

GetGrowth 0.2

Rasmus Ågren (RAAG), 150923

Purpose

GetGrowth is a program for calculating (exponential) growth rates from BioLector files. The main advantage compared to using spreadsheet software is that GetGrowth lets the user group biological replicates and estimate the growth rate for the group as a whole. This is achieved by using a random effects model.

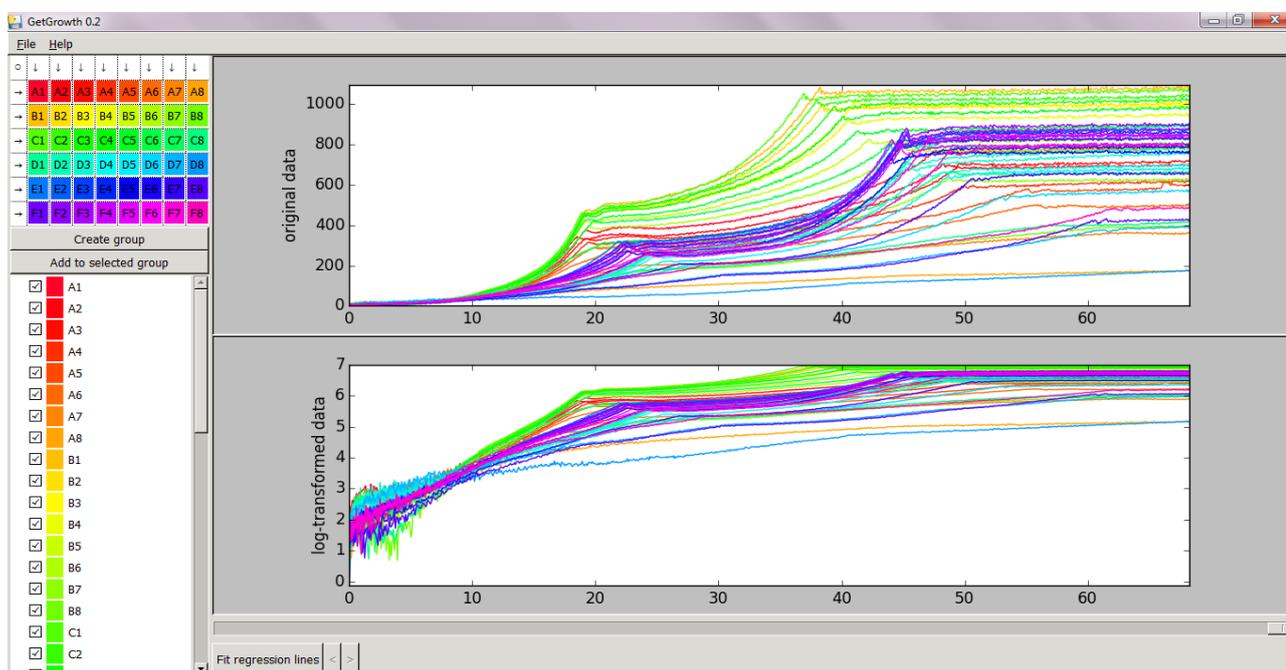
The resulting growth estimates and their fitting statistics (R^2 , 95% confidence interval and time span) can be exported as a CSV file. In addition to normal least-squares linear regression, GetGrowth also calculates the statistics using a robust regression algorithm called RANSAC. The main purpose for this is that it can be difficult to visually define the linear region in the log-transformed data. RANSAC will identify points which are unlikely to belong to a linear model and exclude them from the fitting.

NOTE: This is software under development. It is likely there are some bugs when it comes to the user interface. Please contact RAAG if you experience anything weird or if you have suggestions for improvements. If the program freezes or starts to behave unexpectedly then restart and try again.

