



Emerging Technologies in Sample Analysis

4 April 2002

New England Bioterrorism Preparedness Workshop
MIT Lincoln Laboratory

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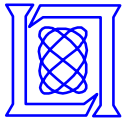
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Outline

- **Current techniques in sample analysis**
 - Clinical (subject of yesterday's talk)
 - Environmental
- **Challenges associated with environmental sampling**
- **Examples of technologies in use and in development**

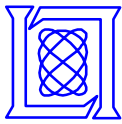


CDC's Sample Analysis Guidelines

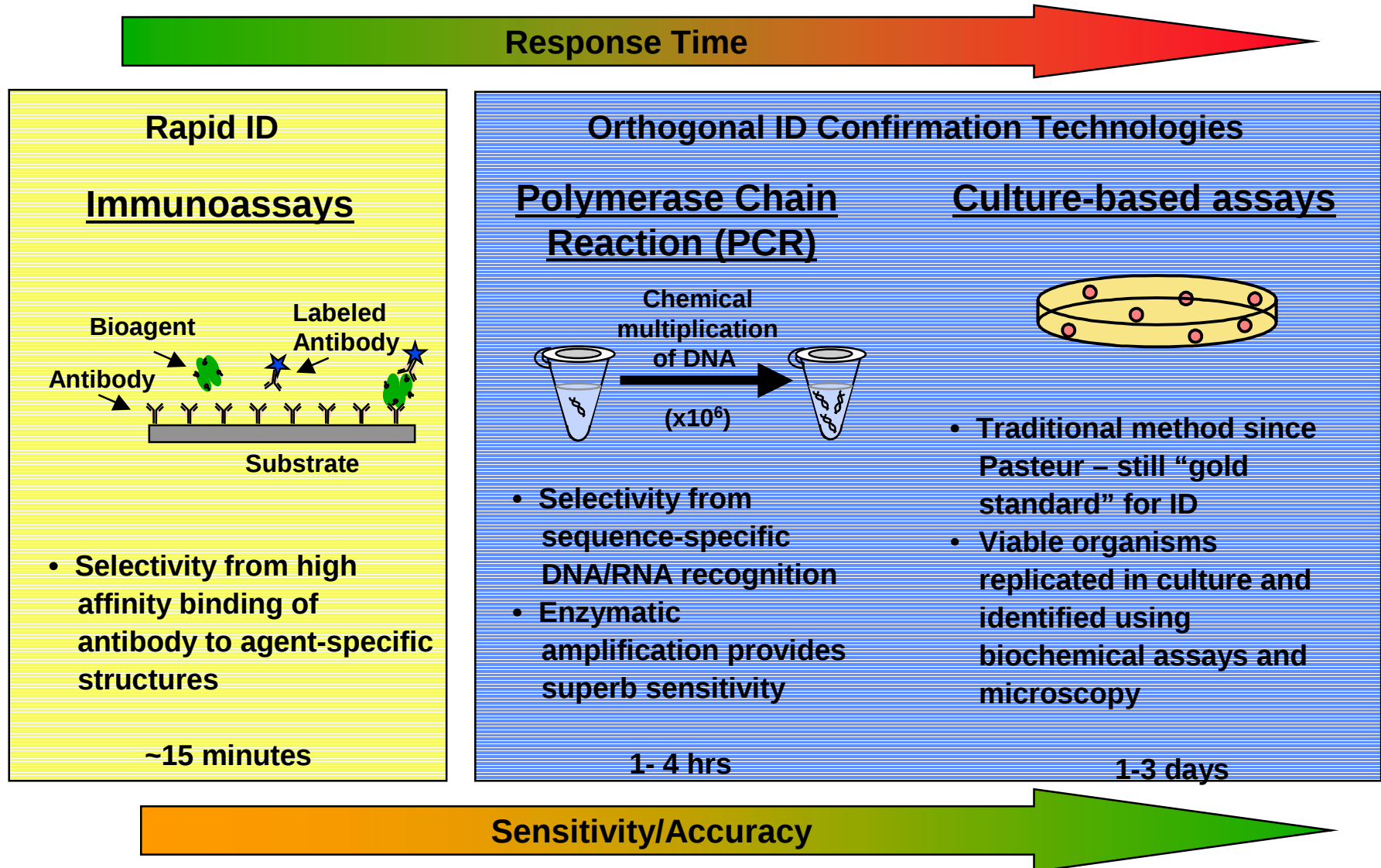
(example: *B. Anthracis*)

- **Persons suspected of exposure/infection**
 - Cultures of blood and spinal fluid
 - Cultures of tissues or fluids from affected areas
 - Microscopic examination
 - PCR
 - Nasal swab (occasionally for exposure, but not for diagnosis)
 - Antibody testing (exposure, not validated for diagnosis)

- **Environmental contamination**
 - Cultures of air samples, surface swabs, suspicious powders
 - Microscopic examination of suspect material
 - Evaluation of growth properties of suspect agent
 - PCR
 - DFA (direct fluorescent assay) to detect key bacterial proteins
 - Specialized tests, such as immunoassays (SMART)



How Do These Techniques Compare?





