Documentation updated February 21, 2018

GrowthRates 3.0 Documentation

Current version as of February 21, 2018: Version 3.0
Barry G. Hall

What the **folders** contain:

GrowthRates 3.0 Mac: A Macintosh OS X command-line program: ***GrowthRates*** plus an Academic License Agreement and this Documentation

Formatters Mac: Universal OS X command-line programs: ***FormatGR_Bioscreen, FormatGR_Biotek, FormatGR_Tecan, FormatGR_Perkin_Elmer, FormatGR_Biotek_PowerWaveHT***, ***FormatGR_SpectraMaxM2,*** plus the data files ExampleData1.txt and ExampleData2.txt

GrowthRates 3.0 Linux: A Linux 64-bit command-line programs: ***GrowthRates*** plus an Academic License Agreement and this Documentation

Formatters Linux: Linux 64-bit command-line programs: ***FormatGR_Bioscreen, FormatGR_Biotek, FormatGR_Tecan, FormatGR_Perkin_Elmer, FormatGR_Biotek_PowerWaveHT***, ***FormatGR_SpectraMaxM2,*** plus the data files ExampleData1.txt and ExampleData2.txt

GrowthRates 3.0 Windows: A Windows 64-bit command-line programs: ***GrowthRates.exe*** plus an Academic License Agreement and this Documentation

Formatters Windows: Windows 64-bit command-line programs: ***FormatGR_Bioscreen.exe, FormatGR_Biotek.exe, FormatGR_Tecan.exe, FormatGR_Perkin_Elmer.exe, FormatGR_Biotek_PowerWaveHTk.exe*** , ***FormatGR_SpectraMaxM2,*** plus the data files ExampleData1.txt and ExampleData2.txt

Citation

Please cite Hall, B. G., H. Acar, A. Nandipati, and M. Barlow. 2014 Growth Rates Made Easy. Mol. Biol. Evol. **31**: 232-238 doi:10.1093/molbev/mst197

Table of Contents

Introduction

Measurements of bacterial growth rates have been made considerably faster and easier by the use of micro-plate readers that simultaneously monitor the densities of cultures in, typically, 96 or 384 wells. Plate readers turn what used to be a week-long tedious chore of data collection into a one-day experiment that requires less than an hour of the investigator's time. What is not, automated, however is the business of turning that data into a reliable growth rate determination. The typical growth curve consists of a lag period, followed by an acceleration period during which the growth rate increases, followed by an exponential phase during which the growth rate is constant (and maximum for the conditions), followed by a deceleration period during which the rate declines until the culture enters stationary phase during which little or no growth occurs, the cells undergo considerable physiological change, and cell death begins (Figure 1).

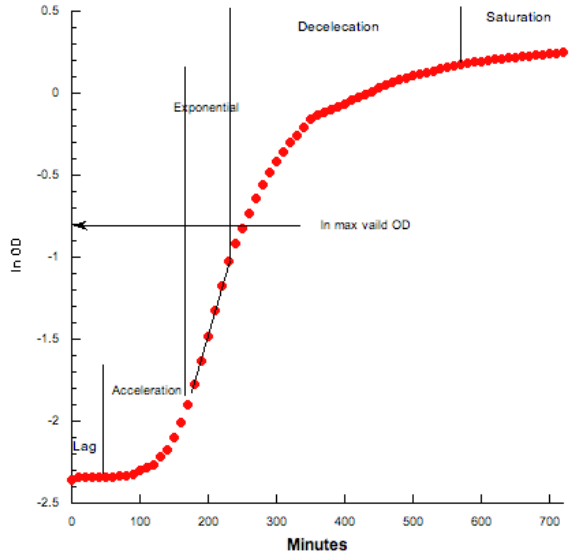


Figure 1

The growth rate is based only on the Exponential phase. Manually determining the Exponential portion of the growth curve, then calculating the growth rate as the slope of ln OD vs time during the Exponential phase, requires investigator time – for 96 or 384 wells, considerable time – and is quite tedious.

The program ***GrowthRates*** automates the entire process and requires only a few minutes of investigator time.

Installation

You should download both the **GrowthRates 3.0** folder and the **Formatters** folder that are appropriate for your computer platform.

Macintosh OS X: Put ***GrowthRates*** into the /usr/local/bin folder. That folder is invisible, so you will need to choose **Go to folder…** from the Go menu in the Finder and enter /usr/local/bin in the dialog box. That will open the folder in a new window. Just drag ***GrowthRates*** and whichever formatting program is appropriate for your microplate reader into that folder then provide the requested Administrator password.

If you don't have a usr/local/bin folder just create one. From the Go menu enter `/usr/local`, then create a bin folder within **/usr/local**. You will be asked for a password when you try to create the bin folder.

Linux: Put ***GrowthRates*** into the /usr/local/bin folder. Just drag ***GrowthRates*** and whichever formatting program is appropriate for your microplate reader into that folder then provide the requested Administrator password.

If you don't have a usr/local/bin folder just create one. From the Go menu choose Location…, then enter `/usr/local`, then create a bin folder within **/usr/local**. You will be asked for a password each time you try to create the bin folder.

Windows: See Appendix I

Input File

The input Data file is a tab-delimited text file that contains the output from the microplate reader. Depending on the make of plate reader, its output format may or may not include tab-delimited text, but to the best of my knowledge all makes can save their output or export it as a Microsoft Excel file. If the data are in an Excel file, open that file in Excel and export it the text (tab-delimited) format.

Caution! The input files must have line endings that are appropriate for your platform. Your microplate reader may not use the same platform as the computer on which you are running ***GrowthRates***, so be sure to check the line endings format of your data file. A portion of a data file is shown in Figure 2 below. For Mac OS X **Unix** line endings are required. The Mac provides a particular problem because Excel for the Mac exports text files with Classic Mac, not Unix, line endings. Use a text editor to change line endings. See Appendix II for recommended text editors.

The format of the input data file is that the first row is a header row that lists the well IDs, with the first column being headed Min (minutes) and the remaining columns being headed with the well IDs. The remaining rows contain the time in the first column and the OD readings in the remaining columns. Figure 1 shows part of a typical data file. Although it is not required, I usually use the extension '.txt' for the data file, e.g. `Example1.txt`.

