GeneGnome Imager training guide

PAB 2/10/2015

The GeneGnome Imager measures chemiluminescence generated from a Western blot.

Helpful suggestions:

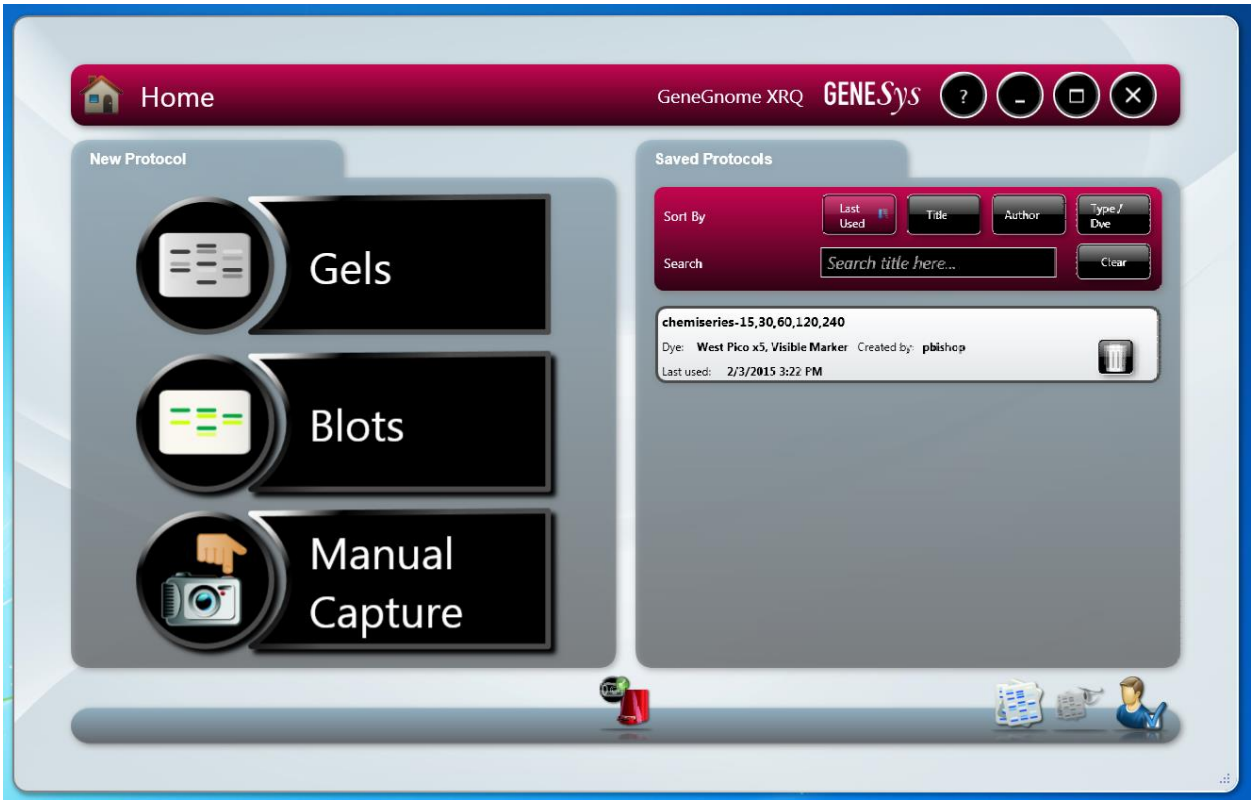
1. If saturated signals are a problem, try incubating with less secondary antibody (decrease by 100 fold for a 1 second film exposure).
2. Soy milk can give a lower background than cow milk.
3. Low background is more important than high chemi signal.
4. The background should be no more than twice the counts of the non-background (off the gel).
5. Binning is a way to decrease the time it takes to collect data. However, increasing the binning will lower resolution.
6. Increase exposure times and decrease binning to enhance quality of images.
7. The blot needs to be no larger than 8 x 11 cm and can be placed directly on the tray.
8. The camera is a CCD (charge coupled device) and needs to be cooled to -60 C. This will occur automatically when the software is opened and takes about two minutes. The camera icon is in the bottom center of the screen and will turn from pink to green when ready.
9. The white light is used to take an image of the standards.

