DNA Fingerprinting – Your Bioremediation "Taq"ometer

Annemarie L. Douglas[1], Michael J. Borda[2], and J. Stephen Goudey[1]

[1]HydroQual Laboratories Ltd. and [2]Golder Associates Inc.

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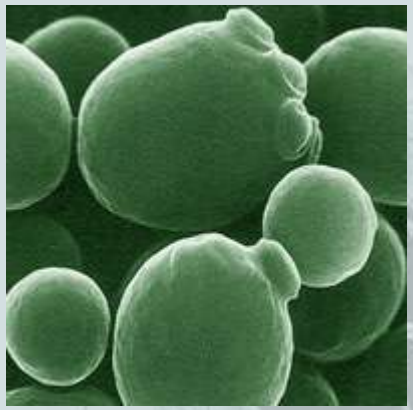
Outline

- Who I am and who I work for
- Explanation of DNA profiling
- Examples of how it can be used
- Other possible uses
- New tests that are being developed



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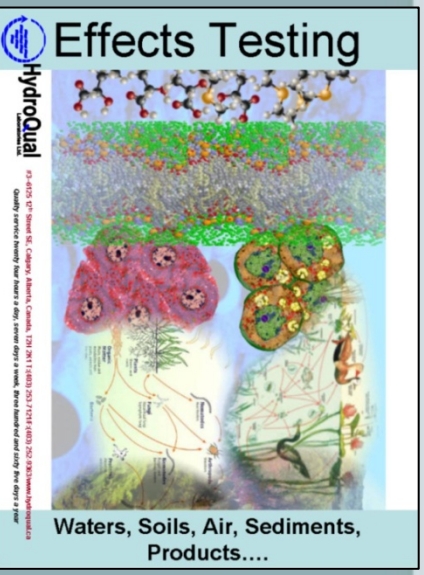

- HydroQual Laboratories Ltd. (HydroQual) does applied biology for environmental management.
  - We are a wholly-owned subsidiary of Golder Associates Ltd.
  - Our talented team of professionals all have degrees and expertise in the natural sciences (microbiology, biochemistry, toxicology, ecology, botany and zoology).
- We measure effects for assessing environmental quality.

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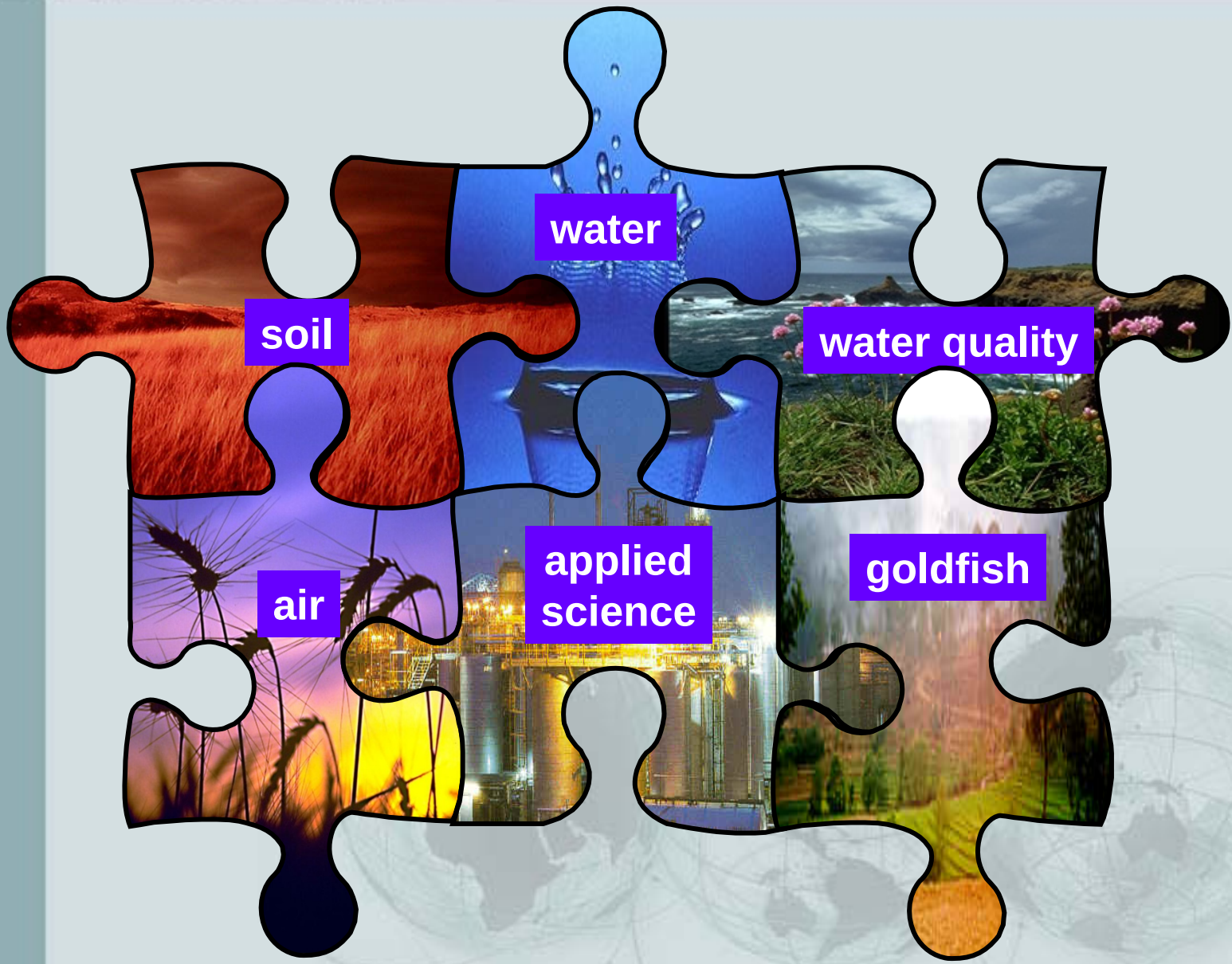
Groups



