TWO

Anderson, Cynthia

From: Katz, Arthur [Arthur.Katz@science.doe.gov]
Sent: Thursday, September 13, 2001 10:18 AM
To: 'cynthia.anderson@ch.doe.gov'
Subject: Final Report

U. of Florida
DE-FG02-98ER62623

Dear Ms. Anderson,

Attached below is Dr. Trevor Hawkins final report. It is fully satisfactory.

Sincerely,

Arthur Katz

At the start of this award, it was decided to modify and focus the specific aims to better match those of the DOE Joint Genome Institute. The current goals for the three years of funding are:

1. Development of 1536 well based DNA sequencing platform. This will include small scale reaction set up, thermal cycling in a custom built cycler, and sample clean up prior to loading.
2. Integration of the device above into the CuraGen MicroNiagara system, PerSeptive BioSystems Voyager MALDI Mass Spec and validation of results from the ABI 377 DNA sequencer.
3. Collaboration with the JGI to build Sequatron-like systems for DNA purification and DNA sequencing. Also work with JGI to implement 1536 well system if required.
4. Demonstrate use of robotic systems through genomic sequencing of up to 1Mb DNA.

Over the past year we have focused on :

1. DNA purification methods. Two main projects were undertaken. The first was the development of the SPRI method to be performed in 384 well plates to replace the more traditional 96 well plates. This involved the design and construction of a 384 well magnet array and the testing of commercially available 384 pin plate washers. We have now been able to show the SPRI purification of PCR products in this format and hope to have a system ready for production in the second year of funding. This will not only increase the throughput but also decrease costs associated with the plastic plates used for the process. The second application was the use of SPRI for the clean up of sequenced products after thermal cycling and prior to electrophoresis. We have found the method to be ideal for the removal of dye terminators for both slab gel and more importantly for the clean up of samples prior to electrokinetic injection into the CE machines such as the MD MegaBACE. The advantage of the SPRI approach is that it removes the small primers as well as desalts the product prior to loading. We plan to have a fully operational protocol within the next few weeks.

2. Module Development: 1536 well thermal cycling, Mass Spectrometry, 96 lane development & Automated gel loading. In the first phase of our desire to construct a third generation Sequatron, we have focused on the development of modules that can be used on the new system.
1536 thermal cycler. One such device is a 1536 well thermal cycler. Using water as our thermal transfer media, we have constructed a prototype device which accepts the two commercially available 1536 well plates. The plates are sealed using a Marsh plate sealer and then places in a chamber into which water is fed. We have obtained even thermal heating and cooling with ramp temperatures slightly better than 1 degree per second. This prototype has now been used for the generation of PCR amplification products and we now plan to move ahead with its use in DNA sequencing.

96 Lane ABI system. The second project was a method for turning the ABI 377 to run 96 lanes without the expensive ABI upgrade. We noted that several

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years ago many of the genome centers were running the ABI 377 at 192 pixels but at 48 lanes. When ABI upgraded to the 64 lanes they increased the pixels to 388 but at this density it should have supported 96 lanes. One issue was the problem with loading 96 lanes into a gel that was 0.2mm thick. The combs were so fragile and the wells so small that this was a seriously difficult task to load and use in production. We decided to try using a wedge spacer similar to the ones used for radio-isotope gels but with the thicker end at the top of the gel. We had the Gel Company (San Francisco, CA) to make us a spacer that could be used on the ABI 377 that was 0.4mm at the top and 0.2mm at the bottom. We then had a 0.4mm 96 lane comb made which was on the normal 9mm spacing. The extra thickness of the comb and the fact that the wells were now 0.4mm thick meant that this was far easier to load than even the old 48 lane gels. The system worked fine and is now in production at several sites and being sold by the Gel Company.

MALDI TOF Mass spectrometry. The third project was the use of MALDI TOF mass spectrometry for the analysis of DNA. We focused on developing methods and approaches that would enable us to use the speed and low cost of the mass spec but for DNA analysis in genomic sequencing projects. To date we have developed methods for single and two base extension from custom or standard primers as well as the development of DNA sequencing with reads in the 30-40base length. We are now scaling this up and are planning to re sequence sub clones that have been sequenced on the ABI machines as a test of this method and approach.

Automated Gel loading system for the ABI. One final project has been the development of an off platform gel loading and pre-running system. We have built a prototype using a cavro XYZ arm and a custom made gel rig that holds the ABI cassette at a 20 degree angle from vertical. This angle increases the area that the tips have to hit in order to load the sample. In addition, we used thin flexible tips which aided loading.

3. Helping the JGI with Sequatron-like systems. In an effort to help the JGI, we have sent drawing and timing schedules to the R&D group at the JGI PSF. In addition, we have be helping and advising on the number and type of modules used in a future system.