Method Sheet 20

Background correction and normalisation of enzyme activity data

Overview

This method sheet explains how to subtract the background absorbance values from a kinetic assay of β-galactosidase enzyme activity as part of a 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.

The second part of the method sheet explains how to normalise the enzyme activity values to a control condition of incubation 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.

Background correction of absorbance data

1) For the standard β-galactosidase screening project, 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 30 minutes incubation, or another similar timepoint.
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 an analysis file.
3) Paste the values for the absorbance measurements relating to one of the plates measured at the 0 hour timepoint near the top of the sheet.
4) Paste the values relating to the absorbance measurements for **the same plate**, but at the timepoint you want to correct for background lower down in the same sheet.
5) Type text similar to this in a cell just above where you want the corrected data to go: “Background corrected data (30 minute timepoint)”.
6) 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.
7) 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
```

8) 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`.
9) 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.
10) 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.
11) 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.

12) 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	Phytotitre plate 1 exp 1: Time = 0 min												
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	Phytotitre plate 1 exp 1: Time =30 min												
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 (30 minute 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

13) 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.

14) 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.

15) 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 absorbance measurements to percentages of the control condition, which is enzyme activity in the absence of any inhibitor.

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

3) All other activity values are then calculated as a percentage of this maximum.

4) First we must calculate the mean (average) of the maximum enzyme activity values, which should be in wells A1 to H1 in the plate you set up.

5) Type “Max activity” in the first well 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)
```

6) 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.

7) Now, just below this, type a label for the normalised data table, for example: “Normalised enzyme activity (30 minute timepoint)”

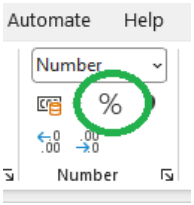
8) In the first cell of your new table, insert the following formula:

```
=B26/$C$35
```

9) This example assumes the first cell in your background corrected data table is at position `B26`, and the cell containing the `AVERAGE` max activity 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.

10) Note also that this formula uses the **absolute cell reference** for the max activity value, so it is essential to place the dollar symbols before both letter and number of the max activity 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 activity values.

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

24	Background corrected data (30 minute 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 activity:		0.632										
36													
37	Normalised enzyme activity (30 minute 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%

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

15) If your experiment and normalisation have gone well, you should see that the percentage activity values in column 12 of your table, which should have received galactose treatment as a positive control for enzyme inhibition, should all be low percentages.

16) As we are seeking inhibitors of β-galactosidase in this screen, the cells showing values with the lowest percentages of enzyme activity represent the “hit” extracts or compounds we want to take forward for further investigation.

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.
- If you have taken aborbance readings of your assay plates at multiple different timepoints, you should repeat the same methods as shown above for each timepoint, subtracting the zero hour timepoint for background correction each time.
- 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% activity or over 100% activity, this is normal and reflects normal variation in the assay and measurements.