

Colorimetric markers – how do I see these on my chemiluminescent blots to determine molecular weights?

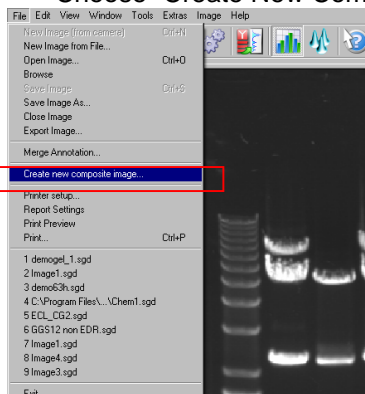
A common life science application using western blot membranes involves labelling standard lanes with a colorimetric marker, and unknown sample protein bands with a chemiluminescent substrate such as the GE Healthcare (Amersham) ECL⁺ Kit.

The role of the colorimetric marker is to check the efficacy of protein transfer from the gel to the membrane and to measure the position of sample bands. There is a noticeable lack of good, stable commercially available chemiluminescent markers. Consequently the user requires the ability to image the colorimetric markers and combine this image with the chemiluminescent component of the blot. In the past this has been difficult to achieve and often involved the simple labelling of the membrane with fluorescent pens.

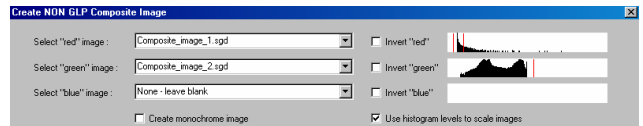
The latest GeneSnap software allows separate images of the colorimetric markers and the chemiluminescence to be captured, background and display corrected and combined to allow for molecular weight /Rf measurements to be calculated.

How?

1. Capture an image of the colorimetric marker using epi-reflective white lights.
2. Choose 'Save Image As' from the File menu and save this image with an appropriate file name.
3. Leaving the membrane in situ, open the iris fully, select 'No filter' and "No Lights" from the drop down menus. Expose the blot for as long as necessary until the desired image is achieved.
4. Choose 'Save Image As' from the File menu and save the image with an appropriate file name.
5. Leave "Open" both the images you wish to overlay so they are visible in the GeneSnap Image Window.
6. To overlay the two images, perform the following functions:
 - On the main GeneSnap software Header Bar, click on FILE.
 - Choose "Create New Composite Image".

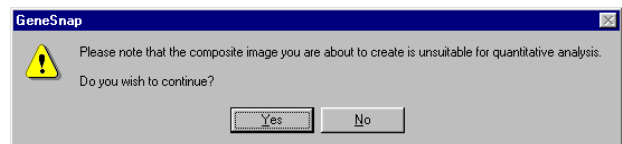


- Select images by using the drop down arrows. In the first channel choose the image with the markers on and due to it being 'dark' bands on a white background choose "invert" by ticking the box opposite. Select the chemiluminescent image for the second box, leave the third as "none".



- Select "Create monochrome image" by ticking the box at the bottom.
- Select "Use histogram levels to scale images" if you wish to adjust the individual contributions made by each image to the composite image. If you do not select this option then GeneSnap creates a composite image using equal contributions from each component image.
- Click OK.

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



The resulting combined image can now be used for accurate molecular weight /Rf calculations using GeneTools software. It does not affect band position or migration, however, these combined images should not be used for quantitative analysis since these are based on pixel intensity data.

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