FAQ

About MYcroarrays

What equipment will accommodate MYcroarray slides? MYcroarrays are synthesized on standard microscope slide-sized glass substrates (26mm x 75mm). Any equipment (fluidics stations, hybridization stations, scanners, centrifuges, etc.) developed to process "spotted" arrays or *in situ* synthesized arrays made on standard microscope slide-sized substrates should accommodate our MYcroarrays.

Are MYcroarrays compatible with Agilent Technologies' gasket slides and hybridization cassettes? Yes, MYcroarrays can be used with Agilent Technologies gasket slides and hybridization cassettes. All MYcroarray formats fit the Agilent hybridization cassettes. The choice of which Agilent gasket slide depends on the MYcroarray format. Six by 5K (or 7K) arrays use Agilent's 8-gasket backing slide. Three by 15K (or 20K) use Agilent's 4-gasket backing slide. Thirty or 40K arrays use Agilent's 2-gasket backing slide. All other formats (60, 80 and 90K) use the one gasket backing slide.

Does MYcroarray sell hybridization cassettes or backing slides? No, we do not sell hybridization cassettes or backing slides. Agilent Technologies' hybridization cassettes and backing slides can be found here. If you would prefer other options, please contact techsupport@mycroarray.com.

Are MYcroarrays compatible with Agilent Technologies' scanners? Yes, our MYcroarrays can be *scanned* with an Agilent scanner. However, the gal files we provide cannot be used for data extraction. Gal files can be converted to the appropriate Agilent scanner software file type but MYcroarray does not support this process. We recommend the Axon 4000 series of scanners.

When I order a MYcroarray, what do I get? In the package with your MYcroarray slides, you will receive a spike-in control oligo mixture. In addition, we will send, by email, a .gal (GenePix Alignment) file that describes the location and probe information for each feature. The control oligos are added to the hybridization solution (1μl control oligo per 100μl of hybridization solution). These fluorescent oligos will bind to control probes on each MYcroarray and will help determine if the hybridization and washing stringency were sufficient.

What is the probe length on MYcroarrays? MYcroarray probes can be of any length the customer desires up to 50 bases. Catalog MYcroarrays have probe lengths of 45-47 bases (the slight variation in probe length insures that all probes fall within a very narrow Tm range).

In which orientation are probes synthesized? We synthesize probes 3' > 5'. This orientation will tether the 3' end of the probe to a spacer molecule and the 5' end of the probe will be free.

Can probes be synthesized with a free 3' hydroxyl? In theory, yes, as reverse phosphoramidites are available for 5' > 3' synthesis. However, the reverse amidites are extremely expensive. Consequently, microarrays with 3' free ends would be almost prohibitively expensive.

How are the probes attached to the glass substrate? All probes are synthesized on amine-functionalized glass substrates such that the 3' end is covalently coupled to a spacer moiety (see below), which is covalently linked to an amine. The probe sequence is pushed away from the glass by synthesizing a lawn of poly deoxy Thymidine spacer everywhere. Typically the T-spacer is 15 nucleotides in length. Longer linkers can be requested for shorter custom probes.

How do I know what side of the glass slide has my probes? We use glass substrates that have a notch in one corner (see **Figure 1**) for unambiguous orientation. When the slide is held so that the long axis is vertical and the notch is in the upper right hand corner, the surface containing probes is facing you. In addition, there will be a sticky label with a barcode on the top of the slide closest to the notched end. The probe surface will be facing the user when the label is in reading frame and at the top of the slide.

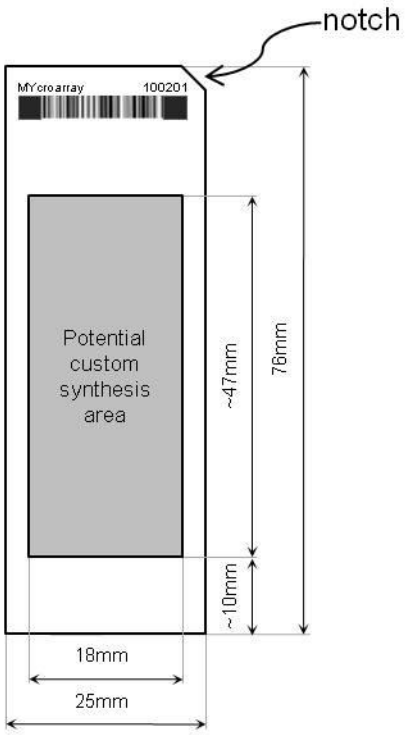

Figure 1. Schematic diagram showing the dimensions of the maximum potential synthesis area of MYcroarrays. Note the notch in the upper right corner for unambiguous orientation.

How many features (spots) can I get in one array? Of course this depends on the format. Currently, we can synthesize from 5K to 90K features per array.

How many arrays can I get on one microscope slide? Some array sizes are replicated on one slide (e.g., 5K and 7K arrays have six replicate arrays on one slide). Our standard formats can be found here.

Custom formats are also available but must fit within the synthesis area. The synthesis area (see **Figure 1**) is ~18x~47mm, centered relative to the short axis (26mm) of the slide and can begin ~10mm from the bottom of the array (opposite end of the notched corner, which is the upper right corner of the slide with the probes on the surface facing the observer).

How many technical replicates can I get of each unique probe sequence? Catalog arrays have at least three technical replicates of each unique probe sequence within each array. Some catalog arrays are available with 5 or more replicates. For custom designs, the extent of probe replication is up to the customer.

