

Multiplexing gel samples

Introduction

Multiplexing dyes is becoming increasingly common within the life science laboratory to use more than one fluorescent label within the same gel. Multiplexing is especially common for laboratories using Alexa Fluor and Qdot dyes (Invitrogen, UK). Often up to three different stains will be used to identify different samples on the same gel. In order to image these stains, additional specific emission filters must be used.

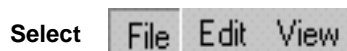
GeneSnap software allows a colour image to be generated by acquiring 3 images using red, green and blue emission filters. Once saved, these images can be combined producing either an RGB true image or a monochrome image.

Image Capture

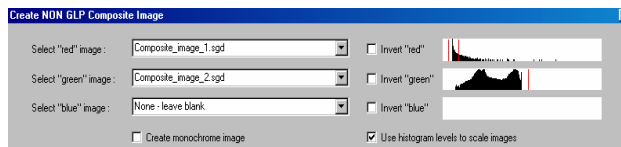
1. Capture images of the gel (make sure the same position is maintained) with the Syngene image capture system, simply use the software to select the lighting (excitation source) and emission filter appropriate for each stain used (See Application Note 10).
2. Save all images captured by choosing the 'Save Image As' command from the File menu, and save each image with an appropriate file name.
3. Open all of the images you wish to overlay (up to three may be selected) so they are visible in the GeneSnap image window.

Generating a composite image

1. To overlay the images, perform the following functions:
2. On the main GeneSnap software Header Bar, click on the FILE menu. Select "Create New Composite Image".



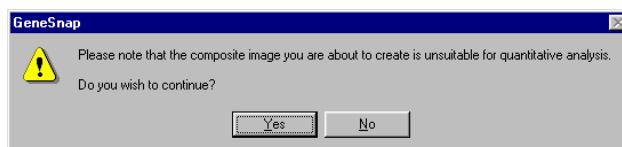
3. A box will appear asking you to select images for each of the 3 channels. If you are only multiplexing two images, simply leave one of the windows on 'None - leave blank'.



4. Use the drop down menu to choose one of the open images for each colour. You can choose to "invert" any image by ticking the box along side it.
5. To adjust the individual contributions made by each image to the composite image choose "Use histogram levels to scale images". If this option is not selected then GeneSnap creates a composite image using equal contributions from each component image.
6. If you are looking at Gel images and wish to proceed to analyse your composite image you should also choose "create monochrome image".
7. Click OK at the bottom of the box and the new composite image will appear in the GeneSnap Image Window.

This new composite image can now be treated as any other and enhanced, annotated or sent to GeneTools and analysed.

N.B. When combining the images, a warning will appear to alert the user that the multiplexed image is not suitable for quantitative analysis.



The combined image should not be used for quantitative analysis as it is only based on pixel intensity data. However, it is acceptable to use the composite image for determining molecular weights (MW) or Rf data.

Syngene reserves the right to amend or change specifications without prior notice. This Application Note supersedes all earlier versions.

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May 2010

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