LLNL MBARI ASTEP project update

4/6/2007

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Background

LLNL is working for MBARI on a NASA-sponsored project "An Environmental Sample Processor for Deep-Sea Seep and Hydrothermal Vent Applications" from the solicitation "Astrobiology Science and Technology for Exploring Planets" (ASTEP). The goal is to develop a polymerase chain reaction module for oceanographic microbiology in MBARI's ESP by 3/2008. The work areas are:

Task 1: Design the PCR module, 4/2006 – 9/2006.

Task 2: Fabricate and test a prototype, 10/2006 – 3/2007.

Task 3a: Fabricate and integrate production modules into the ESP, 4/2007 – 9/2007.

Task 3b: Support production modules, 10/2007 – 3/2008.

Summary of recent activity (3/2/2007 – 4/6/2007)

We completed assembly of the prototype ESP flow-through PCR module, electronics and software debugging, initial thermal and optical testing, integration with an APDS-style fluidics workstation (Figure 1), and ultimately demonstration of real-time PCR (Figure 2).

The successful reaction was a duplexed TaqMan PCR with only positive control DNA added. Channels 2 and 3 were loaded optically for TAMRA and show positive control PCR with a cycle threshold around 34. Channels 1 and 4 were loaded for FAM. Channel 1 shows that there is no optical cross-talk, but Channel 4 has an electronics problem. We have now met the single biggest milestone on this project. There was much rejoicing.

This has not been an easy month on ASTEP, and there has been a lot of debugging and hacking to get through the demonstration. We have come across items that we need to fix and items we would like to change.

We just sent a functional digital board to MBARI. This will allow testing the communications between the ESP core controller and the PCR module controller. There will certainly be some software changes, but those will have little or no impact on the communications.

We also worked with MBARI on some of the technology transfer issues like fluidic integration, methods of nucleic acid extraction, and the PCR recipes.

Upcoming activity

We will be doing extensive experimentation to characterize the prototype module and fix some known problems.

We need to schedule a CDR for late April or early May. The agenda items will include

- Data from the characterization of the prototype

- Known problems with the prototype and proposed solutions
- Additional potential redesign for the production version and risks
- Outstanding change request or interface control items
- Fluidics integration into the ESP
- PCR and extraction chemistry

It probably makes the most sense to have this at LLNL again, since this is where the PCR hardware is for now.

Schedule and budget

We are about two months behind schedule. As of 3/30/2007, the total spent was 374 k$ (81 k$ in March) and 75 k$ remained in the account. The Year 2 budget is 166 k$, so the spending rate needs to be greatly reduced. This will affect how many design changes can be pursued for the production module.

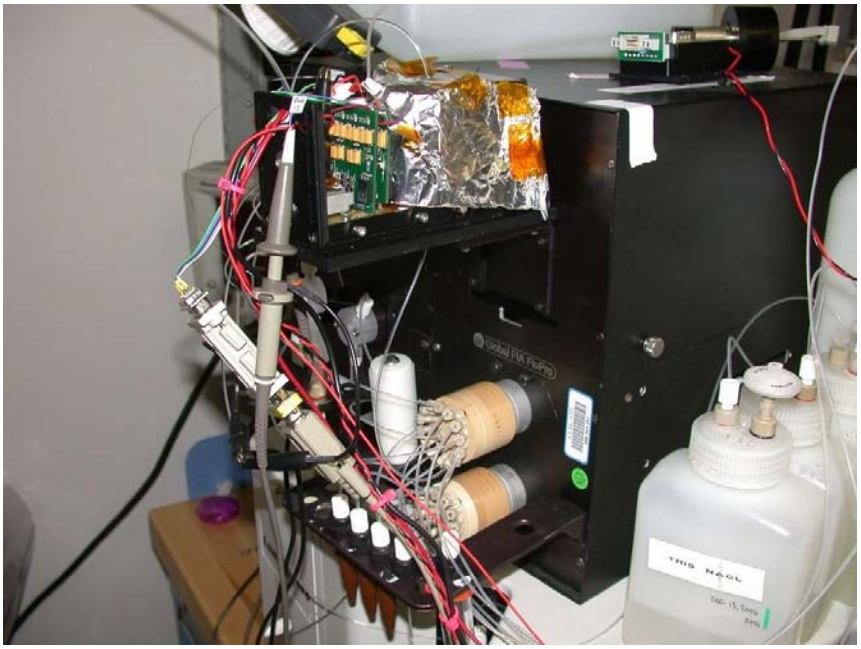

Figure 1. ESP flow-through PCR module integrated with an APDS-style (modified Global Flo-Pro) fluidics workstation. We had to fly some power lines in to the electronics boards and block light with foil because the cover is off. It isn't pretty, but it works.

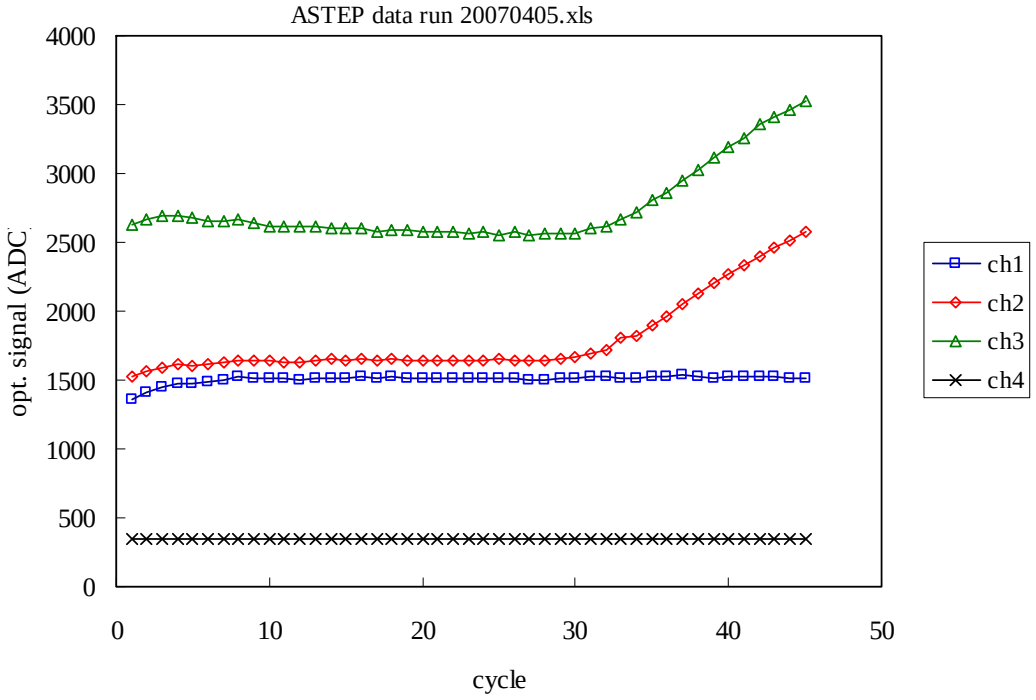

Figure 2. Optical data *versus* cycle number for the first real-time PCR demonstration in the prototype ESP flow-through PCR module, 4/5/2007. This was a duplexed TaqMan PCR with only positive control DNA added. Channels 2 and 3 were loaded optically for TAMRA and show positive control PCR with a cycle threshold around 34. Channels 1 and 4 were loaded for FAM. Channel 1 shows that there is no optical cross-talk, and Channel 4 has an electronics problem.