

Recombinant DNA Techniques

We will be looking at our microarray data so that we can see how we did in the procedure. We will then use data from another lab for our analyses.

Installation of TIGR Spotfinder

1. Insert the Genomics CD into a drive.
2. If the computer does not open a window to show the drive contents (or ask what it should do):
 - a. Select “My computer”. It may be on the “Start” menu at the bottom of the screen.
 - b. Select the CD drive.
3. Double click on the “Spotfinder311win” zip file.
4. Follow the directions. If you are using WinZip as the unzipper:
 - a. Click on “Next”.
 - b. Say “No” on adding the directory to your favorites.
 - c. Click on “Next”.
 - d. Click on “Unzip now”, then “Close”.
 - e. The files will be in “c:\unzipped\Spotfinder311win”. You can run the program from that directory. However, you can move the files to another directory such as the “c:\Program files” directory by copying the Spotfinder311Win folder and pasting it into the “c:\Program files” directory.

Using TIGR Spotfinder to examine our data

Load the data

1. Double click on the Spotfinder311Win directory (e.g., in the “c:\unzipped” directory).
2. Double click on “Spotfinder3.1.1.exe” (or “Spotfinder3.1.1” if no exe is seen). The program should start.
3. Load microarray data into the program. Select “Image”, then “Open images”. Select the CD drive, then “Recombinant arrays”. You select two files by selecting one, then press the “Ctrl” key and the other file. Note that each set of files has a similar name (DR 352_532 and DR 352_635). All the files we generated start with DR, and then the slide number is listed, and then the last three numbers are the wavelength used to read the microarray.

Adjust the image

4. At the top left of the program are two vertical sliders with yellow and black circles/half circles. Move the sliders up and down to see the effects. These change the brightness and contrast of the images.
5. Check all of the sets of slides. Number 653 has visible grids for color 635, but they are not good enough to do analyses.

Installation of MagicSpot

Note: Installation of MagicSpot isn't necessary, as it runs from the CD. However, if you put it on your computer, you won't need to insert the CD every time you use the program.

1. Insert the Genomics CD into a drive.
2. If the computer does not open a window to show the drive contents (or ask what it should do):
 - a. Select "My computer". It may be on the "Start" menu at the bottom of the screen.
 - b. Select the CD drive.
3. Copy the MagicTool directory.
4. Select the directory where you want the program to reside, for example "c:\program files".
5. Paste the directory.

Using MagicSpot to analyze data

Tutorial

1. In the Genomics CD, double click on the MagicTool directory.
2. Double click on the MAGIC_Users_Guide.pdf file.
3. Go to page 6, Getting Started.
4. To start the MagicSpot program, select MagicTool.bat.
5. Go through the tutorial. The following files are the ones mentioned in the tutorial:
 - a. When you start a new project, enter any name that you can remember.
 - b. The files listed below are located on the CD in the RecomMicroarrayData directory.
 - c. The control R and G files are green1.tif and red1.tif (for the single grid).
 - d. The gene list file is Y01godlist1grid.txt. (for the single grid).
 - e. Go through step 25. This will give you a basic understanding of how to compile the data for the microarray.

On your own

1. Repeat the steps in the tutorial for the 4 grid microarray. Use the same file names as in the tutorial, except that the 1's are changed to 4's (e.g. red4.tif instead of red1.tif).