Min	A1	A2	A3	A4	A5	A6	A7	A8
0	0.088	0.091	0.091	0.089	0.091	0.093	0.09	0.089
10	0.088	0.091	0.09	0.089	0.09	0.091	0.09	0.089
20	0.088	0.091	0.089	0.089	0.089	0.089	0.09	0.089
30	0.088	0.092	0.092	0.089	0.088	0.089	0.09	0.089
40	0.088	0.092	0.093	0.089	0.088	0.089	0.091	0.089
50	0.088	0.093	0.093	0.09	0.089	0.089	0.091	0.089
60	0.088	0.094	0.094	0.091	0.089	0.089	0.092	0.089
70	0.088	0.096	0.096	0.092	0.09	0.09	0.093	0.09
80	0.088	0.098	0.099	0.094	0.091	0.091	0.095	0.09
90	0.088	0.1	0.103	0.096	0.093	0.091	0.097	0.091
100	0.088	0.104	0.108	0.101	0.095	0.093	0.101	0.092
110	0.088	0.11	0.117	0.108	0.1	0.096	0.106	0.093
120	0.088	0.119	0.131	0.12	0.108	0.099	0.114	0.095
130	0.088	0.133	0.152	0.138	0.121	0.105	0.124	0.098
140	0.088	0.153	0.182	0.167	0.143	0.114	0.139	0.103
150	0.088	0.184	0.226	0.207	0.177	0.128	0.16	0.11
160	0.088	0.227	0.282	0.265	0.228	0.15	0.186	0.12
170	0.088	0.283	0.326	0.304	0.282	0.183	0.219	0.135
180	0.088	0.331	0.391	0.37	0.334	0.229	0.259	0.155
190	0.088	0.389	0.455	0.44	0.403	0.28	0.304	0.18

Figure 2

Note: each row ***must*** begin with the time in minutes. If there is no time entry for a row the program will crash with a message directing your attention to the defective row.

Some plate readers output data as Excel files, but in other formats; e.g. time is given in hours:minutes: seconds format (1:20:13), additional information is added before and/or after the data block, or data blocks are interleaved. Even when converted to tab-delimited text files GrowthRates cannot read such non-standard data files. Those files must either be manually edited to conform to the format above or must be formatted with a ***FormatGR*** program. ***FormatGR_Biotek*** automatically formats the output from the **Biotek Eon** plate reader. ***FormatGR_Biotek_PowerWaveHT*** automatically formats the output from the **Biotek PowerWave HT** plate reader. ***FormatGR_Tecan*** automatically formats the output from the **Tecan** plate reader. ***FormatGR_Perkin_Elmer*** automatically formats the output from the **Perkin Elmer** plate reader. ***FormatGR_SpectraMaxM2*** automatically formats the output from the **SpectraMax M2** p-late reader. Contact me at barryghall@gmail.com to discuss adding additional versions of ***FormatGR*** . See Appendix III for details on using ***Format GR***.

Running *GrowthRates*

GrowthRates is a classical command line program that is run from within the Terminal program (Mac OS X and Linux, found in the Utilities folder under Applications) or from within the Command Prompt program (Windows, found in the Accessories folder under All Programs in the Start menu).

Correcting for background absorbance and drift in the absorbance readings

It is ***essential*** to correct the absorbance readings for any absorbance by the growth medium. Background is particularly a problem when the growth medium is a dark broth such as L-broth or 2xYT. There are two ways to correct for background absorbance.:

1. Include a blank well during the growth experiment. A blank well is not an empty well. Instead it is a well that contains the same volume of the same medium (uninoculated) used in the experiment. A blank well almost always has some OD, which is the background. That background, the OD due to the medium, refraction, etc., ***must*** be subtracted from the OD reading for each well in order to correctly calculate growth rates, lag times and maximum OD. Typically the absorbance readings from the blank well will remain constant over the course of the experiment, but they may change as the result of precipitate formation, bleaching or instrument drift over the course of long experiments. Inclusion of a blank well is the most reliable way to compensate for background absorbance. When a blank well is included ***GrowthRates*** automatically compensates to the background absorbance at each time point in calculating growth rates. While it is the most accurate, this method uses one of the well, which may be inconvenient for the experimental design. When a blank well is included in the experiment you must provide the identity of the blank well when ***GrowthRates*** is run as described in the next section.
2. Immediately prior to the experiment read the absorbance of several wells containing the same volume of the same medium to be used in the experiment. Record the mean background absorbance, then run the experiment. When you have *experimentally* determined the background absorbance you must provide that background absorbance when ***GrowthRates*** is run as described below. Users are cautioned against using the same background absorbance in all experiments. Variation in the growth medium means that it is important to determine that background before each experiment.

Running *GrowthRates*

To run the program, start Terminal or Command Prompt and navigate to the folder that contains your input file by typing "`cd` ", then dragging that folder into the Terminal or Command Prompt window and pressing "enter". Don't forget the space after "`cd`".

Now that you have navigated to the folder that contains the input file you are ready to run ***GrowthRates***.

<u>If a blank well has been used during the growth experiment</u>

Enter `GrowthRates -i myFile -w wellNumber,` where myFile is the name of the input data file and wellNumber is the column in which the data for the blank well is found. Column number refers to the column in the input file for the blank well. The Time column is column 0, the sample A1 column is column 1, etc. For ease of identifying the column that corresponds to a particular well it is suggested that well A1 be used as the blank.. You may use any well you like as the blank, but which case you must enter the number of the data column corresponding to that well on the command line. Assuming the your input file is named myfile.txt, to specify well A4 as a blank well enter:
`GrowthRates -i myfile.txt -w 4` then hit the return key to complete the entry.

<u>If a blank well has *not* been used during the growth experiment</u>

In this case, you must provide the experimentally determined background absorbance on the command line. Enter `GrowthRates -i myFile -b background`, where myFile is the name of the input data file and background is the mean background absorbance. For instance, if the background absorbance is 0.088 enter **`GrowthRates -i myFile -b 0.088`**, then hit the return key to complete entering the command.

If you fail to enter either a blank well number or a background absorbance value > 0 ***GrowthRates*** will terminate with an error warning.

You cannot provide both a blank well number and a background absorbance. If you do so ***GrowthRates*** will terminate with an error warning.

