Method Sheet 19

Background correction and normalisation of mammalian cell viability screening data

Overview

This method sheet explains how to subtract the background absorbance values from mammalian cell viability data as part of a natural product screening project. This process is necessary to establish the percentage of the maximum potential growth the cells exhibit in the presence of each plant extract or compound.

The second part of the method sheet explains how to normalise mammalian cell growth values to a control condition in the absence of any inhibitor. These methods assume Microsoft Excel will be used to perform the calculations, but any other spreadsheet software should work equally well. They can be used for data from either crystal violet assays or MTT assays of cell growth or viability.

Method for background correction

1) For mammalian cell growth experiments, some wells on each plate should have been challenged with the vehicle alone (DMSO) as the negative control, and a positive control for cell killing (e.g. SDS) in a series of separate wells, in addition to the remaining wells which were challenged with extracts or compounds of interest.
2) Copy and paste as values the data from the primary absorbance read files from each of the relevant 96-well plate reads into a single Excel file for data analysis, using a separate tab for each plate, and name it to distinguish it as a separate analysis file.
3) First we must calculate the mean background absorbance caused by the plastic, medium and residual cell debris, in the positive control wells.
4) Type "Positive control" in the first well of a row below the table of 12 x 8 raw data values, and then in a separate cell to the right of it, insert the following equation:
```
=AVERAGE(M5:M12)
```
5) This example assumes that the 8 positive control wells are in the right-most column of your raw data, and run from cells `M5` to cell `M12`, but check your own data as the locations may be different.
6) Now to perform background correction, we must subtract this value from each of the values in the raw data table.
7) Type "Background corrected data" into a cell just below the average positive control cell.
8) Just below this, prepare your table of background-corrected data by typing the following function into the first (top left) cell of your new data table:
```
=B5-$E$14
```
9) This example assumes that the first cell in your raw data table is at position `B5`, and the cell containing the `AVERAGE` positive growth calculation is in cell `E14` - make sure to check that you insert the correct cell references for your own data as the locations may be different.

10) Note also that this formula uses the **absolute cell reference** for the positive control, so it is essential to place the dollar symbols before both letter and number of the positive control cell reference (but not the first cell reference).

11) Copy this formula and paste it into all 96 cells in a new table of 12 x 8 cells to yield the background corrected data.

12) This is done most easily by dragging the lower right green cross on the bottom right of the first cell down 8 rows, then letting go of the mouse button, then clicking the green cross again and dragging the cell 12 columns to the right to fill the table.

13) Your two blocks of data for the same 96-well plate should now look something like this, with the raw data in the upper table, and the background-corrected data in the lower table:

Phytotitre set 1: extracts 001 - 080

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.887	0.935	0.504	0.967	1.036	1.019	1.046	0.914	0.933	0.845	0.914	0.138
B	0.890	0.835	0.835	0.954	1.021	0.967	0.959	1.000	0.765	0.949	0.897	0.137
C	0.977	0.901	0.916	0.886	0.939	0.922	1.016	0.979	0.877	0.899	0.834	0.140
D	0.950	0.947	0.872	0.891	0.946	0.900	0.567	0.989	0.951	0.870	0.604	0.139
E	1.031	0.671	0.863	0.862	0.921	0.442	0.890	0.916	0.849	0.872	0.935	0.136
F	1.005	1.010	1.019	0.874	0.935	0.992	0.921	1.004	0.958	1.014	0.990	0.135
G	1.049	0.973	1.001	0.922	1.020	1.121	0.955	1.103	1.139	0.968	1.105	0.139
H	1.085	0.941	1.018	0.971	1.059	0.911	0.965	1.072	1.165	1.000	1.100	0.134

Average of positive control values 0.137

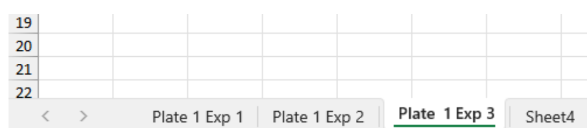
Background corrected absorbance values

	1	2	3	4	5	6	7	8	9	10	11	12
A	=B5-E14	0.798	0.367	0.830	0.899	0.882	0.909	0.777	0.796	0.708	0.777	0.001
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
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
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
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
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
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
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

14) Now repeat the same process for data from any other plates you have measured, ensuring that the absorbance values used for one background correction calculation are always from the same plate.

15) To help ensure you do not mix data from one plate with another plate during this process, it can be helpful to insert a new worksheet for each individual plate you have data for.

16) Double click the tab at the bottom of the page to change the name of the worksheet to indicate which plate each sheet refers to, e.g. "Plate-1 Exp 1", "Plate-5 Exp4" etc., as shown below:



Normalisation of background-corrected data

1) Once you have background-corrected the raw absorbance values using the method above, 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.

2) We assume that cell growth in this condition is maximal, and we therefore assign it a value of 100% growth.

3) All other growth values are then calculated as a percentage of the maximum growth.

4) 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.

5) 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)
```

6) 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.

7) Now, just below this, type a label for the normalised data table, for example: “Normalised growth data”

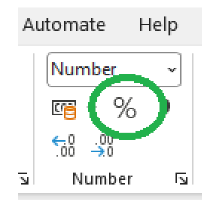
8) In the first cell of your new table, insert the following formula:

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

9) 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.

10) 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).

11) 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.



12) 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.

13) 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%

14) 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.

15) 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.

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.

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