Enter> to proceed.

The file will be copied to the target directory and the copy will be given the new name. Its data will also be entered into the database for that user folder.



Next

If you proceed, after a short wait, you'll then be asked what you wish to do next, and can repeat the process of renaming with a different file, or move on to create a combined file:

COMBINE/VISUALIZE RENAMED FILES

This section of the program will join together data from multiple input files into a new combined file. This combined data is stored in the database, and stored as a tab delimited text file, and various graphical presentations of the data are generated (see “VISUALIZATIONS” on pg. 5).

Combined files are given a code that combines the user initials and experiment number, and the individual file letters that comprise it, so that TST1a + TST1b becomes TST1ab.

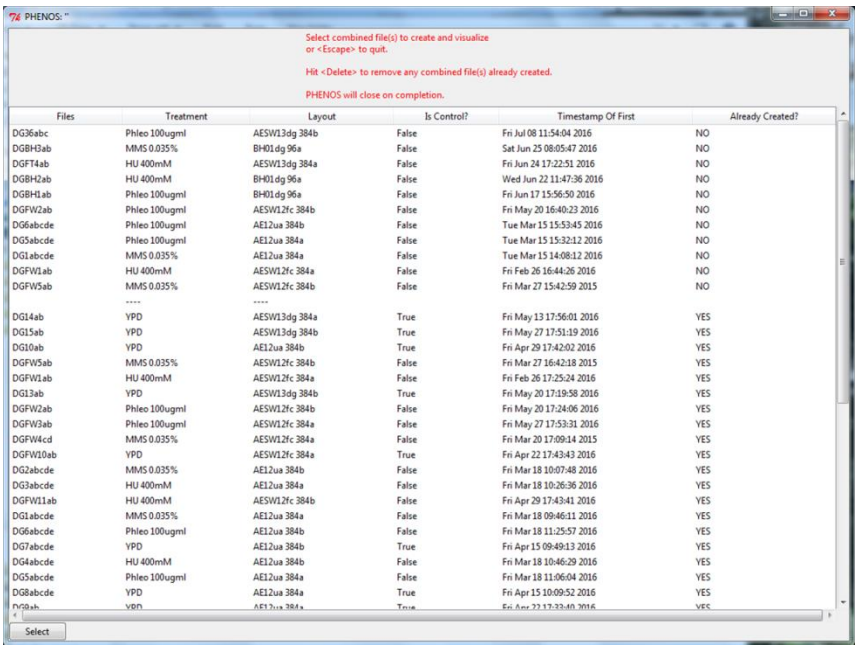
First PHENOS asks you which user folder (and database) to use and then it searches through the available files to see which combined files have already been created, and which ones can be created. The latter are displayed at the top.

Select combined file

If you select a combined file that has already been created (by clicking or hitting <Enter> when it is highlighted), then PHENOS will open an explorer window showing the visualizations that have already been created for it.

If you select a combined file that hasn’t been created yet, PHENOS will create it and add it to the database.

If you hit <Delete>, the selected combined file will be removed from the database, and so will any controlled experiments based on it. PHENOS will not delete any visualizations or data files however.

