Troubleshooting Forum

Molecular Biology Techniques Q&A

Microarray: Working with Cy3 and Cy5

This month's questions from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) come from the "Microarray" section. Entries have been edited for concision and clarity. Mentions of specific products and manufacturers have been retained from the original posts, but do not represent endorsements by, or the opinions of, *BioTechniques*.

How can I improve Cy5 signal strength? (Thread 2283)

Q I am having significant problems with low Cy5 signals in microarray experiments. I have tried different lots of Cy5, but each time the signal is extremely low, especially compared to Cy3. I tried swapping dyes, but saw the same result. I know the Cy3 dye is typically stronger, but my arrays look completely green most of the time. I don't know what could cause such a bias and would appreciate any suggestions for resolving the problem.

A What method are you using? I had similar problems with poor Cy5 signals, but then I changed some steps in the preparation protocol and now Cy5 is as strong as Cy3.

Q I mainly use Superscript. At one point, I did change to CyScribe, which yielded slightly better results, but the improvement here was not enough to make up for the large imbalance. What did you change that finally corrected your problem?

A Our facility is also experiencing issues with Cy5. Oddly, it seems to be correlated with high-ozone days. I discussed this with some of my European colleagues, who are having similar problems. They recommended using Dye Saver from Genisphere. I have not tried this method yet, but I will soon. You could also try an indirect labeling method.

A I have seen a strong correlation between Cy5 stability and humidity. Dye Saver tends to add background, so it may not be a good solution.

A Is there any literature suggesting environmental effects on Cy-3 similar to those you mention with Cy5? I am actually having tremendous difficulty getting the Cy3 labeling to work well, although Cy5 looks normal.

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How can I correct high background staining in microarrays? (Thread 17323)

Q2 I just started working with oligo microarrays, and I'm experiencing an issue with high background staining. In most of my experiments, half of the glass in the Cy5 channel is covered with intense red stain. The spots are surrounded by tiny black circles and are only weakly hybridized. We use the epoxy slides recommended by the oligo supplier. The mystery is that the second glass from two slides processed together is always much better than the first. The only difference between the two slides is reciprocal labeling. The glass does not dry out during hybridization, so what could be causing such problematic staining?

A My colleague experienced a similar problem using a CGH array, although in his case, the background staining was green. After much troubleshooting, we found the problem was introduced at the final washing step and during drying with compressed air. He solved the problem by repeating the final washing and drying steps after seeing the stain until the stain eventually disappeared.

Q I tried the additional wash steps as you suggested, but, unfortunately, our stain is pretty resistant to additional washing. I didn't see any visible change in stain intensity or shape after three additional wash cycles. I did consider whether there was a problem with blocking the epoxy surface of the slide, but if that is the case, why does one slide stain properly while the other does not?

A I too saw bizarre background staining similar to what you describe here. However, after trial and error, I believe the problem came during the hybridization step. You must be very careful during hybridization. Do you use hybridization chambers? If so, did you check your o-rings to be sure they weren't contributing to the problem? Do you coverslip the slides? If so, did any solutions leak from the sides? I don't know which technique you are using.

Q I am using a hybridization chamber and the o-rings look okay. I load several drops of SSC into the holes below the slide. I then place the packed chamber on a box filled with wet cotton, which is put into the hybridization oven with slow agitation. I cover all of the slides with coverslips. The volume of hybridization probe is enough to reach all areas of the coverslip and sometimes slightly leaks from the sides.

Do you think there could be too much probe? Or maybe agitation during hybridization leads to problems? Do you have any other suggestions?

A I started running my arrays before it was common to use swinging or other motion to uniformly distribute probe. If I found a rare chip with background staining, I would put the hybridization chamber into a water bath warmed to the correct hybridization temperature. To prevent leaking, I put the chamber in a zipper-topped bag and vacuumed out the air; this generally improved the results. When I saw background, I always assumed leaking was the cause, but it could have been something else. Preventing leaking did resolve the background in those cases.

A How do you dry your slides before testing? How long after drying do you wait before testing?

Q Are you asking how we dry our slides after spotting the arrays? We dry slides in a controlled atmosphere inside the spotter. After spotting and drying, I did not check or test the slides before using them for hybridization. During hybridization and afterward, I dry the slides in a centrifuge.

A I wonder if your background might be caused by uneven heating during the drying steps which might account for the two different slides returning different staining results.

Q There doesn't seem to be a problem with overheating before, or during, scanning and both slides are processed at room temperature. The second slide (with nice staining) is all right even after multiple rounds of scanning, while the slide with background staining is always bad.

Selected and edited by Kristie Nybo, Ph.D.

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