

Emerging Technology and Forensic Microbiology



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Attribution: Our Job

- **From Various Dictionaries**

- **Attribution noun/adjective**

- **at tri bu tion :**

1: the act of “attributing”; the ascribing of a work to a particular author or artist.

2: an ascribed quality, character, or right; an attribute.



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Interagency Collaboration



National Strategy for Countering Biological Threats

National Security Council
November 2009

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Interagency Collaboration



National Research and Development Strategy for Microbial Forensics

Implementation Plan

National Science and Technology Council

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National Research and Development Strategy

- **Goal 1: Develop a strategic microbial forensics research agenda that will produce a national microbial forensic capability that is ultimately capable of high confidence, robust detection, characterization and comparison of biological agents in forensic samples.**
- **Goal 2: Promote interagency communication, coordination and information sharing on microbial forensics research and development efforts.**
- **Goal 3: Develop effective interagency education and training on microbial forensics designed to inform policymakers and scientific and technical personnel.**



Forensic Microbiology

The Challenge is Microbial Diversity

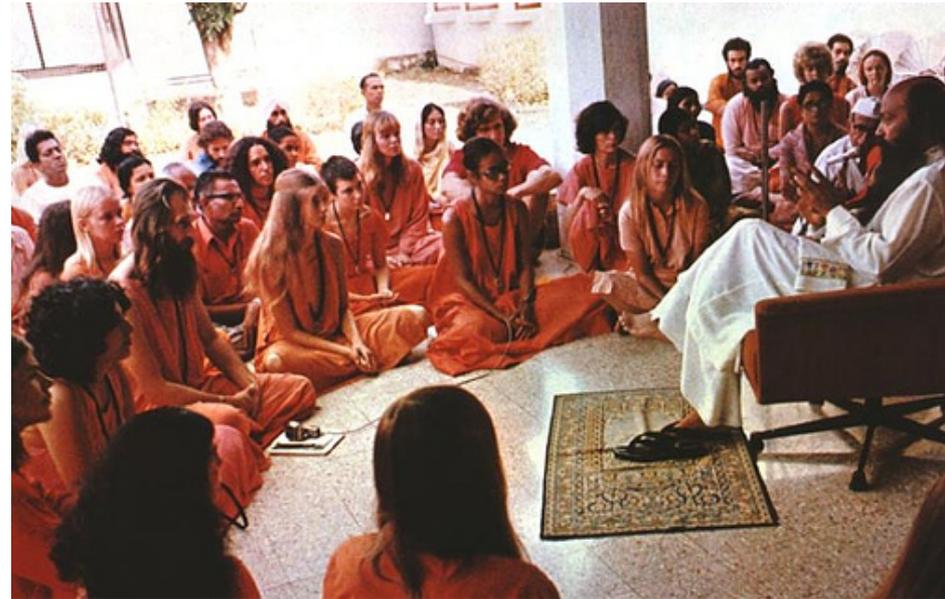
- Basic Microbial Genetics:
 - Bacterial Diversity
 - Mixed Microbial Communities, Competition
 - Transformation
 - Conjugation
 - Transduction
 - Mobile Genetic Elements
 - Random Mutations
 - Viral Diversity
 - DNA genomes, RNA genomes (+, -)
 - Co-infections, recombination
 - Replication errors
 - Host Ranges – multiple host species?
 - Immune pressure



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Salmonella Attacks: 1984 The Dalles Oregon

- 2 members of the Bhagwan Rajneesh Ashram Spiked Salad Bars at 10 Area Restaurants using *Salmonella enterica ser. Typhimurium*
- The attack was an attempt to disrupt local elections. The Town and Ashram were at odds with each other.
 - Over 750 people became ill.



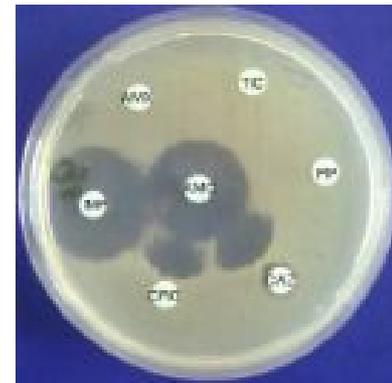
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Microbial Epidemiology in 1984

- Biochemical Phenotyping
- Salmonella Serotyping
- Antibiotic Susceptibility Testing



