FAQs

Who do I contact with questions?
The best way to get a quick answer is to use the nciarrays-r@mail.nih.gov email address. This is monitored daily and any question will be answered as soon as possible by the appropriate group or person.
If your question is about any array business (or Array Tracker) you should always email nciarrays-r@mail.nih.gov.
If your question is particular to uploading data problems on the mAdb website or to set up privileges you should contact madb-support@bimas.cit.nih.gov .

What are the microarrays composed of and how are they manufactured?
Our microarrays are composed of 70-mer oligos immobilized on Corning UltraGAPS™ coated slides. The oligos are spotted onto the slides using a GeneMachines microarrayer. The arrayer can print up to 100 arrays at a time. Arrays are typically printed in 32 or 48 sectors (or "blocks" of spots) depending on the number of printing tips used. The printing tip works much like a quill pen. It wicks up a fraction of a microliter of oligo and deposits a fraction of a nanoliter of oligo onto each slide. Each spot diameter can vary but averages ~100 um. Spots are printed with center-to-center spacing ranging from 160-220 um depending on the size of the array and density requirements. Printing with a 32-tip configuration, it takes approximately 18 hours to manufacture 100 microarrays each consisting of 22464 spots for the Operon Human Version 2.0 oligo set. Printing with a 48-tip configuration, it approximately 22 hours to manufacture 100 microarrays each consisting of 34,580 spots for the Operon Human Version 3.0 long oligo set. And printing with a 48-tip configuration, it approximately 24 hours to manufacture 100 microarrays each consisting of 38,467 spots for the Illumina Mouse MEEBO long oligo set.

How do the microarrays work?
Fluor-labeled cDNA is generated from purified RNA (mRNA or total RNA). One RNA sample is labeled with Cy3 (emits green fluorescence at 532 nm) and the other is labeled with Cy5 (emits red fluorescence at 635 nm). The labeled cDNAs are combined and hybridized to the same microarray. The cDNA hybridizes to the probes via complementary interaction. The ratio of Cy5 signal to Cy3 signal can be calculated for each spot on the array and indicates the relative ratio of gene expression. These ratios can be normalized and converted to color for visualization purposes. Traditionally, a yellow spot indicates a 1.0 (or a 1:1) expression ratio which means no difference in expression levels between the two samples while a red spot (>1.0) indicates that gene is expressed at higher levels in the sample labeled with Cy5. Conversely, a green spot (<1.0) indicates that gene is expressed at higher levels in the sample labeled with Cy3. (The expression ratio is generally reported as Cy5/Cy3, or red over green.)

How should the arrays be stored and how long do they last?
The arrays at the NCI Microarray Facility are stored at room temperature in a desiccant chamber for minimizing humidity. We recommend that the slides be stored in slide mailers, at room temperature, in a sealed container with desiccant to keep out dust and humidity. We have found that the arrays stored this way last for 6 months and still deliver optimal performance.

Where do the genes represented on the microarrays come from?
Currently, we are printing the Qiagen Human Version 2.0 set of oligos for human arrays and Compugen Mouse Release 2.0 for mouse arrays. Each company has their own proprietary bioinformatic algorithms for selection of oligos. However, oligo design and optimization criteria include incorporating alternative splicing structure by selecting probes from regions that are common to a maximal number of predicted splice variants, minimizing cross-homology, maintaining sequence quality by avoiding sequencing errors and polymorphic sites, normalizing GC content / Melting Temperature across the collection, minimizing the distance from the 3' end, avoiding oligo secondary structures, and considering genomic information.

How can I find information for an oligo on the array?
First go to the Qiagen's Oligo Microarray Database at http://oligos.qiagen.com/arrays/omad.php. Follow the link for the appropriate oligo set. Currently, we are printing Human Version 2.1 and Version 3.0.
For mouse arrays information can be found at http://www.labonweb.com/chips/libraries.html regarding the Compugen Mouse Release 2.0 oligo library. For more information on the Mouse MEEBO set visit http://www.illumina.com/products/dna/genomesets/meebo_mouse.ilmn.

How long can labeled cDNA be stored prior to hybridization?
Some users have successfully kept labeled cDNA if stored at –20ºC under dark conditions for up to one month. Store labeled cDNA without SSC, SDS and before the final denaturation step.

How soon after washing should the arrays be scanned?
It is best to scan them as soon as they are washed. As a general rule, the arrays should be scanned the same day they are washed – preferably within a few hours of washing. Be aware that during the summer months when ozone levels are high, signal decay can occur quickly.

How much total RNA is required for labeling?
For maximum signal detection and reproducibility, we recommend at least 10μg of total RNA. The amount of total RNA required for labeling is dependent on the quality of the RNA and the level of mRNA in the sample.

How do I increase my hybridization signal?
There are a number of ways to approach this question. If your signal is consistently weak, try doubling the amount of total RNA used when labeling. Often the mRNAs can account for a much smaller fraction of your total RNA sample then expected. By increasing the amount of template RNA used in the RT reaction, you can often increase the amount of labeled target. However, it should be noted that this is not always the solution. Degraded RNA is a problem that accounts for low signal as well. In some tissue types, isolating high molecular weight RNA is inherently difficult. If RNA quality is in question, we recommend running a few micrograms out on a gel or using Agilent's Bioanalyzer to check for degradation. In many cases, higher quality RNA is necessary for better array hybridizations. Also, certain reagents that go into the RT reaction can go bad overtime. DTT and dNTPs are particularly sensitive to multiple freeze/thaw cycles. Fresh RT enzyme can sometimes make a positive difference, too. New methods in probe labeling are emerging that promise to increase signal sensitivity.

