Research Report

Microarray Assistant Clone Organizer and Array Simulator

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ABSTRACT

Microarrays are extensively used in molecular biology experiments. While several vendors offer microarrays on a variety of platforms, many researchers prefer to use custom microarrays with a selected list of clones for their experiments. Many research centers have established core facilities for the production of custom microarrays. Microarray production involves a number of steps, including maintaining a master list of stock clones, selecting required clones for custom microarrays, subculturing selected clones, amplifying inserts, recording results, and identifying the orientation of clones in the microarray. We have created a simple, user-friendly, and versatile Microsoft® Excel® spreadsheet-based software, Microarray Assistant, which can assist the user in all the steps of microarray design and synthesis. In addition, the program gives options to insert, delete, or interchange clones during various steps. The program also gives a visual picture of the locations of the clones in the plates, as well as in the microarray. The program can also be used to assist in the transfer of clones between plates of different configuration.

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.

The major tasks of a microarray facility include maintaining a master list of stock clones, selecting required clones for custom microarrays and subculturing them (inoculating the stock cultures into new culture tubes or plates and regrowing them), performing PCR amplification of the inserts, recording the subculture and PCR results, and figuring out the orientation of clones in the microarray to correlate with hybridization data.

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 differ-

ent 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 (alphanumeric) 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

Select	Plate Number	Row Number	Column Number	Clone ID	Name
0	2304	1	1	1	1 zinc finger protein, X-linked
x		1	1	2	2 RAN binding protein 2
x		1	1	3	3 sigma receptor (SR31747 binding protein 1)
x		1	1	4	4 wingless-type MMTV integration site family, member 5A
x		1	1	5	5 villin 2 (ezrin)
x		1	1	6	6 ring finger protein 4
x		1	1	7	7 Homo sapiens mRNA for Neuroblastoma, complete cds
x		1	1	8	8 v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
x		1	1	9	9 v-akt murine thymoma viral oncogene homolog 2
x		1	1	10	10 uroporphyrinogen III synthase (congenital erythropoietic porphyria)
x		1	1	11	11 v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma)
x		1	1	12	12 tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
x		1	2	1	13 Human protein phosphatase 2A regulatory subunit alpha-isotype (alpha-PR65) mRNA
x		1	2	2	14 placental protein 11 (serine proteinase)
x		1	2	3	15 benzodiazepine receptor (peripheral)
x		1	2	4	16 carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacti

Figure 1. *Master List* sheet of *Microarray Assistant*. Note the clones selected with an “x” in column A and the total number of selected clones displayed in cell A2.

BioComputing/BioInformatics>>>>

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 copies all Clone IDs from the *Selected List* sheet into the *Subculture Plates* sheet.

Subculture Plates Sheet

The *Subculture Plates* sheet now displays the Clone IDs of the selected list of clones in a row and column format reflecting their position in real plates. In addition, adjacent plates are colored differently and marked with an outline around the plates (Figure 3). In addition, plate numbers are displayed on the right side of each plate. This sheet can be printed out and kept on the bench side as a guide while inoculating clones for subculture.

The user can add, delete, or interchange clones in this *Subculture Plates* sheet. However, make sure that the plates maintain their continuity to each other (i.e., there are no empty rows between any of the plates). None of the wells in the plates should be left empty, and also there should be no duplicate IDs. If you want to leave some wells empty, fill up the wells with Clones IDs such as Empty1, Empty2, etc. After culturing the clones, execute the command *Subculture List* from the *Microarray Assistant* menu. After a brief delay, you will see that the Clone IDs from the *Subculture Plates* sheet and their corresponding plate, row, and column information from the *Master List* sheet is copied 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

	B	C	D	E	F	G	H	I	J	K	L	M
4												
5												
6		SUBCULTURE PLATE INFORMATION					Number of plates		8			
7							Number of rows in a plate		12			
8							Number of columns in a plate					
9												
10		PCR PLATE INFORMATION					Number of plates		8			
11							Number of rows in a plate		12			
12							Number of columns in a plate					
13												
14		MICROARRAY PLATE INFORMATION					Number of plates		8			
15							Number of rows in a plate		12			
16							Number of columns in a plate					
17												
18		PIN CONFIGURATION					Number of rows in the pin					
19							Number of columns in the pin					
20												
21							Number of rows in a satellite					
22		ARRAY CONFIGURATION					Number of columns in a satellite					
23							Number of subarrays in a row					
24							Number of subarrays in a column					
25												
26												
27												