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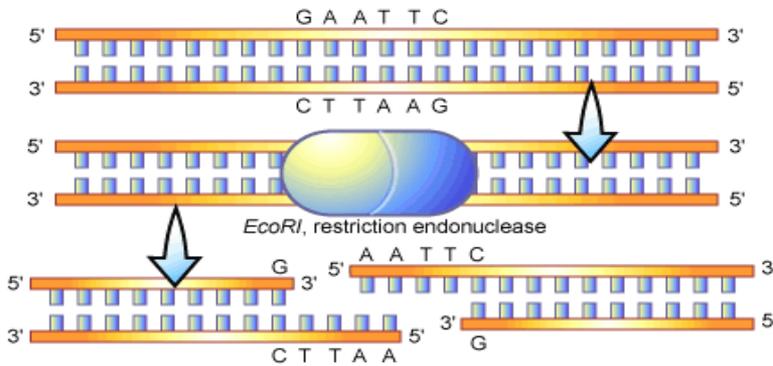
Molecular Genetic Techniques 1980's

- Restriction Endonuclease Enzymes
- Polymerase Chain Reaction (PCR)
- DNA Hybridization Techniques

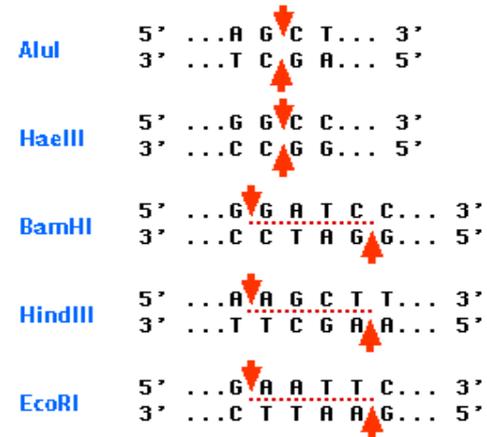
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Restriction Endonuclease Typing



<http://biotech.ubc.ca/MolecularBiology/RestrictionEndonucleases>



AluI and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RestrictionEnzymes.html>



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Pulsed Field Gel Electrophoresis PFGE

- In PFGE, first described in 1984, the polarity of the electrodes is switched back and forth, so that the molecules are pulled first forward, then back. The timing is such that the net movement is forward. introducing a new way to separate DNA.
- In particular, PFGE resolved extremely large DNA for the first time, raising the upper size limit of DNA separation in agarose from 30-50 kb to well over 10 Mb (10,000 kb).

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Pulsed Field Gel Electrophoresis PFGE

- PFGE is commonly used in epidemiological investigations.
 - Food born outbreaks
 - Nosocomial infections
 - Antibiotic resistance tracking
 - Public Health PulseNet
 - Forensics

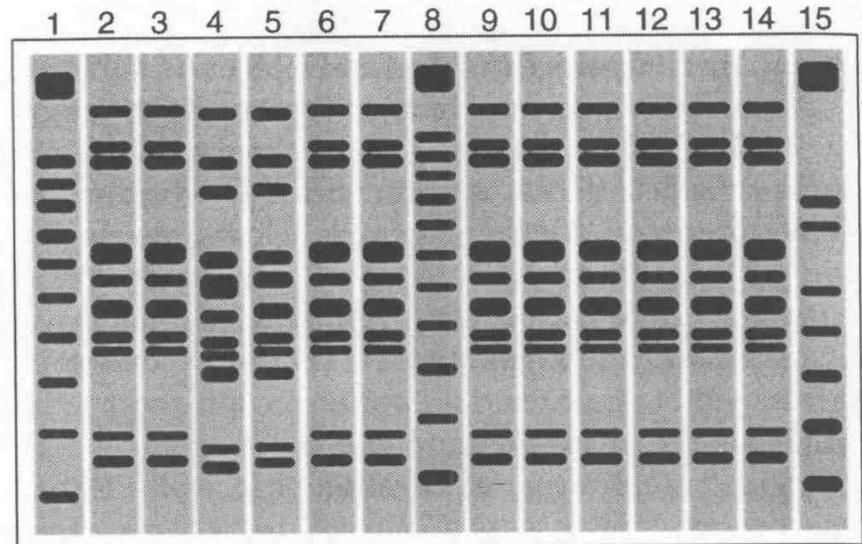
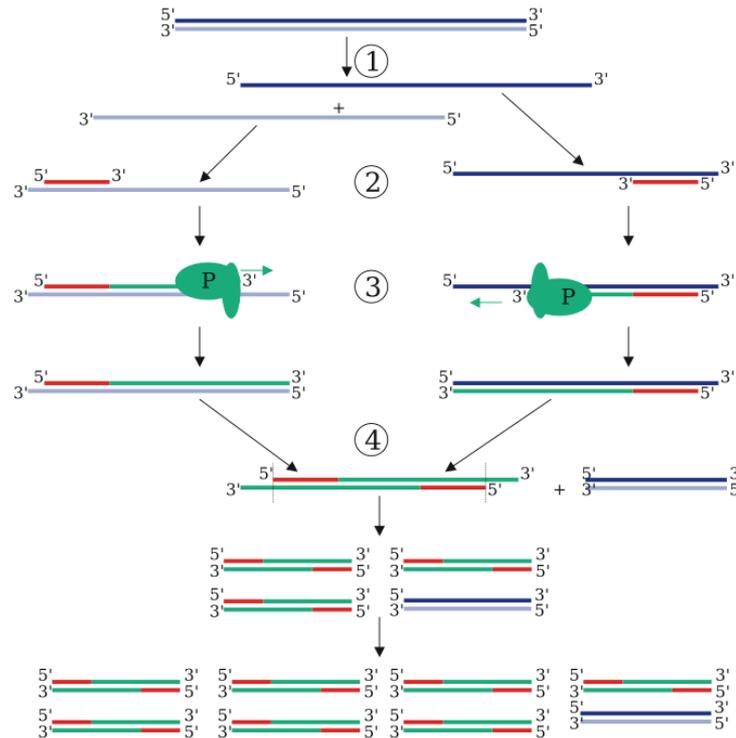


