**INTRODUCTION**

Microarrays are extensively used in molecular biology experiments. Commercial vendors market different kinds of microarrays. While many vendors incorporate only named genes in their microarrays, others include a mixture of named genes and expressed sequence tags. Microarrays with a small number of targeted lists of genes from different gene families or specific biological pathways have been marketed by some other vendors. However, many researchers like to use their own custom list of clones in their microarray based on the area and scope of their study. Many research centers have established core facilities for the production of custom microarrays.

We have developed a number of programs for molecular biology experiments based on Microsoft® Excel® (1,2). Here we describe a simple, user-friendly, and versatile spreadsheet-based software, Microarray Assistant, which can assist the user during different stages of microarray design, synthesis, and analysis. In addition, the program gives options to insert, delete, or interchange clones during various steps of the microarray design. The program can also be used to assist in the transfer of clones between plates of different configuration.

**MATERIALS AND METHODS**

Obtaining and Opening the Program

The program works with Microsoft Excel 2000 and is available free of charge for nonprofit use under a Material Transfer Agreement (MTA) with the Johns Hopkins University School of Medicine. It is also available for exclusive licensing for commercial use. Please e-mail the author to obtain a copy of MTA. The program files will be e-mailed after receiving the completed copy of MTA.

The program files, maa.xls and maa.hlp, need no special installation. Simply copy these two files to a new directory in your hard drive. The program can be started by opening the program file maa.xls. A dialogue box appears, asking to either enable or disable macros. Click on the Enable Macros button. When the program opens, a new menu, named Microarray Assistant, is added to the program menu bar. This menu has 11 menu items, namely New Microarray Workbook, Selected List, Subculture Plates, Subculture List, PCR...
Plates, PCR List, Microarray Plates, Microarray List, Make Microarray, Final List, and Microarray Help. These menu items can be executed to perform various functions of the program.

Opening a New Workbook

When the program is opened, it automatically creates a customized workbook with 14 sheets. You can also create new customized workbooks by executing the command New Microarray Workbook from the Microarray Assistant menu. The first 10 sheets of this customized workbook are used for recording, manipulating, and visualizing clones. These sheets are named Master List, Selected List, Subculture Plates, Subculture List, PCR Plates, PCR List, Microarray Plates, Microarray List, Microarray, and Final List. The program automatically creates column headings in the Master List sheet. The sheet named Configuration Data provides a simple form for entering information about plate, pin, and array configuration. The last three sheets, named Subculture Plate Template, PCR Plate Template, and Microarray Plate Template, are meant to provide templates for use. The program automatically enters default data in these template sheets.

Usually, many clones are rearranged and/or replaced in various steps during microarray design, and they may also be transferred from 96-well plates to 384-well plates. The worksheets in the Microarray Assistant program are organized in a sequential order to help through these different stages of microarray design and synthesis.

Master List Sheet

This first worksheet, named Master List, is used to maintain a list of stock clones (Figure 1). This sheet also assists in selecting clones for making custom microarrays. The first cell in the first column of this sheet (A1) displays the label “Select” and the cell below that shows a “0”. The remaining cells in this column are meant for marking the clones that would be selected for subculturing. The next three columns (B, C, and D) are meant for recording information about plate, row, and column numbers of the clones in the stock plates, and column E is for recording clone identification numbers (Clone ID). In addition to these five columns, the user can fill up any number of additional columns with data pertaining to the clones, but they should be added only after the Clone ID column. Also, it is mandatory that Clone IDs should be unique for each clone in the master list. In case there are duplicate clones in the list, please make sure that each one is given a unique Clone ID.

The user can select the clones for a custom microarray by putting a mark in column A in the corresponding row. Either a single character or multiple characters (alphanumerical) can be used to mark the selected clones. The cell A2 initially shows a “0”, indicating that no clones have been selected. This cell is automatically updated by the program each time a clone is marked for selection or unmarked by the user. This cell indicates the total number of clones marked by the user at any given time.

Configuration Data and Plate Template Sheets

Before proceeding to the next step, have a brief look at the Configuration Data sheet and the three template sheets towards the end of the workbook. The Configuration Data sheet displays a user form that has to be filled up by the user with plate, pin, and array configuration data (Figure 2). The total number of subculture plates, PCR plates, and microarray plates are to be manually entered by the user in the appropriate columns. The program automatically enters a set of default row and column values, 8 and 12 respectively for these plates. These values can be left as such or can be modified by the user appropriately. For example, if the user performs PCR in a 384-well plate, then the data regarding PCR plate in the Configuration Data sheet and the corresponding template in the PCR Plate Template sheet should be modified accordingly. The form also provides space for entering data related to pin and array configuration.

The plate template sheets (Subculture Plate Template, PCR Plate Template, and Microarray Plate Template) are meant to provide templates to the program to indicate the order in which the clones should be transferred from one plate to the other. Each of these templates represents four 96-well plates, and they are automatically filled up with a set of numbers from 1 to 384. If the user performs all the steps in 96-well plates, then these templates can be

![Figure 1. Master List sheet of Microarray Assistant](image)

Note the clones selected with an “x” in column A and the total number of selected clones displayed in cell A2.
left unchanged. If the user transfers the clones from a 96-well plate to either 384-well plate or any other plate format, then the corresponding template should be modified to indicate the order of transferred clones in the new plate. When the program performs transfer of clones between different plates, it reads this template and performs the transfer in the same orientation as indicated in the template. Some of the most commonly used templates are included in the help file. These can be copied and pasted into the template sheet, or the user can create new templates.

