Method Sheet 103

Normalisation of assay measurements to a control condition

Overview

This method sheet explains how to normalise data to a control condition (such as a negative or positive control treatment) for the purpose of minimising variation between different experiments.

Two methods are given, depending on whether your data analysis project is for antibiotic discovery (looking at bacterial cell growth) or anti-cancer drug discovery (crystal violet assays for mammalian cell viability). Follow the method appropriate for the type of data analysis project you have chosen. These methods assume Microsoft Excel will be used to perform the calculations, but any other spreadsheet software should work equally well.

Normalisation of bacterial cell growth screening data

1) Open your *Phytotitre* or *Puretitre* screening data analysis file.
2) Once you have background-corrected the raw absorbance values using the approach given in Method Sheet 102, you should progress to normalising the growth measurements to percentages of the control condition, which is growth of bacteria in the presence of vehicle alone (DMSO), and the absence of any inhibitor.
3) We assume that bacterial growth in this condition is maximal, and we therefore assign it a value of 100% bacterial growth.
4) All other growth values are then calculated as a percentage of the maximum growth.
5) First we must calculate the mean (average) of the maximum growth values, which should be in wells A1 to H1 in the plate you set up.
6) Type “Max growth” in the first cell of a row below the background corrected data table, and then in a separate cell to the right of it, insert the following equation:

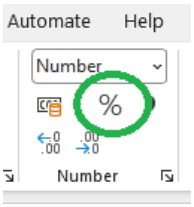
   `=AVERAGE(B26:B33)`

7) This example assumes that the left-most column of values in your background corrected data table run from cell `B26` to cell `B33`, but check your own data as the locations may be different.
8) Now, just below this, type a label for the normalised data table, for example: “Normalised growth data (21 hour timepoint)”
9) In the first cell of your new table, insert the following formula:

   `=B26/$C$35`

10) This example assumes the first cell in your background corrected data table is at position `B26`, and the cell containing the `AVERAGE` max growth calculation is in cell `C35` - make sure to check that you insert the correct cell references for your own data as the locations may be different.
11) Note also that this formula uses the **absolute cell reference** for the max growth value, so it is essential to place the dollar symbols before both letter and number of the max growth cell reference (but not the first cell reference).

12) Now click on the percentage icon in the Home ribbon near the top of the page to reformat the cell to show the new calculation as a percentage.



13) Now copy and paste, or drag and fill, this formula into every cell of a 12 x 8 table to give all of your normalised growth values.

14) Your max growth average calculation cell, and normalised data table should now look something like this:

24	**Background corrected data (21 hour timepoint)**											
25		1	2	3	4	5	6	7	8	9	10	11	12
26	A	0.576	0.630	0.532	0.531	0.650	0.729	0.713	0.730	0.710	0.628	0.610	0.000
27	B	0.643	0.654	0.806	0.778	0.811	0.688	0.767	0.677	0.635	0.685	0.797	0.000
28	C	0.613	0.761	0.780	0.783	0.798	0.790	0.695	0.783	0.721	0.584	0.548	0.000
29	D	0.656	0.780	0.731	0.786	0.798	0.784	0.766	0.686	0.716	0.606	0.518	0.001
30	E	0.657	0.816	0.791	0.823	0.828	0.471	0.728	0.691	0.633	0.648	0.512	-0.001
31	F	0.657	0.674	0.748	0.832	0.754	0.766	0.747	0.581	0.652	0.587	0.664	-0.001
32	G	0.701	0.556	0.790	0.779	0.761	0.724	0.621	0.576	0.576	0.482	0.547	0.002
33	H	0.555	0.572	0.651	0.655	0.643	0.568	0.558	0.591	0.572	0.529	0.494	0.000
34													
35	**Max growth:**		0.632										
36													
37	**Normalised growth data (21 hour timepoint)**												
38		1	2	3	4	5	6	7	8	9	10	11	12
39	A	91%	100%	84%	84%	103%	115%	113%	115%	112%	99%	96%	0%
40	B	102%	103%	127%	123%	128%	109%	121%	107%	100%	108%	126%	0%
41	C	97%	120%	123%	124%	126%	125%	110%	124%	114%	92%	87%	0%
42	D	104%	123%	116%	124%	126%	124%	121%	109%	113%	96%	82%	0%
43	E	104%	129%	125%	130%	131%	74%	115%	109%	100%	102%	81%	0%
44	F	104%	107%	118%	132%	119%	121%	118%	92%	103%	93%	105%	0%
45	G	111%	88%	125%	123%	120%	115%	98%	91%	91%	76%	87%	0%
46	H	88%	90%	103%	104%	102%	90%	88%	93%	90%	84%	78%	0%
47													

15) You may notice that some values show more than 100% growth - this is normal, and reflects natural variation around the mean for the control wells, or enhancement of growth for some of the natural extracts or compounds.

16) If the experiment and normalisation processes have gone well, you should see that the percentage growth values in column 12 of your table, which should have received antibiotic treatment as a positive control, should all be close to zero.