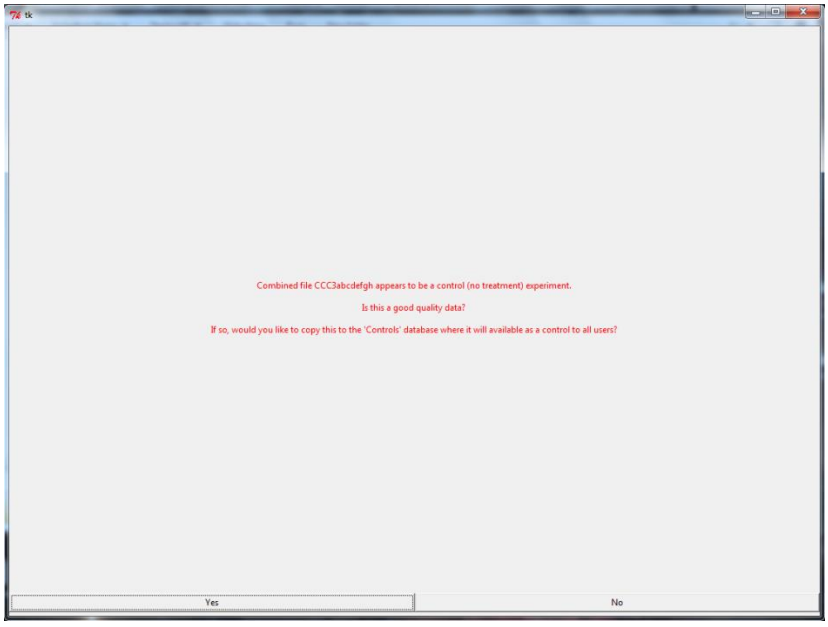


Create visualizations

After this you will be asked if you wish to create visualizations (the types created will be determined by the config.txt configuration file, see “CombiFileVisualizations” on pg. 20), or just the combined data tab delimited text file on its own (quicker), or neither (which will take you back to the previous screen):

Visualizations take some time, particularly Animations and ReplicatePlots. As long as you can see the new text appearing in the cmd window (with a black background), you can be confident the program is still working and hasn’t crashed. In the event of a crash, that window will shut down.

Copy to Controls/All



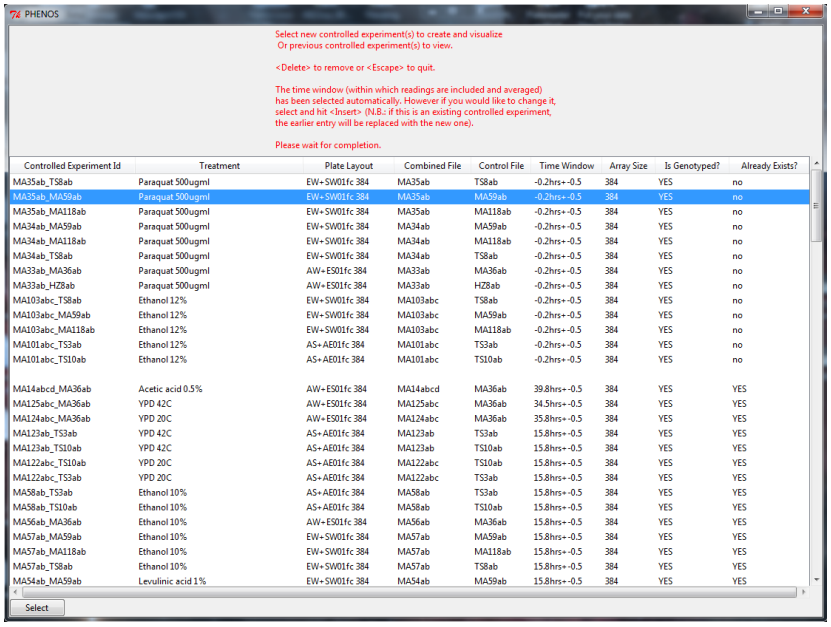
If all goes well, whether you create visualizations or not, you will then be asked if you want to copy the data to the Controls database (if it is a control) or the All database (if not).

If it is added to the Controls database, it will be available as a potential control to other experiments in other user folders (see below).

After this you will be returned to the starting screen.

GENERATE R/qtl INPUT FOR CONTROLLED EXPERIMENTS

This final section allows you to compare a combined file from a treatment experiment with a matching combined file from a control experiment. This creates a new kind of database object called a 'controlled experiment', which allows for cross-file calculations such as the 'TreatmentRatio' phenotype calculator (see below).