Workflow

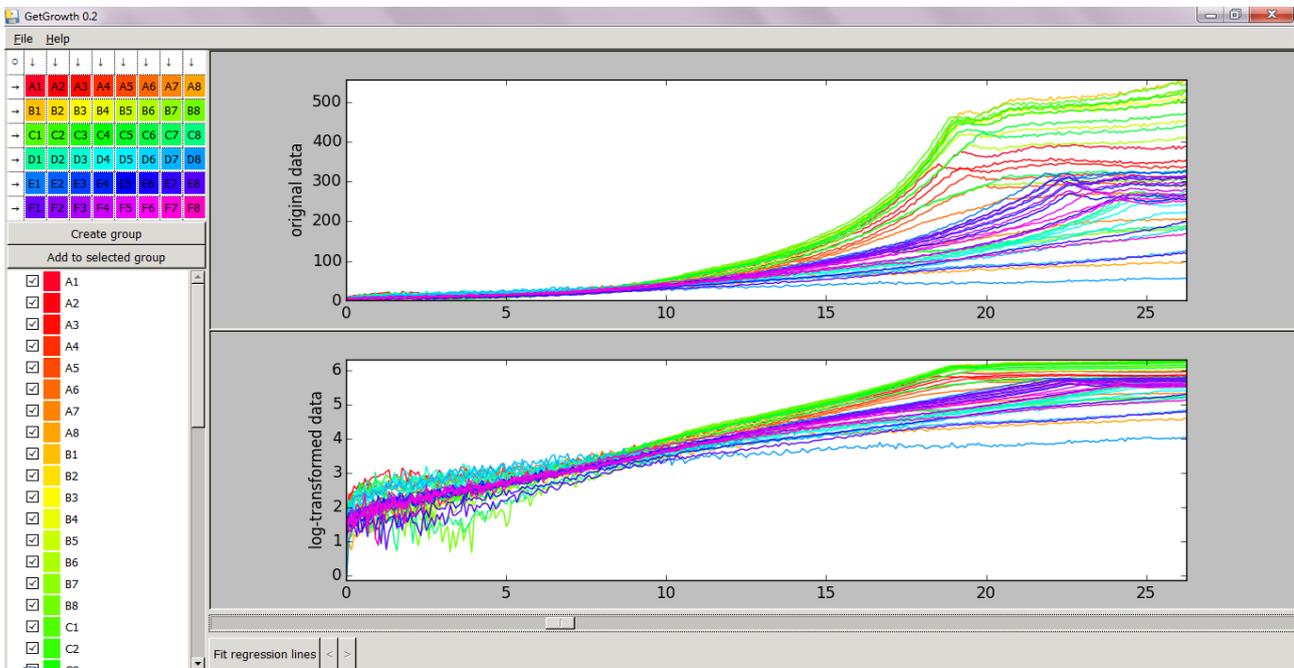
Select "File->Load file" to import a BioLector file. It currently only supports files in the transformed format, so you have to export it from BioLector like that. GetGrowth will try to determine if the file is comma- or semicolon-separated and use the same format when exporting. Do not export as "US comma-separated values"!

You can choose "Help->Load sample" to load a sample file.



In the upper left corner are the 48 wells used in our BioLector plates. Below then is a list of the wells (and/or groups, see below). The upper plot shows the original data and the lower shows the log-transformed data.