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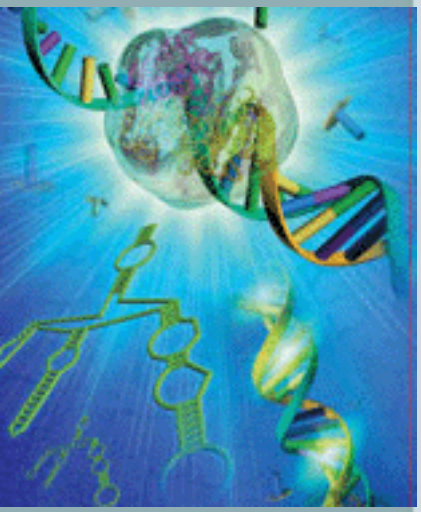
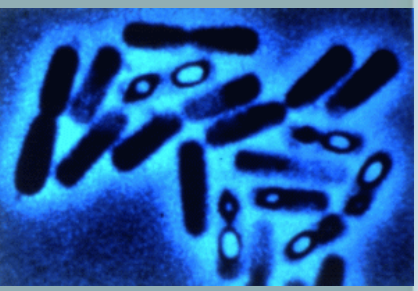
DNA Profiling





- Sampling
- How it is performed
- Results

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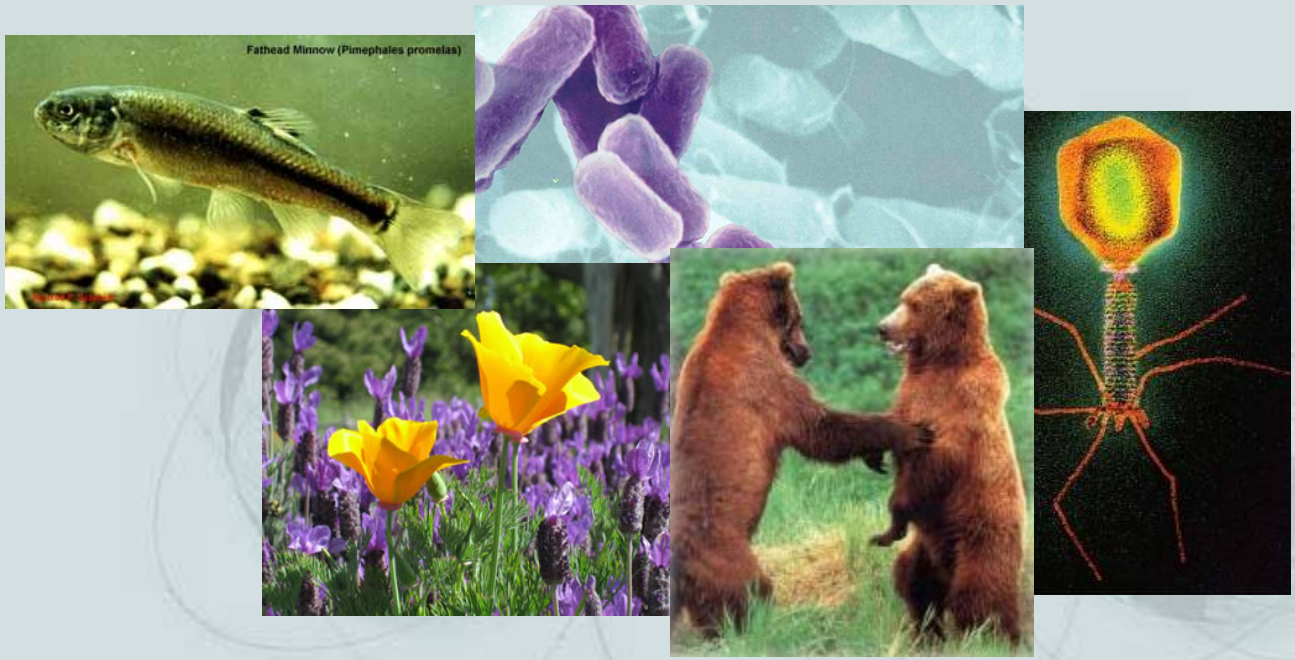
All living things have DNA

- DNA encodes for all cellular processes
  - Proteins and enzymes
  - Cellular machinery
  - Cellular structure



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Fingerprinting

- Identify individuals within a population
  - CSI, genetic testing, genealogy
- Create a fingerprint of the population
- Monitor population changes

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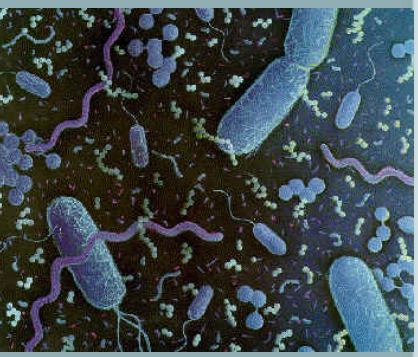
Conventional Approach