Collecting data:

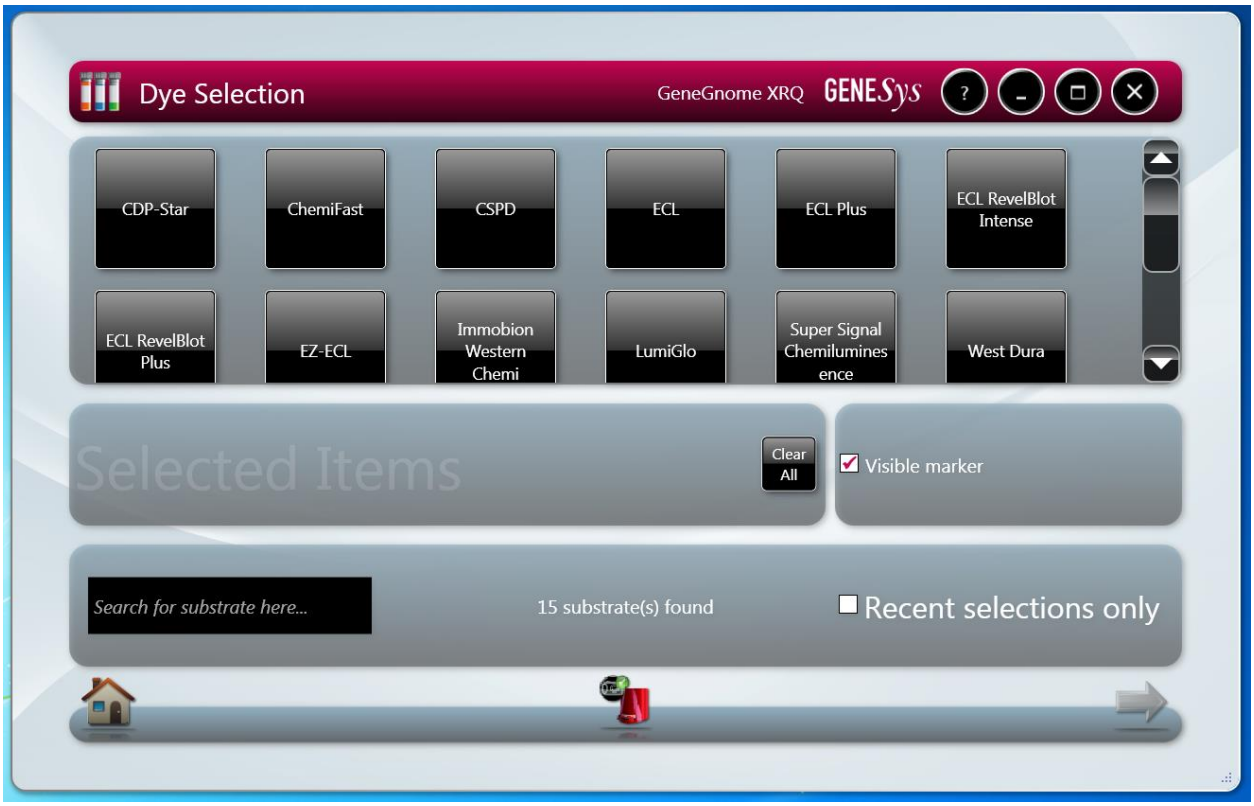
The Gels icon is inactive.

Using the Blots icon will give data with more of the parameters automatically set.

