Method Sheet 22

Calculation of Z' factor from screening assay data

Overview

This method sheet explains how to calculate the Z' factor from screening assay data. This metric is the gold standard for assessing the quality and robustness of a high-throughput screening (HTS) assay. It essentially measures the "gap" between your positive and negative controls while accounting for the noise (variability) in each set of measurements within a single 96-well plate. The Z'-factor tells you three things about your assay:

1) If your positive and negative controls are working correctly
2) If the difference between your "hits" and the assay "background" is large enough to be statistically reliable
3) If your pipetting and assay set up is reproducible and reliable

The methods shown below assume that Microsoft Excel will be used to perform the calculations, but any other spreadsheet software should work equally well.

Method

1) You should begin by completing the background correction and normalisation processes with respect to your raw absorbance measurements for each plate you want to analyse (see Method sheets 18-21).
2) Identify the locations of your positive and negative control wells in your assay plate.
3) From these, we must calculate the mean (average) and the standard deviation (SD) of both the positive and negative control measurements.
4) The Z' factor value for the plate is then calculated using the following equation:

$$Z' = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

Where:

- σ_p and σ_n are the standard deviations of the positive and negative controls
- μ_p and μ_n are the means of the positive and negative controls

5) In the first cell of each of 4 rows below the normalised data table, you should type the following headings: Average positive, Average negative, SD positive, SD negative (see below for what this should look like).
6) In a separate cell to the right of the 'Average positive' title, type the following formula:

```
=AVERAGE(M4:M11)
```

7) Note that this example assumes your positive controls are in positions `M4` to `M11`, check this is correct for your own data, as you may have these in different locations.
8) Now do the same for the negative control values, for example:

```
=AVERAGE(B4:B11)
```

9) Note that this example assumes your negative controls are in positions `B4` to `B11`, check this is correct for your own data, as you may have these in different locations.

10) Next, calculate the Standard Deviation (SD) for the positive controls as follows:

```
=STDEV(M4:M11)
```

11) Then calculate the SD for the negative controls using this formula:

```
=STDEV(B4:B11)
```

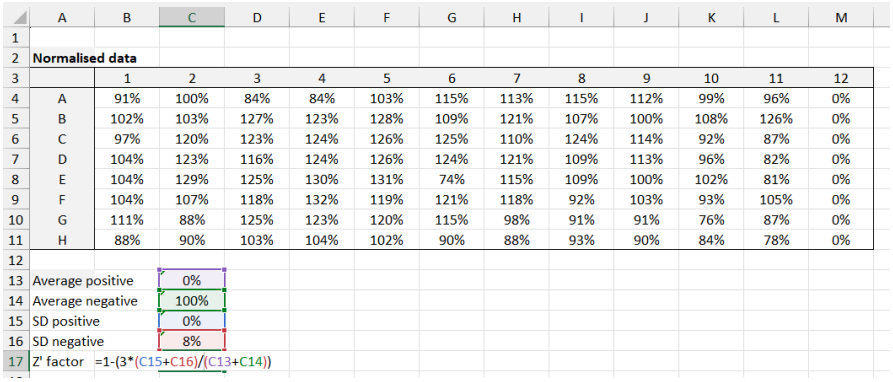
12) As before, make sure these cell references are correct for the positions of the positive and negative controls in your own data.

13) Now calculate the Z' factor using the following formula:

```
=1-(3*(C15+C16)/(C13+C14))
```

14) Note that this assumes the Z' factor will be in cell `C17`, and the means and SDs are in the cells as shown in the formula above (and the image below), make adjustments as necessary if you have any of them in different locations.

