# PUMA for homemade arrays

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Homemade arrays get split and rotated while scanning.
Using SFTP, upload the red and green tiff files for each array into your incoming directory on loader. SFTP them onto the PC.

## Gridding

Open Genepix 5.1 in Analysis Only mode. Emulate scanner 4000B.
Click the Options button (on right, image of little checkboxes).
In "File Open" tab, under scaling, make sure pixel size is set to 5 µm.
Click the little disk icon on the right and "load array list."
Select the .gal file that goes with this print (can download from PUMA if needed).
Under the disk icon, select "open images" and select the 2 images for an array.
Tell it which one is red (635 nm) and green (532 nm).
The exclamation mark button (on left) autoscales the image. The gain and brightness sliders can also be adjusted.
Go to block mode (B).
Select all blocks by dragging or with ctrl-A.
Position them roughly in the center of the array. Use the magnifying glass to zoom in if need be.
Under the spot target icon (on left), select find array, find all blocks, find all features.
Use the greater than/less than keys to cycle through all the blocks and make sure they're aligned.
If a block isn't aligned, select the block and reposition it. Hit "find features in selected block" under the spot target icon (shortcut F5).
Once all the blocks are aligned, switch to Feature mode (F).
Select all the features in a block and hit L to cLear the not found flags (the ones with the bar through the middle).
Move and/or resize any misfound spots. To resize, hit crtl and the arrow keys. If the program has mis-sized a lot of spots, you can change the settings under the Options button.
To flag as bad, hit A (for Awful, I suppose). Flag obviously bad spots like dust flecks, scratches, bubbles, etc. If a spot just doesn't look right, go ahead and flag it. The most important thing is to decide on your standards and stick to them.
Under the disk icon, select "save settings" to save a .gps file with the grid settings.
Once you're done flagging, hit the Analyze button (on right, looks like a table of numbers).

A big table of numbers will open. Save this under the disk icon with "save results" to get a .gpr file.

FTP the .gps and .gpr files to loader.

**Database**

Log in to the database. Under Enter Data, click Experiments and Results.
Click Enter a New Experiment into the Database.
In the database, select Spotted as array type, Genepix as analysis software, and Saccharomyces cerevisiae from the 3 pulldown menus.
Click Enter a New Experiment.
Fill out the experiment description form. Choose the print from the menu. Make sure all the files match. Pick a logical category and subcategory. If the reference was in the red channel, click the dye swap box. Select your collaborators from the list to give them access. Put in as much information as possible. You will probably not go back and add in extra documentation later. Do it now.
Submit the form to the database.
You will get an email confirming that the files have been submitted to the database, then another one when they have been actually placed in the database. Go to the URL in the second email and look at the submission data. Make sure everything looks OK.

To look at the data, go to the search section, select any subcategory or user filters, and hit data retrieval and analysis.
Select the experiments you want to analyze from the list.
Choose Data Retrieval and Analysis.

I typically retrieve data by SUID, which averages all the spots that are for the same gene, as long as they pass your filters. On occasion, you may want to retrieve by spot to make sure your replicates are actually correlating.
Select any biological annotations you want.
Choose what label you want on each experiment.
Proceed to Data Filtering.
I usually retrieve the Log (base2)(REDsignal/GREENsignal).
Invert any dye swaps.
Choose your filtering criteria. I usually use 2.5X>background in either channel (i.e, 2 OR 3).
If you would like to actually inspect the spots, click the box next to retrieve spot coordinates.

Download the resulting .pcl file, or proceed to filtering/clustering as desired.  Or add it to your repository.