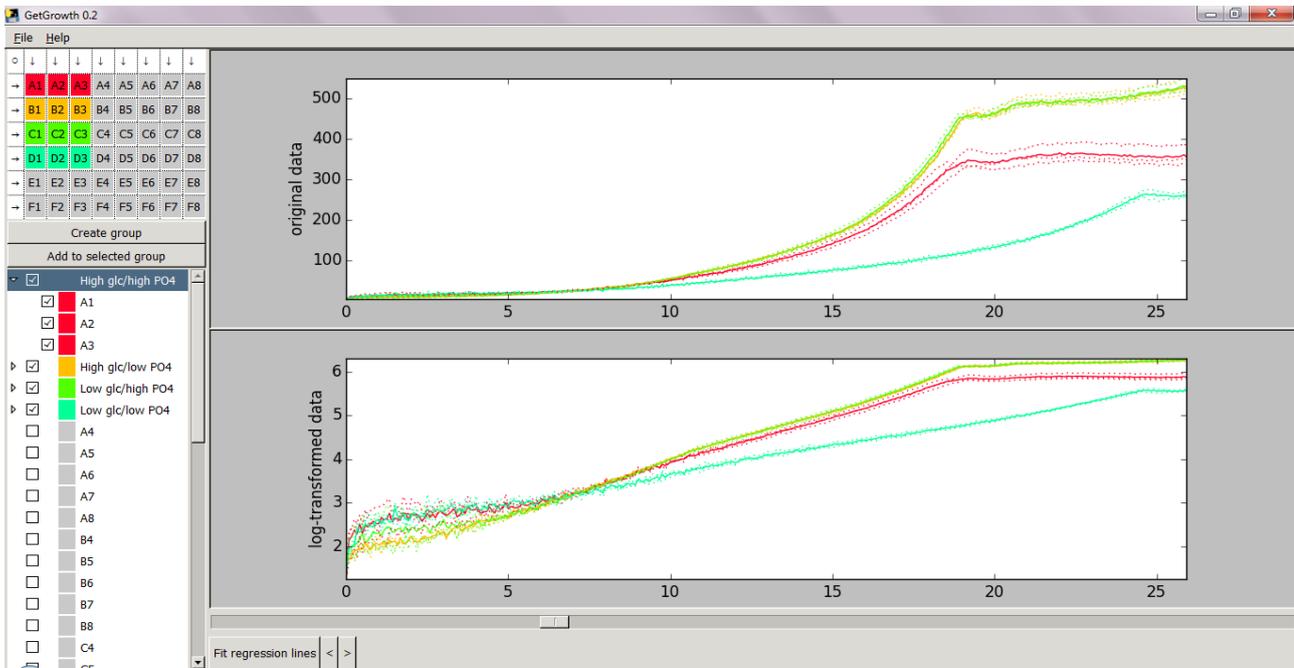
Since we're interested in the exponential growth phase in the beginning we can use the slider below the plots to adjust the upper x-axis limit.



By clicking on the well array or the checkboxes in the list you can include or exclude wells from the analysis/plotting.

Group wells by first clicking "Create group", then select that group and click "Add to selected group". You then add wells to that group by selecting them in the well array. Note that the group colour will be based on the first well added to it. Use this to select colours which make it easy to differentiate between groups. The mean of the individual wells in a group is shown as a solid line and the individual wells are shown as dotted lines. Note that this is just for illustration; the mixed effects model uses all data without any averaging.

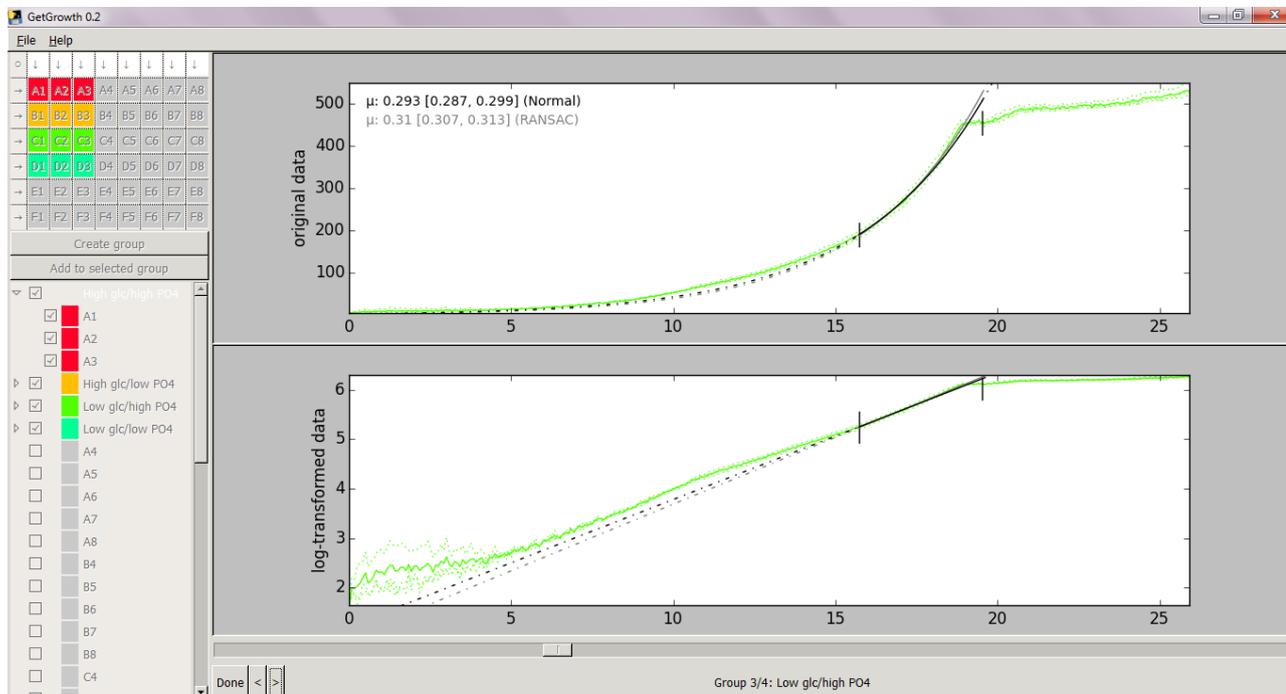
You can also rename groups and wells. In the figure below there are four groups (high/low glucose, high/low PO₄³⁻) with three wells each.



