New Hybridization Apparatus For In Situ Synthesized Microarrays Which Improves Experimental Performance.

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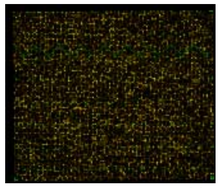
ABSTRACT

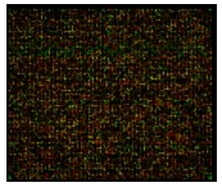
The standard 1" x 3" microscope slide is the most widely used microarray format. However, sample hybridization to the microarray slide has traditionally been a poorly optimized step using home-made hybridization apparatus that lead to lowered microarray performance. We have developed and characterized a new apparatus and method which improves the following properties of our existing system: Quality of hybridization, Consistency of hybridization, and Ease of use.

Hybridization quality has been improved by providing a hybridization chamber made from inert materials that enable easy hybridization buffer mixing. Consistency has been improved via material quality and high-quality manufacturing. Finally, ease-of-use has been improved by protocols that employ standard lab equipment in conjunction with a straightforward hybridization chamber assembly.

The apparatus has been validated for use with Agilent's *in-situ* synthesized oligonucleotide microarrays by comparison to Agilent's current standard hybridization method. It exhibited equivalent or superior experimental performance in these studies.



A1



A2

EXPERIMENTAL SET-UP

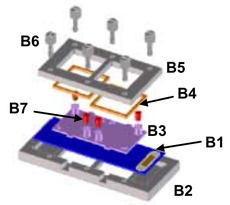
All experiments were performed with Agilent's Yeast Oligo microarray which contain 60-mer yeast probes. The total number of features on the microarray is 10,807 representing 6,256 yeast ORFs.

Labeled targets were prepared using the Agilent Linear Amplification Kit with S. cerevisiae poly A+ RNA. A final concentration of 0.5ug/ml of cRNA for each dye in 200ul of hybridization buffer. A statistically significant number of microarrays for each condition was hybridized according to Agilent's standard procedure.

Homologous comparisons (A1) were performed using starting material from Clontech (Cat. No. 6999-1) . Differential comparisons (A2) were carried out in fluor reversed pairs using cRNA obtained from yeast grown in synthetic complete (SC) medium versus yeast grown in sporulation (Spo) medium.

Arrays were randomly assigned to the different conditions, loaded in random order, washed in random order and scanned in random order, to reduce any systematic biases.

Microarrays were scanned using the Agilent dual laser DNA microarray scanner (Cat. Number G2565AA) and data was extracted using Agilent's Feature Extraction software (Cat. Number G2566AA). The data was analyzed using Rosetta Resolver®, and Microsoft® Access in conjunction with Microsoft Excel.

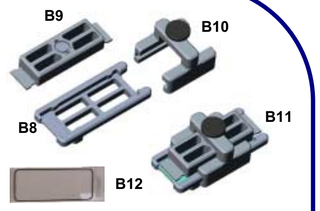


THE LEGACY PRODUCT

Agilent's legacy hybridization chambers (G2530A, G2531A, and G2533A) are comprised of multiple pieces that the user is required to assemble. The microarray slide (B1) is placed on the chamber bottom (B2) with the microarray side up, a polypropylene backing (B3) is placed on the slide, a soft gasket (B4) is placed on the backing. Next, the chamber top (B5) is applied and fixed to the chamber bottom with six screws (B6). To complete the assembly, the rubber septa are applied to the cylindrical septa ports (B7).

The hybridization fluid is added to the chamber via a hypodermic syringe through a septa port and a syringe tip through the other port which serves as the chamber vent.

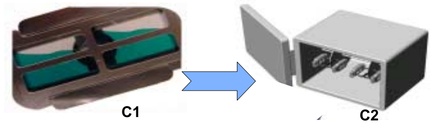
Disassembly is similar with the exception that the chamber is immersed in wash solution.



THE NEW PRODUCT

Agilent's new G2534A hybridization chamber (B11) is comprised of four pieces: three interlocking metal pieces, and a glass backing slide with a gasket adhered to the surface of the glass. The gasketed backing-slide (B12) is placed in the chamber bottom (B8), the hybridization fluid is introduced onto the backing-slide via pipette. Next, the microarray slide is placed microarray-side-down onto the backing slide. To close the chamber, the chamber top (B9) is applied, and the clamp (B10) is applied.

Disassembly is similar to assembly with the benefit that the chamber does not need to be disassembled with the chamber immersed in wash solution: the gasket adheres to the microarray until the gasket-microarray sandwich is placed into the wash solution.



BUBBLE MIXING THE OLIGO MICROARRAYS

Agilent's standard protocol for hybridizing *in-situ* synthesized oligonucleotide microarrays utilizes a mixing bubble to mix the hybridization fluid during the 17-hour incubation process. During incubation, the hybridization chamber rotates in a rotator rack in a dry incubation oven (C2 – Robbins Scientific) and the bubble is allowed to rotate freely inside the chamber which promotes microarray signal uniformity. For illustration purposes, a blue dye has been added to the solution in figure C1 to aid visualization.

