SameSpots Example Analysis Report

Analysis of example data on the effects of drug candidates

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Introduction

This report demonstrates the use of SameSpots for differential expression analysis between four conditions – three drug treatments and one control - using an example data set of 4 DIGE gels with internal standards. The images and data can be quality checked throughout the analysis process and statistically significant changes between specific spots of each condition can be identified and investigated further.

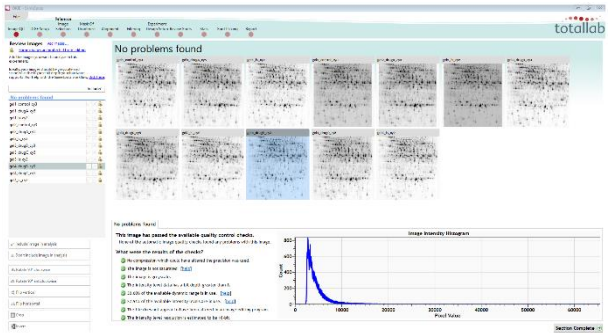
Key Features of SameSpots:

SpotCheck • Objectively validate gel running quality within your lab.
Image QC • Check the quality of your images before you start your analysis.
Alignment and Automatic Spot Detection • Remove positional variation introduced during the gel electrophoresis and imaging process to allow a single spot pattern to be automatically detected resulting in 100% spot matching with no missing values.
Statistical Analysis • Multiple experimental designs can be created and all measurements and statistics are automatically calculated for each design.
Data Reporting • Reports can be created and all images in the analysis can be exported easily using the clip gallery.

Method

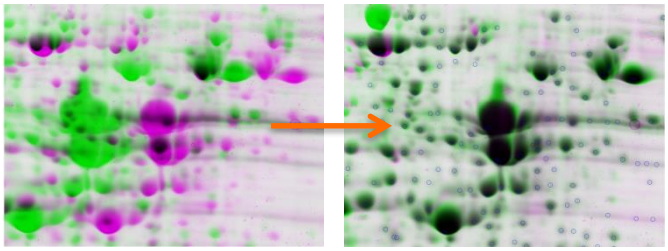
Image QC:

Images were uploaded to SameSpots and automatically quality checked. There were no quality issues with these images.
Images were then grouped by gel and the internal standard identified.



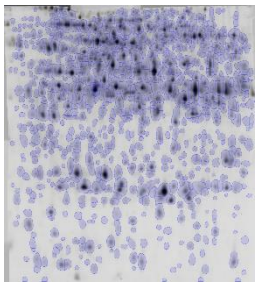
Alignment:

Each internal standard image was aligned to the reference image, each image was then aligned to the representative internal standard ("gel4_is_cy2" was automatically selected as the reference). Alignment removes the positional variation introduced during the gel electrophoresis process, ensuring 100% spot matching across all images.



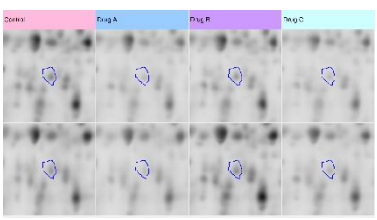
Spot Detection:

Automatic spot detection created a spot pattern representative of all images in the analysis. Spots too small to reliably pick were removed from the pattern by filtering spots with an area ≤ 200.



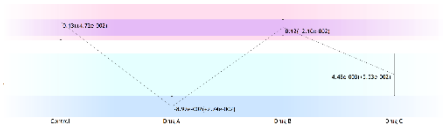
Experimental Setup:

Multiple experiments can be created, a between subject design was used: images were grouped into their conditions. The ANOVA p-values and fold change of each spot were then automatically calculated between the conditions of each experiment.



Results:

Spots with an ANOVA p-value ≤0.05 and a fold change ≥2 were identified using the quick tags option, spots were then filtered to include only those which had both tags. 52 spots with a statistically significant difference between each condition and a fold change greater than 2 were identified.



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QC

Image QC: Images were automatically checked against a range of criteria and no quality issues were identified.

Number and direction of Alignment vectors per image: It is expected that a similar number of automatic alignment vectors would be required for each image. In this analysis an average of 910 vectors ± 7% were added.

PCA: Principle component analysis (PCA) helps to identify any potential outliers within the data by checking that the images group as expected. Figure 1 shows the PCA for the comparison of all drug treatments and the control. Images of each condition are represented by coloured dots, an experiment with little biological variation is expected to show clear groupings of the images. The control (pink), Drug A (blue) and Drug B (purple) can be easily grouped together, Drug C (sky blue) does not have good clustering compared to the other conditions.

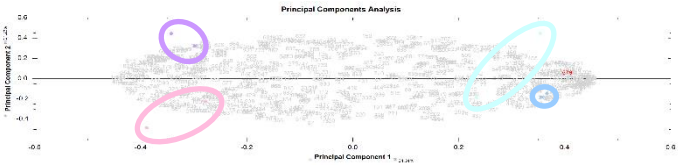

Figure 1. PCA analysis of all spots. Pink spots identify images of the control condition and blue , purple and sky blue represent the gels of Drug A, B and C respectively.

Power Analysis: ANOVA p-values are the chance that a result is a false positive. The power is the probability that a difference would be observed if it was real, it takes into account the number of replicates. Of all conditions only 18% of the data has a power >0.8. 12 replicates would be required to give 80% of the data with a power .0.8.

Other Quality Checks: Other quality checks are available within the software to ensure your results are robust. q-values are calculated and expression profiles of each spot are presented. SpotCheck is an additional feature of SameSpots which allows you to check the quality of the gel running within the lab.

Results

Control vs Drug A conditions were chosen to investigate further.

Top 10 Spots: The top 10 spots with the lowest ANOVA p-values and a fold change ≥ 2 were identified and are listed in Table 1. The expression profiles of the spots are shown in Figure 2, all spots were upregulated in the treated with drug A condition.

Table 1. Top 10 statistically significant spots with a fold change greater than 2 between the conditions.

#	Anova (p)	Fold change	Average Normalised Volumes		Images	
			Control	Drug A	Control	Drug A
735	8.587e-005	4.0	0.554	2.233		
715	0.001	16.8	0.153	2.579		
718	0.001	4.2	0.509	2.111		
423	0.002	8.0	0.339	2.706		
553	0.002	2.5	0.374	0.946		
725	0.002	2.9	0.651	1.859		
719	0.003	7.0	0.366	2.572		
733	0.003	3.5	0.528	1.829		
736	0.003	3.7	0.554	2.029		
716	0.003	3.0	0.544	1.638		

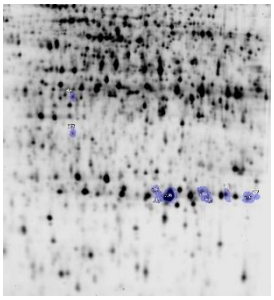

Figure 3. Picking image showing the location of top 10 spots and labelled with spot #.

Conclusions:

SameSpots is a simple software tool for analysis of differential expression. Images and results can be quality checked throughout the analysis and statistically robust results can be obtained. Alignment and automatic spot detection allow reproducible results with no missing values.