A Macintosh running a 3.4 GHz Intel Core i7 processor completes the analysis of a data file for 384 wells in slightly under 3 seconds. The results are written to two files with the same name as the data file, but with the extensions '.results' and '.summary'; i.e if your data file is Exp47b.txt the output will be saved to Exp47b.results and summarized in Exp47b.summary.

ExampleData files and brief tutorial

The file ExampleData1.txt is an input file to *GrowthRates* in the required format from an experiment in which well A1 was blank.. Copy ExampleData1.txt into some folder (directory) of your choosing. After installing *GrowthRates* navigate to that folder and run *GrowthRates* as described above by entering **`GrowthRates -i ExampleData1.txt -w 1`** If the run is successful files named ExampleData1.results and Example1Data.summary will appear in that same folder. Open those files with a text editor and look at them to see what the GrowthRates output looks like.

The file ExampleData2.txt is an input file to *GrowthRates* in the required format from an experiment in which there was no blank well but the background absorbance was experimentally determined to be 0.08.. Copy ExampleData2.txt into some folder (directory) of your choosing. After installing *GrowthRates* navigate to that folder and run *GrowthRates* as described above by entering **`GrowthRates -i ExampleData2.txt -b 0.08`** If the run is successful files named ExampleData1.results and Example1Data.summary will appear in that same folder. Open those files with a text editor and look at them to see what the GrowthRates output looks like.
If the run is not successful you failed to install GrowthRates exactly as described above.

If ***GrowthRates*** runs the example data correctly, but fails to run your own input files the most likely explanations are (1) your file is not formatted correctly. Look at it. Does it look exactly like the format shown on page 3? If not, it won't work. (2) The line endings of your file are incorrect for your version of ***GrowthRates***. See the caution on page 3. (3) there is more than one blank line at the end of your input file.

The output

GrowthRates writes two output files, a .results file and a .summary file. The output file names are taken from the name of the data file. If your data file was Exp47b.txt the two output files would be named Exp47b.results and Exp47b.summary

The .results file

The .results file looks like Figure 4:

```
GrowthRates v3.0 copyright 2018 Barry G. Hall and The Bellingham Research Institute.

Fri Jan 12 12:39:42 2018
Data file: Test.txt.
**********************************************************
           Well A1
6 points from 140 through 190 minutes were used to estimate the growth rate.
The growth rate +/- s.e. is 0.03461 +/-0.000393 per minute.
This is equivalent to a doubling time of 20.0 +/-0.23 minutes.
The correlation coefficient R is 0.99974
The maximum OD is 1.324
The lag time is 111.9 minutes
**********************************************************

**********************************************************
           Well A2
6 points from 120 through 170 minutes were used to estimate the growth rate.
The growth rate +/- s.e. is 0.03332 +/-0.000379 per minute.
This is equivalent to a doubling time of 20.8 +/-0.24 minutes.
The correlation coefficient R is 0.99974
The maximum OD is 1.233
The lag time is 82.6 minutes
**********************************************************

**********************************************************
           Well A3
6 points from 110 through 160 minutes were used to estimate the growth rate.
The growth rate +/- s.e. is 0.03426 +/-0.000270 per minute.
This is equivalent to a doubling time of 20.2 +/-0.16 minutes.
The correlation coefficient R is 0.99988
The maximum OD is 1.345
The lag time is 74.9 minutes
**********************************************************
```

Figure 4

The growth rate is reported with its standard error both as a first-order rate constant in units of reciprocal minutes and in doubling times in minutes. The correlation coefficient R is reported. Typical correlation coefficients are around 0.998 to 0.999. If the correlation coefficient is <0.995 it is wise to look at a graph of the data for anomalies and to look at the data itself. The program GRplot can be used to easily look at a graph of the data. See Appendix IV.

The .summary file

The .summary file is a tab-delimited text file that just lists the well ID and the growth rate, correlation coefficient R, maximum OD and lag time for each well. That file can be imported into a spread sheet or a statistics program for analysis of the growth rates. Part of a .summary file is shown below.

Well	Growth Rate	R	Max OD	lag time (minutes)
A1	0.03461	0.99974	1.324	111.9
A2	0.03332	0.99974	1.233	82.6
A3	0.03426	0.99988	1.345	74.9
A4	0.03803	0.99986	1.341	80.5
A5	0.04047	0.99900	1.342	96.9
A6	0.03556	0.99935	1.320	112.4
A7	0.02796	0.99975	1.156	76.2
A8	0.02965	0.99975	1.202	109.0
A9	0.02866	0.99983	1.152	117.8
A10	0.02763	0.99982	1.152	129.2
A11	0.02841	0.99974	1.186	135.0
A12	0.02505	0.99991	1.179	141.9
B1	0.00			

How *GrowthRates* calculates growth rates

The program first converts OD values to ln OD. Starting at time zero, it considers a window of 5 time points (points 1-5) and calculates the slope of ln OD vs time and saves that value. It then moves one time point and considers the next window of five (points 2-6) and saves the product of the slope and the correlation coefficient, R. After it has calculated all 5-point slope x R products up through the highest OD it uses the time points set whose slope x R product was highest to determine the initial time points from which the growth rate will be determined. It then attempts to extend those 5 time points by adding successive time points as long as the slope x R product remains at least 95% of the maximum slope x R product. The extended set of time points are used to determine the final growth rate. The principle is that as points begin to fall off the exponential growth line (Figure 1) the slopes over the 5-point window begin to decrease below the maximum.

The use of slope x R to determine which time points to use was introduced in version 3.0; previously only the slopes were used to determine which time points to use. Why use the product of the slope x R? Occasionally a spurious OD reading will fall significantly above the line through the exponential region. The slope of a 5-point line that includes that spurious point will be high and result in an over-estimate of the growth rate if that set of points were used. At the same time, inclusion of that spurious point will result in a low correlation coefficient R. By using the product of slope x R as the criterion for choosing which time points to use in most cases the spurious time point will not be used and the growth rate estimate will be more accurate. In practice such spurious time points are quite rare and growth rates calculated by version 3.0 are identical to those calculated by earlier versions.

How GrowthRates calculates lag times

Figure 5 shows how ***GrowthRates*** calculates lag times. The calculation is somewhat unrealistic because it assumes that there is no growth at all until the lag time, at which point growth instantly begins at the reported growth rate. Nevertheless, the calculated lag time is useful for relative comparisons among wells.