Sample → Extract bacteria → Grow in media → Identify



Disadvantages

- No detection of bacteria that can't grow
- Lots of time - 3 to 21 days or longer

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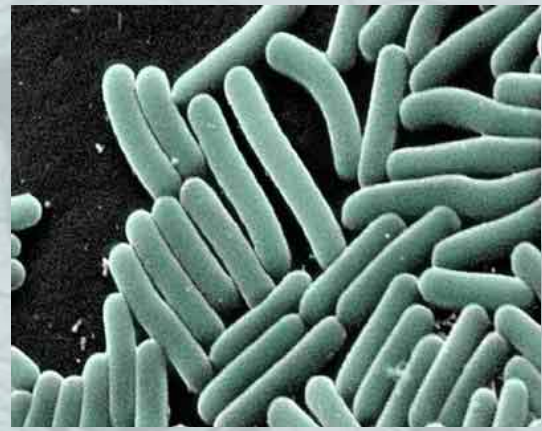
Fingerprint Approach

Sample → Extract DNA → Fingerprint

Advantages

- No culturing = Fast
- Sensitive
- Small sample size



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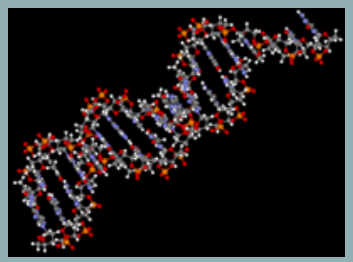
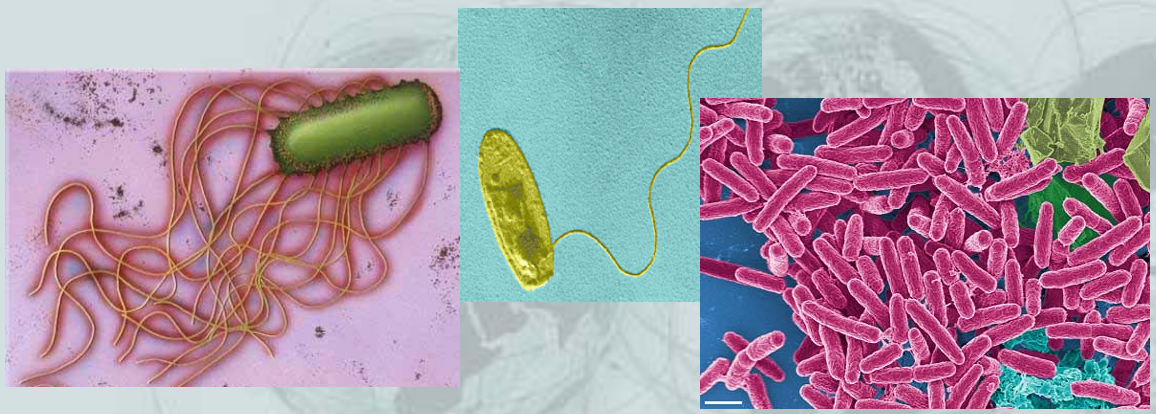
How Do You Fingerprint DNA?

Sample → Isolate DNA → PCR → DGGE



- Break open the bacteria
- Specifically isolate DNA from everything else



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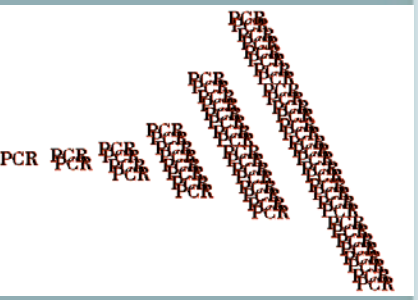
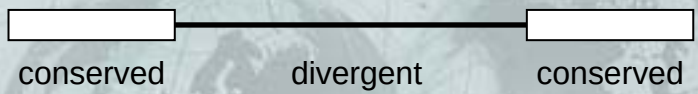
Polymerase Chain Reaction

Sample → Isolate DNA → PCR → DGGE



- Need just a small piece of DNA, but lots of it.
- What section of the chromosome?
  - Ribosomal RNA
    - Involved in protein synthesis
    - Used for taxonomy