IMPROVING EASE-OF-USE

The new hybridization tool represents an improvement in the ease-of-use as measured by an improvement in the loading efficiency: typical times for setting up the legacy hybridization chamber and loading the sample are ~6min. Conversely, typical times setting up and loading the new chamber are ~1.5 min.

The disassembly takes ~1.5 min for the legacy chamber and ~ 15 sec for the the new chamber.

Together the assembly and disassembly of new configuration takes about 25% of the time compared to that of the legacy configuration.

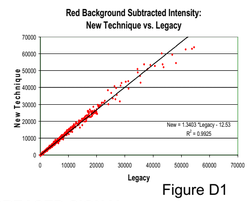


Figure D1

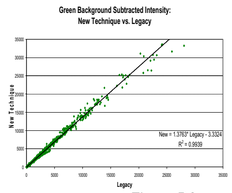


Figure D2

INCREASED SIGNAL

In a 12 microarray self vs. self experiment, a background subtracted signal comparison was perform to identify if the new hybridization apparatus offered any improvement in sensitivity (figures D1 and D2). The following table summarizes the findings. Overall, the signal increased an average of 36%.

Channel	Signal Increase Factor
Cy3	1.38
Cy5	1.34

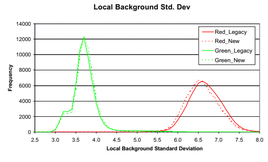


Figure D3

UNCHANGED LOCAL BACKGROUND

The standard deviation of all the non-flagged local backgrounds were plotted in a histogram frequency distribution to detect any shift in the background std dev. that might indicate a change in the background noise (Figure D3). The histogram curves nearly overlap indicating no significant change in the local background.

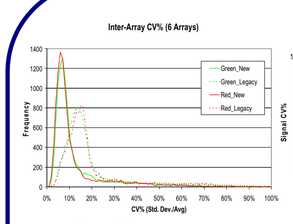


Figure E1

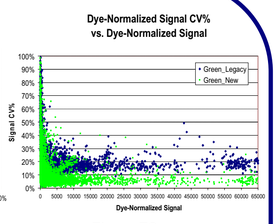


Figure E2

INTER-MICROARRAY REPRODUCIBILITY

In the same 12 microarray self vs. self experiment in which 6 microarrays were hybridized using the legacy hybridization tool, and the other 6 were hybridized using the new tool, the CV%s (SD/Avg.) were compared on a feature-by-feature basis. Each feature on the microarray was compared to the corresponding feature on the 5 other microarrays in the group. Any saturated feature, or any feature that was identified by the Feature Extraction software program as a 'Feature Non-Uniformity Outlier' was removed from the calculation. The CV% of the Dye-Normalized Signal was calculated and plotted as a histogram (frequency of occurrence) plot in figure E1, and versus Dye-Normalized Signal in Figure E2.

The plots show a clear difference between the legacy and the new apparatus indicating a 2 fold reduction in the mode and median CV%s.

	Mode CV%	Median CV%
Legacy	15%	14.5%
New	7%	7.4%

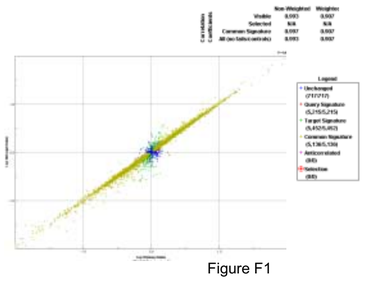


Figure F1

COMPARISON OF LOG RATIOS

The log ratios for the differentially expressed ORFs, using eight microarrays for each hybridization tool, were compared using the Rosetta Resolver system (Figure F1). At the 99% confidence level, 5,452 ORFs for the new hybridization tool vs. 5,215 ORFs for the legacy tool were detected. Of these, 5,136 ORFs were common between the two tools with zero anticorrelated suggesting no change in the log ratio for these ORFs. This results in 316 unique signature ORFs (green) vs. 79 unique ORFs (red) for the new and legacy hybridization tools respectively.

SUMMARY AND CONCLUSION

The new hybridization apparatus represents a dramatic improvement in ease-of-use as measured by the time required to assemble & load, and disassemble and wash. This improvement should translate into the ability for a single user to process more microarrays in the same period of time.

An improvement in hybridization reproducibility is realized through lower microarray-to-microarray variability as demonstrated by the self-self experiment in which the signal CV% (mode method) were reduced by more than half from 14-16% to 6-7%.

An improvement in hybridization sensitivity is achieved by using this new tool. An increase between 34-38% in background subtracted signal is observed in conjunction with no change in the background noise as measured by the local background standard deviations. As a result, the signal-to-noise, the most common indicator of sensitivity reports a 34-38% increase in sensitivity.

Finally, the differential expression study shows that while most of the ORFs in the experiment were equally differentially expressed in both data sets, more unique signature ORFs were detected while using the new hybridization apparatus.

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5989-0354EN November 1, 2003