Examples of In-use and **Developmental** Immunoassay Devices



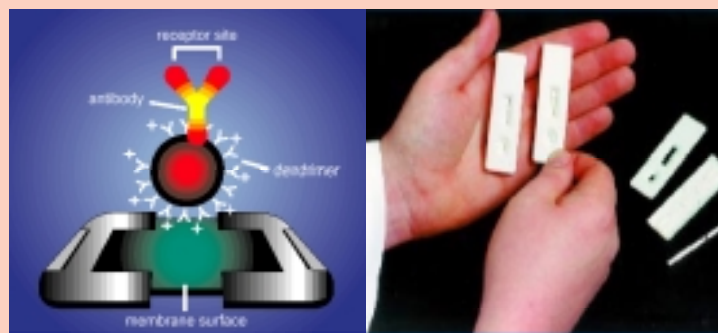
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**Ticket cartridges and reader
for lateral-flow immunoassay
in Joint Biological Point Detection System
(JBPDS)**

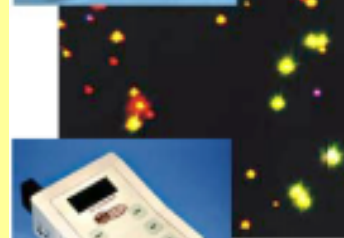


**Response Equipment Co.
Bio-HAZ Biodetector**



Dendrimer-Based Alert Ticket (ARL)

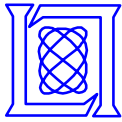
Disposable Test Strip



Prototype Design

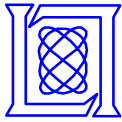


**Upconverting
Phosphors (SRI)**

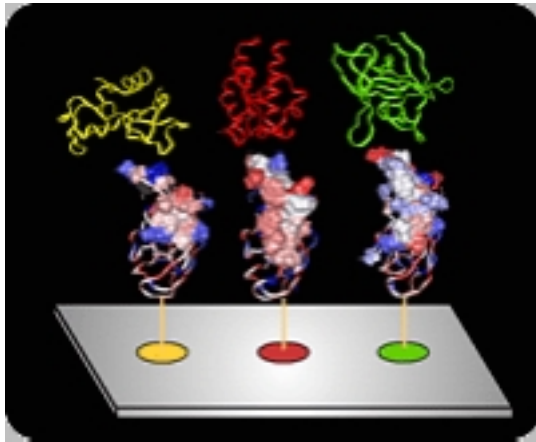


Features of Immunoassay Analysis

- Can be used on environmental samples with little or no preparation
- Readout is fast (~ 15 minutes) and simple (colorimetric or fluorimetric)
- Sensitivity modest (~10,000 - 100,000 particles)
 - Depends on antibody-antigen binding affinity and readout scheme
- Specificity reasonably good
 - Depends on antibody construct and antigen specificity
- Current IAs are not multiplexed; development of protein microarrays may lead to sensitive, multi-assay analysis tools



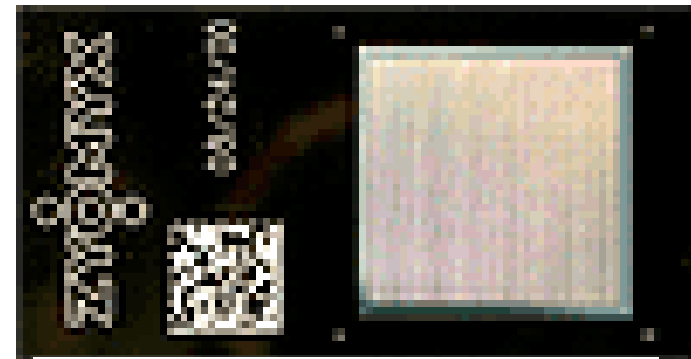
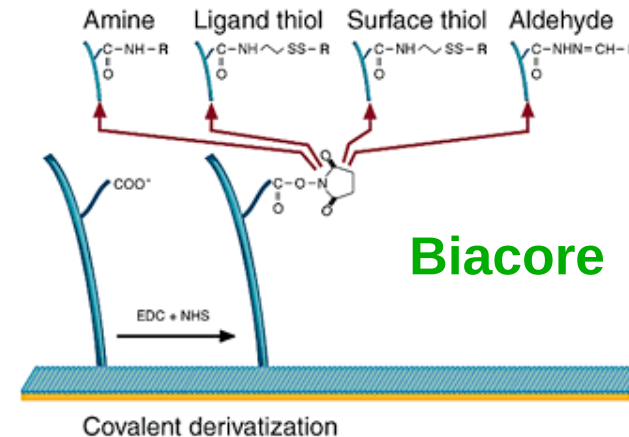
Examples of Existing Protein Microarrays



Phylos (2000 element)

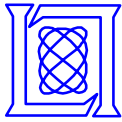


Ciphergen (multiple classes of proteins)