Figure 3–6 PFGE fingerprints of *S. javiana* isolates from a suspected food-borne outbreak. Digestion of the DNA with *Xba*I generated several large fragments of DNA. Lanes 1, 8, and 15, standard DNA markers; lanes 4 and 5, *S. javiana* unrelated to the outbreak; lane 2, isolate from leftover chicken sandwich; lanes 3, 11, 12, 13, and 14, samples from patients; lanes 6, 7, 9, and 10, isolates from food handlers. (Reprinted with permission from R. Lee, J. Peppe, and H. George, *J. Clin. Microbiol.* 36:284–285, 1998.)



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Genotyping by Polymerase Chain Reaction (PCR)

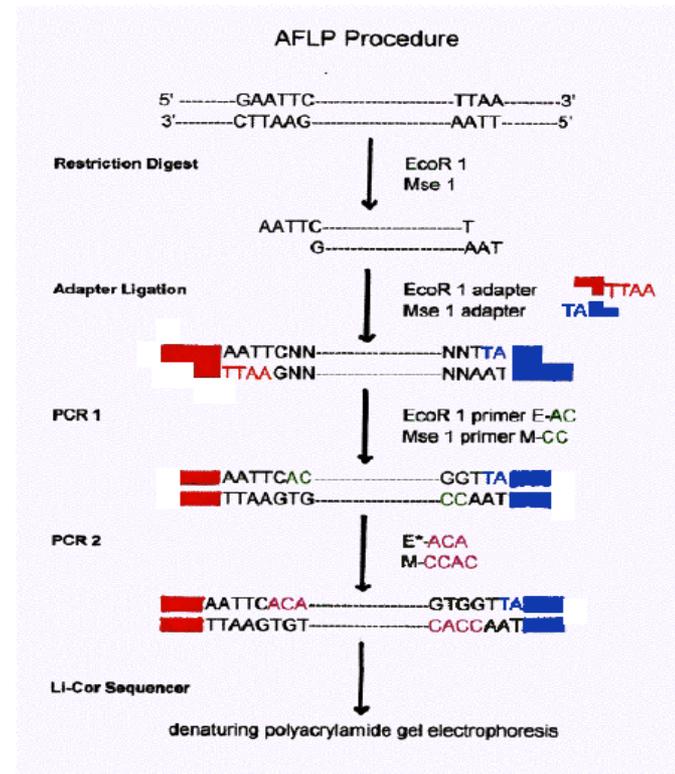


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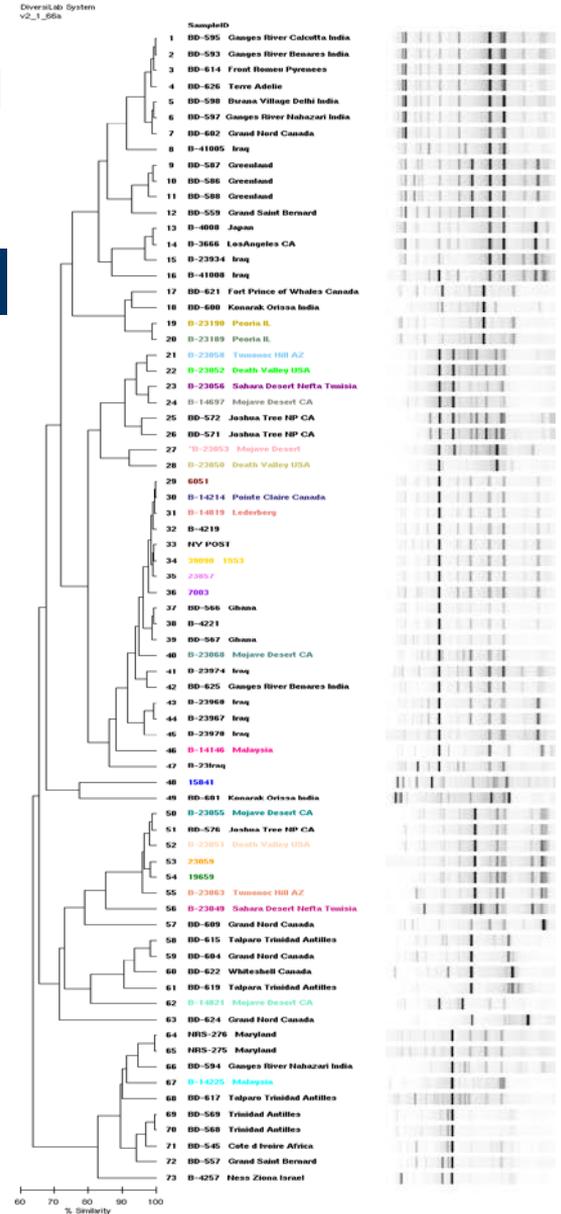
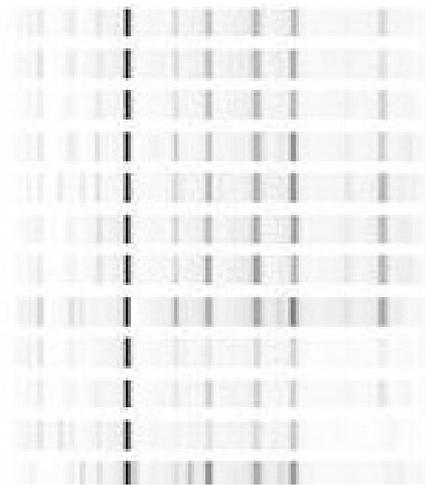
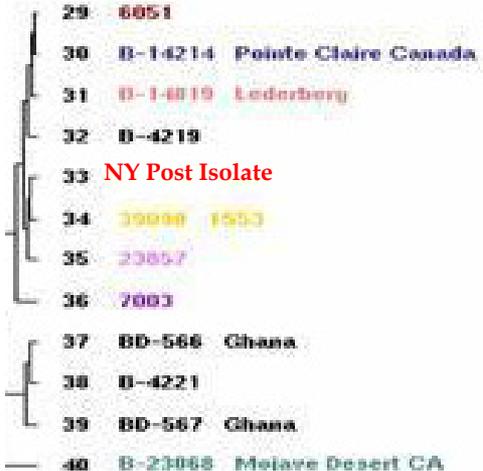
Amplified Fragment Length Polymorphisms (AFLP)

- Relies on sequence differences reflected in restriction sites.
 - Restriction cut DNA
 - Add PCR Linkers to specific ends of DNA
 - PCR amplify fragments using primers specific to the linker sequences.
 - Analyze by electrophoresis





Repetitive Element PCR Typing





Genotyping *B. anthracis* using VNTR sites

- Keim, P. et al. Molecular diversity in *Bacillus anthracis*. J Appl Microbiol. 1999 Aug; 87(2):215-7.
- Used to differentiate *B. anthracis* strains.

Fingerprinting a killer

Having identified all 5.1 million "letters" of genetic code inside the bacterium that causes anthrax, scientists hope to use genetic "fingerprinting" techniques to trace the origins of the spores used in last fall's terrorist attacks.

Part of the circular DNA strand of *Bacillus subtilis* (a close relative of the anthrax bacterium, whose genome is not yet published) is shown. Each bar represents one gene.