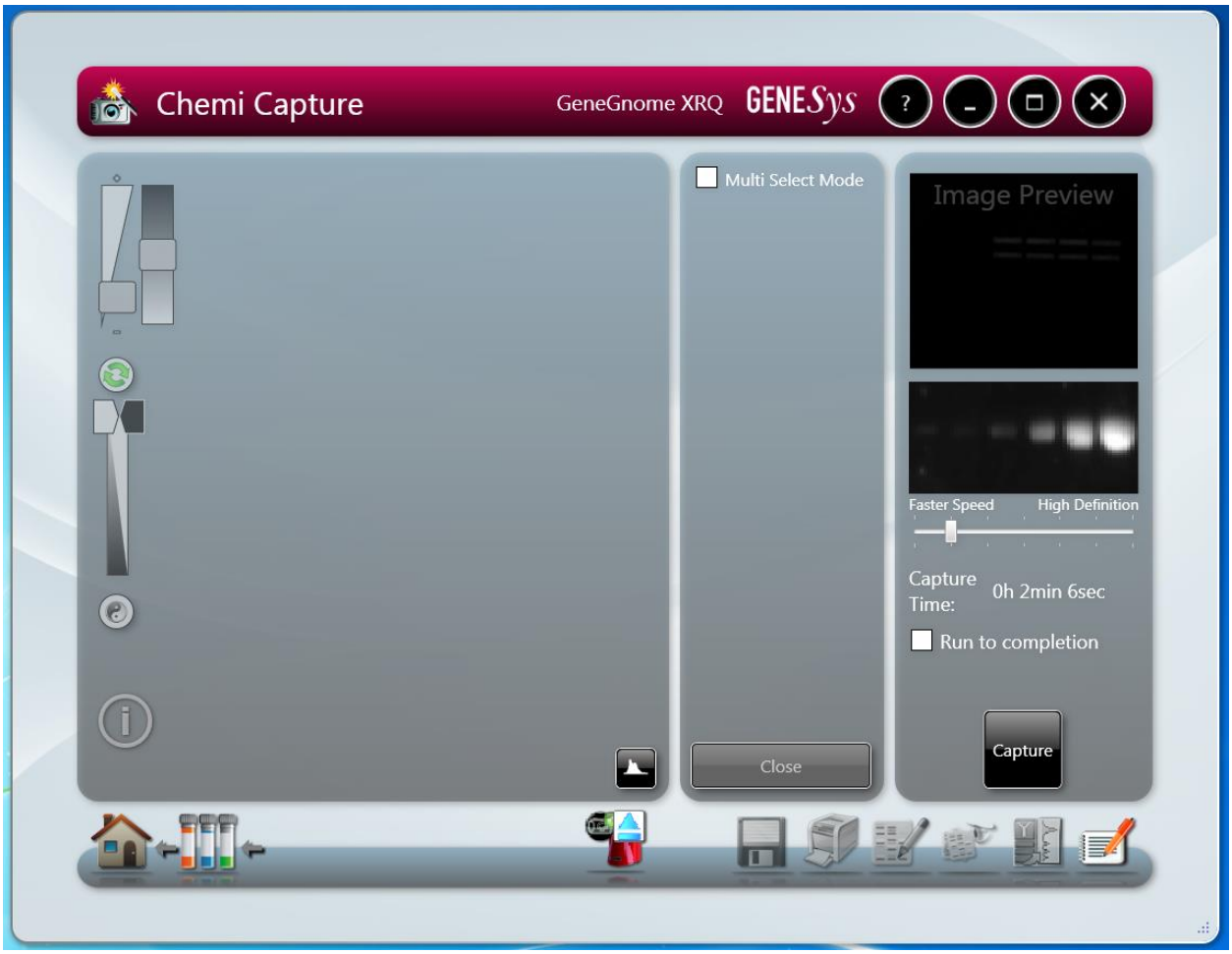
Using the Manual icon requires the user to set all experimental parameters.



Blots:
When the Blots icon is clicked, a menu will appear with two choices: a single exposure or a series of exposures.
A. If "Single" exposure is selected, the following screen appears:



1. The top panel will ask you to select the dye. The software will save the dye you selected previously – if it is a different dye, uncheck the "recent selections only" box in the lower right to display all dye choices.
2. Check the "Visible marker" box if you have visible standards. A white light image of the blot will be taken.
3. Place the blot on the tray, insert the tray in the machine and then click the green arrow in the lower right.
4. Refer to the figure on the next page. The instrument will take a quick scan and calculate the time for optimal exposure. The will be displayed on the slidey bar on the right panel directly under the two black boxes. You have the option of increasing or decreasing the exposure time by moving the slidey bar.
5. Never check the "Run to completion" box. This will make the camera collect data until all chemiluminescence is gone. This could take hours.
6. Click on "Capture".
7. The instrument will take two pictures. One will be the chemi picture and the second will be the while light (only if you checked that box on the previous menu).
8. On the top left side is the zoom button.
9. Under the zoom is the brightness and contrast buttons. Slide the button up and down. If you'd like to return to the computer optimized value, click the green circular arrows button.