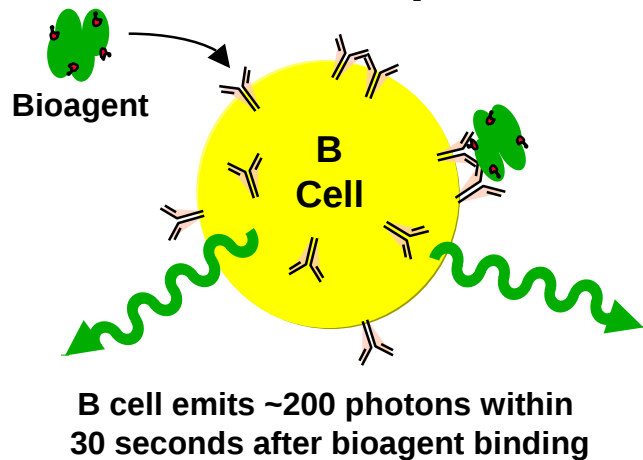
Zyomyx (10,000/cm²)

- Protein microarray technology development driven by drug screening and disease-marker investigations
 - Diagnostics (clinical and environmental) still developmental



Developmental Antibody-Based Sensor: CANARY

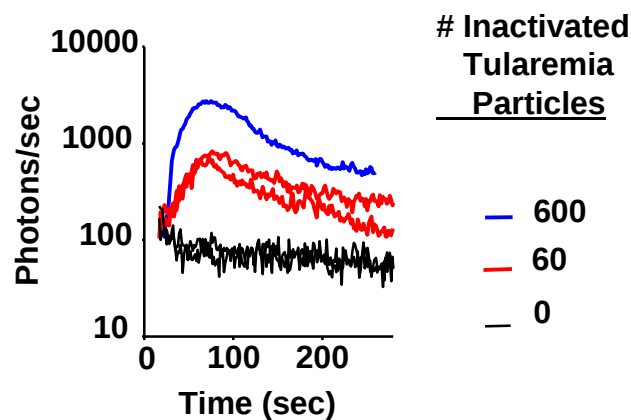
Concept



Prototype microcentrifuge device



Tests Against Killed Tularemia (Collab. with NMRC)



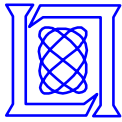
Status of B-Cell Lines

Complete

FMDV
VEE
Vibrio cholera
Orthopox viruses
Yersinia pestis
Brucella spp
Francisella tularensis

In development

Coxiella burnetti
Bacillus anthracis
E. coli O157:H7



PCR-Based Analysis Tools

- **Systems being developed (and deployed) that provide agent ID within 30 minutes of introduction of prepared sample**