Geneticists are learning how to differentiate among similar strains and isolates of the anthrax bacterium by looking at "variable number tandem repeats" (VNTRs) on the bacterium's DNA. DNA is a long molecule made of four kinds of subunits, abbreviated A, T, C and G. VNTRs are repetitive patterns of those subunits that are unique to a particular strain or bacteria.

Variable Number Tandem Repeats

The "Sterne" strain of the anthrax bacterium contains the pattern CAATATCAACAA four times in a row in one part of its DNA:

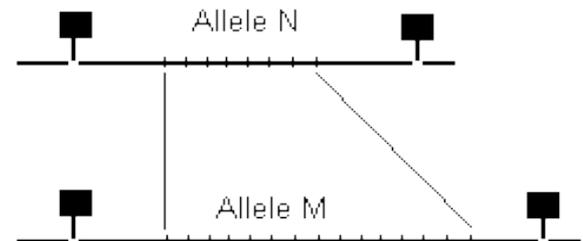
...GTTACGG CAATATCAACAA CAATATCAACAA CAATATCAACAA CAATATCAACAA GACT...

The "Vollum" strain of the anthrax bacterium contains the same pattern only twice in a row in the same part of its DNA:

...GTTACGG CAATATCAACAA CAATATCAACAA GACTAGGACTTAATAATAACGTAGGTCA...

Image courtesy of the Institute for Genomic Research

Washington Post

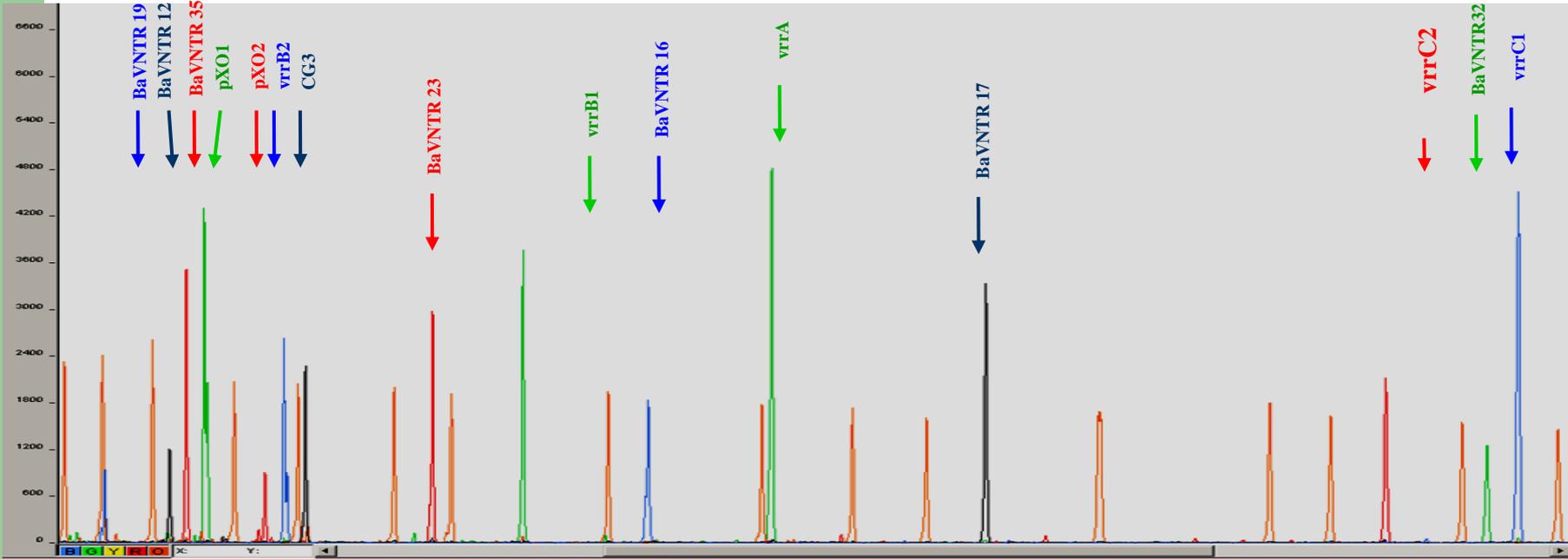




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MLVA-15

A 15 locus 4-color Genotyping System



B. Anthracis Ames Strain

P. Keim Lab, NAU

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MLST: Sequence Level Discrimination at Multiple Loci Across the Genome