15) Your mean, SD, and Z' factor calculations should now look something like this:



	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2	**Normalised data**												
3		1	2	3	4	5	6	7	8	9	10	11	12
4	A	91%	100%	84%	84%	103%	115%	113%	115%	112%	99%	96%	0%
5	B	102%	103%	127%	123%	128%	109%	121%	107%	100%	108%	126%	0%
6	C	97%	120%	123%	124%	126%	125%	110%	124%	114%	92%	87%	0%
7	D	104%	123%	116%	124%	126%	124%	121%	109%	113%	96%	82%	0%
8	E	104%	129%	125%	130%	131%	74%	115%	109%	100%	102%	81%	0%
9	F	104%	107%	118%	132%	119%	121%	118%	92%	103%	93%	105%	0%
10	G	111%	88%	125%	123%	120%	115%	98%	91%	91%	76%	87%	0%
11	H	88%	90%	103%	104%	102%	90%	88%	93%	90%	84%	78%	0%
12													
13	Average positive		0%										
14	Average negative		100%										
15	SD positive		0%										
16	SD negative		8%										
17	Z' factor	=1-(3*(C15+C16)/(C13+C14))											

Interpretation of the Z' factor values

The Z' factor for a bioassay is a value between -infinity and +1. The closer you can get to 1, the better, as this indicates a robust and reliable assay. However, your Z' values will not reach 1, as this is a theroretical maximum that we cannot achieve in practice, since it would mean an ideal assay with no variability at all. Here's how to interpret the Z' values for your own plates:

Z' greater than 0.5

This is the "gold standard" value we are seeking to achieve. If your Z' value is greater than 0.5, your assay is robust and reliable. You can confidently identify hits because the "noise" (well-to-well variation) is much smaller than the "signal" (the effect of your extract or compound). You can have strong confidence that your "hits" are real.

Z' greater than 0, but less than 0.5

Z' values in this range are sub-optimal. You can still find hits, but you'll likely need to perform more replicates and fewer of the hits are correct and likely to replicate in follow-on studies. The assay exhibits quite high variation, and could benefit from improvements to protocol or technique.

Z' values below 0

If your Z' value is negative, it means your controls are too variable. The standard deviations are so wide that they "bleed" into each other, making it impossible to distinguish a true result from random noise. We cannot rely on the results of such an assay to identify any hits, and it will have to be improved and/or repeated.

Pro tips

1) You can save lots of time and typing by copying the cells containing the mean, SD and Z' factor calculation formulas into another worksheet and Excel will automatically calculate the Z' factor for the new plate data, so long as you paste the calculation cells in exactly the same orientation relative to the plate you are copying from (e.g. two cells directly below the bottom left cell of the normalised data table).
2) If you feel like trying something more ambitious, it is possible to calculate the Z' factor within a single Excel cell using a more complex formula that calculates the SDs and means within one long formula, as follows:

```
=1-(3*(STDEV(M4:M11)+STDEV(B4:B11))/(AVERAGE(M4:M11)+AVERAGE(B4:B11)))
```

3) Note that this example assumes the cell spacings and locations are the same as those given in the above methods.
4) Be warned, making a single typo in the formula will likely ruin the calculation, so if you are just starting out, it may be better to begin with the longer method as shown in the previous section.

Notes

- If your Z' values are consistently low for each of your plates, the following are some possible reasons for poor reproducibility that you can consider when attempting to improve the reliability of your assays:
- Fluctuations caused by pipetting errors - ensure you pipette slowly using correct technique and mix all solutions before applying them to cells.
- Air bubbles in wells - look at the plate before inserting it in the microplate reader, if there are large bubbles in the wells, you should pop them with a thin piece of rolled up tissue, and try not to introduce them while pipetting in future.
- Plate edge effect - a common issue seen in 96-well plate assays is that the outer edges may have higher or lower than normal absorbance readings, try warming your media and cell suspensions before seeding and placing your plates individually (not in a large stack) in the incubator to allow them to get to temperature quickly, also try humidifying the plates in a tupperware container with water soaked tissue if there is excessive evaporation from the outer wells.
- No difference between positive and negative controls - if these values are very close to each other, it is likely that there was a problem with the positive control compound (e.g. an antibiotic or enzyme inhibitor), so you should repeat the assay paying careful attention to the preparation and use of this reagent.