- Target population

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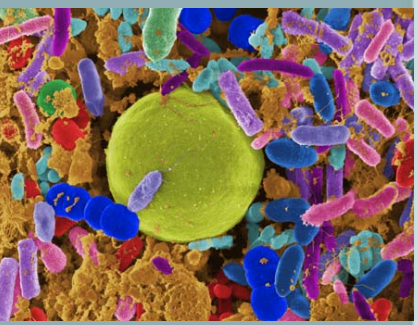
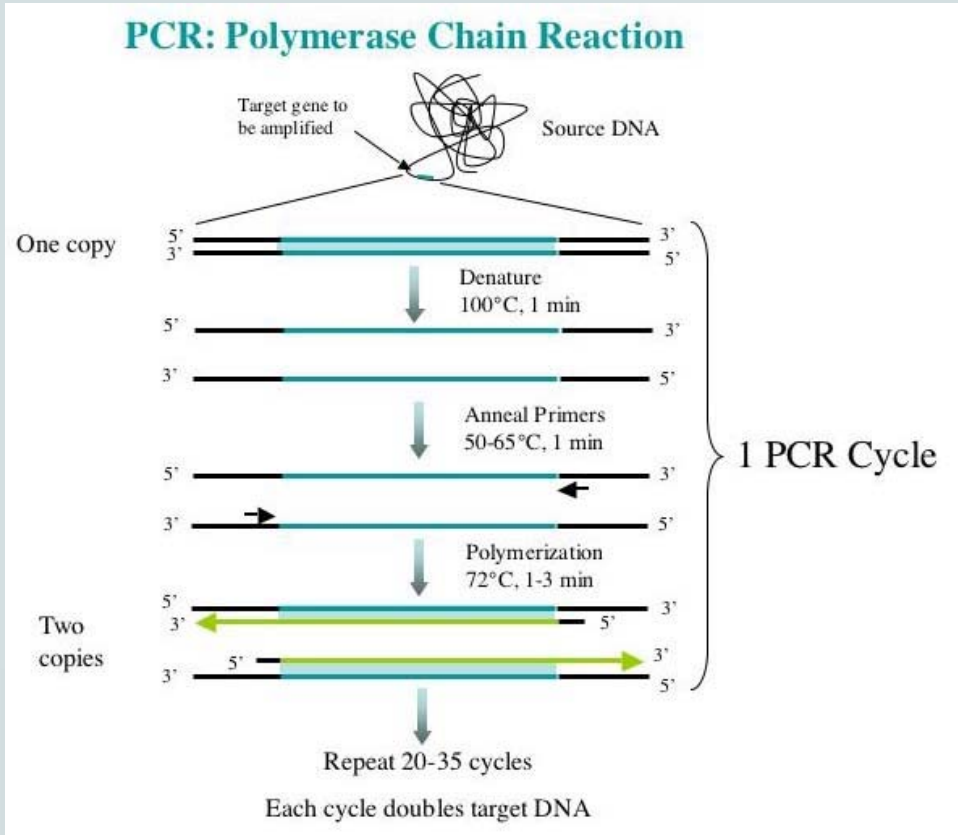
PCR

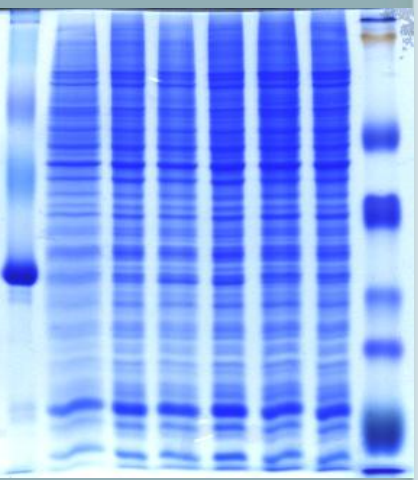
- What's needed
  - Thermophilic enzyme = Taq
  - DNA bases – AGCT
  - Primers – DNA targets





Denaturing Gradient Gel Electrophoresis

Sample → Isolate DNA → PCR → DGGE



- To separate the PCR DNA
  - Special gel
  - Special chemicals to denature DNA
  - Gradient of chemicals

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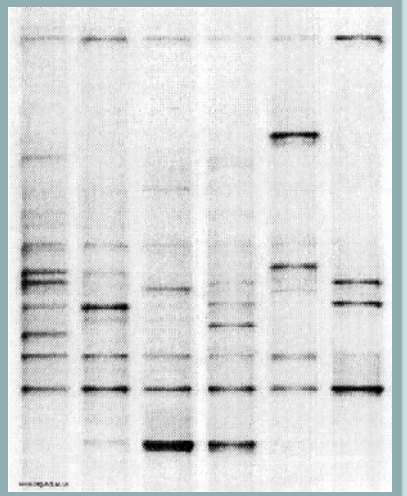
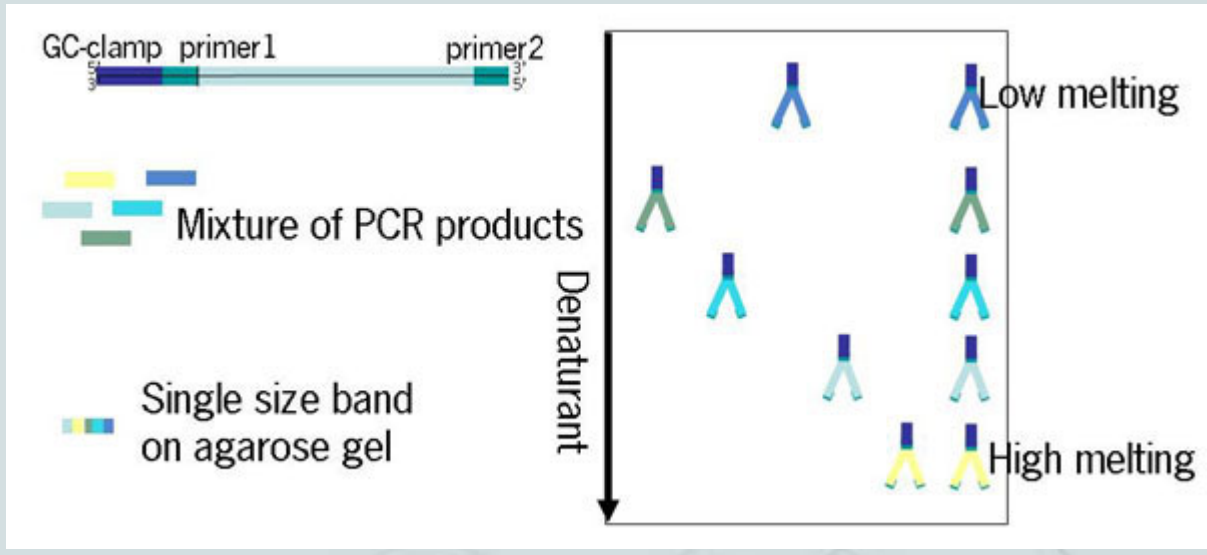
DGGE





- A,T – separates at lower conc.
- G,C – separates at higher conc.

