Technical Note

UHN Microarray Centre

Stability of cyanine-labelled cDNA stored at -20°C

- cDNA labelled with cyanine dyes can be stored at minus 20°C for up to 4 weeks with no appreciable loss of quality

Introduction

The UHNMAC's standard aminoallyl (indirect) labelling protocol indicates that users may freeze the cDNA or labelled-cDNA after either of the purification steps for several days. While we normally label cDNA and hybridise it to an array on the same day, we have assessed the stability of labelled-cDNA stored at –20°C for about 4 weeks. This stability information is particularly useful for large projects performed over several weeks and for quality control experiments (testing different batches of slides with the same labelled-cDNA).

The goal of this experiment was to evaluate the stability of indirectly labelled Universal Human Reference RNA (UHRR; Stratagene) that was stored at –20°C.

Method

Following the standard aminoallyl (indirect) labelling protocol, 60 µg UHRR was labelled with aminoallyl and half was conjugated with Cy5 and half with Cy3 monofunctional dyes and frozen at –20°C for 3 days. After 3 days ("week 1"), the samples were thawed and 5 µL (of the 15 µL initial volume) was hybridised to Hum19k8 cDNA microarrays (2 arrays; face-to-face hybridisation). The remaining labelled-cDNA (10 µL) was frozen and the hybridisations were repeated after 10 and 26 days ("week 2" and "week 4", respectively). The slides were washed after the overnight incubation, scanned using the Agilent DNA microarray scanner and the images were quantified using ArrayVision (Imaging Research/GE Healthcare).

Results & Discussion

The data shows that the mean raw intensity fluctuated slightly over the 3 time intervals but did not drop off as the time stored at –20°C increased. The maximum Cy3 and Cy5 signal intensities were similar over the 3 time intervals (data not shown). Figure 1 shows the mean Cy3 and Cy5 signal intensities and number of flagged spots (flagged due to spot signal intensity less than preset threshold of 100) were similar over the 3 time intervals.

Figure 2 shows a comparison of the M versus A plot for two of the slides, where M is computed as $\log_2$ (Cy3 Intensity/Cy5 Intensity) and the A value is ($\log_2$[Cy3 Intensity x Cy5 Intensity])/2; one array hybridised with 1-week-old labelled-cDNA and the other hybridized with 4-week-old labelled-cDNA. M versus A plots allow for the identification of skewed data and since UHRR labelled-cDNA was in both channels, it appears

the cDNA was stable for 4 weeks at -20°C as the plots appear similar with very few outliers.

The coefficient of variance (the standard deviation divided by the mean) was similar when comparing data from week 1 and week 2 with data from week 1 and week 4 (data not shown). This further suggests that labelled-cDNA can be stored at –20°C for up to 4 weeks.

Conclusion

It appears that UHRR is stable for up to 4 weeks when labelled with aminoallyl and then cyanine dye and stored at -20°C. The UHNMAC only freezes labelled-cDNA for QC testing and certain R&D projects; experiments in which the exact ratio between experimental and reference sample is not critical. Since each RNA sample is different, one cannot conclude that all labelled-cDNA samples would be stable at -20°C for any length of time, and thus, the UHNMAC recommends hybridising labelled-cDNA as soon as possible and only freezing samples whenever necessary.

