Method Sheet 102

Background correction for bioassay absorbance values

Overview

This method sheet explains how to subtract the background absorbance values from a data set arising from a bacterial cell-growth or tumour cell-growth natural product screening project. This process is necessary to account for the potential impact of pigments and other coloured compounds in plant extracts that may otherwise confound the correct identification of hits from the screen.

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.

Background correction of bacterial cell growth screening data

1) Open the *Phytotitre* dataset Excel file you have chosen to analyse and save it with a different name it to distinguish it as an analysis file (e.g. 'Bacterial Growth Data Analysis').
2) For bacterial growth data, each plate should have been measured on at least two occasions: once at zero hours (i.e. straight after preparing the plate) and then again after 21 hours incubation, or another similar timepoint.
3) In a cell somewhere below the data tables in the worksheet, type the following text: "Background corrected data (21 hour timepoint)".
4) Take a note of the cell reference ID (e.g. `A3`, `C15`, etc.) for the top left cell of each table of plate absorbance values (e.g. the tables at zero hours and 24 hours).
5) Type the following formula into the upper left cell of the new table of values you are forming just below the "Background corrected data" title:

```
=B15-B4
```

6) This example assumes that the first (top left) cell of the 24 hour data is in cell `B15`, and the first cell of the 0 hour data is in cell `B4`.
7) Check to make sure that the formula you type uses the correct cell references for your own data, as they may be in a different location from the example above.
8) Copy this formula and paste it (as normal with a relative cell reference WITHOUT dollar signs) into all 96 cells in a new table of 12 x 8 cells to yield the background corrected data.
9) This is most easily done 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.
10) Your three blocks of data for the same 96-well plate should now look something like this:

	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2	**Experiment # 1**		Time = 0 h										
3		1	2	3	4	5	6	7	8	9	10	11	12
4	A	0.041	0.043	0.050	0.046	0.043	0.041	0.041	0.042	0.040	0.040	0.042	0.041
5	B	0.040	0.048	0.047	0.045	0.045	0.041	0.045	0.044	0.041	0.043	0.044	0.040
6	C	0.042	0.044	0.043	0.045	0.045	0.042	0.041	0.042	0.042	0.044	0.049	0.040
7	D	0.042	0.045	0.047	0.046	0.045	0.043	0.044	0.047	0.042	0.044	0.050	0.040
8	E	0.043	0.045	0.044	0.045	0.045	0.049	0.045	0.047	0.045	0.043	0.044	0.041
9	F	0.044	0.048	0.047	0.048	0.047	0.045	0.045	0.048	0.048	0.045	0.046	0.041
10	G	0.044	0.053	0.045	0.047	0.047	0.046	0.046	0.046	0.044	0.048	0.046	0.040
11	H	0.044	0.049	0.048	0.048	0.045	0.046	0.045	0.047	0.047	0.046	0.048	0.040
12													
13	**Experiment # 1**		Time = 21 h										
14		1	2	3	4	5	6	7	8	9	10	11	12
15	A	0.617	0.673	0.582	0.577	0.693	0.770	0.754	0.772	0.750	0.668	0.652	0.041
16	B	0.683	0.702	0.853	0.823	0.856	0.729	0.812	0.721	0.676	0.728	0.841	0.040
17	C	0.655	0.805	0.823	0.828	0.843	0.832	0.736	0.825	0.763	0.628	0.597	0.040
18	D	0.698	0.825	0.778	0.832	0.843	0.827	0.810	0.733	0.758	0.650	0.568	0.041
19	E	0.700	0.861	0.835	0.868	0.873	0.520	0.773	0.738	0.678	0.691	0.556	0.040
20	F	0.701	0.722	0.795	0.880	0.801	0.811	0.792	0.629	0.700	0.632	0.710	0.040
21	G	0.745	0.609	0.835	0.826	0.808	0.770	0.667	0.622	0.620	0.530	0.593	0.042
22	H	0.599	0.621	0.699	0.703	0.688	0.614	0.603	0.638	0.619	0.575	0.542	0.040
23													
24	**Background corrected data (21 hour timepoint)**												
25		1	2	3	4	5	6	7	8	9	10	11	12
26	A	=B15-B4	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

11) Now repeat the same process for data from all other plates that have been measured at the 21 hour timepoint, ensuring that the absorbance values used for one background correction calculation are always from the same plate.

12) You are now ready to proceed to the next stage, which is normalisation of the data.

Background correction of mammalian cell viability screening data

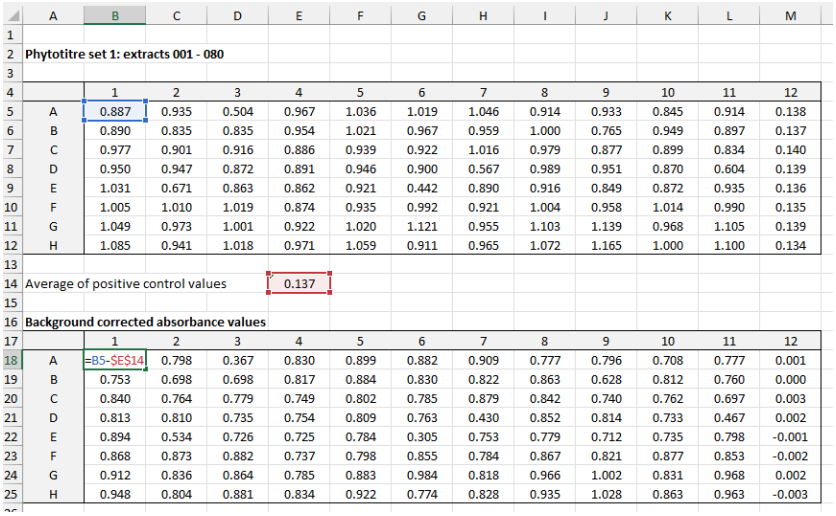
1) Open the *Phytotitre* dataset Excel file you want to analyse and save it with a different name it to distinguish it as an analysis file (e.g. 'Cell Growth Data Analysis').
2) For mammalian cell growth experiments, some wells on each plate should have been challenged with the vehicle alone (DMSO) as 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.
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:



	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2	**Phytotitre set 1: extracts 001 - 080**												
3													
4		1	2	3	4	5	6	7	8	9	10	11	12
5	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
6	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
7	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
8	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
9	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
10	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
11	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
12	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
13													
14	Average of positive control values				0.137								
15													
16	**Background corrected absorbance values**												
17		1	2	3	4	5	6	7	8	9	10	11	12
18	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
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

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

15) You are now ready to proceed to the next stage, which is normalisation of the data.

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.