Semi-automated field-portable
PCR devices



RAPID - Idaho Technologies



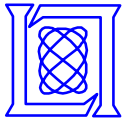
***SmartCycler
XC System - Cepheid***

Example of handheld PCR
device

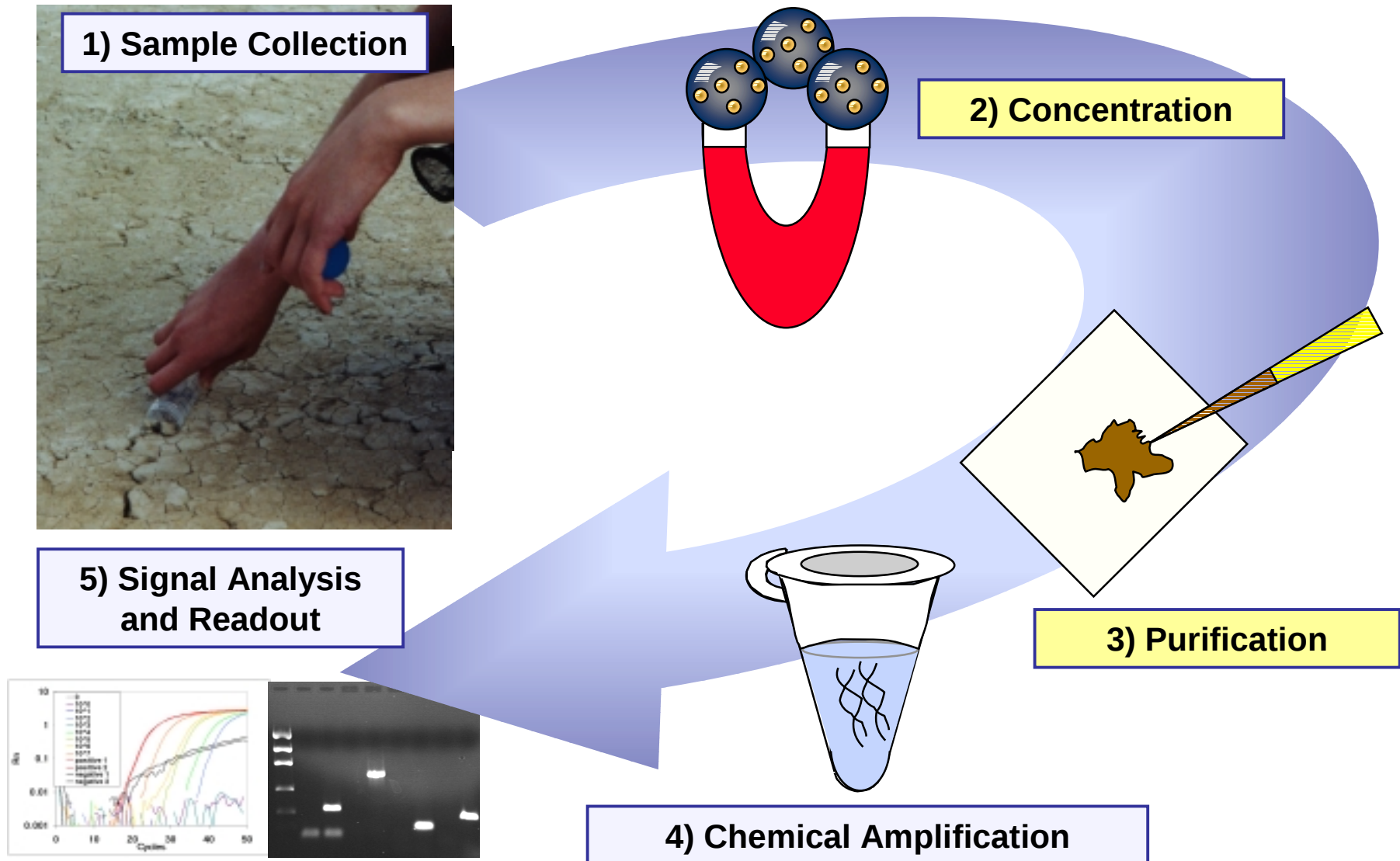


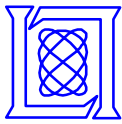
***HANAA - Handheld Nucleic
Acid Analyzer, developed by
LLNL, Cepheid, and ETG, Inc.***

- **Challenge remains in automating sample preparation and analysis**
 - Pathogen cells or spores must be ruptured to liberate the DNA/RNA
 - DNA/RNA must be separated from protein debris/environmental impurities

