

## **Title: Evaluation of the Diversilab System for Typing *Escherichia coli* by rep-PCR**

**Project Chief:** Don Stoeckel

**Project Support:** Chris Kephart

**Cooperators:** Internal pilot project

**Project Duration:** October 2003 through March 2004

### **Introduction and Problem**

Repetitive DNA sequence-based polymerase chain reaction (rep-PCR) has been used to type *Escherichia coli* for tracking sources of fecal contamination to environmental waters. In our application, the rep-PCR typically used (as described in Dombek and others, 2000) has the following characteristics: throughput of about 100 isolates per week; 25% failure to produce adequate PCR fragment patterns; data reproducibility limited to about 90% pattern-to-pattern similarity. An alternative protocol, the Diversilab System, developed and operated by Bacterial Barcodes, Inc., Houston, Texas, claims to be speedy, more reliable, and have better reproducibility compared with the traditional protocol. We were able to obtain a 6-month loan of a Diversilab system for comparison of these two protocols.

### **Goals and Objectives**

The goal of this pilot project was to determine throughput, reliability, and accuracy of data generation from the Diversilab system for comparison with the traditional rep-PCR protocol.

- Enable collection of data with enhanced resolution
- Reduce need for sample reruns
- Build microbial source tracking capability

### **Approach**

1. Obtain training on the Diversilab System from Bacterial Barcodes
2. Certify operators of the system using the Bacterial Barcodes certification panel
3. Measure data reproducibility by comparing data for a single isolate
  - Same DNA extract, same PCR reaction, same chip
  - Different DNA extract, same PCR reaction, same chip
  - Same DNA extract, same PCR reaction, different chip
  - Different DNA extract, same PCR reaction, different chip
  - Same DNA extract, different PCR reaction, different chip
  - Same DNA extract, different PCR laboratory, different chip
4. Confirm reproducibility results using different isolates

The results of this evaluation are currently being reviewed and will be written into an internal summary memo. Based on these results, a decision will be made on whether to purchase the Diversilab system for use in our laboratory.

The system is being evaluated for application in a limited microbial source tracking study as described in the project "Relation of Septic-System Construction and Site Characteristics to Shallow Ground-Water Quality in Ohio."

*Written 12 January, 2004 DMS*