MP B.s. groEL align.doc - Microsoft Word

File Edit View Insert Format Tools Table Window Help

Final Showing Markup

100% Courier New 10

23857_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

6051_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

7003_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B14214_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

B14819_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

B23068_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGC CTTGCAACAC

B23967_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B23968_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B23974_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B23978_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B3666_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B4008_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B4219_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

B4221_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC TACAC

BD566_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

BD567_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

BD625_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

Query_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGCACTTGC AACAC

B14821_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGC TCTTGCTACAC

B23189_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGC TCTTGCTACAC

B23190_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGGCGAAGC TCTTGCTACAC

BD545_groEL 1 AGCAAGGCAAACCATTGCTTCTGATCGCTGAGGATGTTGAAGCTGAAGCTCTTGCTACAC

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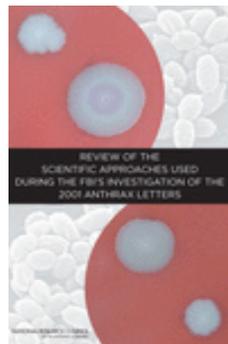
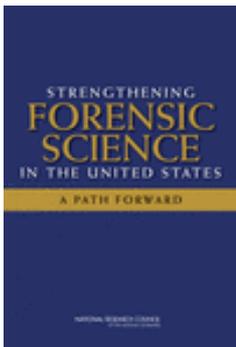
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Public and Scientific Interest

Doubts Persist About FBI's Anthrax Investigation 10 Years Later Amid New Bio-Terror Concerns

By Catherine Herridge, FoxNews

Published October 18, 2011



Expert Panel Is Critical of F.B.I. Work in Investigating Anthrax Letters

By SCOTT SHANE

Published: February 15, 2011

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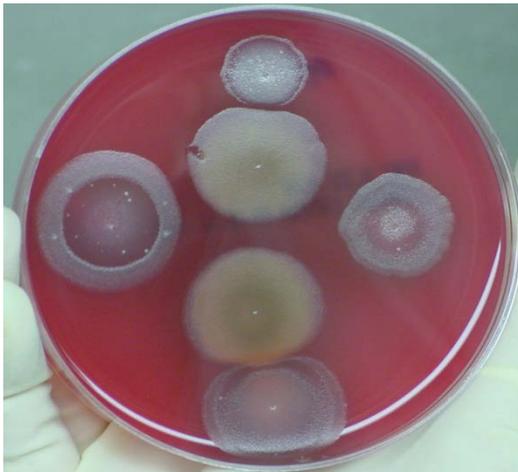


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Basic Microbiology Examinations



- Isolation of Contaminating Microorganisms



- Isolation of Phenotypic Variants of Threat Agent.

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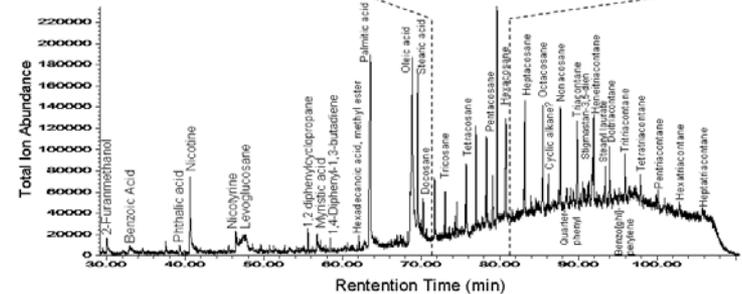
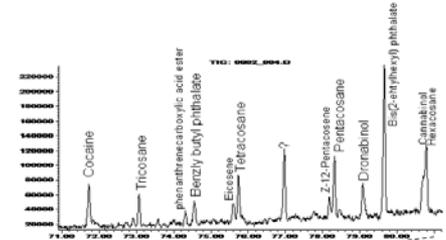
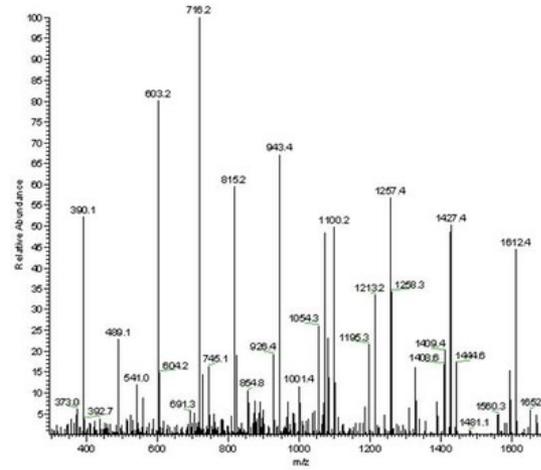
Evaluation of Production Methods: Potential Effects Upon Evidence Characteristics