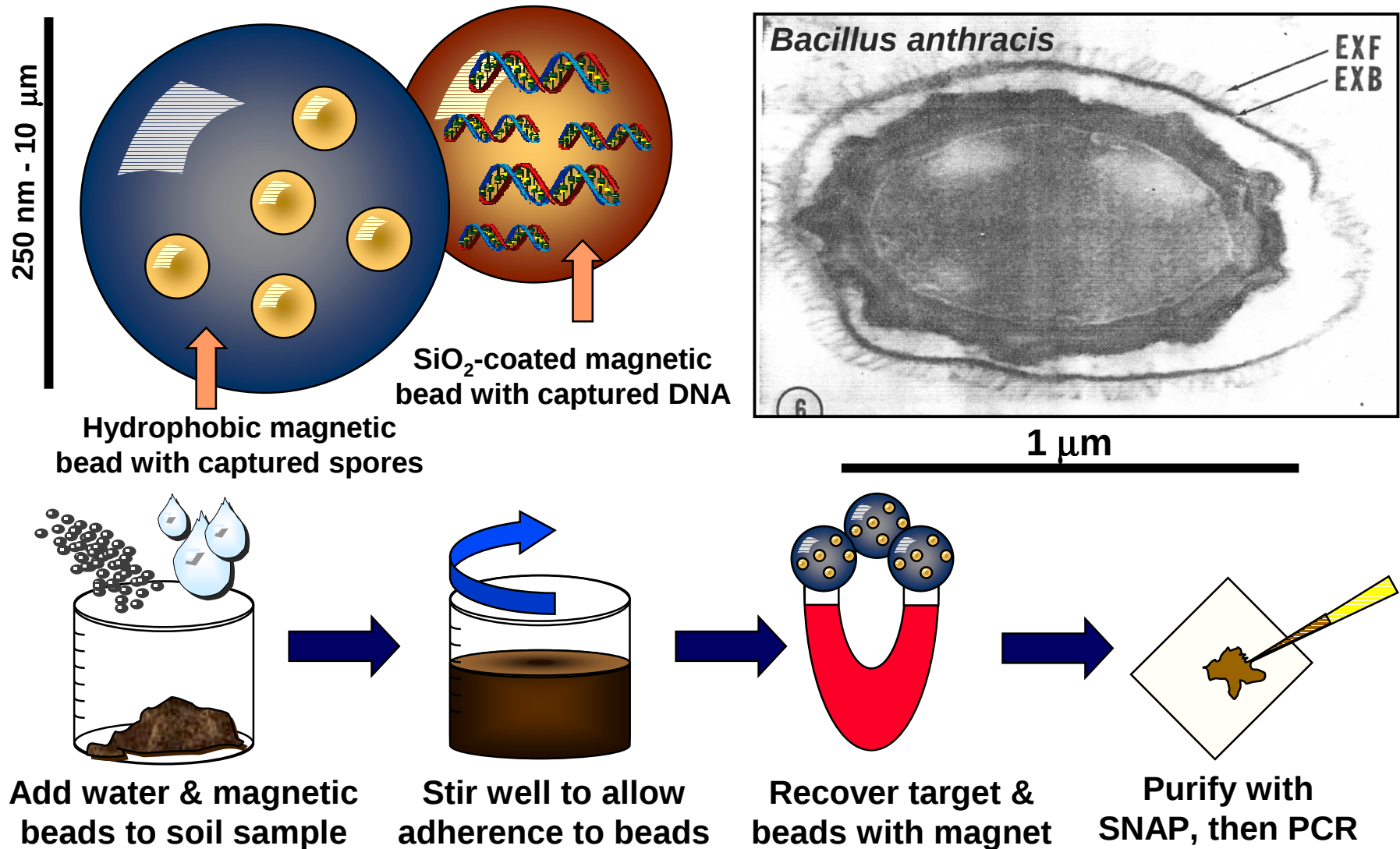


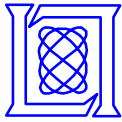
Overview of Sample Preparation





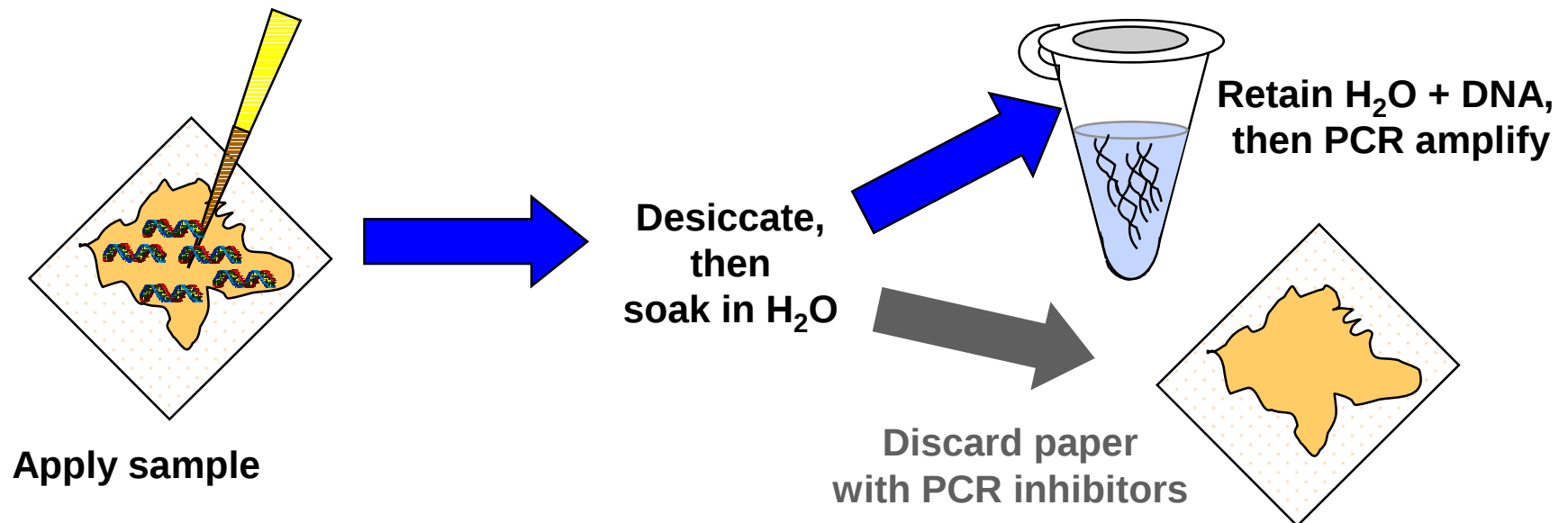
Target Concentration: Affinity Magnet Protocol





DNA Purification: Simple Nucleic Acid Prep (SNAP)

- Chemically treated paper is the key component of SNAP
- Lyses cells, binds PCR-assay inhibitors, and purifies DNA
- **Advantages:**
 - Fast and easy (1/5th the time of other published protocols)
 - Water is only added reagent (no phenol, chloroform, or alcohol)
 - Lightweight, compact, enables archiving
 - On-site fixation: preserves DNA & kills pathogenic organisms

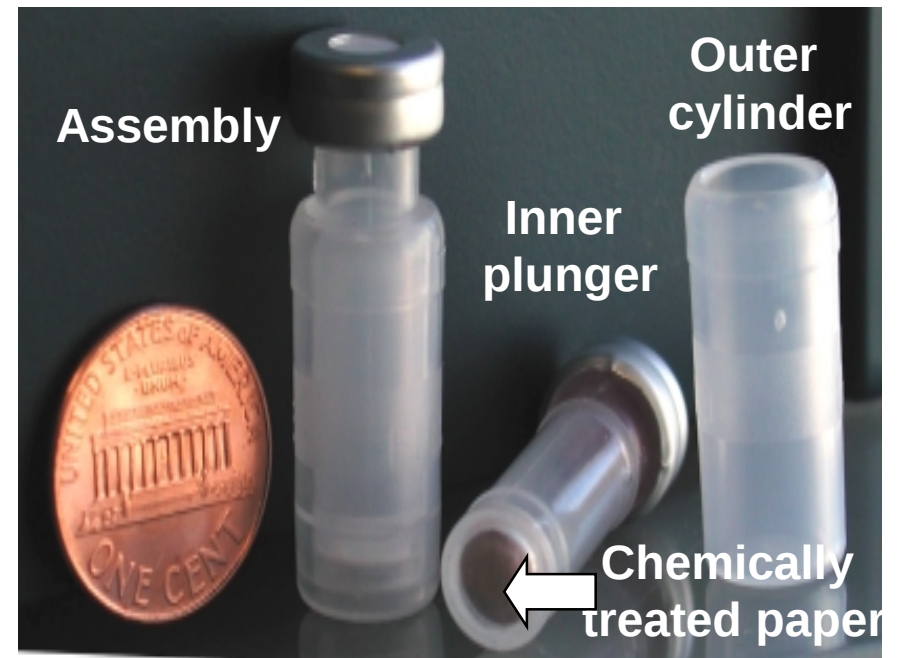




Lincoln Interim Nucleic-acid Kit (LINK)

