EPA NEW ENGLAND
REGIONAL LABORATORY

ENVIRONMENTAL
SCIENCE FACT SHEET

POLYMERASE CHAIN REACTION

INTRODUCTION

Almost one quarter of the 2,840 listed polluted waters of New England do not meet water quality criteria for bacteria. Traditional microbial test methods limit state, federal and/or tribal regulators' ability to implement appropriate and timely control measures and/or to assess human health risk. Traditional methods only identify and quantify fecal pollution in water. New analytical tools are needed to identify fecal pollution sources. The polymerase chain reaction method is just such a tool, providing an innovative way to assess microbial water pollution.

The goal of this method is to develop rapid, routine, and cost effective regional analytical capability to discriminate between fecal pollution sources in fresh and marine waters. To meet this goal, the New England Regional Laboratory (NERL) is developing state-of-the-science rapid, quantitative, library-independent Real-Time Polymerase Chain Reaction (PCR) methods to differentiate and quantify human and non-human sources of fecal pollution.

WHAT IS PCR?

PCR is a repetitive biochemical technique that mimics the natural cellular process of Deoxyribonucleic Acid (DNA) replication for synthesizing copies of genetic material from either DNA or Ribonucleic Acid (RNA). DNA, present in all organisms, whether plant or animal, is made up of genes comprised of unique sequences of nucleotide bases. These unique base sequences are used to identify a specific organism, a species or group of related species, or a particular strain within a species. In three cyclically repeating steps, **Denaturing, Annealing,** *and* **Extension**, *PCR amplifies or makes copies of targeted fragments of the DNA. After 30 to 40 of these repetitive cycles, copying each of the copies produced in the previous cycle, millions of copies of the replicated nucleic acid are produced.*

The product of PCR is called **amplicon** *and* **Real-Time PCR** *measures the amount of amplicon being copied by labeling the DNA strands with a fluorescent marker and measuring, in real time, the change in sample fluorescence as the copies are produced.*

Each of the three steps of the PCR process is controlled by heating and/or cooling the PCR reaction. EPA's Regional Lab currently uses a high-speed thermocycling instrument, controlling the repetitive process and both detecting and quantifying the targeted products of interest.

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PARTNERS & RELATED PROGRAMS

- EPA New England, Boston, MA
- EPA Office of Research & Development, Cincinnati, OH
- USGS
- UMASS/Boston, Dept. of Biology
- UMASS/Amherst, Dept. of Civil Engineering
- MA Dept. of Environmental Protection
- MA Water Resource Authority
- MA Dept. of Conservation & Recreation

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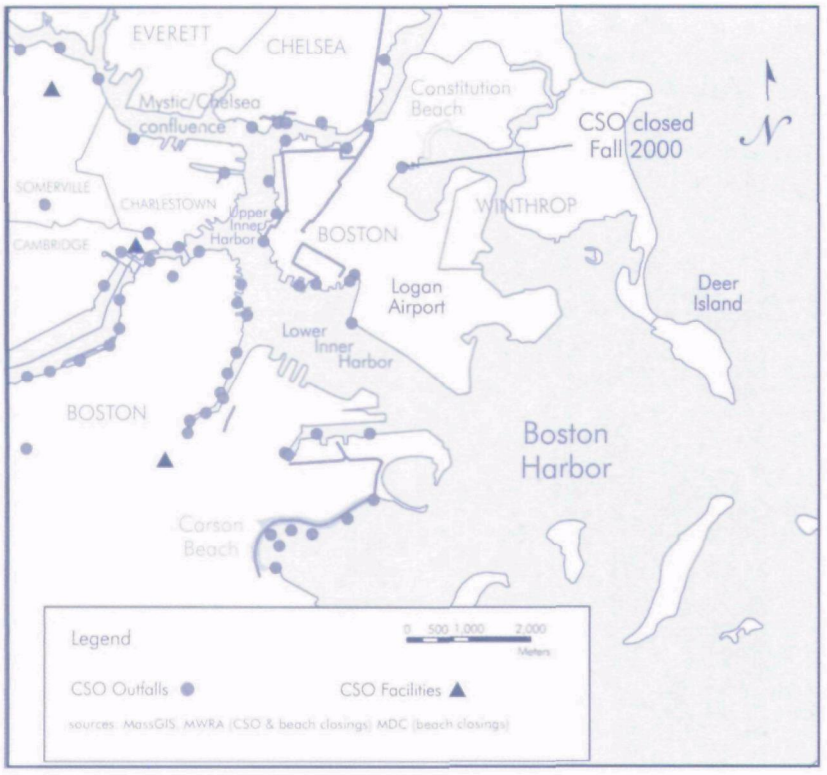
Environmental Forensics

Like phenotypic tests where colored or sheening colonies grow on agar plates allowing identification and enumeration of bacteria, genotypic PCR techniques amplify portions of gene sequences to enable quantification and identification of the original DNA from a specific progenitor organism, or strain of an organism. Thus, PCR allows analysts to test whether or not samples contain DNA from known sources, asking questions such as "Is this the suspect's DNA?" or "Does this person have a specific disease?" or "Is the fecal pollution in a water sample from human waste?"



Progress to Date

In 2003, NERL established and validated three different Real-Time PCR techniques to analyze water samples from two **urban beaches** *(Carson and Wollaston) in Boston and Quincy, MA for bacterial indicators; a hydrolysis probe technique for E. coli, a hybridization probe technique for E. coli, and a hybridization probe technique for Enterococcus.*

These ongoing Total Maximum Daily Load-funded projects focused on removing PCR inhibitors from environmental samples and rapid sample processing using NERL's DNA extraction and purification robotic instrumentation. The automation enables relatively high sample through-put and precise handling of environmental DNA samples as compared to manual sample processing techniques.

What's in the Future

Future efforts will build upon previous PCR accomplishments to develop a Real-Time PCR F+RNA Coliphage quantitation and differentiation procedure. Applied research and development in 2004 will include field assessments conducted primarily on Charles River water samples collected between Waltham and Boston, MA, in dry and wet-weather conditions. Viral PCR results will be compared to more traditional membrane filtration and MPN techniques.

The goal of this method is to develop rapid, routine, and cost effective regional analytical capability to discriminate between fecal pollution sources in fresh and marine waters.

Microbial Source Tracking Background

Bacterial F+Coliphage viruses (BCV) are an excellent indicator of viral contamination in both marine and fresh waters and can more effectively differentiate between human and animal sources of fecal contamination than simple E. coli- based techniques. Application of NERL's BCV methods will determine simultaneously the relative contribution of human versus non-human fecal contamination from different sources and indicate the potential for human viral pathogen contamination in water bodies.

Key Factor

Regulators must exercise caution when applying new methods to environmental questions. Until now regulators have not had the ability to discriminate between specific sources of fecal pollution and must be careful to determine how best to interpret the PCR signal derived from human and non-human pollution. In addition to developing these analytical techniques, the New England Regional Laboratory will work with its PCR program partners to learn how best to apply this new technology to solve environmental problems.