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LLNL-TR-740340

Development of DNA Pillar Chip Final Report CRADA No. TSB-2035-01

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October 20, 2017

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This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Development of DNA Pillar Chip

Final Report
CRADA No. TSB-2035-01
Date Technical Work Ended: November 21, 2001

Date: November 20, 2001 Revision:

A. Parties

This project was a relationship between Lawrence Livermore National Laboratory (LLNL) and Tetracore.

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B. Project Scope

This was a collaborative effort between The Regents of the University of California, Lawrence Livermore National Laboratory (LLNL) and Tetracore, to demonstrate a proof of principal device for the capture and controlled release of DNA moving within a flow stream.

This project consisted of the following three tasks and deliverables:

Task 1: Fabrication (LLNL)

A cut *F. tularensis* plasmid will be used as the target DNA sample. A micromachined columnar array will be used to broadly capture the DNA sample in solution. The captured DNA sample will then be eluted from the chip and quantitated by TaqMan assay. The quantity of DNA will be compared to the amount spiked by quantitative DNA, and this will determine the efficiency of the techniques. The protocol will be advanced to Task 2, if it is able to produce a minimum ten-

fold concentration of the target. If it is unable to do so, it will not be advanced to Task 2. (Month One to Month Two)

Task 2: Device Testing (LLNL/Tetracore)

The micromachined columnar array will be tested against "dirty water" spiked with autoclaved F. tularensis culture material based on the specimens evaluated in Task 1. All testing is to be done at Tetracore.

The efficiency of the capture techniques will be evaluated by quantitative PCR. The quantity of captured DNA will be compared to the amount spiked. The protocol will be advanced to Task 3 if it is able to produce a minimum ten-fold concentration of the target. If it is unable to do so, it will not be advanced to Task 3. (Month Two)

Task 3: Data Analysis (LLNL/Tetracore)

The method developed in Task 2 will be evaluated against autoclaved *B. thuringiensis* cultures spiked into dirty water. The protocol will be accepted if it is able to produce a minimum ten-fold concentration of the target. If it is unable to do so, it will not be accepted.

The micromachined columnar array technology is proposed as an early step in the process of capturing DNA from environmental samples. It is a method designed to nonspecifically capture DNA as a preparative step for either hybrid capture or other technology. (Month Two to Month Three)

LLNL will generate a report summarizing the results of this evaluation. (Month Two to Month Three)

Deliverables:

1. LLNL will deliver multiple chips to Tetracore.

2. LLNL will deliver a report summarizing the results of the project evaluation to Tetracore.

3. LLNL and Tetracore will submit a Final Report due upon completion of the project.

Project Dates: September 7, 2001 through December 7, 2001. The technical work for this project was completed ahead of schedule.

C. Technical Accomplishments

Two wafers consisting of 64 pillar chips with integrated heaters were successfully fabricated and tested. A chip fixture, based on patented LLNL fluidic interconnects, was assembled to harbor the chip and allow sample and reagents to be passed through the chip. The chips were demonstrated to extract and concentrate microbial genomic DNA from relatively large volumes of pure water and wastewater. The work provided confirmation to the sponsor that LLNL has

the capability to fabricate these chips. Most importantly, the sponsor is now interested in funding a new phase of the project to pursue new concepts. In addition, other government agencies are now interested in the technology.

D. Expected Economic Impact

No immediate economic impact is anticipated. Further chip development is required to decrease the cost and make the fluidic process automated. This project will help increase the detection of various bio/chemical warfare agents through the DNA signature.

E. Partner Contribution

Tetracore provided the published information describing the chip, technical advice for mask and fixture design, and all reagents and samples for biotesting. Tetracore designed the biotesting experiments and executed the biotesting. Biotesting consisted of running samples through the chip, collecting the purified nucleic acids, and subjecting the nucleic acids to real-time PCR analysis. Tetracore provided the biological expertise to interpret the data.

F. Documents/Reference List

Reports

The results were submitted as progress reports to the US government agency sponsoring the research.

Copyright Activity

No copyright activity resulted from the work performed under this CRADA.

Subject Inventions

No Subject Inventions resulted from the work performed under this CRADA.

Background Intellectual Property

No Background Intellectual Property was declared for this project.

G. Acknowledgement

Participant's signature of the final report indicates the following:

1) The Participant has reviewed the final report and concurs with the statements made therein.
2) The Participant agrees that any modifications or changes from the initial proposal were discussed and agreed to during the term of the project.
3) The Participant certifies that all reports either completed or in process are listed and all subject inventions and the associated intellectual property protection measures generated by his/her respective company and attributable to the project have been disclosed and included in Section E or are included on a list attached to this report.
4) The Participant certifies that if tangible personal property was exchanged during the agreement, all has either been returned to the initial custodian or transferred permanently.
5) The Participant certifies that proprietary information has been returned or destroyed by LLNL.

3 Dec 2001

Dr. Gary W. Long, Vice President — Date
Tetracore

Kevin Ness — 12-09-01

Kevin D. Ness, Principal Investigator — Date
Lawrence Livermore National Laboratory

Karena McKinley — 1/16/03

Karena D. McKinley, IPAC Director — Date
Lawrence Livermore National Laboratory

Attachment I – Final Abstract

Attachment I

Final Abstract

The goal of this CRADA was to design and evaluate a silicon chip based system for purification of nucleic acids form aqueous specimens. High surface-area micromachined silicon chips and microfluidic pump interfaces were designed, manufactured and tested. Aqueous solutions containing either autoclaved *Francisella tularensis* cultures or DNA plasmids containing a *Bacillus thuringiensis* gene sequence were treated with a binding solution and passed through the chips. DNA was eluted and quantitated using probe hydrolysis polymerase chain reaction assays for the respective targets. The results demonstrated that the chip bound DNA from solution and that this DNA could be eluted and analyzed directly by PCR without further processing or purification steps. The chips easily handled as much as five ml of sample and concentrated the DNA target by as much as 25 to 50 fold.