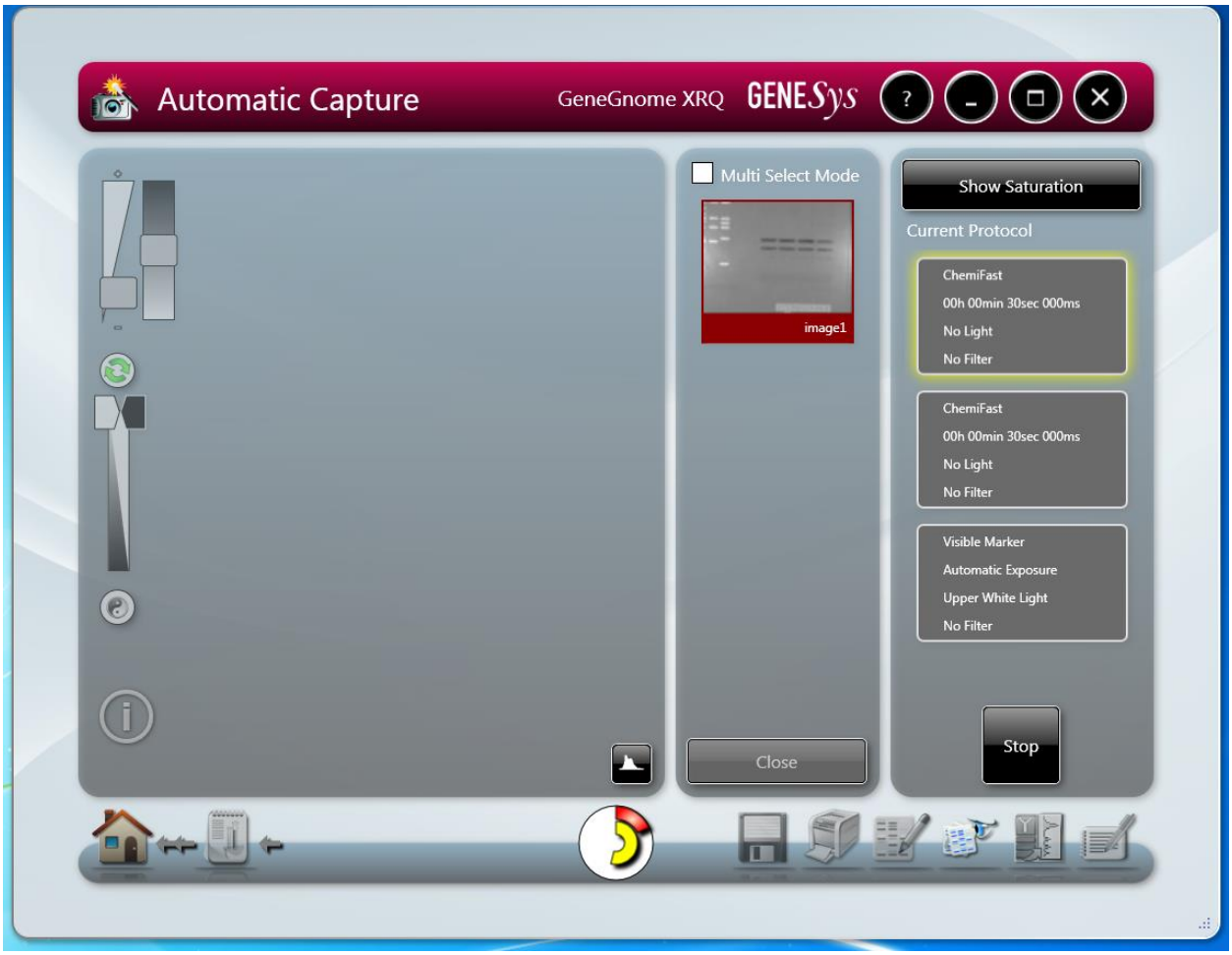


10. For information regarding the image, click on the "i" button in the lower right. Hover over various bands and this will provide the relative counts for each.
11. The histogram button in the lower right of that panel will give information regarding overexposure. Inspect the right side of the histogram.
12. On the very bottom of the window are the navigation buttons: if you'd like to return to previous screens, click on the appropriate icon to the left. If you want to save the data or open the data in GeneTools, these icons are on the right.
13. If a second exposure is desired, change the exposure with the slidey bar and click on "Capture".
14. To remove the blot, click on the blue arrow button directly in the center of the lower panel.

