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Automation of cDNA microarray hybridization and washing yields improved data quality

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Abstract

Microarray technology allows the analysis of whole-genome transcription within a single hybridization, and has become a standard research tool. It is extremely important to minimize variation in order to obtain high quality microarray data that can be compared among experiments and laboratories. The majority of facilities implement manual hybridization approaches for microarray studies. We developed an automated method for cDNA microarray hybridization that uses equivalent pre-hybridization, hybridization and washing conditions to the suggested manual protocol. The automated method significantly decreased variability across microarray slides compared to manual hybridization. Although normalized signal intensities for buffer-only spots across the chips were identical, significantly reduced variation and inter-quartile ranges were obtained using the automated workstation. This decreased variation led to improved correlation among technical replicates across slides in both the Cy3 and Cy5 channels.

Keywords: cDNA microarrays; Automated hybridization; Signal intensities; Microarray technology

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1. Introduction

DNA microarrays have become a core technology in many research centres. Several papers have demonstrated numerous sources of variation that affect the measurement of gene expression [1–6], some resulting from sub-optimal hybridization and washing. Variation may be minimized by the implementation of automated workstations. Automated washing and hybridization have been integrated into the Affymetrix approach; the high quality data produced by Affymetrix is due primarily to automation of all procedures from start to finish, which yields highly reproducible data with minimal technical variation. Despite this, most other commercially available microarray manufacturers have not implemented automated workstations into their protocols. Presently, automated methods are used mainly with in-house printed microarrays, and not with commercial platforms. Furthermore, automation should significantly improve image quality and reproducibility, minimize labour and increase throughput.

We used a HS4800 liquid handling system (TECAN, Research Triangle Park, NC, USA) to develop a hybridization and washing protocol for an academically available cDNA microarray (University Health Network [UHN], mouse NIA 15k cDNA microarrays, Toronto, ON, CA), and compared data quality to an analogous manual protocol.

2. Materials and methods

2.1. RNA extraction and probe preparation

RNA was extracted from three cultures of a confluent cell line [7] using TriZol (Invitrogen Life Technologies, Burlington, ON, CA) and RNEasy columns (Qiagen, Mississauga, ON, CA). Twenty micrograms of cell line, or reference total RNA (Stratagene Universal mouse reference RNA; Stratagene, La Jolla, CA, USA) was amplified using Amersham Cyscribe first strand cDNA labelling kit in the presence of Cy3 or Cy5 (Amersham Biosciences, Piscataway, NJ, USA). Details of the protocol can be seen on the UHN website (http://www.microarrays.ca/support/proto.html). Probes were purified using Microcon columns (Millipore, Nepean, ON, CA) with 4 mM EDTA, 400 mM sodium hydroxide, and 400 mM acetic acid. We hybridized cell line against reference RNA and performed dye-swap experiments (6 chips in total for automated hybridizations and 6 chips in total for manual hybridizations) so that each sample was analyzed on two chips, one with Cy3 (and reference in Cy5) and one with Cy5 label (and reference in Cy3). Pre-hybridization (3XSSC, 0.1%SDS, 2 μg/ml BSA solution at 65 °C for 45 min) was added to both the manual and automated protocols to reduce background. Hybridization for both procedures was carried out using DIG Easy hybridization solution (Roche Diagnostics, Indianapolis, IN, USA) in the presence of 50 μg sonicated calf thymus DNA (Sigma-Aldrich, Oakville, ON, CA) and 50 μg yeast tRNA (to reduce non-specific hybridization).