A. HOW TO INSTALL Fs

You need to have the MATLAB software (www.mathworks.com) installed on your system to run Fs. Please follow the following steps:

1. Download the file Fs.email and save it at: Matlab\work.
2. Start MATLAB and chose work as your current directory.
3. Right-click on the file Fs.email within MATLAB, and choose rename.
4. Rename the file as Fs.dll. This will generate an MEX file that is visible only within MATLAB. This MEX file is now ready to use.

B. THE FUNCTION Fs

To execute the function Fs, write at the MATLAB prompt:

[M] = Fs('S1', 'S2', 'E', sub, format, fig)

The inputs and outputs of Fs are defined below.

C. WHAT YOU NEED TO EXECUTE Fs

1. Your current directory should include tab-delimited text files containing the expression data from dye swapping cDNA microarray experiments. Fs reads the data from the text files. Each text file must contain the expression data of duplicate spots listed consecutively (see cartoon 1 below). This experimental design generates 4 replicate spots per gene/sample
2. Name your files as detailed below in section D.
3. Prepare the text files to fit the rules listed below in section E.
4. To know the number of spots per subarray (see cartoon 2 below).

D. HOW TO NAME YOUR FILES

A dye swapping experiment comparing two samples S1 vs. S2 generates 4 text files per slide (see cartoon 1 below). Please name the files as:

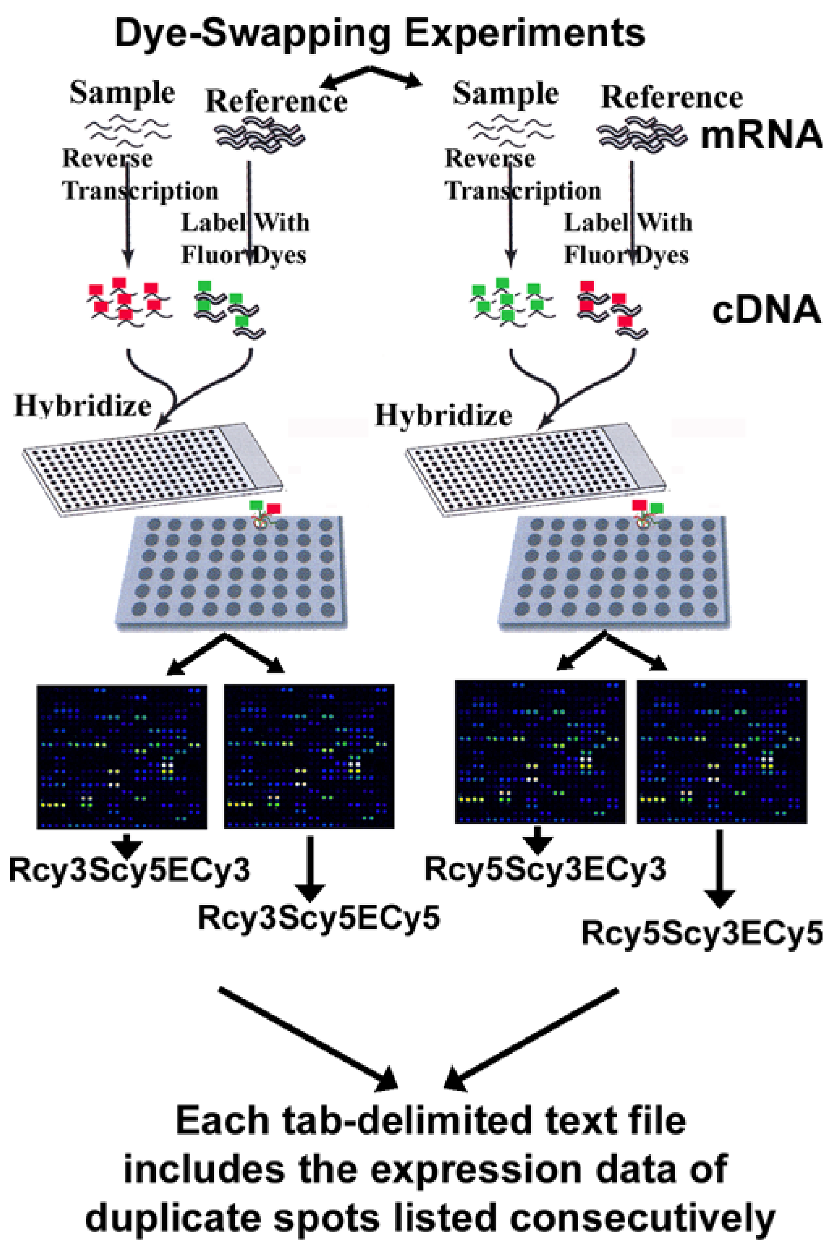
S1cy3S2cy5ECy3
S1cy3S2cy5ECy5
S1cy5S2cy3ECy3
S1cy5S2cy3ECy5

The terms cy3 and cy5, located between S1 and S2 and S2 and E, <u>must be</u> lower case. The C of the Cy3 and Cy5, located at the end, <u>must be</u> capitalized.

E may be any term to help you characterize the experiment. For example, if you are comparing a reference (R) to Tumor 1 (T1) using 19K P1 slides, the four files may be named:

Rcy3T1cy519KP1Cy3
Rcy3T1cy519KP1Cy5
Rcy5T1cy319KP1Cy3
Rcy5T1cy319KP1Cy5

Fs measures log2(background-subtracted intensity in S2/ background-subtracted intensity in S1). In experimental designs, where multiple samples are compared to a single reference, it is advisable to use S1 and S2 to refer to the reference and samples, respectively.