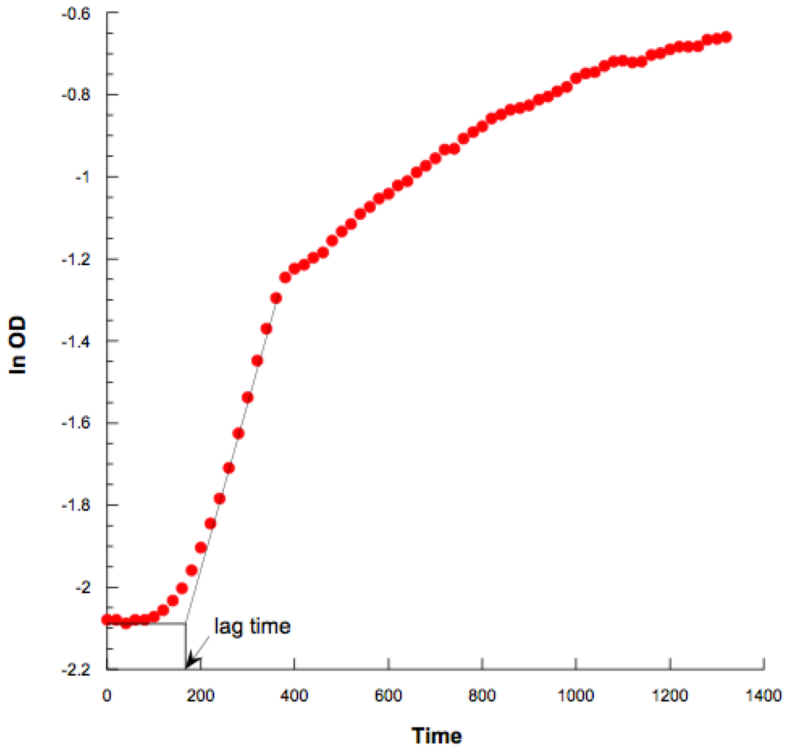


Figure 5

Some questions that might arise

What about non-linearity between OD and culture density at higher ODs?

Because bacterial cell suspensions scatter light rather than absorbing it, the relationship between OD and culture density is linear only up to a point. Above that point OD increases more slowly than does culture density. As a result, the slope of ln OD vs time may decline even though the culture may still be growing at the maximum exponential rate. Because the growth rate is based only on the maximum slope, it doesn't matter whether that slope declines because the growth rate declines or whether it declines because OD isn't linear with culture density; points above the point where the slope declines will not be included in the growth rate measurement.

What if there is diauxic growth so that there are two real, but distinct, growth rates?

GrowthRates will report the higher of those two rates. You will have to look at a graph of all the points to detect diauxic growth and you will need to calculate the lower growth rate manually. Use GRplot (Appendix IV) to detect diauxic growth and to calculate the growth rate in the second exponential phase.

How does sample volume affect the measurements?

Sample volume affects the relationship between OD and culture density. A volume of 300µl will give an OD reading that is 1.5x that of 200µl of the same culture. As long as the volume does not change during the experiment, for instance by evaporation, the volume will not affect the determination of growth rate.

On which platforms has ***GrowthRates*** been tested?

Macintosh OS X version 10.13.1, Ubuntu Linux 16.04, Windows 10. There is no reason to expect problems with different versions of OSX, but if problems appear on later versions please let me know at barryghall@gmail.com.

GrowthRates doesn't read my data file or wells file correctly and it gives me weird error messages. What can I do?

Be sure that the format of your data file conforms exactly to that shown above and that there is no extraneous information before or after the data. If the format is correct, the most likely source of the problems is that the line endings of the data or wells files are not correct for your platform. Use a text editor to check line endings and set them correctly. Remember that the Mac OS X platform requires Unix, not classic Mac, line endings.

Is there more than one blank line at the end of the input file? You need exactly one blank line.

GrowthRates reports some negative growth rates with negative lag times. What does that mean?

If there is no growth and the cells are actually dying over time the O.D. may gradually (or abruptly!) decrease. Growth rates will be negative and the way lag times are calculated will cause the lag times to be negative as well. In almost all such cases the correlation coefficient will also be very low. The numbers themselves can safely be ignored and you can report "no growth".

What if the initial ODs are at or slightly below the background OD and the lag is very long?

The initial OD should be **at least 0.01** above the background. The difference between measured OD and background OD is the OD attributable to the cells. When the OD attributable to cells is very close to the limit of detection there is a lot of scatter in in the background-corrected OD readings, which can in some cases result in unrealistic growth rate estimates.

How can I tell if well-to-well variation in growth rates for the same strain or condition is excessive; i.e. how much should I trust the growth rates of replicate samples?

The program **CGR** is a statistic program specifically designed to analyze results of growth rate experiments generated by GrowthRates. See Appendix IV