How do I decrease the background signal on my array?
Background often results from high salt and detergent concentrations during the hybridization. Most often, high salt and detergent concentrations occur as the result of evaporation of the hybridization solution. Evaporation drives the salt and detergent concentrations up and may facilitate the precipitation of target onto the glass surface (particularly Cy5-labelled target). Evaporation can occur rapidly at two particular steps in the protocol: 1) when the target is first pipetted onto the array, and 2) following the hybridization when the hyb chamber is opened and the array is exposed to cold, low-humidity air. Point number 2 is potentially the most critical. Most hyb chambers have aluminum bases. When they come out of the water bath, they remain hot for several minutes. Once the cover is removed, the array is essentially sitting on a hot plate in a cold environment. At this point evaporation of the target around the edges of the coverslip can occur almost instantaneously. Therefore, you must work quickly to remove the array from the hyb chamber and submerge it in the first wash buffer.

Sometimes the background actually results from fluorescence emanating from the opposite side of the array. We routinely clean the backside of the arrays (after washing and drying) with moist breath and a Kim wipe – just prior to scanning.
In cases of particularly weak signal, we often have to scan at near-maximum PMT voltage that results in relatively high background levels. To combat this phenomenon, you should try to increase signal (i.e. probe intensity) and scan at lower PMT voltage.

It has been reported that organics used during RNA extraction can contaminate RNA samples and contribute to background fluorescence. Likewise, benzene which is used to dehydrate 95% EtOH to 100% EtOH, has been implicated in background artifacts. For this reason, many researchers avoid using EtOH preparations made with 100% (200 proof) EtOH.
We have seen that re-soaking the arrays in each wash buffer for several minutes can reduce some forms of high background. Specific signal can remain while background diminishes.

How do I get rid of the "scars" that I occasionally see on my arrays?
Sometimes we observe symmetrical and parallel scratches on arrays that would seem to indicate mechanical forces acting on the surface. The most likely culprit is the coverslip. To avoid coverslip scratching, try a more gentle approach when removing the coverslip. We recommend inverting the array (coverslip down) in the first wash buffer (containing SDS which helps loosen the coverslip) and gentle rocking up and down until it starts to move. Then slowly tilt downward and let the coverslip fall away on its own volition.

How do I get rid of all those tiny "specks" of fluorescence I see on my arrays?
We call this speckling "schmutz" and it comes in a variety of flavors. It can be particulate from the air (such as dust and lint fibers) that settles onto the array surface or into the target during preparation and binds to the array surface during the hybridization. It can be precipitate such as SDS or cDNA aggregates that result from aberrant salt and detergent concentrations or unknown contaminants. Sometimes this phenomenon seems to be associated with a particular sample of RNA, which can be corrected by RNA purification. If this phenomenon occurs with frequency, you can clean the target on a 0.20-0.45 um filter, which retains the particulates on the surface and allows the probe to pass through.

How do I minimize the "dim signal" areas on my array?
The dim areas often have a circular boarder suggesting a bubble is the culprit (although obvious bubbles do not always result in dim areas). Though we try to minimize bubble formation when applying the coverslip, bubbles that were not initially apparent can form during the hybridization as a result of de-gassing. Sometimes the dim areas display a pronounced signal gradient suggesting nonuniformity of hybridization stringency across the surface of the array. We and others think that this phenomenon arises due to initial volume gradients under the coverslip. Volume gradients can result from warped coverslips and uneven distribution of probe beneath the coverslip. When applying a standard coverslip to the hybridization solution (pipetted onto the array surface), we recommend a centered and level release such that the target spreads beneath the coverslip in a radial fashion converging on all four corners of the coverslip simultaneously. Coverslips that possess "vertical lifts" on two or more edges can potentially reduce volume gradients and eliminate dim areas.

How do I just get better array results?
Think critically about what you are doing when you are isolating your RNA and making your probe. Quality is everything when it comes to microarray science. Pure, high-molecular weight RNA is absolutely required for good array results. Fresh reagents and meticulous technique can make the difference between good probe and no probe. Be observant every step along the way and always consider the molecular and physical consequences with each manipulation of your RNA, labeled cDNA, and the array or coverslip. Everyone has a learning curve, which usually begins with consistently sub-average hyb outcomes and culminates in consistently stellar hyb outcomes and array data. All it takes is a little patience and a lot of critical thinking.

Some Commercial RNA Amplification Kits:
Many amplification kits/protocols have come to market within the past year.

1. Ambion http://www.ambion.com/catalog/SubApps.html?4
2. Acrturus http://www.arctur.com/research_portal/products/
3. Genisphere http://www.genisphere.com/
4. Nugen http://www.nugentechnologies.com/
5. Epicentre http://www.epicentre.com/item.asp?ID=454&CatID=12&SubCatID=26

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