Technical Note

UHN Microarray Centre

Use of Cot-1 DNA as hybridisation competitor for CGI microarrays

- Using Cot-1 DNA as a hybridisation competitor for CpG island (CGI) arrays can decrease non-specific hybridisation which will result in fewer false positives

Introduction

Regions rich in the CpG dinucleotide pattern are known as CpG islands (CGIs). It is estimated that about 60% of human genes and 47% of mouse genes are associated with CGIs, usually in the 5'-end[1], and that the vast majority of CGIs are within -500 to +1500 bp of the transcription start site[2]. It is generally accepted that CGIs are at least 200 base pairs in length, have a G/C content greater than 50%, and the CpG percentage exceeds 60% of that expected in a random sequence[3]. The significance of CGIs is that they are strongly associated with gene regulatory regions[4].

CGI microarrays enable researchers to identify regulatory regions that may be affected by a particular protein, using Chromatin Immunoprecipitation (ChIP)-on-chip hybridisations, and to identify changes in DNA methylation patterns, using the Differential Methylation Hybridisation (DMH) technique.

One of the inherent problems with CGI arrays is the presence of interspersed repeats in the clones. Most hybridisation protocols employ competitor DNAs and RNAs to prevent non-specific hybridisation. One such commonly used competitor, Cot-1 DNA, which is restriction-digested human genomic DNA that has been enriched for repetitive elements, is potentially an ideal competitor for use with the CGI arrays. We therefore tested the use of Cot-1 DNA at varying ratios as a potential competitor to reduce false-positive signals and increase specificity.

The UHNMAC has updated the Human 12k CGI clone set, adding an additional 9k clones and then removing redundant clones. The resultant Human 8k CGI array has been released. As part of this process, we compared the original Human 12k clone set and the additional Human 9k CGI clone set. This analysis revealed that upon hybridisation, the 9K set had, on average, lower signal intensities than the 12k set. We hypothesise that this is likely because the 9K set was produced using a subtractive method to reduce the presence of interspersed repeats and that the higher level of fluorescence in the 12k set comes from the hybridisation of interspersed repeat elements, which may not necessarily be hybridising to clones containing homologous genomic regions. We used the signal differential between these two sets as a means to test the effect of Cot-1 competitor to determine if the signals between these two sets could be equalised by removal of non-specific hybridisation.

Method

An array was printed containing 768 clones from each of the 9K and 12K Human CGI sets. Varying amounts

of Cot-1 DNA (10, 20, and 40 μg) was added to the standard hybridisation solution used in the UHNMAC Protocols (DIG Easy Hyb, yeast tRNA (0.45mg/mL; final concentration)) and slides were hybridised for overnight at 37°C (visit www.microarrays.ca for standard protocol). Slides were washed and scanned as usual, and the resultant images were quantified.

Results & Discussion

When Cot-1 DNA was used as a hybridisation competitor, there was much less signal intensity for both the 9k and 12k clone sets than when Cot-1 DNA was absent (Figure 1). This decrease in signal intensity indicates how much non-specific hybridisation is occurring on the CGI arrays. The changes in signal-to-noise by the addition of varying amounts of Cot-1 DNA to the hybridisation solution are outlined in Table 1. Upon the addition of 10 μg Cot-1 DNA to the hybridisation solution, the signal-to-noise ratios for the 12k set decreased from 40 (without the addition of Cot-1 DNA) to 9, while the signal-to-noise ratios for the 9k set also decreased, but less drastically, from 18 to 8. The addition of more Cot-1 DNA (20 μg and 40 μg) further reduced the signal-to-noise ratios to 4-6 for both the 12k and 9k clones sets.

It is likely that some clones, especially in the Human 12k CGI set, contain interspersed repeats and these repeats can cause the labelled-DNA to hybridise to the wrong clone. The prevention of non-specific hybridisation by adding Cot-1 DNA as a hybridisation competitor will reduce the number of false positives. Ongoing work with the CGI arrays will determine if the addition of 10 μg Cot-1 DNA to the hybridisation solution is an appropriate amount.

Prior to this study, the standard protocol included the addition of calf thymus DNA and yeast tRNA (each 0.45 mg/mL; final concentration) to the hybridisation solution. The current recommendation, based on this study, is the addition of Cot-1 DNA at a final concentration of 0.125 mg/mL (10 μg Cot-1 DNA in 80 μL hybridisation solution) and the omission of calf thymus DNA, as to not dilute the pool of labelled-DNA.