**Selected List Sheet**

After marking the required number of clones in the Master List sheet, go to the Microarray Assistant menu and execute the menu item Selected List. After a brief delay, the program activates the Selected List sheet. This sheet now displays all the selected clones copied from the Master List sheet. In addition, the program inserts four new columns just before the Clone ID column. Three of these new columns (E, F, and G) are meant for recording the position of clones in the subculture plates (plate, row, and column numbers). The program automatically fills up these columns based on the subculture plate configuration data from the Configuration Data sheet. The user can organize the selected clones in the subculture plates using the values in these columns. After the list of selected clones is finalized, execute the command Subculture Plates from the Microarray Assistant menu. The program reads the Clone IDs and copies the relevant data from the Selected List sheet into the Subculture List sheet.

**Subculture List Sheet**

Before copying data into the Subculture List sheet, the program checks to make sure that there are no duplicate Clone IDs or empty wells in the Subculture Plates sheet. If there are duplicate Clone IDs or empty wells, then the program displays an error message. The program reads each Clone ID in the Subculture Plates sheet and copies the relevant data from the Selected List sheet into the Subculture List sheet. In addition, the program adds a new column named “Subculture Result” just before the Clone ID column. This column is meant for recording the results of the subculture.

**PCR Plates and PCR List Sheets**

If the configuration of the subculture plates and PCR plates are the same (i.e., if both the plates are of 96-well format), then the user can proceed with the default values in the Configuration Data sheet and PCR Plate Template sheet. In other situations (using 384-plate or any other plate format), these values should be modified appropriately. You can use the templates provided in the help file or create a new template for this purpose. Then execute the command PCR Plates from the Microarray Assistant menu.

The program reads the Clone IDs from the Subculture Plates sheet and transfers them to the PCR Plates sheet. The transfer is performed in accordance with the information provided in the Configuration Data sheet and PCR Plate Template sheet. After transferring the Clone IDs, the program marks the plates with different colors and draws an outline around each plate as described earlier. At this stage, the user again has

[Figure 2. Configuration Data sheet showing the user form filled up with plate, pin, and array configuration data.]
the option to add, delete, or interchange the clones in the PCR Plates sheet. However, precautions should be taken to avoid duplicates and empty wells, as described previously. After performing PCR amplification, execute the command PCR List. The program reads the Clone IDs in the PCR Plates sheet and copies all relevant data from the Subculture List sheet into the PCR List sheet. In addition, the program inserts three more columns for PCR plate data and another column for recording the PCR results.

Microarray Plates and Microarray List Sheets

While in most cases the PCR plates with their products are directly processed for arraying, the PCR products are occasionally transferred to new plates. The two sheets Microarray Plates and Microarray List are meant to facilitate recording data relevant to the final plates used for arraying. The corresponding commands Microarray Plates and Microarray List can be executed to perform these functions. The program uses the data provided in the Configuration Data sheet and Microarray Plate Template sheet to perform these functions. All options for altering the Clone IDs in the Microarray Plates sheets and restrictions are similar to the ones described above.

Microarray and Final List Sheets

Before proceeding to the next step, make sure that relevant pin configuration (number of rows and columns in the spotting pin) and array configuration data (number of rows and columns in a satellite, number of subarrays in a row, and number of subarrays in a column) are properly entered in the Configuration Data sheet. Then, execute the menu item Make Microarray from the Microarray Assistant menu. After a brief delay, the Microarray sheet displays the clone IDs of the PCR products in the final array format. The position of each Clone ID in this sheet represents its position in the microarray. In addition, the program marks the satellites with different colors and draws an outline around each subarray. Before executing this command, the program checks to see if the plate, pin, and array configurations match with each other and displays an error message if there is any mismatch.

To get a final list of all the clones in the microarray and data regarding their location, execute the menu item Final List from the Microarray Assistant.

Figure 3. Subculture Plates sheet showing the Clone IDs displayed in a plate configuration. Note the different background shades for adjacent plates and the plate numbers on the right side.
menu. This will create a list of all clones in the microarray in the Final List sheet with complete information about each spot on the microarray, including the location of the respective clones in the master plate, subculture plate, PCR plate, microarray plate, and microarray (Figure 4).

The program comes with its own help file maa.hlp, which can be opened by executing the Microarray Help command from the Microarray Assistant menu or by directly clicking on the help file. The first page of the help file shows a list of topics for which help is available, and individual topics can be visualized by clicking on the topic names.

DISCUSSION

The major advantages of the program are that it employs a well-known interphase of the Excel spreadsheet, and it is very user friendly. Thus, the learning curve required to master the program is very much reduced. The program combines all relevant data required for the microarray design and synthesis in a single workbook. The program helps to prepare different lists and also gives a visual display of all plates, as well as the microarray. The program also gives options to insert, modify, delete, or replace the clones during various steps.

In addition to the commands provided by the Microarray Assistant menu, the user can also make use of all the built-in Excel commands. For example, if the Microarray sheet is too large to view, the user can execute the built-in “zoom” command from the Excel menu to visualize a large area. Additional enhancements for easy visualization of the data in different worksheets can be achieved using different background colors, font colors, or by using special outlines.

The program might be a little slow in executing commands that create Subculture List, PCR List, and Microarray List. This is because the program searches and combines the relevant data while executing these commands. Users are advised to try these commands with a small number of clones (1000 or 2000) to get an idea about the time it takes to complete the execution of these commands. Attempts will be made to improve the speed of these commands in future versions.

Researchers can utilize the Microarray Assistant to integrate their work related to microarray design and synthesis. They can use the program if they find it easy to use when compared with commercial software or if some of the features found in Microarray Assistant are not available in their commercial software. This program also will be very useful to researchers who perform their procedures manually (e.g., transfer of clones from 96-well plates to 384-well plates).

Further improvements of the program will depend on the feedback from the users. While critical suggestions would be incorporated, the emphasis on a simple, user-friendly interphase will be maintained.

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REFERENCES


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