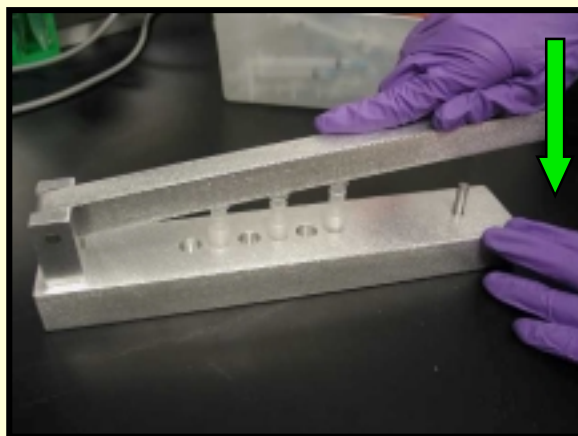
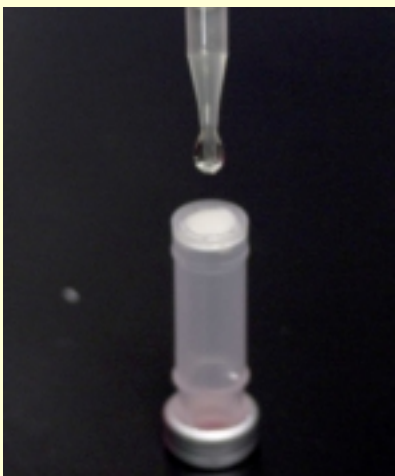
(Developed in response to October 2001 events)

- **LINK as a solution:**
 - Incorporates SNAP paper but in a more user-friendly format
 - Faster processing than basic SNAP
 - Easier to sample, handle, and process
 - Enables on-site fixation
 - Outside can be decontaminated
 - 6 minute processing time
 - Single-step processing
 - Results equal to or better than basic SNAP