Conclusion

Although the addition of Cot-1 DNA to the hybridisation solution decreases the level of spot intensity on both the Human CGI 12K and 9K clone sets, the intensities of the 12K clones decrease more than the 9K. The addition of an appropriate amount of Cot-1 lowers the intensities of the 12k clone set to the same level as on the 9k clone set. It is likely that this decrease in hybridisation to clones with repetitive elements will result in fewer false positives and less noisy data. Based on this study, the UHNMAC recommends the addition of Cot-1 DNA (0.125mg/mL, final concentration) to the hybridisation solution when using Human and Mouse CGI arrays.

References:

1. Antequera, F. and Bird, A. Number of CpG islands and genes in human and mouse. Proc. Natl. Acad. Sci. USA, 1993, 90:11995-11999.
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3. Gardiner-Garden, M. and Frommer, M. CpG islands in vertebrate genomes. J. Mol. Biol. 1987, 196:261-282.
4. Heisler, L.E. et al. CpG Island microarray probe sequences derived from a physical library are representative of CpG Islands annotated on the human genome. Nucleic Acids Research. 2005, 33(9):2952-2961.

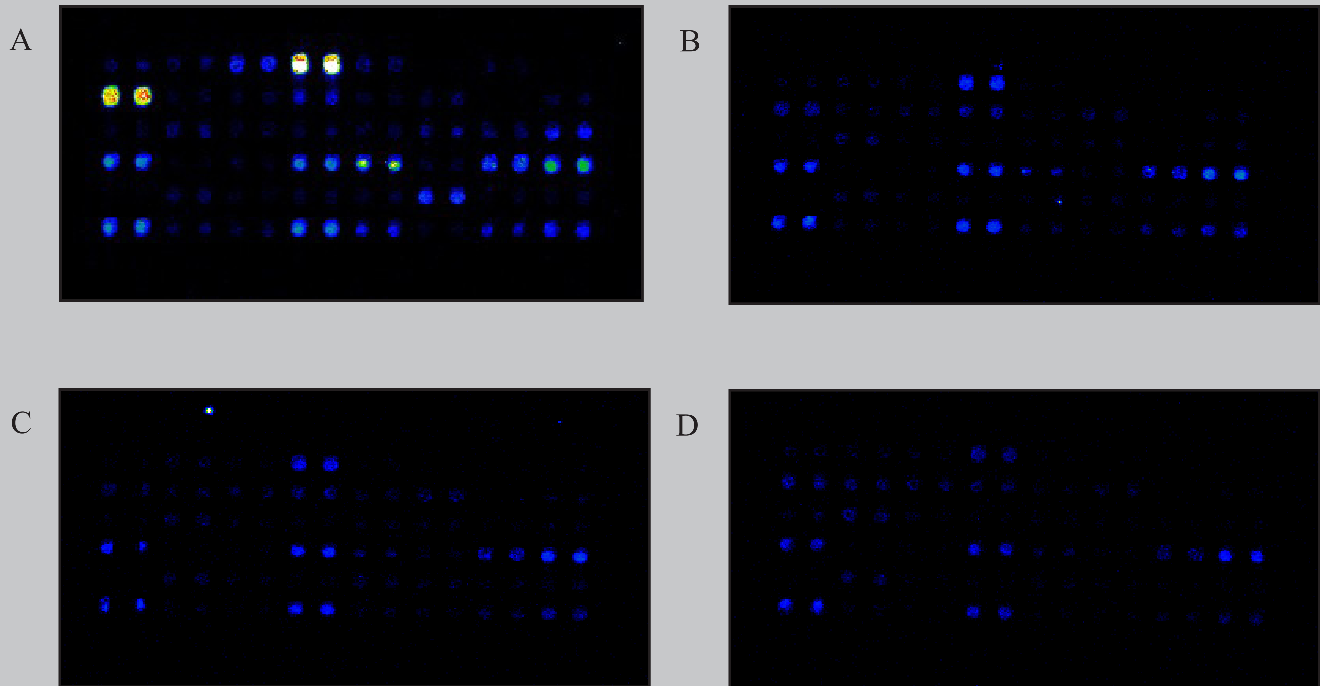

Figure 1. Image of the top left subarray of Human CGI array hybridised with A) 0 µg, B) 10 µg, C) 20 µg, and D) 40 µg Cot-1 DNA added to the hybridisation solution. As the amount of Cot-1 DNA added increased, the signal intensity decreased due to reduced non-specific hybridisation.

Table 1. As the amount of Cot-1 DNA added to the hybridisation solution increased, the signal due to non-specific hybridisation decreased. The 12K set is currently being used for array manufacture. The 8.1K set is a sub-set of the 12K set that has had redundant clones removed. The revised 8.1K set has replaced the current Human 12K CGI array.