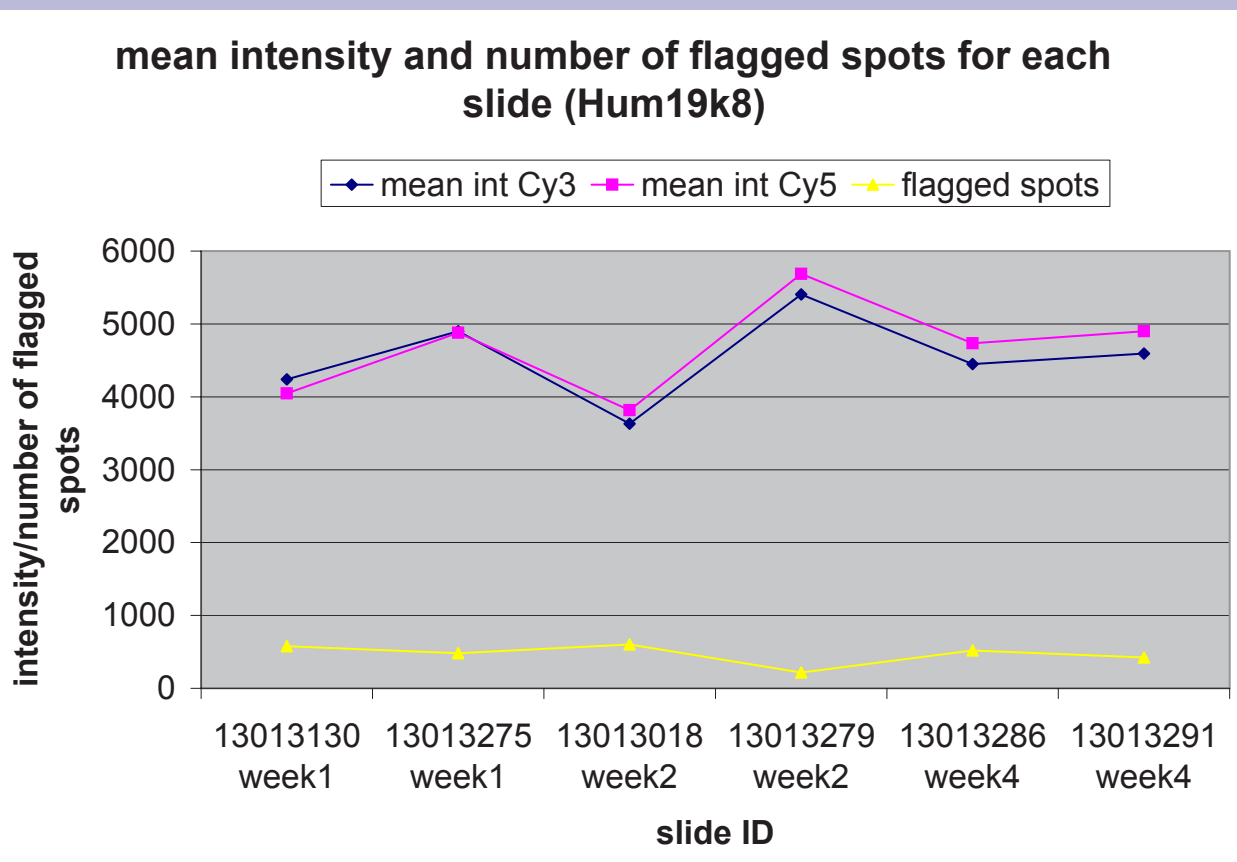
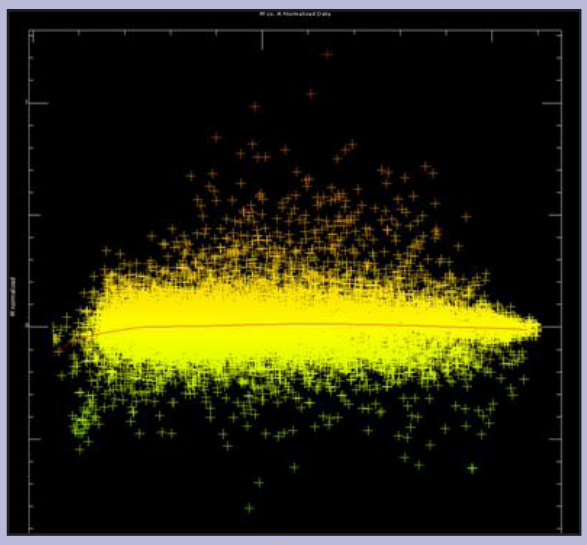


Figure 1. The mean signal intensities for Cy3 and Cy5 and the number of flagged spots (signal intensity less than the preset threshold of 100) for the two Hum19k8 arrays **hybridised with the same** labelled-cDNA at three different time points (after being frozen for 3, 10 and 26 days).

A



B

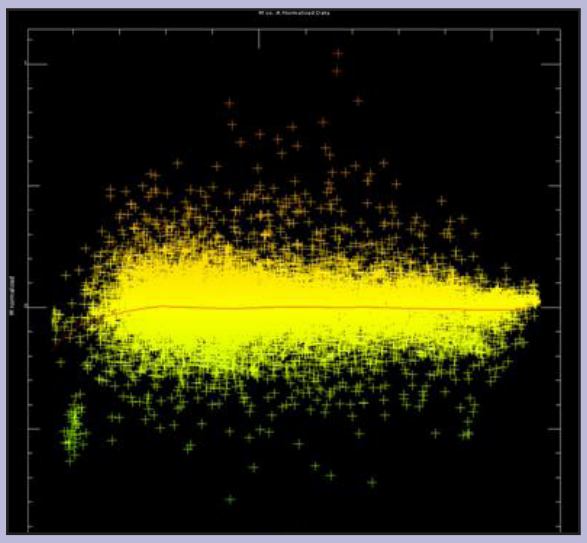


Figure 2. M versus A plot for slides hybridised with cyanine-labelled UHRR stored at -20°C for one week (image A) and approximately four weeks (image B). The plots are similar and show that the data is not skewed.