Controlled Experiment Id	Treatment	Plate Layout	Combined File	Control File	Time Window	Array Size	Is Genotyped?	Already Exists?
MA35ab_TS8ab	Paraquat 500ugml	EW+SW01fc 384	MA35ab	TS8ab	-0.2hrs+-0.5	384	YES	no
MA35ab_MA59ab	Paraquat 500ugml	EW+SW01fc 384	MA35ab	MA59ab	-0.2hrs+-0.5	384	YES	no
MA35ab_MA118ab	Paraquat 500ugml	EW+SW01fc 384	MA35ab	MA118ab	-0.2hrs+-0.5	384	YES	no
MA34ab_MA59ab	Paraquat 500ugml	EW+SW01fc 384	MA34ab	MA59ab	-0.2hrs+-0.5	384	YES	no
MA34ab_MA118ab	Paraquat 500ugml	EW+SW01fc 384	MA34ab	MA118ab	-0.2hrs+-0.5	384	YES	no
MA34ab_TS8ab	Paraquat 500ugml	EW+SW01fc 384	MA34ab	TS8ab	-0.2hrs+-0.5	384	YES	no
MA33ab_MA36ab	Paraquat 500ugml	AW+ES01fc 384	MA33ab	MA36ab	-0.2hrs+-0.5	384	YES	no
MA33ab_HZ8ab	Paraquat 500ugml	AW+ES01fc 384	MA33ab	HZ8ab	-0.2hrs+-0.5	384	YES	no
MA103abc_TS8ab	Ethanol 12%	EW+SW01fc 384	MA103abc	TS8ab	-0.2hrs+-0.5	384	YES	no
MA103abc_MA59ab	Ethanol 12%	EW+SW01fc 384	MA103abc	MA59ab	-0.2hrs+-0.5	384	YES	no
MA103abc_MA118ab	Ethanol 12%	EW+SW01fc 384	MA103abc	MA118ab	-0.2hrs+-0.5	384	YES	no
MA101abc_TS3ab	Ethanol 12%	AS+AE01fc 384	MA101abc	TS3ab	-0.2hrs+-0.5	384	YES	no
MA101abc_TS10ab	Ethanol 12%	AS+AE01fc 384	MA101abc	TS10ab	-0.2hrs+-0.5	384	YES	no
MA14abcd_MA36ab	Acetic acid 0.5%	AW+ES01fc 384	MA14abcd	MA36ab	39.8hrs+-0.5	384	YES	YES
MA125abc_MA36ab	YPD 42C	AW+ES01fc 384	MA125abc	MA36ab	34.5hrs+-0.5	384	YES	YES
MA124abc_MA36ab	YPD 20C	AW+ES01fc 384	MA124abc	MA36ab	35.8hrs+-0.5	384	YES	YES
MA123ab_TS3ab	YPD 42C	AS+AE01fc 384	MA123ab	TS3ab	15.8hrs+-0.5	384	YES	YES
MA123ab_TS10ab	YPD 42C	AS+AE01fc 384	MA123ab	TS10ab	15.8hrs+-0.5	384	YES	YES
MA122abc_TS10ab	YPD 20C	AS+AE01fc 384	MA122abc	TS10ab	15.8hrs+-0.5	384	YES	YES
MA122abc_TS3ab	YPD 20C	AS+AE01fc 384	MA122abc	TS3ab	15.8hrs+-0.5	384	YES	YES
MA58ab_TS3ab	Ethanol 10%	AS+AE01fc 384	MA58ab	TS3ab	15.8hrs+-0.5	384	YES	YES
MA58ab_TS10ab	Ethanol 10%	AS+AE01fc 384	MA58ab	TS10ab	15.8hrs+-0.5	384	YES	YES
MA56ab_MA36ab	Ethanol 10%	AW+ES01fc 384	MA56ab	MA36ab	15.8hrs+-0.5	384	YES	YES
MA57ab_MA59ab	Ethanol 10%	EW+SW01fc 384	MA57ab	MA59ab	15.8hrs+-0.5	384	YES	YES
MA57ab_MA118ab	Ethanol 10%	EW+SW01fc 384	MA57ab	MA118ab	15.8hrs+-0.5	384	YES	YES
MA57ab_TS8ab	Ethanol 10%	EW+SW01fc 384	MA57ab	TS8ab	15.8hrs+-0.5	384	YES	YES
MA54ab_MA59ab	Levulinic acid 1%	EW+SW01fc 384	MA54ab	MA59ab	15.8hrs+-0.5	384	YES	YES

