Technical Note

UHN Microarray Centre

Purification of Labelled-cDNA

- The UHNMAC standard protocol for the purification of labelled-cDNA recommends the use of CyScribe™ GFX™ columns (GE Healthcare)

Background

The goal of this study was to evaluate several methods for the purification of cDNA labelled with fluorescent dyes such as cyanine or Alexa (Invitrogen). The optimal purification method would efficiently remove enzyme, salt, excess primer, unincorporated dNTP, unincorporated fluors/dyes, and minimise the loss of labelled-cDNA.

The CyScribe™ GFX™ purification kit was optimised for the purification of Cy-labelled cDNA. The GFX™ purification columns are comprised of a glass fiber matrix to bind labelled-cDNA in the presence of chaotropic salt while removing contaminants with an ethanol wash buffer. The labelled-cDNA is then eluted using a low ionic strength buffer. Manufacturers specifications indicate that recovery of labelled-cDNA after purification should be greater than 60% and removal of unincorporated dye nucleotide should approach 99.9%[1]. The columns also remove labelled fragments less than 100 base pairs in length, thus reducing background[1].

The goal of this study was to compare the CyScribe™ GFX™ purification columns, as described in manufacturer's protocol, to the UHNMAC standard protocol which, at the time, recommended using Amicon® Microcon®-PCR Centrifugal Filter Devices (Millipore) for the purification of labelled-cDNA.

Method

Briefly, the CyScribe™ GFX™ column protocol involves combining the cDNA sample with binding buffer, adding the sample to the column and centrifuging, washing three times with ethanol wash buffer and eluting with elution buffer. The Microcon® Filter Device protocol involves adding the sample and water to the sample reservoir, centrifuging (labelled-cDNA retained on filter surface), adding water to filter surface and incubating for 30 seconds, then inverting the reservoir into a clean tube and centrifuging to collect the purified labelled-cDNA.

The GFX™ columns were used as recommended by the manufacturer for purification of directly-labelled cDNA. Modifications were made to the standard UHNMAC aminoallyl (indirect) labelling protocol to account for the fact that aminoallyl-labelled cDNA needs to be in a 0.1 M sodium bicarbonate (final concentration) buffered solution for the conjugation reaction with monoreactive cyanine or Alexa fluors. Therefore, the elution of aminoallyl cDNA from the GFX™ column with 60 µL of 0.017 M sodium

bicarbonate buffer, pH 9, followed by drying down to 5 µL by vacuum centrifugation, allowed for a quick set-up of the conjugation reaction.

Results & Discussion

In the past, purification methods including isopropanol precipitation and the Amicon® Microcon®-PCR Centrifugal Filter Devices (Millipore) were used. As the UHNMAC assesses new microarray-related products, standard protocols are revised whenever improvements are made.

The evaluation of the CyScribe™ GFX™ purification columns led to improved image results (brighter signal, less background) following a quick straightforward protocol. Besides image quality, the GFX™ purification method offered other improvements over previous methods. The isopropanol precipitation method had a lengthy incubation time and sometimes the precipitated cDNA pellet was difficult to see in the tube. The Amicon® Microcon®-PCR Centrifugal Filter Device (Millipore) protocol was modified by the UHNMAC by adding a smaller volume of elution buffer than recommended by the manufacturer. This change was made so that the eluted sample could be added directly to the hybridisation solution without the need for further reducing the sample volume. The problem, however, was that the eluted volume could range anywhere from 5 µL (ideal) to 20 µL (requiring additional step to reduce volume to 5 µL).

It is also interesting to note that the way the microarrays themselves are processed (after printing) can greatly influence the image quality for certain methods of labelled-cDNA purification. For example, we have found that labelled-cDNA purification using the High Pure PCR Purification kit (Roche) will result in excellent images if hybridised to arrays that have been processed with n-methyl-pyrrilidinone (M2P) but will result in images with very high background if hybridised to arrays that were processed with SDS solution or not processed at all. Labelled-cDNA purification using the CyScribe™ GFX™ columns results in excellent images whether the slides are processed with M2P, SDS, or not processed after printing and UV cross-linking.

Conclusions

The current UHNMAC standard protocol for the purification of labelled-cDNA recommends the use of CyScribe™ GFX™ columns (GE Healthcare). For directly-labelled-cDNA, the manufacturer's protocol is recommended. For indirectly-labelled-cDNA, the UHNMAC has modified the protocol slightly by eluting the cDNA using diluted dye conjugation buffer.

References

1. Wang, H. et al. Glass fiber matrix purification of CyDye labelled cDNA probes for use in microarray hybridizations. Life Sciences News 11, 2002, Amersham Biosciences. (Technical Note).