An Advanced Approach to Simultaneous Monitoring of Multiple Bacteria in Space

Interim Report

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Probe Selection

During this period we developed probes specific to the waterborne contaminant Pseudomonas aeruginosa and P. putida. We found that one probe was sufficient for P. putida but P. aeruginosa required two. The next step is to test these probes with RNA isolated from these organisms. We are awaiting test results on probes designed earlier to determine if refinement is required.

Development of spacecraft-compatible methods of nucleic acid preparation and cleanup continued this month. In testing a widely-known protocol from the literature, we found that its performance could be greatly enhanced by addition of a sizing step to eliminate acids; testing of our alternative adsorptive methods is now underway. We also confirmed and extended our observation of precipitation of DNA by condensing agents such as spermine at low ionic strength. The phenomenon does not occur at higher ionic strength. Most importantly, linear and circular DNA is precipitated, but RNA is not, raising the possibility of a selective purification process.

Surface Chemistry

During January, we have completed the set of studies initiated several months ago to investigate and exploit surface charge effects to speed the hybridization of high molecular weight DNA and RNA to microarray surfaces. A scientific paper is currently in preparation.

Briefly, we have completed studies with the streptaviden surface model to verify that amplified DNA target hybridization rates can be increased by a factor of 10 - 20 by introducing a weak positive charge onto the underlying surface. We have also shown that the same surface effects lead to high affinity hybridization signals in the absence of added solution state cations, which greatly reduces the distorting effect of target secondary structure on hybridization data.

We have also initiated studies, employing combinatorial chemistry and double printing with the Genometrix array printer, to develop a rapid way to screen many new hybridization surface coatings for improved kinetics. The goal is to develop a family of surface coatings which can be optimized to obtain the correct balance between kinetic increase, affinity and selectivity with respect to target binding. The feasibility of this double printing approach has been confirmed and a first candidate surface coating has been discovered which emulates the general attributes of the charged streptaviden surface. During the next month we will extend the surface exploration studies and will begin to test kinetic enhancement on the various 16S RNA targets which the UH lab has provided to Genometrix.

It is possible that the surface principles and surface chemistry which have been discovered thus far could prove to be a routine improvement to the design and fabrication of microarrays for 16S RNA mixture analysis. Thus, continuation of this work will be a high priority for the Baylor lab.

Instrumentation

A graphical user interface was developed for the microarray printer offering easier user control. Now the user simply chooses from a menu the particular microarray print pattern for microscope slide or microtiter plate formats. The interface also obviates the need to reprogram the system if the capillary position changes.

Hybridization of test rRNA samples isolated and purified at the UH lab were performed on the newly printed 8x5 NASA microarrays. Unfortunately, the hybridization SNR was low due to the high ELF background which swamped many of the weaker signals originating from the actual hybrids. The large ELF background was due to the double label positive controls which appear to have been printed at too high concentration (10μM). The experiment will be repeated with arrays reprinted with significantly low (concentration to reduce background and hence provide an opportunity to detect the hybrids.