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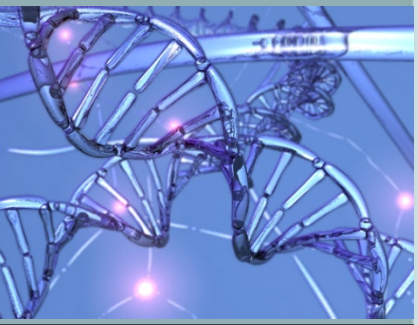
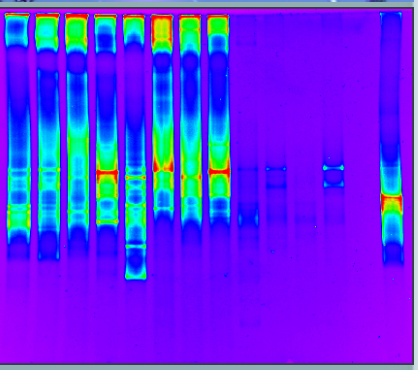
Case Studies





- 5 case studies
  - Range of issues
- Demonstrate the capabilities of DNA profiling

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Study 1

- Effects of nickel on plant growth
  - 5 types of plants
  - 11 concentrations of Ni^{2+}
    - 0 to 5 g/kg
- Soil samples were analyzed by fingerprinting

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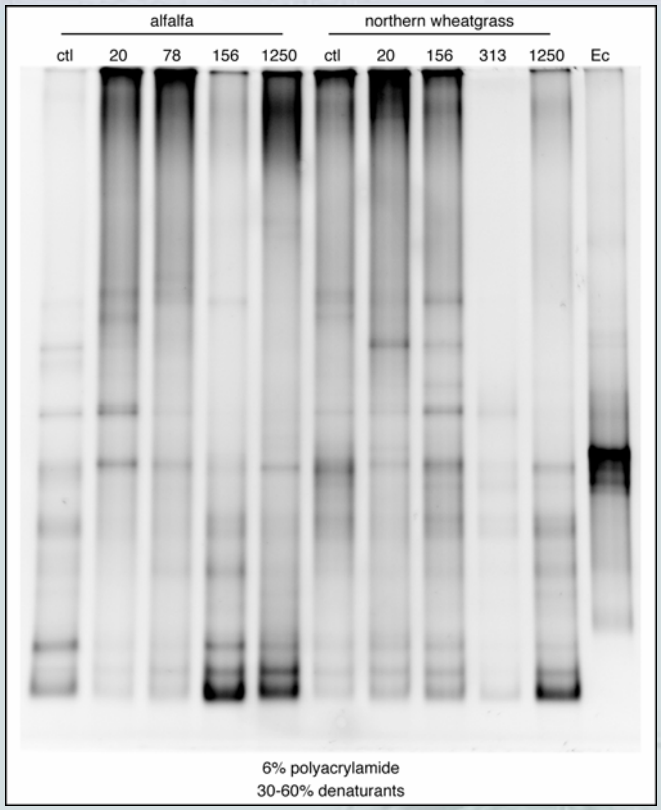
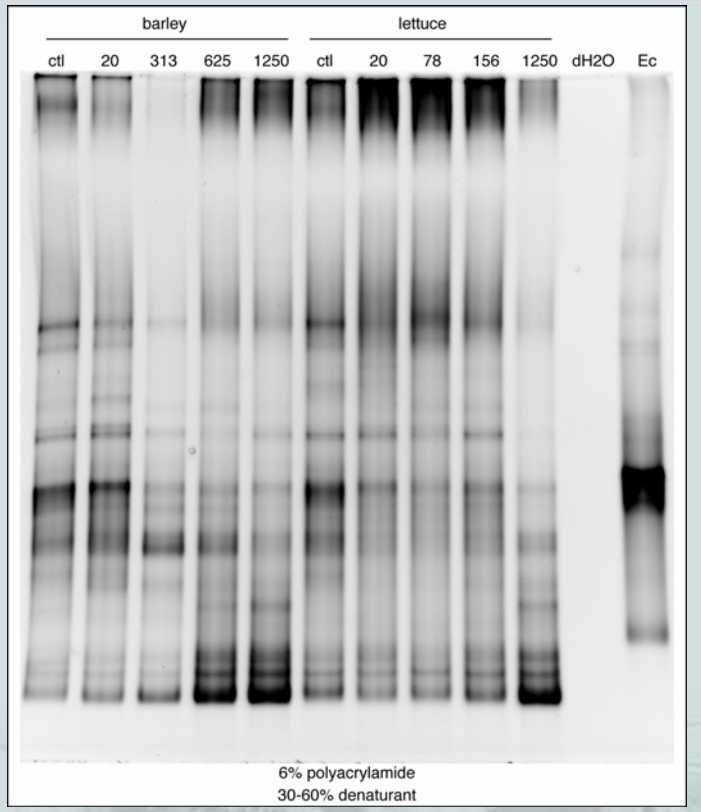
Ni^{2+} Effects on Plant Growth





- Large changes in bacterial population when plants can't grow
- Smaller changes when Ni^{2+} concentration is lower

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Study 2

- Biofouling of Bag Filters

GW → Bag Filter → Zero-Valent Iron Wall → Air Stripper

- Question: Was bacterial growth in the bag filters caused by the remediation system?

