

<b>ACGT MICROARRAY FACILITY</b>		
<b>STANDARD OPERATING PROCEDURE</b>		
<b>TITLE: Spot verification using SpotVerify</b>	<b>PAGE 1 of 5</b>	
<b>SOP#: MA015</b>	<b>REVISION LEVEL:. 2</b>	<b>DATE: 15 August 2007</b>
<b>AUTHOR: N. Olivier</b>	<b>REVIEWER: D. Berger</b>	

**Purpose:**

This SOP describes the procedure for using the SpotVerify software to find and verify spotted elements in a microarray image file.

**Procedure**

Access to the program is not automatic, and a user ID and password must first be generated by the site administrator before the program can be used. There are two ways to request access to the server. Firstly you can follow the “Apply for service access” link on the ACGT Microarray Facility website ([www.microarray.up.ac.za](http://www.microarray.up.ac.za)), enter the required information, fill the “Spotverify@UP” checkbox. A message will be sent to the server administrator and a password will be issued to you. Alternatively you must follow the program link (<http://www.bi.up.ac.za:8080/SpotVerify/login.do>) and open the **Register** link. Complete the information and select the **Register** button. A request will be sent to the site administrator for access. Once you have received your access details you can login and proceed using the program.

**Creating an Experiment**

A successful login will open the Experiments page. If this is your first visit it is compulsory to create an experiments folder, since all your data will be saved under this specific experiment.

- Select the **Add** option.
- Complete the required information and select the **Save** button
- The experiment will not be visible to other users, only to yourself and the administrator. If the experiment must be visible to other users, a message must be sent to the site administrator (e-mail [nicky.olivier@fabi.up.ac.za](mailto:nicky.olivier@fabi.up.ac.za) with the details). The permissions will then be set only for the required users.

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### **Editing an Experiment**

Experiments can be edited to change the name, project leader and project leader contact details.

- Select the **Edit** link for the specific experiment in the experiments window
- Change details as required and select the **Save** button.

### **Deleting an Experiment**

Experiments can be deleted if no longer required, or the incorrect experiment was created.

- Select the **Delete** link for the specific experiment in the experiments window
- Select the OK button when prompted for a response

### **Uploading image and data files**

Select the **Manage** link for the required experiment.

Select the **Add** link below the Image Analysis column in the right half of the window.

The relevant image files need to be uploaded to the server prior to analysis. Enter the required information:

#### Name

Enter a name which is sufficiently descriptive to differentiate between different, possibly related analyses.

#### Description

Enter a more detailed description of the specific analysis.

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#### Red channel JPG

Generated when saving the results from Genepix. The red channel jpg file has a \_W1 suffix if created in Genepix. Select the **Browse** button, select the appropriate file and select the **Open** button.

#### Green channel JPG

Generated when saving the results from Genepix. The green channel jpg file has a \_W2 suffix if created in Genepix. Select the **Browse** button, select the appropriate file and select the **Open** button.

#### Combined JPG

Generated when saving the results from Genepix. The combined jpg file has a \_R1 suffix if created in Genepix. Select the **Browse** button, select the appropriate file and select the **Open** button.

#### GPR file

This is the results file (.gpr) generated when saving the results following Genepix analysis. Select the **Browse** button, select the appropriate file and select the **Open** button.

If the required files are selected and the information is correct select the **Upload** button. Following a successful upload the program will return to the Image Analysis window.

### **Performing Image Analysis**

Select the **Analyse** link for the appropriate Image Analysis experiment. A window displaying three images will open. To highlight specific features enter the Name or ID of the required feature in the Highlight spots area. While typing a drop-down list of all the relevant names and IDs will be displayed from which the desired feature name/ID can be selected. Alternatively the Name/ID can be typed in full and the **Highlight** button selected.

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The required feature will be bracketed and can be viewed simultaneously in the combined, red and green images.

If the selected feature is spotted multiple times on the slide all the possible positions will be listed in the window directly below the **Highlight** button. By selecting each of these positions the properties of these features can also be viewed. The position of all the features is listed in brackets, giving the block number, row number and column number respectively.

By moving the cursor over the combined image, holding the mouse button down and dragging the image the displayed section of the image can be changed to a different area of the slide. The feature of interest will still be bracketed even if it is moved out of view.

Above the combined image are several image manipulation options:

Reset

To zoom out to the fullest extent select the **Reset** link.

Fit To Width

To zoom to the width of the image select the **Fit To Width** link.

Zoom In

To zoom in on the image select the **Zoom In** link.

Zoom In X2

To zoom in on the image twice as much as the normal Zoom In select the **Zoom In X2** link.

Zoom Out

To zoom out of the image select the **Zoom Out** link.

Zoom Out X2

To zoom out of the image twice as much as the normal Zoom Out select the **Zoom Out X2** link.

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Once you have completed the image analysis, select the **Back** link to go back to the Image Analysis window. In case the analysis window was open for a very long time without activity the program will automatically log the user out. When you have inserted the relevant login information the Image Analysis window will automatically open.

### **Delete Image Analysis**

If the Image Analysis is completed, the specific analysis can be deleted by selecting the **Delete** link, and by selecting the **Ok** button when prompted by the program. This is advisable to prevent congestion and excess data on the server.

### **Log out**

To leave the program select the Logout link at the top right of any window in the program.