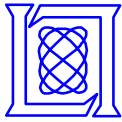
How to Use LINK



1) Apply sample
Sit for 5 minutes

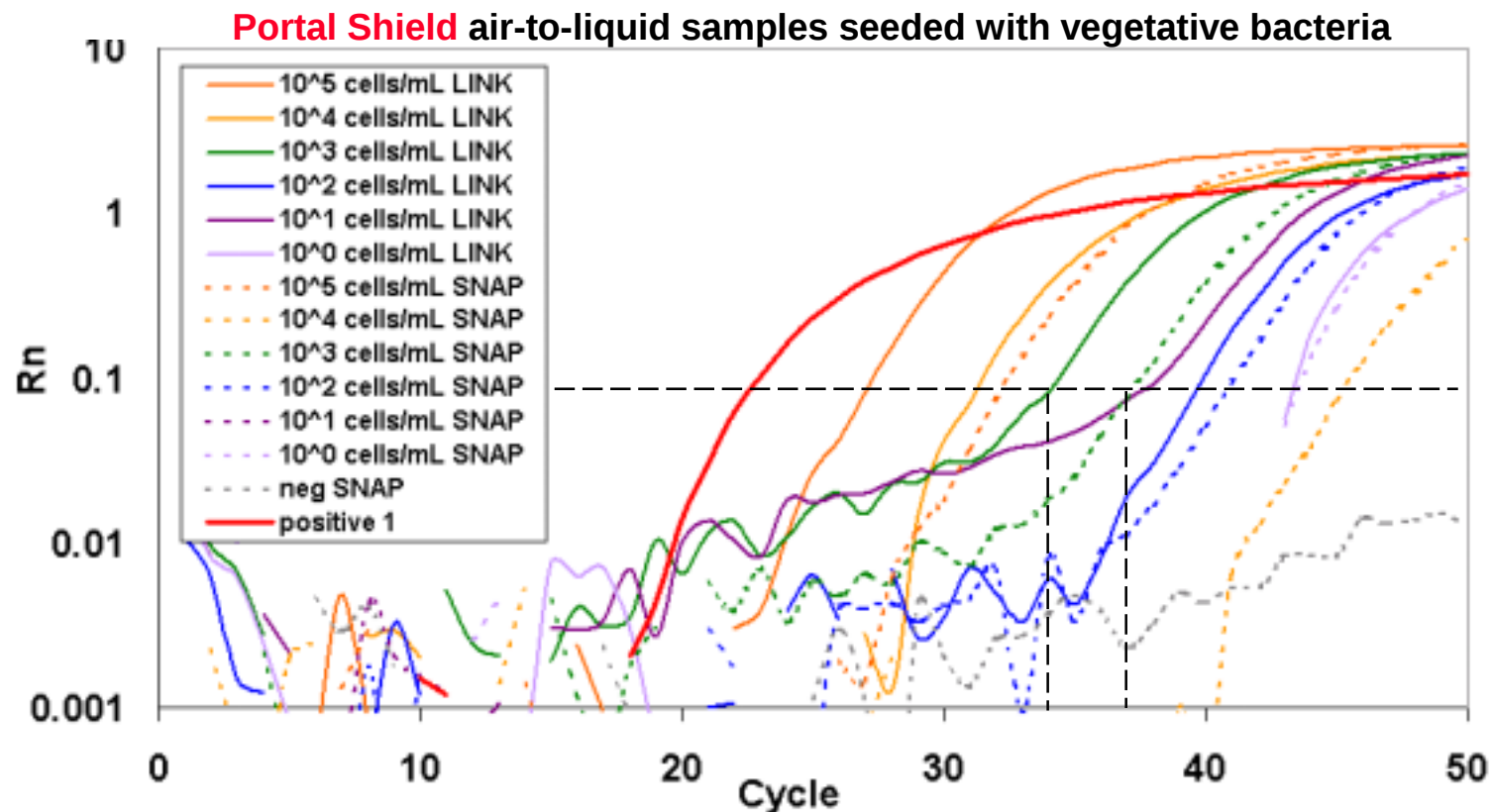
2) Process in **one step**

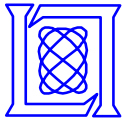
3) Remove DNA
Total time ~6 minutes!



LINK Cartridge Works with Varied Samples

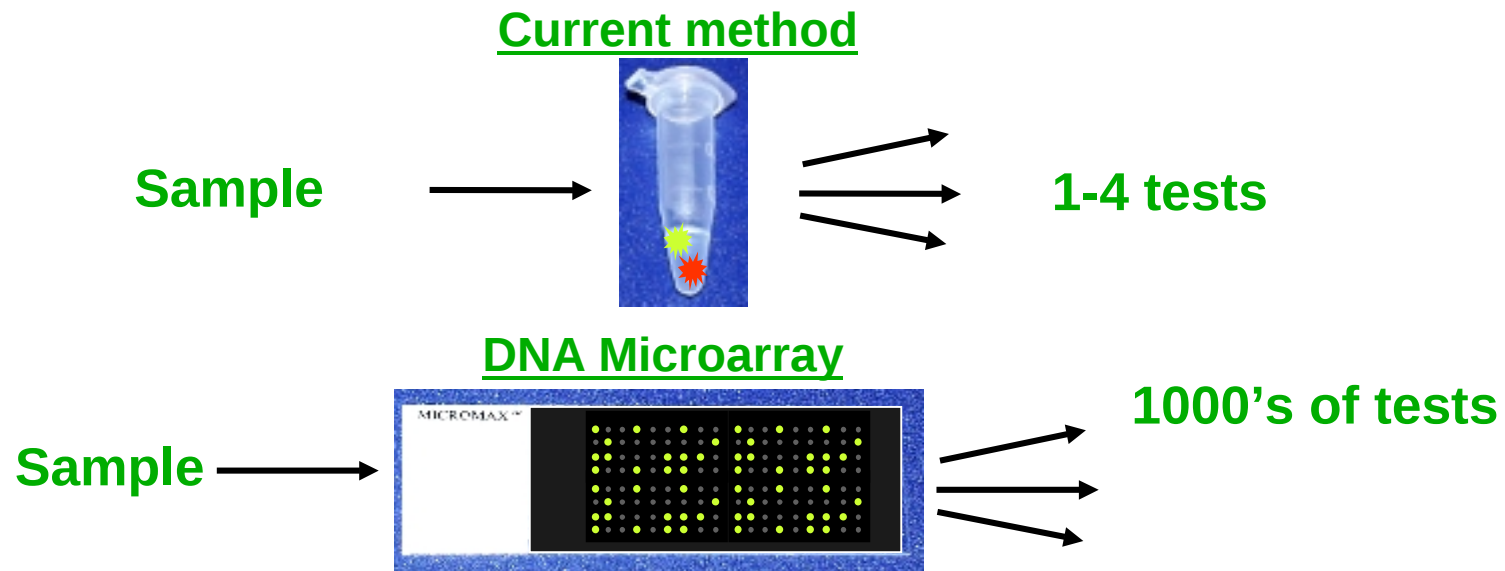
- LINK detection from:
 - **Portal Shield** air-to-liquid samples seeded with vegetative bacteria
 - Untreated domestic sewage (Boston) seeded with vegetative bacteria
 - Paper, envelopes, skin seeded with bacterial spores
 - Air impaction with dry bacterial spores





What About DNA Microarrays?