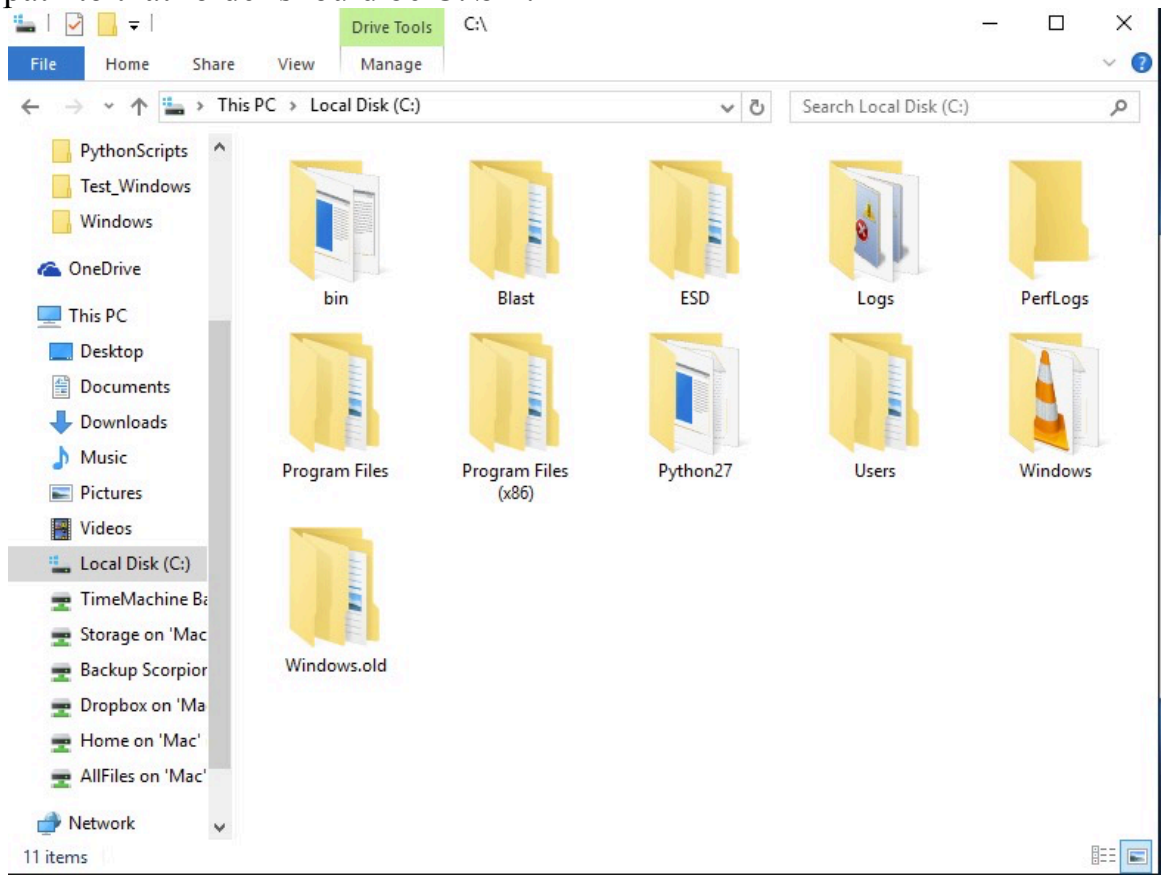
How can I tell if the mean growth rates of two different strains are really different?

The program **CGR** is a statistic program specifically designed to analyze results of growth rate experiments generated by GrowthRates. See Appendix IV

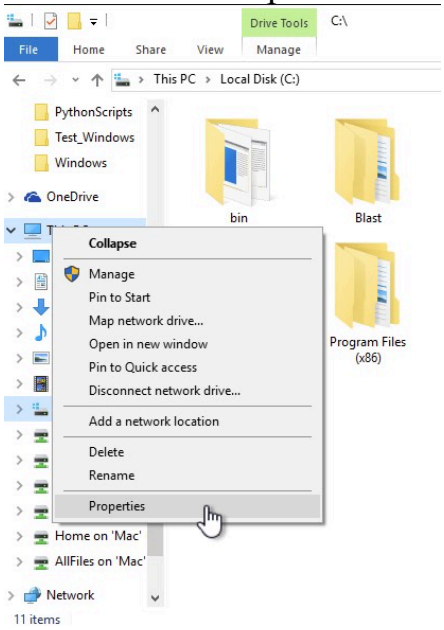
Appendix I Installing programs in Windows 10

To install GrowthRates first create a *bin* folder, next put GrowthRates.exe into that folder, and finally, create a *path* to that folder.

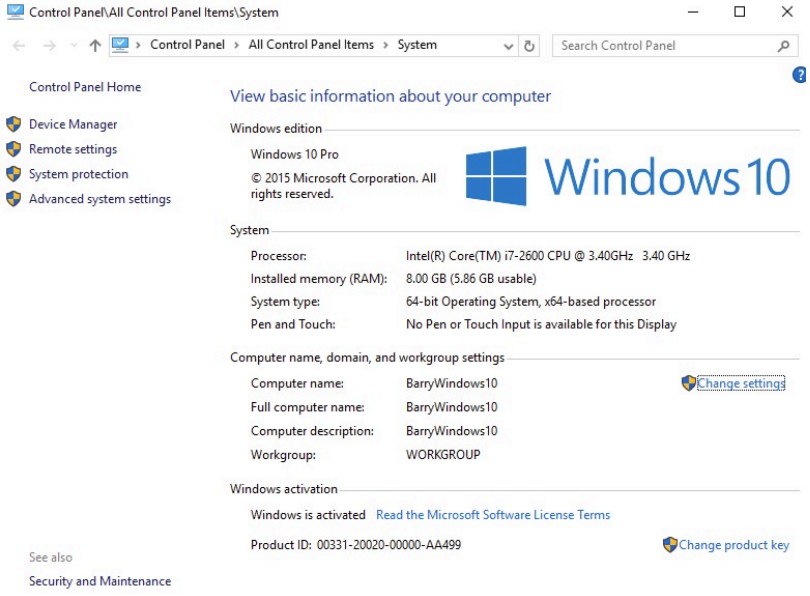
1. Create the *bin* folder directly on your C drive; i.e. not within any other folder. The path to that folder should be C:\bin.



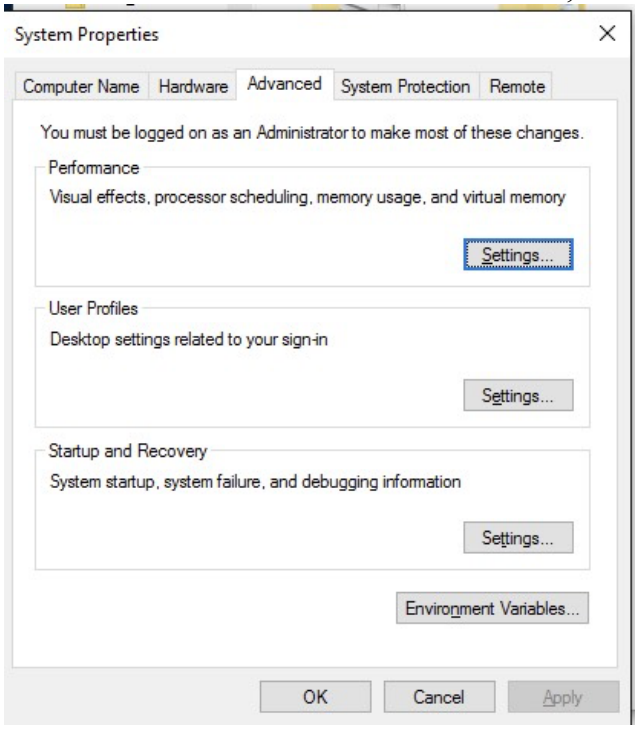
2. In a Windows Explorer window Right-click on This PC and choose Properties.