- Water samples were analyzed by Fingerprinting

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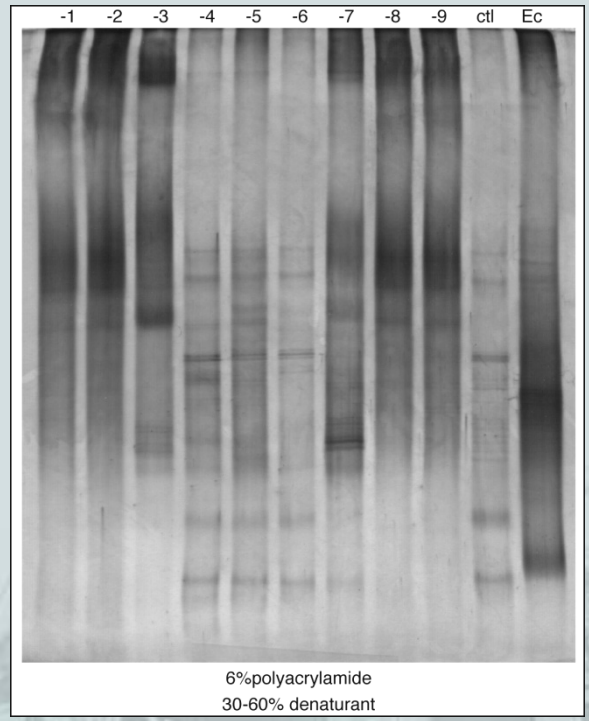
Biofouling of Bag Filters

GW → Bag Filter → Zero-Valent Iron Wall → Air Stripper

6 4, 5 1, 2, 8, 9 3 7



- Bacteria in the bag filters
  - Don't appear to be from the incoming GW
  - Are not leaving the bag filters

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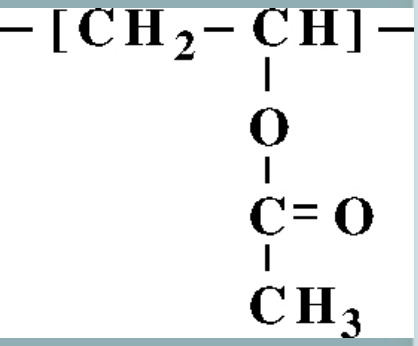
Study 3



- Contamination with vinyl acetate
- Decision: Dig & Dump or Bioremediate
- DNA profiling of soil

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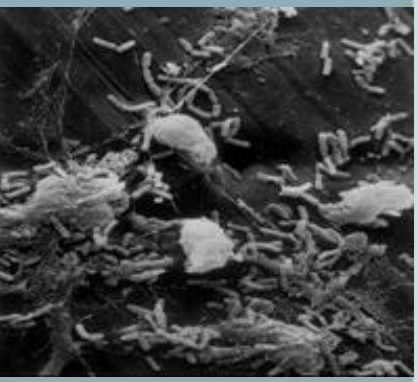
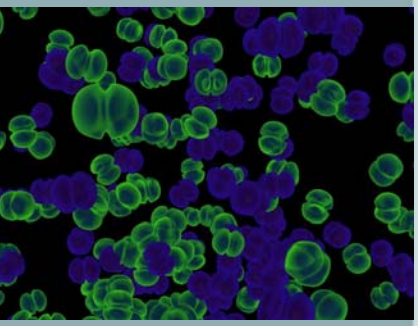
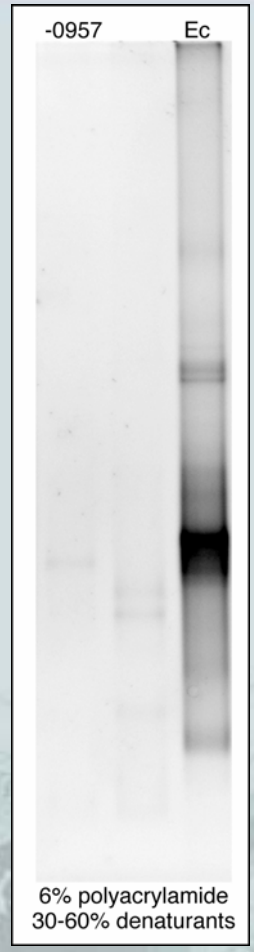
Vinyl Acetate in Soil







- No bacteria are present in the soil.
- Bioremediation is not possible.