- DNA Microarray: Any 2D or 3D substrate having many ($\sim 10^2$ - 10^5) different nucleic-acid capture sites (probes)
- Can identify both strain and drug resistance of pathogens
- Can offer highly multiplexed assay capability



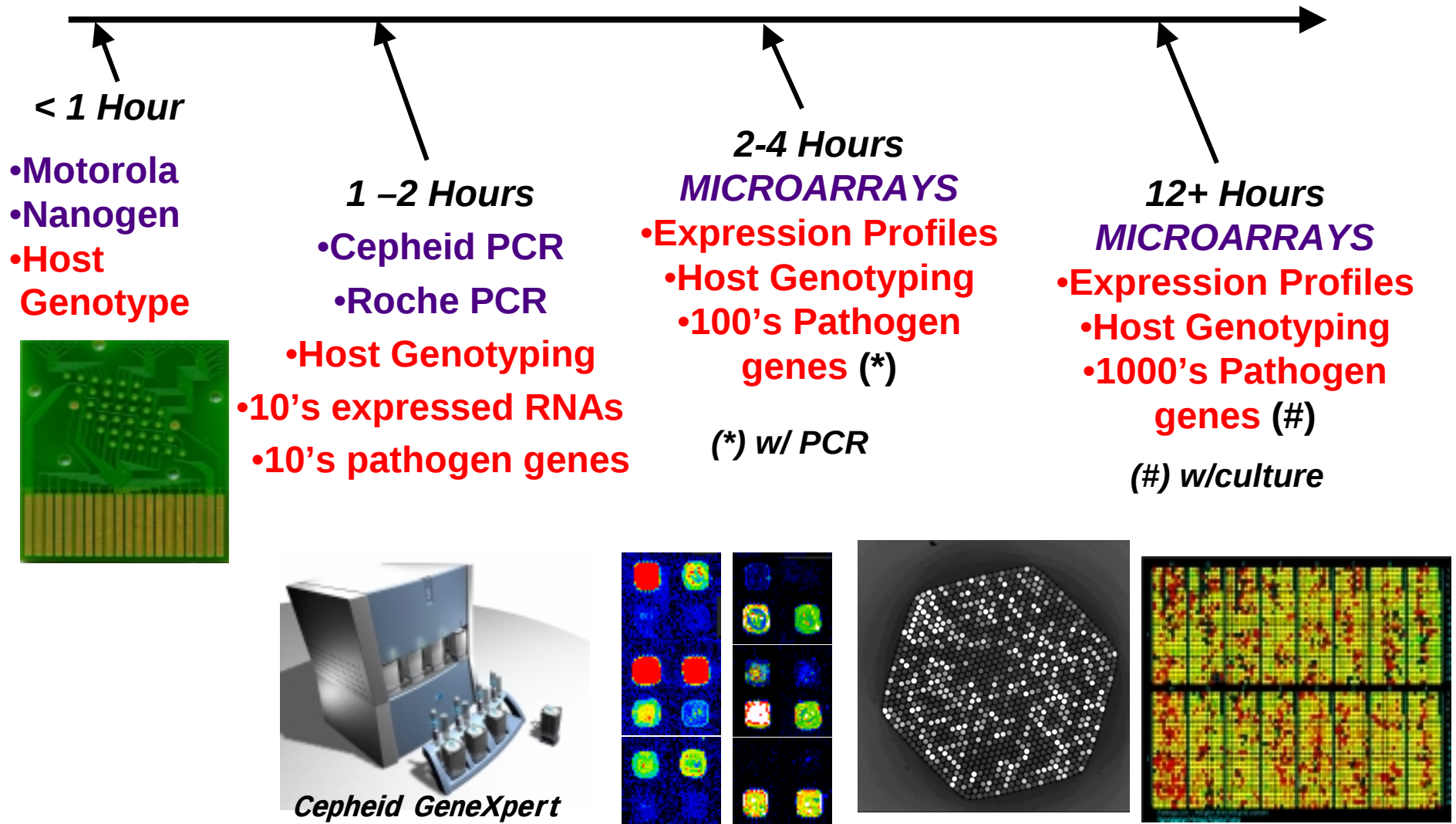


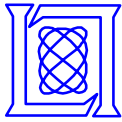
Pathogen Identification via DNA Microarray

- Detect small amounts (<100 copies per ml) of pathogen-specific nucleic acids in environmental sample
- Arrays might provide log orders more information than current PCR-based approaches (e.g. TaqMan)
- Challenges for diagnostic applications:
 - Never demonstrated for environmental (or clinical) samples
 - Amplification may be necessary before micro-array assay
 - Sample preparation required (as in PCR techniques)



Assay Times for Current and Emerging PCR/DNA Systems





Summary

- **Environmental sample analysis parallels methodology developed for clinical sampling**
 - Immunoassays for rapid estimate of exposure (not yet CDC authorized)
 - PCR techniques being deployed in some laboratories to provide strain specificity and drug resistance
 - Culture still used to provide “gold standard” for pathogen ID
- **New technology developments could greatly increase the speed, sensitivity, and multiplicity of environmental assays**
 - Protein microarrays could offer highly multiplexed, rapid ID capability on collected samples
 - DNA microarrays could offer hundreds to thousands of pathogen tests on single-chip format, provided sample preparation can be made compatible