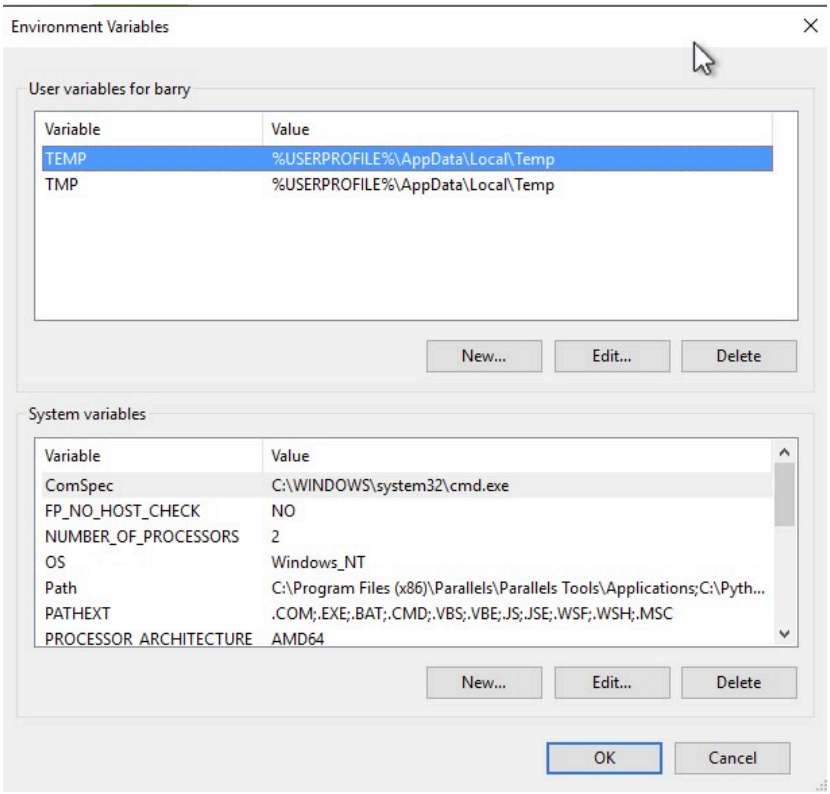
3. In the resulting window click Advanced System Settings to bring up the System Properties control panel.



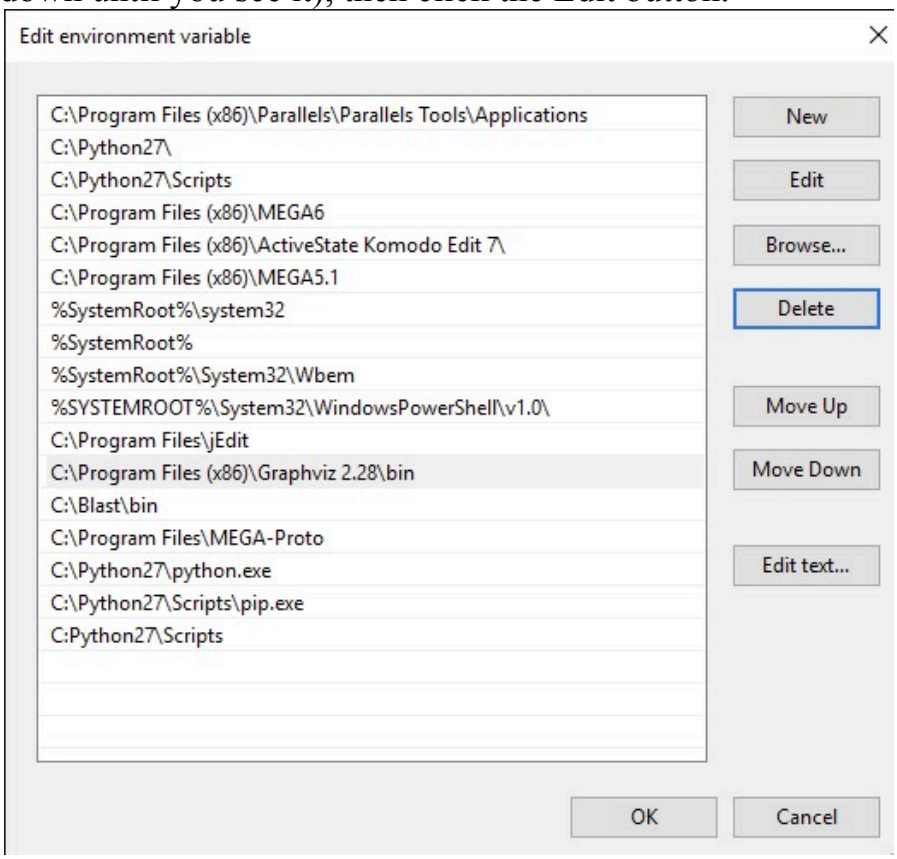
4. The Advanced tab should be selected; if not select it.