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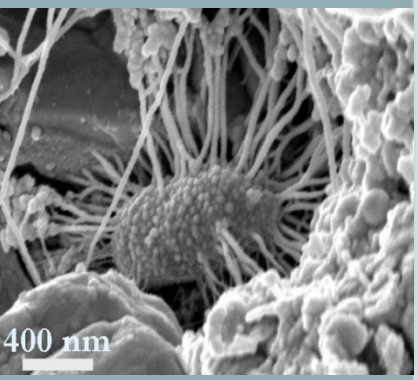
Study 4



- Remediation of chlorinated hydrocarbons with nZVI
  - 6 wells
  - 2 treatments with nanoscale zero-valent iron (nZVI)
- Fingerprinting of water samples before and after treatment

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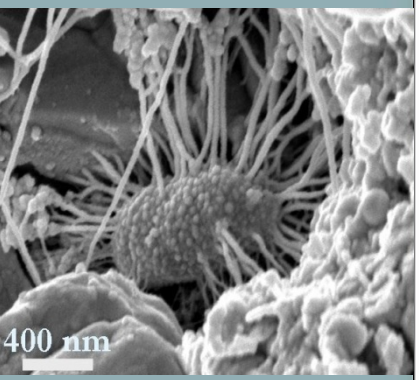
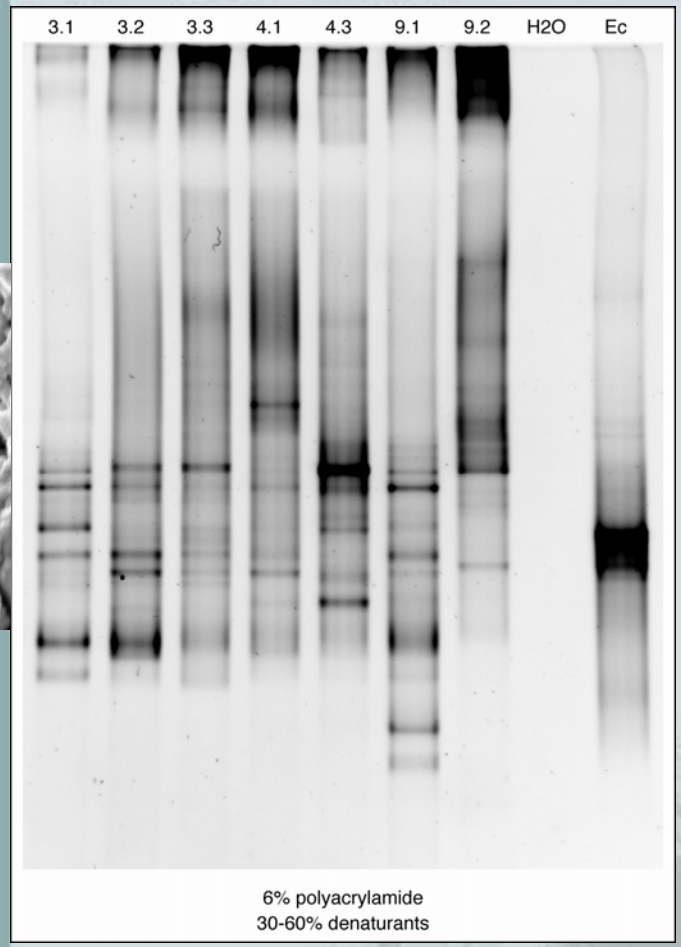
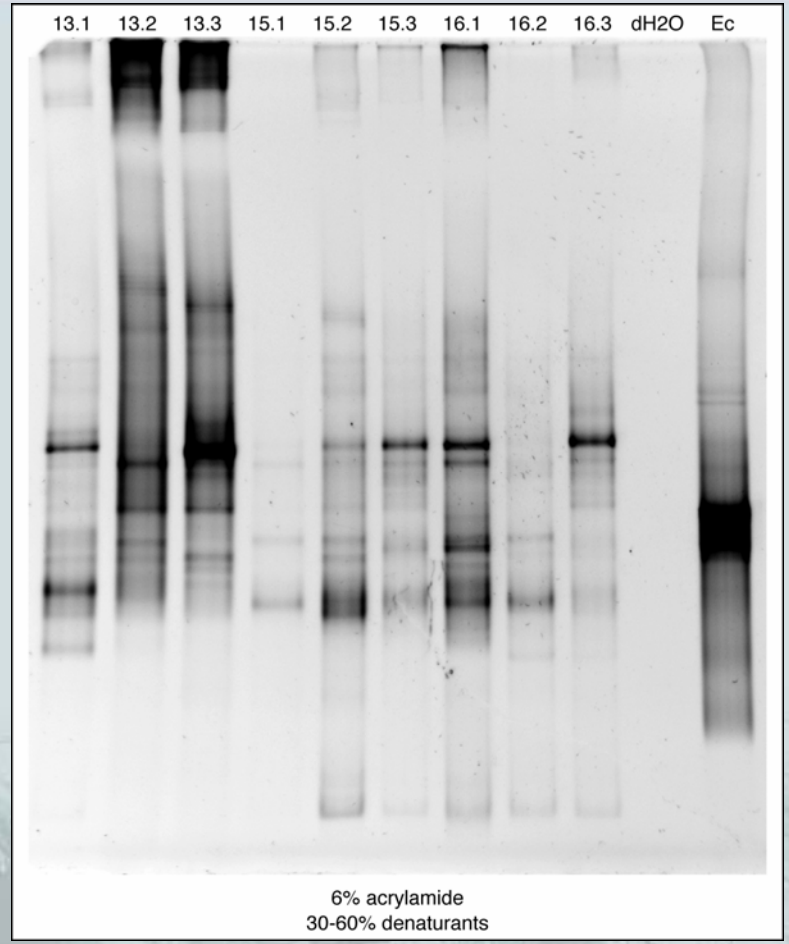
nZVI Remediation