CARTOON 1. A schematic representation of dye swapping in expression profiling by cDNA microarrays. Here, a sample (S) is studied as compared to a reference R. The term E may be used to characterize the experiment. If the cDNA arrays include genes spotted in duplicates, the tab-delimited text files should contain the expression data of duplicate spots listed consecutively.

E. HOW TO PREPARE YOUR FILES

E1. Duplicate spots listed consecutively

If your tab-delimited text file contains the expression data of duplicate spots listed consecutively, then it is ready to use. If your tab-delimited text file contains expression data from single spots, you need to perform 4 separate experiments. For example, if you are comparing S1 and S2, you need to do:

1. Two separate S1cy3 vs. S1cy5, and
2. Two separate S1cy5 vs. S1cy3 comparisons.

Let file1Cy3 and file2Cy3 be the files generated by the “green” laser from the two replicate S1cy3 vs. S2cy5 single-spot experiments. The following codes in MATLAB will generate the file S1cy3S2cy5duplicateCy3, which contains the expression data of duplicate spots listed consecutively:

```
S1cy3S2cy5duplicateCy3(1:2:end) = file1Cy3;
S1cy3S2cy5duplicateCy3(2:2:end) = file2Cy3;
```

This file S1cy3S2cy5duplicateCy3 is ready to use after it is exported to current directory as a tab-delimited text file.

E2. Format

If you text files are generated by the Imagene software (Biodiscovery, Los Angeles, CA), enter format = 1.

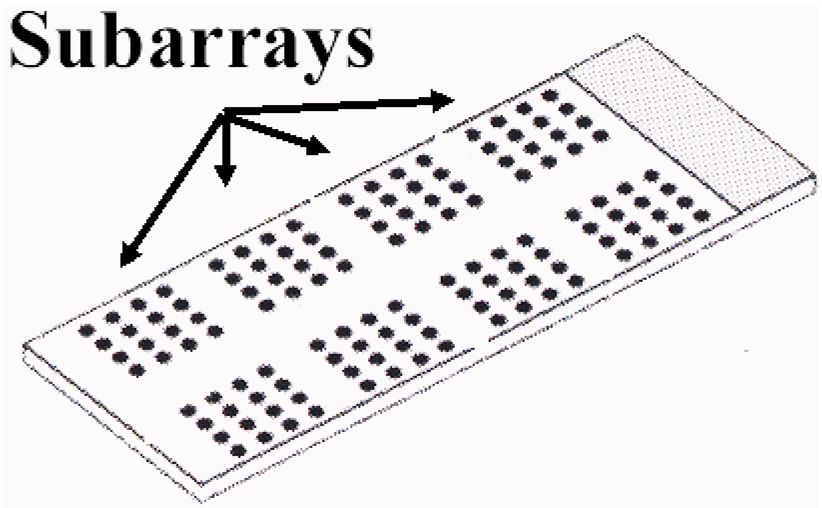
If you are using a different software for signal quantification, enter format = 2 and redesign your text files such that:

1. The columns containing the signal mean and background mean are positioned at columns # 1 and 2 respectively. Delete all columns other than signal mean and background mean. Your tab-delimited text file should include two columns only.
2. The first row of data is preceded only by one row of labeling. Fs will ignore the first row.
3. All rows, which come after the data, should be empty.

F. THE INPUTS OF Fs

1. The terms S1, S2, and E are defined in section D above.
2. The term sub is a number; it refers to the number of spots in each subarray (see Cartoon 2 below). For example, sub = 240 and 600 for the

1.7K and 19K microarrays purchased from the Ontario Cancer Institute, respectively.
3. The term format is a number; it is defined in section E2 above.
4. The term fig is a number. Enter 1 if you want Fs to plot the figures detailed in section G below. Enter 0 if you don't want Fs to generate any figures.



Cartoon 2. A schematic depicting subarrays.

G. THE OUTPUTS of Fs

1. A matrix, M, whose rows correspond to the same order as the input files (see section B above). M includes 9 columns containing the means of the four log2(ratios) of the genes that are resistant to Fs. Columns 1-9 correspond to n = 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6, respectively. A log2 = 0 implies that the gene is <u>not</u> resistant to Fs at a specific n. To compute the total number of genes that are resistant to Fs at n = 2-6, write: sum([M ~= 0]).
2. Figure 1 plots the distribution of log2(ratios) in the third dimensional space whose axes are: 1) ranks in SO1, *x*-axis, 2) ranks in SO2, *y*-axis, and 3) log2(ratios), *z*-axis. Blue and green refer to the spots of dye swapping experiments.
3. Figure 2 plots the distribution of f_4-sensitive log2(ratios) in the third dimensional space whose axes are: 1) ranks in SO1, *x*-axis, 2) ranks in SO2, *y*-axis, and 3) log2(ratios), *z*-axis. Black and red refer to the spots of dye swapping experiments.
4. Figure 3 plots the upper and lower contour surfaces of one of the dye swapping experiments (S1cy3 vs. S2cy5) at n = 3.
5. Figure 4 plots the upper and lower contour surfaces of the other dye swapping experiment (S1cy5 vs. S2cy3) at n = 3