Use of Hidden Correlations in Short Oligonucleotide Array Data Are Insufficient For Accurate Quantification of Nucleic Acid Targets in Complex Mixtures

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Supporting Text

Experimental procedures

Target preparation, hybridization conditions, and construction of the oligonucleotide microarrays.

Target preparation. The targets originated from 12 protozoa species from cultures of the Heterotroph Flagelates Cologne Collection (HFCC, Germany). 28S rRNA templates were derived from the D3-D5 region of the protozoa species. The sequences were amplified using universal primers (28S 485wT7 5'-TAATACGACTCACTATAGGGGACCCGTCTTGAAACACGGA-3'; and 28S 689 5'- ACACACTCCTTAGCGGA -3') of which the forward primer carries a tail with a T7-RNA Polymerase initiation site at its 5' end. PCR products obtained with these primers were directly used for in vitro transcription. The RNA transcript was purified and the quantity and quality of the yield was determined using a spectrophotometer (Thermo Scientific NanoDrop™, USA) and Bioanalyzer (Agilent Technologies, Inc., USA), respectively.

Hybridization. The hybridization was performed following the protocols routinely used for the GENIOM ONE® instrument (Febit GmbH, Heidelberg, Germany). The RNA fingerprint and mixture samples (final volume 16 µL) were denatured before being placed in the arrays and then prehybridized for 30 min. The hybridization was carried out at 45°C for 4 h without agitation (active mixing). After removing the hybridization solutions, the microarray was washed, fluorescently stained, and then washed again to remove unbound dye. The microarray was than scanned using the Geniom device. The images of the hybridized microarrays were recorded by using the autoexposure setting, and the raw data of the signals were converted to MS Excel files. The background intensity of each array was determined using 142 negative controls.

Oligonucleotide microarrays. A set of oligonucleotide probes for the microarrays was generated using a C^{++} program written for a previous study (Pozhtikov et al. 2005). The set consisted of perfect match (25-mer) probes that

were complementary to the 6 rRNA targets as well as several other HFCC species. All probes were replicated two times to provide a measure of intra-array reproducibility. In total, 6,320 oligonucleotide probes were synthesized on each microarray (Febit GmbH, Heidelberg, Germany).

Availability of data. A Microsoft Access database for fingerprint and mixture signal intensities is publicly available at http://faculty.washington.edu/pozhit/default.htm.

References

Pozhitkov, A., Stemshorn, K., Tautz, D. 2005. An algorithm for the determination and quantification of components of nucleic acid mixtures based on single sequencing reactions. BMC Bioinformatics 6, 281.