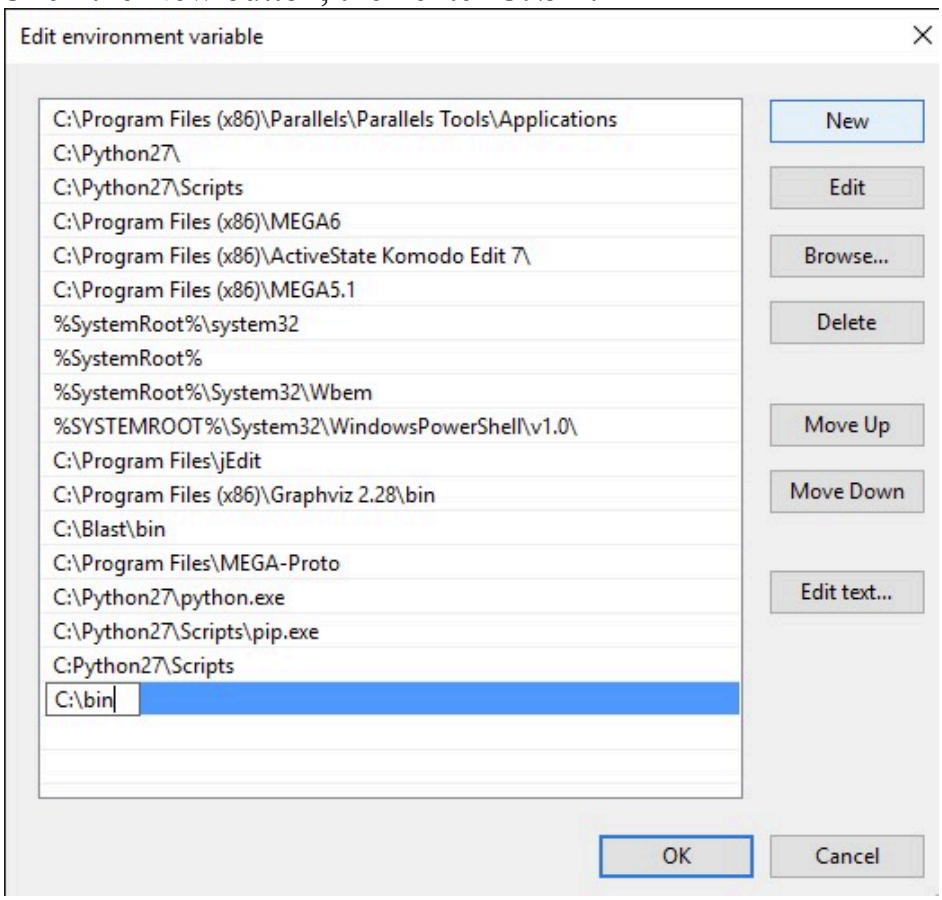
5. Click the Environment Variables button to show the Environment Variables window.



6. In the lower part of the window select the PATH variable (if it is not visible scroll down until you see it), then click the Edit button.



7. Click the New button, then enter C:\bin.



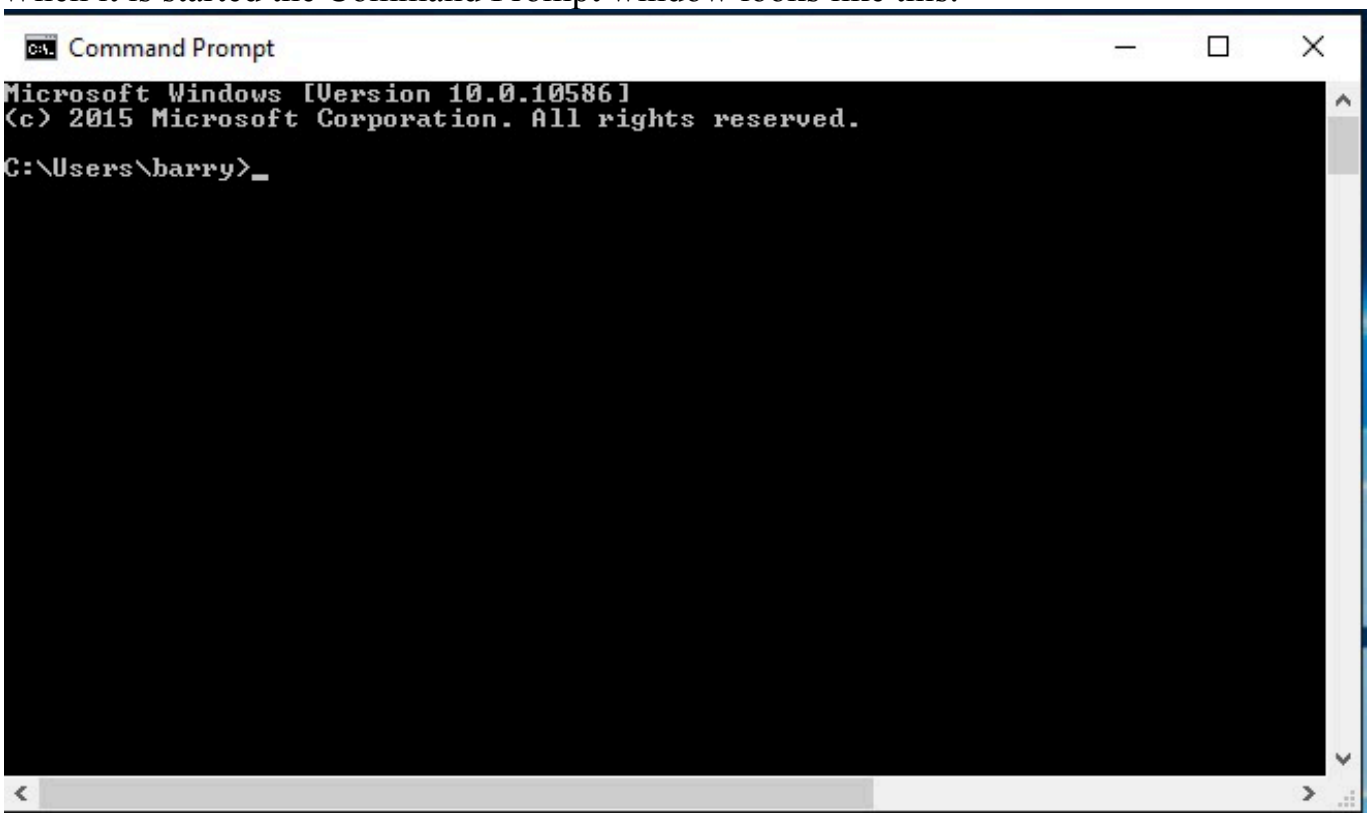
8. Now click OK in each of the windows.

Whew! You are done. Now when you open a Command Prompt window Command Prompt will look in C:\bin for any programs you install there.

Using command-line programs

Command-line programs must be used within the Command Prompt program, a program that resembles the old DOS environment. The Command Prompt program is included among the programs in Windows 10. *It is convenient to put a shortcut to Command Prompt on the desktop or into the task bar*.

When it is started the Command Prompt window looks like this:



It's default size is 80 characters wide, and it displays characters as white on a black background. You can right-click on the window title bar and choose **Properties** to modify the appearance of the window. The **Layout** tab allows you to set the maximum width and height of the window. The Colors tab allows you to set the screen background and screen text colors; e.g. black text on a white background.