PHENOS presents a list of controlled experiments that *have* been created, underneath a list of controlled experiments that *can* be created. To determine the latter, PHENOS first finds all the treatment experiments in the current user folder that have not yet been used to create controlled experiments. Then it looks for control experiments (i.e. experiments with treatments that have been declared controls in the configuration file, see pg. 19) that have the same layout as those treatment experiments. It looks for control experiments in the same user folder, but it also looks for other control experiments that have been copied into the shared 'Controls' database. You can then choose which controlled experiment you want to create.

Time windows

For each of these potential controlled experiments, PHENOS automatically calculates the best time window to use for the TreatmentRatio phenotype calculator: this covers the last set of timepoints that are common to both the treatment and control experiments. If you wish to force PHENOS to use a different time window for a particular controlled experiment, instead of selecting that controlled experiment with <Enter>, hit <Insert> and you will be prompted to enter your own choice of time window.

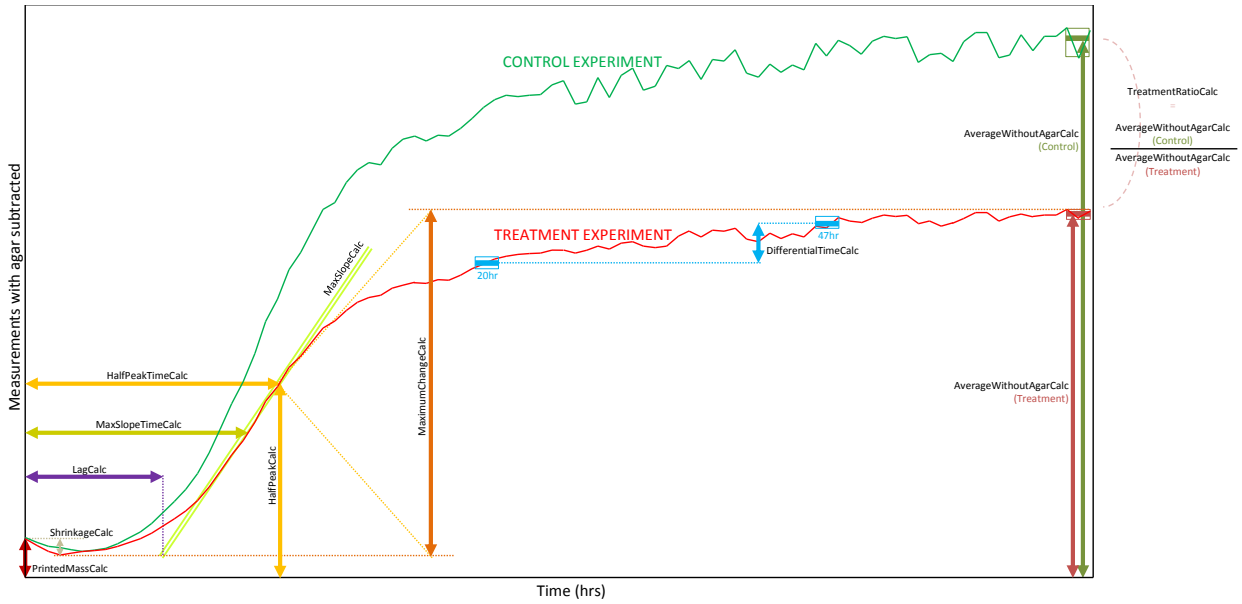
If you do not see the controlled experiment you want to create, it may be because a controlled experiment has already been created for that treatment. You can delete that previous controlled experiment by selecting it and hitting <Delete>. This will remove it from the database, but will not remove any R/qtl files or visualizations that have already been created for it. If you then return to the 'Generate R/qtl' option, you should now see all the possible controlled experiments that can be created from that treatment file.