Custom MYcroarrays

Do custom MYcroarrays cost more? In most cases no! Because our proprietary synthesis is so flexible, we can synthesize arrays that satisfy the customer's specifications without increased cost (or time of manufacture). However, we cannot synthesize features outside of the potential synthesis area (see **Figure 1**) and the maximum number of features we can synthesize on one array remains 90K.

Probe Design

How are probes designed? Probes for gene expression arrays are designed using a proprietary version of publically available software called OligoArray2.1 (see here). Probe sequences for our catalog arrays are deposited in OligoArrayDb (see here)

Can custom probes be designed? Yes. If your organism of interest is not listed on our website, we can design probes for you if the genome has been completely sequenced and the sequence is fully annotated and publicly available.

Is there a set-up or design fee for a custom probe design? Typically probe design is free. Our standard protocol is to attempt to design up to three specific probes per gene. We will likely not be successful for all genes (e.g., some genes will have only 2, 1 or no specific probes). For genes with no specific probes, we may be able to design a probe or probes that are predicted to cross hybridize to other genes. If we include these potentially cross-hybridizing probes on the array, we will disclose the "other" genes that may cross hybridize. Customers may choose the number of specific probes per gene to include on the array (1, 2 or up to 3) and the number of technical replicates per unique probe sequence (minimal recommendation is 3).

Can I submit my own probe sequences? Yes. Probe sequences are submitted as either a tab delimited text file or an Excel file. Only two columns are necessary; an ID column and a sequence column. Please submit only one instance of each unique probe sequence no matter how many technical replicates you may desire within each array. Please submit sequences in the standard 5' > 3' format. We cannot synthesize degenerate bases (e.g., R, Y, M, K, S, N, etc.). Please submit only A, C, G or T as bases. You may submit probes of any length up to 50 bases.

Ordering

How do I order MYcroarrays? Most often customers will request a quote by either contacting sales@mycroarray.com or our microarray product manager, Donald Schwartz, Ph.D. (drs@mycroarray.com). Along with the quote, we will send an order form. To initiate the order, either fill-in and return the order form, or have your institution send a PO by email (drs@mycroarray.com) or fax (734)-998-0750.

Is there a discount for bulk orders? Yes, there are discounts for ordering larger quantities of slides. Currently, there is a 5% discount for ordering 20-49 slides and a 10% discount for ordering 50 or more slides.

What is the typical delivery time? Most orders are shipped within two weeks of receipt of the order form or PO but it may take up to 4 weeks.

Storage

How do I store MYcroarrays? MYcroarrays are shipped in polypropylene slide mailers in a plastic bag under a vacuum. MYcroarrays are very stabile in the original packaging, if the vacuum seal has not been compromised, and if the arrays are stored at the room temperature (~22C) away from moisture and light. Please never freeze or refrigerate MYcroarrays, even after a mailer is opened. Once a shipping mailer has been opened, unused slides are best stored in their mailer and re-vacuum sealed in a pouch.

How long can MYcroarrays be stored before use? If stored properly (see above), MYcroarrays should be stabile for at least 6 months.

Hybridization

What temperature should I use during hybridization? This depends on the length of the probes, salt concentration in the hybridization solution, amount of formamide in the hybridization solution and other factors. We will always recommend a hybridization temperature. For example, for a hybridization solution containing 1M salt and 10% formamide, we would recommend an initial temperature of 50C for a MYcroarray with 45mer probes and a target GC content of 50%.

How many hours should I hybridize? MYcroarrays are compatible with lifter slips (for static hybridizations in water baths) and Agilent Technologies gasket slides (for dynamic hybridizations in hybridization ovens using Agilent Technologies hybridization cassettes). Typically, >20hrs is recommended for static hybridizations and minimally 16hrs is recommended for dynamic hybridizations. These suggestions assume a fluorescent target concentration of ~35ng/uL, mean length of ~150nt and specific activity of ~1fluor moiety per 30 bases.

Will you provide a recommended hybridization solution recipe? Yes. We will provide a recommended target preparation and hybridization protocol (for converting total RNA to fluor-coupled amino-allyl-cRNA). The protocol will have a recipe for the hybridization solution.

Washing and Scanning

What equipment do I need to wash MYcroarrays? MYcroarrays are synthesized on 26mm x 75mm glass substrates. Any washing/hybridization station that accommodates this format should work. However, it is not necessary to have expensive equipment to wash MYcroarrays. Standard 50ml centrifuge tubes can be use. Alternatively, standard microscope washing/staining dishes with metal or glass racks can be used. We prefer the later because it is an inexpensive solution, the dishes are readily available from numerous scientific supply houses that sell histology supplies and, with a standard stir plate and stir bar, wash solutions can be agitated to facilitate washing.

Do I need a proprietary scanner to scan MYcroarrays? No**.** MYcroarrays are synthesized on 26mm x 75mm glass substrates. Any scanner designed to accommodate ~1x3 inch microscope slides should work well. For example, MYcroarrays have been scanned in the GenePix® 4000B and 4000A scanners (Molecular Devices, Sunnyvale, CA).

What PMT setting should I use for scanning? Of course the fluorescent intensity of your target as well as the amount of target hybridized impacts the PMT setting. Most scanners will have an option to auto set the PMT, which may or may not work well. We recommend actively adjusting the PMT during scanning so that the full dynamic range of signal is achieved. Of course this will require a second full scan at the set PMT. The PMT should be set such that only a few features (1-2%) have some pixels (<10%) that are saturated. Under these conditions, the full dynamic range of signal intensity is appreciated.

What scanner resolution is needed to scan MYcroarrays? MYcroarray feature diameter is ~60µm. We suggest using a scanner with 5µm resolution.