- Growth – Agar, Broth Fermentation, Media, Growth Conditions (Temp, time, pH)
- Harvest – Filter, Centrifugation, Density Gradients
- Processed – Detergents, Buffers, Water
- Drying – Lyophilize, Air/Oven, Spray Dry, Acetone or other Solvents
- Sizing – Jet Mill, Grinding, Mortar & Pestle
- Additives – Flow Enhancers, Stabilizers



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Other Analytical Methods



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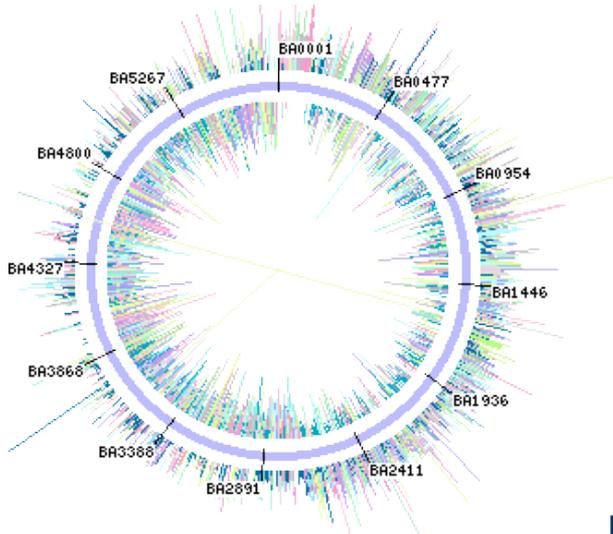
Gas Chromatography/Mass Spectrometry, Spectroscopy, Other Methods?

- Proteomics, Lipids, other Analytes...
- Potential for information from samples when the target is partially degraded or denatured
- Other physiochemical properties related or unrelated to the biological agent

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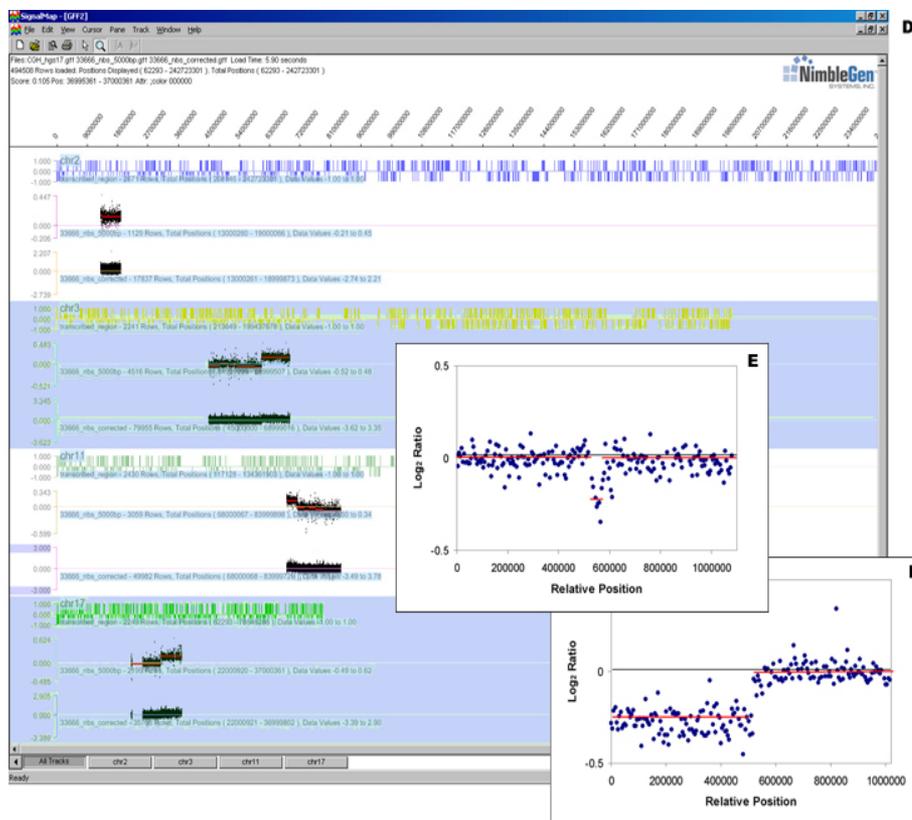
Microbial Genetics/Genomics