You can also directly alter the time window of a previously created controlled experiment by selecting it and hitting <Insert>. This will allow you to enter a new time window, and the previous controlled experiment will be deleted from the database (again, leaving its R/qtl input and visualization files in place) and replaced with the new one.

R/qtl input files & phenotype calculators

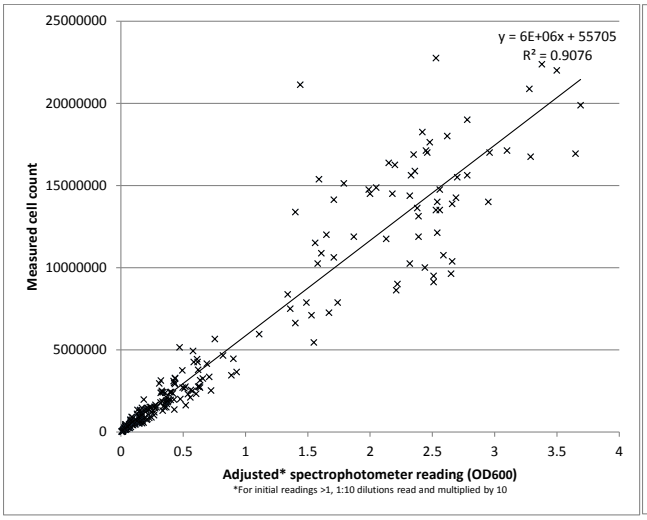
We use PHENOS in concert with an R-based QTL analysis program called R/qtl (http://www.rqtl.org/). PHENOS can generate files suitable for input to R/qtl, provided that there is genotype data, suitably formatted, in the Genotypes folder of the target directory.

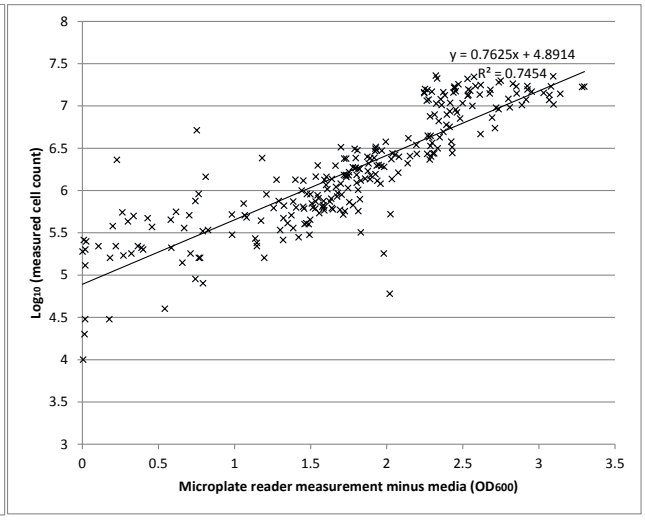
R/qtl input files can incorporate multiple columns of phenotype data. Within PHENOS, each column is generated by a different 'phenotype calculator', each of which extracts a different numerical score from the growth data for a given colony in the array. You can control which phenotype calculators are used (both by default and for particular treatments) by editing the configuration file (pg. 19). The available phenotype calculators (e.g. 'MaxSlopeCalc') are described in the figure below.



