

THE INSTITUTE FOR GENOMIC RESEARCH

Standard Operating Procedure

Proprietary Information

TITLE: **CREATING A MICROARRAY STUDY BINDER**

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SOP #: M015

REVISION LEVEL: 2

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1 PURPOSE

This protocol describes the creation of a microarray study binder containing all study information and hybridization results in a study for the purpose of organization and data quality control.

2 SCOPE

This procedural format is currently used by the Human Colon Cancer, Mouse, and Arabidopsis Microarray projects.

3 MATERIALS

- 3.1 3 ring, 3 inch white study binder (TIGR stockroom, Cat # 6579)
- 3.2 Index dividers, 3 ring, 5 tab set, clear (TIGR stockroom, Cat # 6570)
- 3.3 Standard Excel spreadsheet organizing an entire study (Mouse on Microarray/Project Standards/Excel Template)
- 3.4 An experimental design
- 3.5 All pertinent study information
- 3.6 All pertinent RNA information
- 3.7 A full printout of each hybridization in a study
- 3.8 An RI plot of each hybridization in a study
- 3.9 Printout of the Experiment Report page for each hybridization in a study from Madam Database Program.

4 PROCEDURE

- 4.1 Hybridization Printouts
 - 4.1.1 Once a scan of a hybridization has been completed, use Paint Shop Pro (or some other image program) to capture an image of the full hybridization.
 - 4.1.2 Print the image out on the microarray color printer, P023.

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- 4.1.3 On the printout, clearly label the name of the hybridization, following the standard naming procedure:
3 letter/digit project code-Cy5 probe-Cy3 probe-slide barcode number.

4.2 RI Plots

- 4.2.1 Once a hybridization has been put through Spotfinder, calculate the number of good spots using the Spotfinder TAV output file (in Excel). Sort the data by column P. All spots containing a C in this column are good, all spots containing an A, B, X, or Z in column P are bad.

Note: For mouse 27K arrays, there should be at least 17,000 good spots.

- 4.2.2 Using the Spotfinder TAV file, insert 2 extra columns after column H and before column I. In the first row of the new column I, calculate $\frac{1}{2}$ the log base 2 of the product of Cy3 and Cy 5 $\{1/2\text{Log}_2(G1*H1)\}$. Copy this formula and paste it into the rest of the rows in that column.

- 4.2.3 In the first row of the second inserted column, now column J, calculate the log ratio base 2 of Cy3 and Cy5 $\{\text{Log}_2(H1/G1)\}$. Copy this formula and paste it into the rest of the rows in that column.

- 4.2.4 Using columns I and J, create a scatterplot in Excel. Label and adjust the axes appropriately. For the title, use the standard hybridization naming procedure (see 4.1.3), followed by the number of good spots in that hybridization. Print out a copy of each scatterplot for each hybridization.

Note: Spotfinder now creates an RI plot after analysis. Copy this plot and paste it into Microsoft Word. Spotfinder also provides all spot information on spot quality, so the number of good spots can also be obtained from the program. For the plot title, see 4.2.4. Be sure to save a copy of this plot and print out a copy on the microarray lab color printer.

4.3 Madam Experiment Report Printouts

- 4.3.1 Once all information on a hybridization in a study has been entered into the database using Madam, click on the report tab button in the program.
- 4.3.2 Under the Experiment list, pick the particular hybridization you want, and click on view. A report of all information on that hybridization entered into the database will appear.
- 4.3.3 Click on print, and print out a copy of this report.

4.4 Excel Standard Study Sheet

Locate the Excel standard study sheet under Microarray on NetApp/Project Standards, and complete one for each study. Print out a copy.

4.5 Compiling important study information

- 4.5.1 Printout a copy of the experimental design on the microarray color printer.
- 4.5.2 Printout any other significant study information, such as RNA information, etc.

4.6 Compiling the study binder

- 4.6.1 Obtain a white 3 ring binder and book of index dividers from the shelf in the microarray lab or by ordering from the TIGR stockroom.
- 4.6.2 Use a hole punch to put holes in all printouts suitable for a 3 ring binder.
- 4.6.3 Put experimental design sheet(s), all significant study information and Excel study sheet(s) into the binder first.

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- 4.6.4 Organize the hybridizations in the study according to tissue, treatment, etc.
- 4.6.4 For each hybridization, add the image printout of the hybridization, followed by the RI plot, followed by the experiment report pages from Madam.
- 4.6.5 Label the outside of the binder clearly with the study name and the year.