

Introduction

This report demonstrates the use of SameSpots for differential expression analysis between treated and controlled conditions using an example data set of 12 2D SDS-PAGE images. 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. 10 spots of interest were identified in this analysis and some of the QC features of SameSpots have been highlighted.

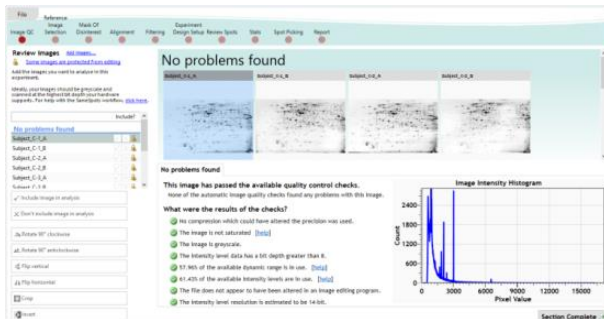
Key Features of SameSpots:

- 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

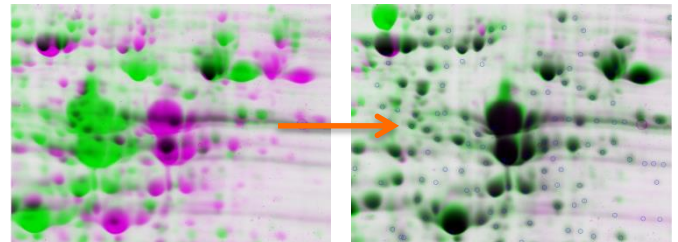
Image QC:

Images were uploaded to SameSpots and automatically quality checked. There were no quality issues with these images.



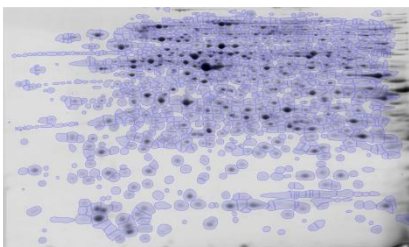
Alignment:

All images were automatically aligned to the reference image ("Subject_C-3_A" was automatically selected as the reference). Alignment removes the positional variation introduced during the gel electrophoresis process, ensuring 100% spot matching across all images.



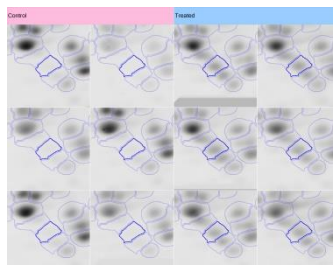
Spot Detection:

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 ≤ 90 .



Experimental Setup:

A between subject design was used, images were grouped into "Control" or "Treated" conditions. The ANOVA p-values and fold change of each spot were then automatically calculated between the conditions.



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. Spots were sorted by fold change to identify the top 10 spots with the lowest ANOVA p-value of the subset.



QC

Results

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

Number of Alignment vectors per image:

A similar number of alignment vectors are expected to be required for each image. An average of 750 +/- 6% vectors were used to align each image to the reference.

PCA: Principle component analysis (PCA) helps to identify any potential outliers within the data and to check that the images group as expected. Figure 1 shows the PCA analysis for this analysis, two clear groups of control and treated can be identified. Each image is represented by a coloured spot to identify the condition. An experiment with a little biological variation is expected to show clear groupings of the images.

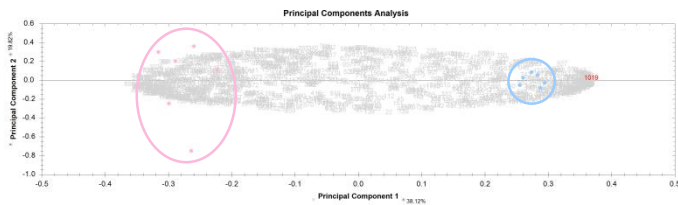


Figure 1. PCA analysis of all spots. Pink spots identify gels of the control condition and blue spots identify gels of the treated condition. There are two clear groupings suggesting there are no outliers.





















Other Quality Checks: Other quality checks are available within the software to ensure your results are robust. Statistical power analysis and 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.



Figure 2. Expression profiles of the top 10 spots. All 10 spots were upregulated in the treated condition.

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 condition. Spots of interest can be identified on an additional picking gel, see figure 3, this can be used to manually pick spots or files can be exported for use with the most common picking robots.

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	Treated	Control	Treated
1019	5.217e-012	6.2	1.456e+005	9.017e+005		
958	1.581e-010	4.2	1.470e+005	6.224e+005		
796	7.718e-010	4.2	4.863e+004	2.043e+005		
709	1.208e-009	3.3	1.398e+005	4.561e+005		
726	1.650e-009	2.6	5.150e+005	1.320e+006		
590	2.370e-009	2.5	2.036e+005	5.004e+005		
265	3.407e-009	3.8	6.169e+005	2.323e+006		
1025	3.830e-009	2.8	2.639e+005	7.445e+005		
983	8.542e-009	3.0	6.330e+005	1.913e+006		
881	1.020e-008	4.8	1.514e+005	7.267e+005		

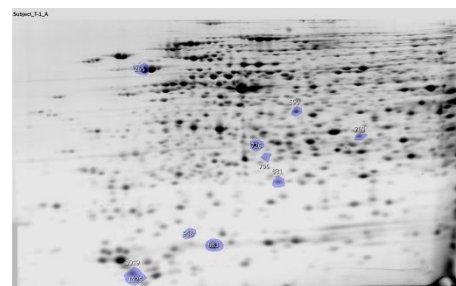


Figure 3. Picking image showing the location of top 10 spots and labelled with spot #.

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.