CONVERTING READINGS TO CELL COUNTS

To validate the PHENOS system, we measured yeast colony cell counts using a cell counting chamber, and compared this to readings taken in a standard spectrophotometer and readings taken using PHENOS, and empirically determined the following conversion formula for haploid yeast cells:





To convert PHENOS measurements (with agar subtracted) 'A' to predicted cell count 'P':

$$\mathrm{Log}_{10} P = 0.7625\ A + 4.9 \qquad \text{or…}$$

$$P = 77900e^{(1.76A)}$$

TROUBLESHOOTING PHENOS

EXPERIMENTAL WORKFLOW TIPS & COMMON PROBLEMS

- Always use the same brand of agar for all your experiments.
- Make sure that the media is at pH ~6.3 before adding agar, or agar may not set properly.
- Make sure that media is well mixed, but free of bubbles, and has not cooled too much before pouring. If necessary, keep incubated at 60°C after autoclaving, and leave a magnetic stirrer in the bottle so that you can easily gently remix it if necessary.
- Make sure that plate lids remain clean and free of condensation: let plates dry with the lids off, in a flow hood, and always wear gloves when handling an experimental plate.
- Practise and optimize your punch-in method before starting proper experiments: make sure that the repad pins push down to the bottom of the agar once and only once. Don't re-use punch-in plates as sources for experimental plates: once they have been punched into once, doing so again won't normalize the cell amounts.
- Make sure that the punch-in plate agar is double the usual thickness (8mm rather than 4mm).
- If using a 384 repad with a 384 array, make sure that you are creating soft agar punch-in plates, as a 384 repad will never push into normal density solid agar.
- Don't try and print to a soft agar plate using a 96 repad, as it will always punch in. You should create your 384 arrays on normal agar beforehand.
- Use soft agar punch-in plates within a week of pouring them, as they do not last.
- Make sure that you have incubated your punch-in plate sufficiently before printing from it, and that all the source colonies have grown big enough to be transferred. If necessary, use the punch-in technique to normalize your stock plate arrays. Letting them grow up more slowly at room temperature may also help slow-growers catch up with fast-growers.
- Make sure that the experimental plate is the right thickness (usually 4mm, requiring 40ml media) and is flat and smooth. Enter your 'emptyplate' readings into the PHENOS software immediately after taking them, and check that the Agar Absorbance visualization looks good before proceeding.
- If adding compounds to the experimental plate solid media, make sure you do not increase the optical density so much that readings get capped (the maximum possible reading is 3.5).
- Leave as little time as possible between incubating the punch-in source plate and punching in to it, and between taking emptyplate measurements of the experimental plate and then printing to it. Plan your experimental timetable down to the hour.
- Make sure that you consistently put your plates into the microplate reader the correct way around, and make a note if you do not.

- Ensure that all your control experiments contain as many timepoints as possible (ideally using the 65hr protocol), so that they can used for as many treatment experiments as possible.
- Don't forget to turn the incubator on in the microplate reader control software where you select protocols.
- Make sure that the microplate reader is kept shaded throughout the protocol as sunlight falling through a window can raise the internal temperature.

PHENOS SOFTWARE TIPS & COMMON PROBLEMS

- Never open multiple copies of the PHENOS software at the same time on the same computer.
- If not getting an option to create the desired controlled experiment in the PHENOS software 'Generate R/qtl input' section, make sure that your control experiment has been entered with the correct layout details, that its treatment is listed as a control in the configuration file, and that it has not already been used to create a controlled experiment that is listed lower down.
- If experiencing trouble with the PHENOS software, consider deleting the problem entries from within the program and putting them in again. If necessary, delete the whole user folder and recreate it, but first try to manually delete each entry from within the program,

GLOSSARY

Array

A rectangular grid of yeast colonies which can be grown and copied between different plates. 96 arrays have 8 rows and 12 columns, with 9mm gaps between the centre of adjacent colonies. 384 arrays have 16 rows and 24 columns, with 4.5mm gaps. 1536 arrays have 32 rows and 48 columns, with 2.25mm gaps.

Combined file

A database object within the PHENO software that combines data from an 'emptyplate' protocol with any number of subsequent protocols taking readings from the exact same plate.