Within the Command Prompt window the mouse does essentially nothing; all commands are entered by typing. The bottom line, showing `C>` is the prompt, consisting of the path to the *current directory* followed by ">". Programs within Command Prompt can only work on input files in the current directory ("directory" is a synonym for "folder"), and all files are saved to the current directory. In the figure above the current directory is "barry". drive. The `cd` command is used to make a different directory the current directory. Type `cd` followed by a space then enter the *path* to the directory of interest. The easy way to

enter that path is to drag that folder (the one that you want to make the current directory) into the Command Prompt window. Doing that automatically enters the path to that folder.

Appendix II Recommended Text Editors

All input file must be *text* files, not word processor files such ass those written by Word or WordPerfect. Text editors should be set number line and ***not*** wrap lines. In order to see column align correctly in output files in be necessary to change the default tab width. All of the recommended text editors allow you to change the line endings.

• For Macintosh I recommend the free TextWrangler from BareBones Software (http://www.barebones.com/) or the more sophisticated commercial BBEdit from the same source. Set line endings to Unix.

• For Windows I recommend the free Notepad++ software from notepad-plus-plus.org. Set line endings to Dos/Windows.

• For Linux I recommend the free Gedit from wiki.gnome.org/Apps/Gedit. By default gedit *does* wrap text and *does not* number lines, so you will need to set the preferences correctly. Set line endings to Unix.

Appendix III Using FormatGR to format non-standard plate reader output

If your plate reader does not output results in the standard format you will need to reformat the file using one of the ***FormatGR*** programs in the table below.

Program	**Instrument**	**Tested on model (if known)**
FormatGR_Bioscreen	Bioscreen	Bioscreen C
FormatGR_Biotek	Biotek	Eon
FormatGR_Biotek_PowerWaveHT	Biotek PowerWave HT	Biotek PowerWave HT
FormatGR_Perkin_Elmer	Perkin Elmer	Victor 3 V 1420 Multilabel Counter
FormatGR_SparkTecan	SparkTecan	Spark 10M
FormatGR_SpectraMaxM2	SpectraMax	SpectraMax, SpectraMaxM2
FormatGR_Tecan	Tecan	Infinite 200Pro

Like ***GrowthRates***, ***FormatGR_X*** programs are command line programs. They are installed exactly like ***GrowthRates***, and are used through Terminal or Command Prompt. You only need to install the version that is appropriate for your plate reader.

If the name of the non-standard plate reader output file is "myfile.txt", enter `FormatGR_X myfile.txt` where X is Biotek, Tecan, Perkin_Elmer, etc..

FormatGR_X programs will write a new file named myfile_formatted_.txt. Use that file for the input to ***GrowthRates.***

Update note, Feb. 12, 2014.
FormatGR_Biotek was formerly known simply as ***FormatGR***. When the need for an additional formatting program for the Tecan plate reader was brought to my attention I wrote ***FormatGR_Tecan*** and renamed ***FormatGR***.

If your instrument's output is non-standard and it cannot be correctly formatted using any of these formatter programs please email me at barryghall@gmail.com and tell me about it. Identify your instrument and attach a tab-delimited text file of the its output. I'll see what I can do to write a FormatGR program for your machine.

Special Note for Mac OSX: Excel exports text files with classic-mac line endings, not unix line endings. You **must** use a text editor to change line endings of the text file to unix or the FormatGR programs will not work on the Mac OS X platform. See Appendix I for recommended text editors.

Appendix IV Additional programs that support GrowthRates

GRplot facilitates trouble shooting unexpected GrowthRates results. Using the same input file as Growth rates, and the same –b or –w options, ***GRplot*** allows the user to specify a well to analyze and to plot either all of the points (to visualize the entire growth curve) or to plot a specific set of time point by specifying the beginning and ending points. It calculates the growth rate and correlation coefficient over those points, and displays the plot with the fitted line. If for some reason the time points chosen by GrowthRates appear to be inappropriate plotting an alternative set of points my produce a better fit (higher correlation coefficient). If plotting the entire curve suggests diauxic growth the user can choose a set of points from the second exponential growth phase to calculate the growth parameters of that phase. The ***GRplot*** packages includes a user guide and a brief tutorial. ***GRplot*** is available for Mac, Windows and Linux at https://sourceforge.net/projects/growthrates/files/?source=navbar.

CGR, standing for Compare Growth Rates, is a statistical package for analyzing and comparing GrowthRates results. It uses three input file: (1) the .summary file from a GrowthRates experiment, (2) the .results file from a GrowthRates experiment, and (3) a *sets* file that defines the wells for each set of replicate wells. It produces two output files: (1) a .matrix file that gives the growth rate of each well in each set, and (2) a .var file that gives the mean growth rate, mean correlation coefficient, and a variability-score (V-score) for each set of replicates. Those values allow the user to assess his level of confidence or trust in each result. Each *set* might be a different strain, or it might be a different nutrient, or it might be a different inhibitor. Members of a set are identical replicates.

The input *sets* file also specifies the sets that make up a group, for instance an experimental and a control group, or all of the sets can be considered as a single group. ***CGR*** generates a bootstrap analysis of each group. When growth rates of some sets are similar the bootstrap analysis allows identification of t the proportion of experiments in which each of those sets would be expected to be the fastest growing set. It also does a pairwise comparison of all sets and identifies the fraction of experiments in which set A would be expected to outgrow set B. The stochastic property of bootstrap sampling gives a much better picture of of the population dynamics of competing strains that is provided by just comparing mean growth rates.

Tne ***CGR*** package also includes the program ***EditReadingIntervals***. When growth is very slow relative to the reading interval there can be enough scatter in the ODs the correlation coefficients are low and the resulting growth rate estimates are unreliable. Counter-intuitively, reading less frequently, e.g. reducing the reading interval from 20 minutes to 60 minutes, can significantly improve relability of the results. It is not necessary to repeat an experiment a lower reading intervals. ***EditReadingIntervals*** edits an input file to delete the unwanted time points and thus to provide a lower-interval input file for GrowthRates analysis.

Tne ***CGR*** package includes ***CGR*** , ***EditReadingIntervals***, a User Guide and a set of example files. It is available for Mac, Linus and Windows at https://sourceforge.net/projects/growthrates/files/?source=navbar. is described in detail in Mira, P., M. Barlow, and B. G. Hall. 2017. Statistical Package for Growth Rates Made Easy. Mol. Biol. Evol. **34:** 3303-3309**.** doi: 10.1093/molbev/msx255.