Once you are done with the naming/grouping click "Fit regression lines" to start the fitting. This will let you step through the groups/wells one by one. Select the interval by clicking in the lower plot. Left click sets lower limit and right click sets upper. The estimate is shown in the upper plot and the fitting statistics in the lower.

In the example below the upper limit has been chosen a bit too high and there is no longer exponential growth there. You can see that the RANSAC fitting compensates for this, and results in a fitting that is somewhat closer to the exponential region (grey curve is above the black one in the upper figure). This is just for illustration; you should of course strive to choose the best region possible.

Since the linear regression is performed on log-transformed data there is typically lots of noise for low OD measurements. If possible avoid including such regions in the interval used for the fitting (although RANSAC to some degree will compensate for that).



Once you're done click "Done" and then "File->Save file" to save the results. The results can be viewed in, for example, Microsoft Excel.

***Groups								
		mu (1/h)	95% CI	R-sqr	mu (1/h) [RANSAC]	95% CI [RANSAC]	R-sqr [RANSAC]	Time interval (h)
3	High glc/high PO4	0.24	[0.229 - 0.252]		0.238	[0.225 - 0.252]		[11.735 - 18.389]
4	A1	0.233	[0.230 - 0.236]	0.997	0.229	[0.226 - 0.231]	0.998	
5	A2	0.252	[0.249 - 0.255]	0.998	0.252	[0.249 - 0.255]	0.998	
6	A3	0.236	[0.233 - 0.239]	0.997	0.235	[0.232 - 0.237]	0.998	
8	High glc/low PO4	0.246	[0.244 - 0.249]		0.237	[0.235 - 0.239]		[11.093 - 18.739]
9	B1	0.246	[0.242 - 0.250]	0.996	0.237	[0.235 - 0.240]	0.998	
10	B2	0.244	[0.240 - 0.248]	0.995	0.235	[0.232 - 0.238]	0.997	
11	B3	0.249	[0.245 - 0.253]	0.995	0.239	[0.236 - 0.243]	0.997	
13	Low glc/high PO4	0.311	[0.305 - 0.318]		0.311	[0.302 - 0.319]		[15.733 - 19.147]
14	C1	0.306	[0.300 - 0.311]	0.998	0.309	[0.304 - 0.313]	0.998	
15	C2	0.31	[0.305 - 0.315]	0.998	0.299	[0.296 - 0.302]	0.999	
16	C3	0.318	[0.313 - 0.324]	0.998	0.318	[0.313 - 0.324]	0.998	
18	Low glc/low PO4	0.156	[0.146 - 0.166]		0.154	[0.136 - 0.173]		[19.235 - 24.284]
19	D1	0.146	[0.143 - 0.150]	0.995	0.145	[0.142 - 0.147]	0.997	
20	D2	0.165	[0.161 - 0.168]	0.993	0.164	[0.160 - 0.167]	0.993	
21	D3	0.157	[0.155 - 0.160]	0.997	0.161	[0.160 - 0.163]	0.999	
***Wells								
24		mu (1/h)	95% CI	R-sqr	mu (1/h) [RANSAC]	95% CI [RANSAC]	R-sqr [RANSAC]	Time interval (h)
25	Control +	0.165	[0.164 - 0.166]	0.999	0.164	[0.163 - 0.165]	1	[15.120 - 21.686]
26	Control -	0.164	[0.163 - 0.165]	0.999	0.164	[0.164 - 0.165]	1	[14.070 - 21.920]