- Evidence Specific Microbial Signatures:
 - phenotypic variants
 - mixed cultures
- Genomic Characterizations:
 - Genotyping
 - Sequence Analysis
 - Comparative Genomics
 - Assay Development



Mutation Discovery and Analysis



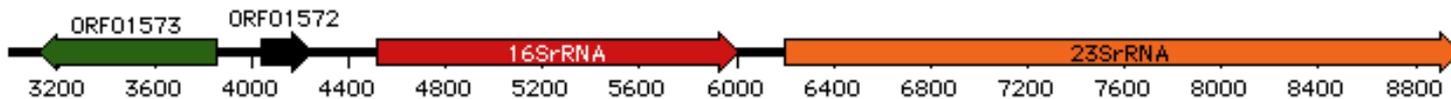
- Insertions
- Deletions
- SNP's
- Genome Rearrangements
- Modifications



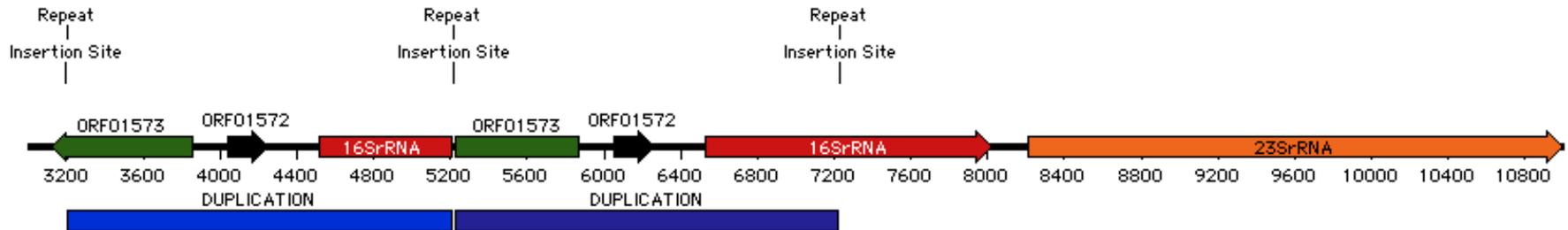
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B. anthracis Mutants from Amerithrax

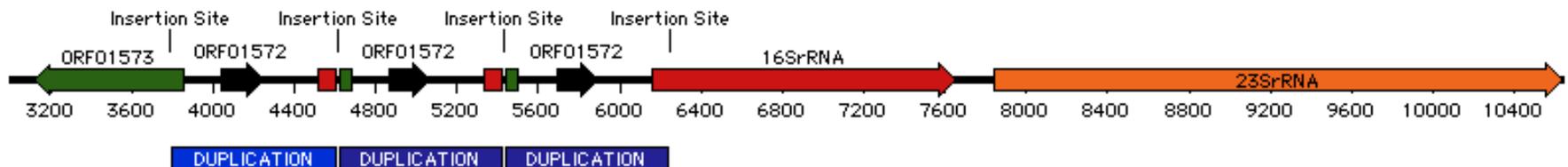
GB6 - Ames Ancestor



GB8 - Morph A Duplication



GB15 - Morph A Daschle Letter



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Sequencing Platforms: Faster, Cheaper... Better?



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Genomics Needs Informatics

- Informatics enabling technologies to:
 - Identify pathogen to the strain level
 - Identify any genetic modification
 - Antibiotic resistance genes
 - Toxin genes
 - Codon optimization
 - Gene with flanking regions that are not appropriate to the gene that has been identified (restrictions sites, species differences, etc.)



Challenges

- Data acquisition and analysis
- Signature to noise challenges
- What are the comparators (references and understanding of the natural diversity)
- Understanding the strengths and limitations of each of the technologies
- Do we have adequate “Standards” by which to evaluate the quality of our work and draw the proper conclusions?



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Meeting Legal and Practical Standards

- Testing & Validation
- Error Rate
- Peer Reviewed
- General Acceptance

These are Important factors:

US Justice System

Investigative Lead Value

Intelligence Value



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Thank You...