17) Now repeat this normalisation process for the data tables for the 21 hour timepoint for all the other plates in your Excel data analysis file.

Normalisation of mammalian cell viability screening data

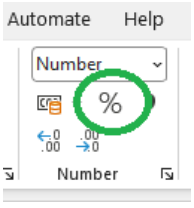
1) Open your *Phytotitre* or *Puretitre* screening data analysis file.
2) Once you have background-corrected the raw absorbance values using the approach given in Method Sheet 102, you should progress to normalising the growth measurements to percentages of the control condition, which we define as the growth of cells in the presence of vehicle alone (DMSO), and the absence of any inhibitor.
3) We assume that cell growth in this condition is maximal, and we therefore assign it a value of 100% growth.
4) All other growth values are then calculated as a percentage of the maximum growth.
5) First we must calculate the mean (average) of the maximum growth values, which should be in wells A1 to H1 in the plate you set up.
6) Type “Max growth” in the first cell of a row below the background corrected data table, and then in a separate cell to the right of it, insert the following equation:

```
=AVERAGE(B18:B25)
```

7) This example assumes that the left-most column of values in your background corrected data table run from cell `B18` to cell `B25`, but check your own data as the locations may be different.
8) Now, just below this, type a label for the normalised data table, for example: “Normalised growth data”
9) In the first cell of your new table, insert the following formula:

```
=B18/$C$27
```

10) This example assumes the first cell in your background corrected data table is at position `B18`, and the cell containing the `AVERAGE` max growth calculation is in cell `C27` - make sure to check that you insert the correct cell references for your own data as the locations may be different.
11) Note also that this formula uses the **absolute cell reference** for the max growth value, so it is essential to place the dollar symbols before both letter and number of the max growth cell reference (but not the first cell reference).
12) Now click on the percentage icon in the Home ribbon near the top of the page to reformat the cell to show the new calculation as a percentage.



13) Now copy and paste, or drag and fill, this formula into every cell of a 12 x 8 table to give all of your normalised growth values.
14) Your max growth average calculation cell, and normalised data table should now look something like this:

	A	B	C	D	E	F	G	H	I	J	K	L	M
15													
16	**Background corrected absorbance values**												
17		1	2	3	4	5	6	7	8	9	10	11	12
18	A	0.750	0.798	0.367	0.830	0.899	0.882	0.909	0.777	0.796	0.708	0.777	0.001
19	B	0.753	0.698	0.698	0.817	0.884	0.830	0.822	0.863	0.628	0.812	0.760	0.000
20	C	0.840	0.764	0.779	0.749	0.802	0.785	0.879	0.842	0.740	0.762	0.697	0.003
21	D	0.813	0.810	0.735	0.754	0.809	0.763	0.430	0.852	0.814	0.733	0.467	0.002
22	E	0.894	0.534	0.726	0.725	0.784	0.305	0.753	0.779	0.712	0.735	0.798	-0.001
23	F	0.868	0.873	0.882	0.737	0.798	0.855	0.784	0.867	0.821	0.877	0.853	-0.002
24	G	0.912	0.836	0.864	0.785	0.883	0.984	0.818	0.966	1.002	0.831	0.968	0.002
25	H	0.948	0.804	0.881	0.834	0.922	0.774	0.828	0.935	1.028	0.863	0.963	-0.003
26													
27	**Max growth**		0.847										
28													
29	**Normalised growth data**												
30		1	2	3	4	5	6	7	8	9	10	11	12
31	A	89%	94%	43%	98%	106%	104%	107%	92%	94%	84%	92%	0%
32	B	89%	82%	82%	96%	104%	98%	97%	102%	74%	96%	90%	0%
33	C	99%	90%	92%	88%	95%	93%	104%	99%	87%	90%	82%	0%
34	D	96%	96%	87%	89%	95%	90%	51%	101%	96%	87%	55%	0%
35	E	106%	63%	86%	86%	93%	36%	89%	92%	84%	87%	94%	0%
36	F	102%	103%	104%	87%	94%	101%	93%	102%	97%	104%	101%	0%
37	G	108%	99%	102%	93%	104%	116%	97%	114%	118%	98%	114%	0%
38	H	112%	95%	104%	98%	109%	91%	98%	110%	121%	102%	114%	0%

15) You may notice that some values show more than 100% growth - this is normal, and reflects natural variation around the mean for the control wells, or enhancement of growth for some of the natural extracts or compounds.

16) If your experiment and normalisation have gone well, you should see that the percentage growth values in column 12 of your table, which should have received a positive control for complete cell killing, should all be close to zero.

17) Now repeat this normalisation process for the data tables for all the other plates in your Excel data analysis file.

Notes

- If you are struggling to follow these instructions, you may find it helpful to first read the advice given in **Method sheet 101**, on Basic Data Handling techniques using Microsoft Excel.
- Remember to never modify the raw data files, but rather copy them into a new, separate analysis file, which should be stored in a separate folder to further protect the integrity of the raw data.
- Don’t worry if you see any values that are slightly below 0% growth, this is normal and reflects typical variation in the assay and measurements.