B. If “Series” is selected from the Blots menu, the following screen appears:

1. Select the correct reagent. Click on the box and a list will appear.
2. Check the Visible Marker box if you have visible standards. A white light image of the blot will be taken.
3. Select the number of images you would like to obtain.
4. Checking the “Add contents of previous image” box will add the exposure times. That is, if exposure time 1 is set for 30 seconds and exposure time 2 is set for 1 minute – then the total exposure time for the second image will be 1:30. If exposure time 3 is set for 2 minutes – then the total exposure time for the third image will be 3:30.
5. Binning is a way of decreasing the exposure time. A 6x6 binning experiment will average the counts in 36 pixels (a 6x6 square). The total exposure time can then be decreased by 36 times. However, increasing the binning will result in decreased resolution.
6. Insert a blot and click on the green arrow in the lower right.
7. The next screen will allow you to set the exposure times of the images. If you check the “Use same exposure” box, then all the exposure times will be the same.
8. If you’d like to do shorter and longer exposures, then uncheck that box and a menu will appear which will allow you to set different the exposure times for each image. Remember, if

the “Add contents of previous image” box from the previous screen was checked, the exposure times are cumulative.
9. Select Capture. The images will appear in the center panel as they are collected. The white light image will be last. Hovering the mouse over each image will give the exposure time of that image.



10. Click on an image to view it. On the top left side is the zoom button.
11. Under the zoom is the brightness and contrast buttons. Slide the button up and down. If you’d like to return to the computer optimized value, click the green circular arrows button.
12. For information regarding the image, click on the “i” button in the lower right. Hover over various bands and this will provide the relative counts for each.
13. The histogram button in the lower right of that panel will give information regarding overexposure. Inspect the right side of the histogram.
14. In the upper right is the “Show saturation” button. If the blot has been overexposed, the bands will be shaded red where the data is no longer reliable for integration purposes.
15. On the very bottom of the window are the navigation buttons: if you’d like to return to previous screens, click on the appropriate icon to the left. If you want to save the data or open the data in GeneTools, these icons are on the right.
16. To remove the blot, click on the blue arrow button directly in the center of the lower panel.

Manual Capture

1. Click on “Capture Setup” in the top right. This will give a menu regarding the types of images that will be captured.

2. Clicking on any of the icons will take you through a series of screens that will ask you to select all the parameters.
"Single Image" will capture one image. You will select the binning and the exposure time.
"Multiplex" is used if two or more dyes are used. This does not apply to our instrument.
"Series" allows you to set a delay time before the first exposure and in between exposures.
"Additive Series" allows you to capture a series of exposures. For each exposure, the contents of all previous exposures are included. You select the number of images, binning, and exposure times.

3. Insert the blot and select "Capture" to begin imaging.
4. Click on an image to view it. On the top left side is the zoom button.
5. Under the zoom is the brightness and contrast buttons. Slide the button up and down. If you'd like to return to the computer optimized value, click the green circular arrows button.
6. For information regarding the image, click on the "i" button in the lower right. Hover over various bands and this will provide the relative counts for each.
7. The histogram button in the lower right of that panel will give information regarding overexposure. Inspect the right side of the histogram.
8. In the upper right is the "Show saturation" button. If the blot has been overexposed, the bands will be shaded red where the data is no longer reliable for integration purposes.
9. On the very bottom of the window are the navigation buttons: if you'd like to return to previous screens, click on the appropriate icon to the left. If you want to save the data or open the data in GeneTools, these icons are on the right.
10. To remove the blot, click on the blue arrow button directly in the center of the lower panel.

Saving the data:

A Multiplex is a combination of two images – typically the white light image showing the standards and the chemi image. When more than one chemi exposures has been captured, you can select which one that will be combined with the white light image (typically the best image) by clicking the "Use in MultiPlex" button.

When the Save icon is clicked, the software will ask whether you want to save "Together" or "Individually." Selecting "Together" will save the Multiplex as one image in .sgd format. This is the format required for further analysis in GeneTools. If you want a jpeg of the chemi image, select "Individually."