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*

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	1	2	3	4	5	6	7	8	9	10	11	12					
2	13	14	15	16	17	18	19	20	21	22	23	24					
3	25	26	27	28	29	30	31	32	33	34	35	36					
4	37	38	39	40	41	42	43	44	45	46	47	48		1			
5	49	50	51	52	53	54	55	56	57	58	59	60					
6	61	62	63	64	65	66	67	68	69	70	71	72					
7	73	74	75	76	77	78	79	80	81	82	83	84					
8	85	86	87	88	89	90	91	92	93	94	95	96					
9	97	98	99	100	101	102	103	104	105	106	107	108					
10	109	110	111	112	113	114	115	116	117	118	119	120					
11	121	122	123	124	125	126	127	128	129	130	131	132					
12	133	134	135	136	137	138	139	140	141	142	143	144		2			
13	145	146	147	148	149	150	151	152	153	154	155	156					
14	157	158	159	160	161	162	163	164	165	166	167	168					
15	169	170	171	172	173	174	175	176	177	178	179	180					
16	181	182	183	184	185	186	187	188	189	190	191	192					
17	193	194	195	196	197	198	199	200	201	202	203	204					
18	205	206	207	208	209	210	211	212	213	214	215	216					
19	217	218	219	220	221	222	223	224	225	226	227	228					
20	229	230	231	232	233	234	235	236	237	238	239	240					
21	241	242	243	244	245	246	247	248	249	250	251	252		3			
22	253	254	255	256	257	258	259	260	261	262	263	264					
23	265	266	267	268	269	270	271	272	273	274	275	276					
24	277	278	279	280	281	282	283	284	285	286	287	288					

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.

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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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	Select Master Plates			Subculture Plates			PCR Plates			Microarray Plates			Sub Array			Satellite			Array			
	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Plate Number	Row Number	Column Number	Clone ID
2	2302																					
3	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	x	1	1	5	1	1	1	1	5	1	1	5	1	1	5	1	1	1	1	1	1	2
5	x	1	1	9	1	2	1	1	9	1	1	9	1	1	9	1	1	1	1	1	1	3
6	x	1	5	1	1	7	1	1	5	1	1	5	1	1	1	1	1	1	1	1	1	4
7	x	1	5	5	1	7	5	1	5	5	1	5	5	1	1	1	1	1	1	1	1	5
8	x	1	5	9	1	8	1	1	5	9	1	5	9	1	1	1	1	1	1	1	1	6
9	x	1	1	2	1	1	2	1	1	2	1	1	2	1	1	1	1	2	1	1	1	2
10	x	1	1	6	1	1	6	1	1	6	1	1	6	1	1	1	1	2	1	1	1	6
11	x	1	1	10	1	2	2	1	1	10	1	1	10	1	1	1	1	2	1	1	1	3
12	x	1	5	2	1	7	2	1	5	2	1	5	2	1	1	1	1	2	1	1	1	4
13	x	1	5	6	1	7	6	1	5	6	1	5	6	1	1	1	1	2	1	1	1	5
14	x	1	5	10	1	8	2	1	5	10	1	5	10	1	1	1	1	2	1	1	1	6
15	x	1	1	3	1	1	3	1	1	3	1	1	3	1	1	1	1	3	1	1	1	3
16	x	1	1	7	1	1	7	1	1	7	1	1	7	1	1	1	1	3	1	1	1	2
17	x	1	1	11	1	2	3	1	1	11	1	1	11	1	1	1	1	3	1	1	1	3
18	x	1	5	3	1	7	3	1	5	3	1	5	3	1	1	1	1	3	1	1	1	4

Figure 4. *Final List* sheet showing data regarding location of each spot in the microarray. In addition, data regarding the location of each clone in the master plate, subculture plate, PCR plate, and microarray plate are also displayed in this sheet.