Configuration file

The 'config.txt' file copied into "C:\Users\[your windows user name here]\AppData\Roaming\PHENOS", which you can edit so as to change settings for the PHENOS software.

Control experiment

An experiment using a particular yeast layout on a particular experimental plate grown in standard conditions: normally YPD agar at 30°C.

Controlled experiment

A database object comparing data between a control experiment combined file and a treatment experiment combined file, which both contained measures of the same yeast array growing under different conditions.

Experimental plate

The plate on which colony growth is measured by the microplate reader. This plate will be put into the microplate reader before anything has been printed to it in order to measure the absorbance of its empty agar, and then an array will be printed to it using the punch-in method to normalize cell quantities, and the plate will be put into the microplate reader again (and usually incubated) while readings are taken.

Installation directory

The folder/directory containing all the PHENOS installation files and subfolders, as downloaded from https://github.com/gact/phenos/.

Layout

A particular set of yeast strains, with the name of the strain in each well recorded in a special layout file in the Layouts folder of the PHENOS target directory.

MTP or microplate

A microtitre plate: a rectangular lidded plastic dish with 96, 384 or 1536 separate wells.

Microplate reader or platereader

The BMG Labtech FLUOstar Omega or similar optical microplate reader, which measures the absorbance of a light beam passing through a sample.

Timepoint

A point in time at which the microplate reader begins measuring the absorbance at each well in the layout. Within the microplate reader software these are also called 'cycles'.

PHENOS

The 'Phenotyping on Solid media' pipeline and software.

PlusPlate™

A proprietary Singer Instruments-branded rectangular petri dish with the same footprint as a microtitre plate, designed for use in the ROTOR. Unlike comparable plates from, e.g. Nunc, Singer's PlusPlates have notches in the edges that ensure they can only be put into the ROTOR in the correct orientation.

Protocols

Microplate reader programs with pre-set instructions for taking a given number of readings at specified timepoints in particular wells of an MTP or PlusPlate™.

Punch-in plate

A special throwaway source plate, with double the usual thickness of agar (and with soft agar if intended for a 384 array) which is used to normalize (equalize) the amount of cells that will be printed to each well in the experimental plate.

Readings file

A set of measurements (or readings) taken by the microplate reader, at each well in the plate, over 1 or more successive timepoints and recorded in a single ASCII data file stored in the source directory.

Repad

A disposable plastic pinned printing block that is used by the ROTOR to print an array from one plate to another.

ROTOR

The ROTOR HDA colony manipulation robot sold by Singer Instruments, which uses plastic 'repads' to print microorganisms from a source plate to a target plate.

R/qtl

An extension package for the statistical programming language R, used for mapping quantitative trait loci, downloadable from http://www.rqtl.org/.

Source plate

Any filled PlusPlate™ or MTP being used as the source to be printed to a target plate or plates.

Source directory

The folder/directory where the microplate reader has been instructed (during set-up) to save its ASCII data output, and the PHENOS software has been instructed to look for that output.

Stock plate

A yeast array growing on solid agar in a PlusPlate™, which is used as the source for creating punch-in plates.

Target directory

The folder/directory where the PHENOS software creates renamed copies of the microplate reader's ASCII data output, and also stores visualizations (in the 'Plots' subfolder) and logs (in the 'Logs' subfolder).

Target plate

Any PlusPlate or MTP that will have microorganisms printed to it from a source plate.

Time window

A time in hours and an additional plus/minus figure. A number of phenotype calculators use an average of measurements from timepoints lying within this window, in order to account for the variance in readings that occurs at high absorbances.

Treatment experiment

An experiment using a particular yeast layout on a particular experimental plate which will be grown under a discriminating condition such as high temperature or with a toxic compound in the agar.

Well

A particular position within a MTP or an array. Individual wells are named with a row letter (A-Z then AA, AB etc) and a column number, e.g. B6 is in second row of the sixth column. AF48 is the last row of the last column in a 1536 array. A1 is always at the top left of an array when you look down on it.