

Imaging chemiluminescent blots: points to consider

Introduction

With the availability of CCD camera systems suitable for chemiluminescence detection, such as G:BOX iChemi XR and XT and GeneGnome systems (Syngene.UK) and the development of chemiluminescence kits scientists are now preferring to image their blots in this way instead of using film.

Some important points to consider when imaging chemiluminescent blots are the sensitivity, dynamic range and binning of the CCD camera and also which chemiluminescent substrate to use.

CCD camera considerations

The dynamic range of a CCD camera is an important performance parameter since it allows data on large and small sample signals to be acquired in a single image.

CCD camera sensitivity is also an important factor to consider. The sensitivity of the camera is dependent on two characteristics, Quantum Efficiency (QE) and 'noise' level. The noise level will increase with exposure time.

Binning can also affect the sensitivity of the camera. Binning involves the combining of the information of adjacent detectors in a CCD camera sensor to create one single pixel in the recorded image. The bigger the pixels, the more sensitive they are to low light levels. To allow maximum flexibility when imaging different sample types, a binning mode has been incorporated into GeneSnap. Within GeneSnap software 2x2, 4x4 and 8x8 binning of pixels are available. The higher the binning will lead to a significant decrease in exposure time required to visualize a sample and a decrease in resolution. When only looking for the presence or the absence of bands, the loss of resolution may not be an issue, but when looking for small differences between bands, it is best to use the highest resolution possible.

Chemiluminescent substrate considerations

Chemiluminescent substrates offer multiple exposures to obtain the best image. There are a range of chemiluminescent substrates currently available including ECL (GE Healthcare, UK), Supersignal chemiluminescence (Piercenet, UK), western breeze chemiluminescence (Invitrogen, UK) and many more.

Chemiluminescent substrates generate light that is then detected as a transient product of the reaction which is only present while the enzyme-substrate reaction is

occurring usually over a period of 10-20 minutes, light output will then start to diminish significantly.

With blots that require longer exposure times, results from ECL may be less satisfactory and therefore an enhanced ECL substrate may be required such as (SuperSignal, Piercenet,UK).

Image capture using GeneSnap software

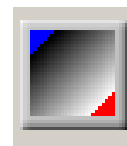
GeneSnap software offers two different methods for capturing images of chemiluminescent blot, the classic mode and the Intelli-chemi mode.

Classic mode

The classic mode allows the user to set their own preferred configurations. The classic mode offers the series capture function where either a single frame or a series of images that may or may not be additive are captured. It is good practice to collect, fewer longer exposures rather than lots of short ones. This reduces the overall background of an image and increases the single to noise ratio.

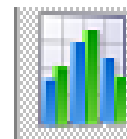
The classic mode is particularly useful for users who have specific imaging conditions that they wish to use e.g. Binning settings, Iris and focus settings.

Using the classic mode the user can watch the images being collected. Once each image is downloaded it can be examined whilst the next image is being collected. If an image is to be used for quantitative analysis, it is important that the greyscale values within it are not saturated. To check this, click the saturation detection button on the right hand side toolbar. Any saturated spots will appear red. Areas shown in blue are areas saturated in black. These areas do not affect the quality of the image as they indicate a clean background.



Saturation Icon

Images captured using the classic mode can be optimised by adjusting the histogram. To view the histogram press the histogram icon.



Histogram Icon

The histogram will be positioned at the base of the capture box. To obtain the best view of your image, adjust the red lines to alter the contrast (**Figure 1**).



Figure 1- Histogram view box

With some chemiluminescent images to clearly see the bands it is often advisable to invert the image so it appears like an autoradiograph. To invert the image press the invert image icon.



Reversal Icon

Intelli-chemi mode

The intelli-chemi mode is a new feature for GeneSnap software. This mode is automated method which will collect the best quantifiable image available as the bands will never saturate.

The intelli-chemi mode is particularly useful for users who are starting a new set of experiments and you are unsure of the optimal imaging conditions and for users who are new to imaging systems as the intelli-chemi mode removes any doubt about whether the correct imaging conditions have been chosen.

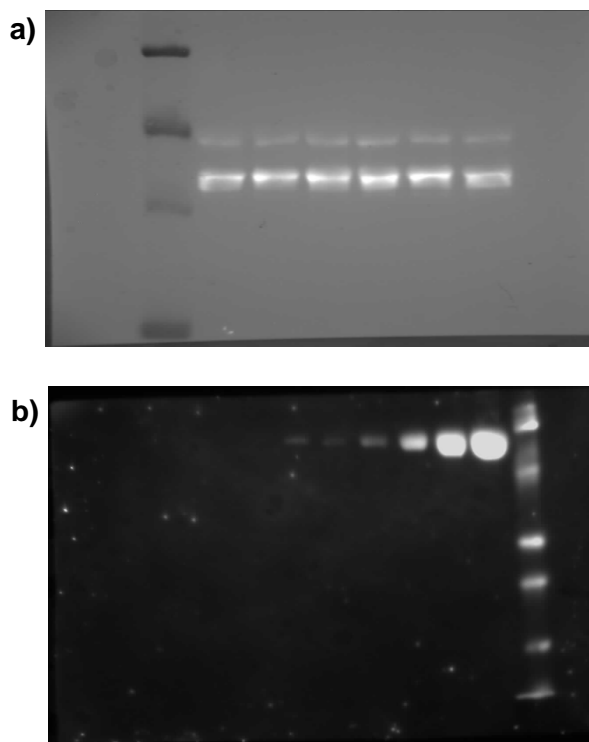


Figure 2-Chemiluminescent blot images captured using the classic and intelli-chemi mode

a) A composite image of a chemiluminescent blot with markers captured using the classic mode. **b)** Image of a chemiluminescent blot captured using the Intelli chemi mode.

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

All trademarks acknowledged.

May 2010

UK tel: +44 (0)1223 727123
 Email: sales@syngene.com

USA tel: 800 686 4407/301 662 2863
 Email: ussales@syngene.com