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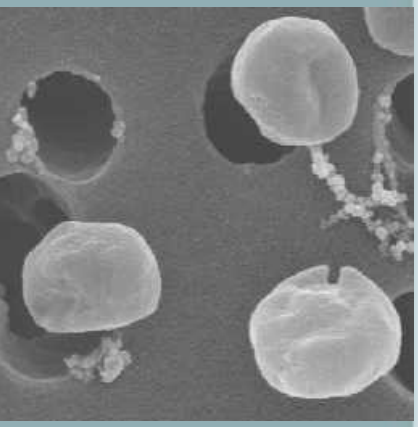
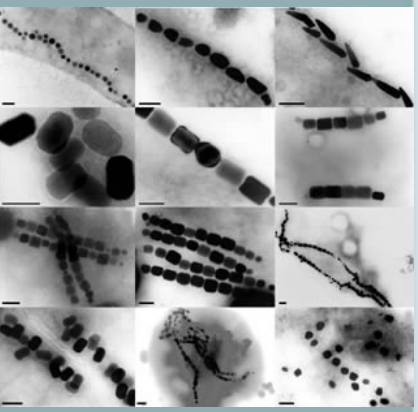
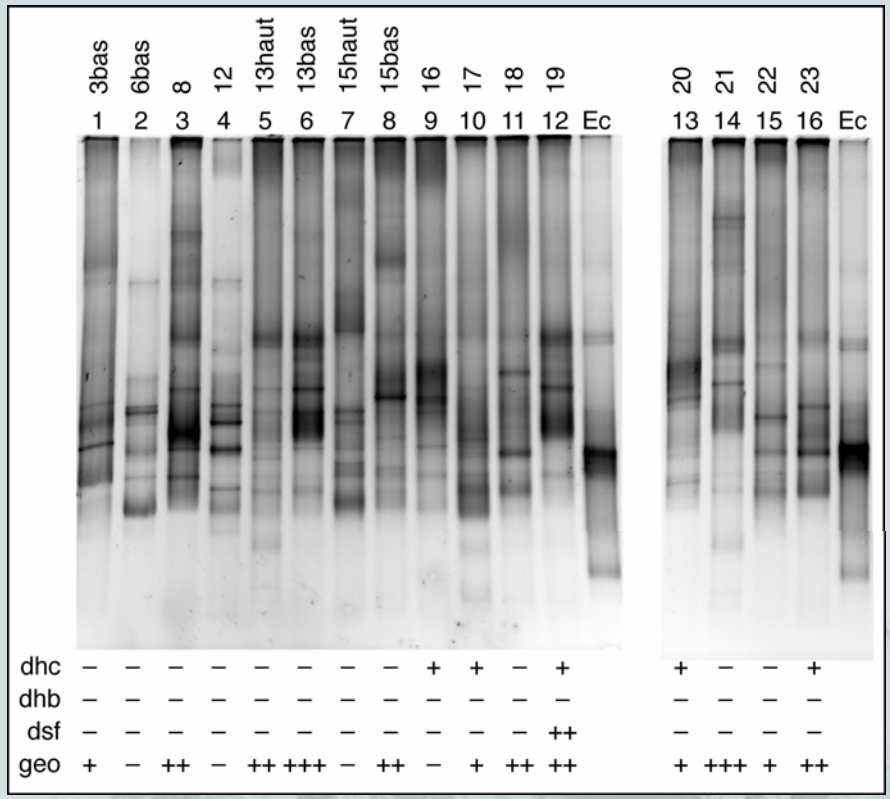
- Treatment affected bacterial populations at each well
- Bacterial populations got less diverse with treatment

6 months later







- Populations are returning to their original state.
- Bacterial populations differ by depth.

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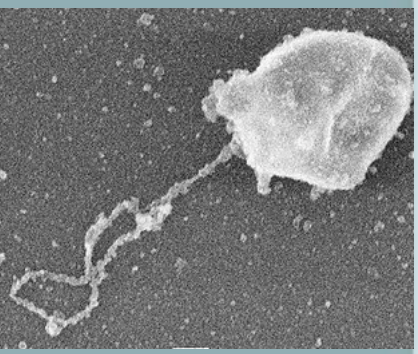
Study 5



- Anaerobic Benzene Bioremediation
  - Previous treatment with nZVI
- Biotreatability study
  - Addition of N and P
  - Nitrate – *Dechloromonas*, etc
  - Sulfate – *Desulfuromonas*, etc
  - Soy protein
- Analyze for chemicals and bacteria
  - DNA profiling for population changes
  - PCR for detection of specific bacteria

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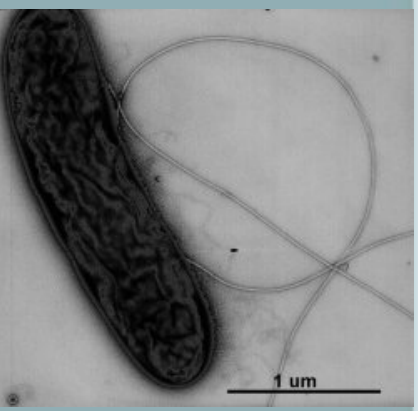
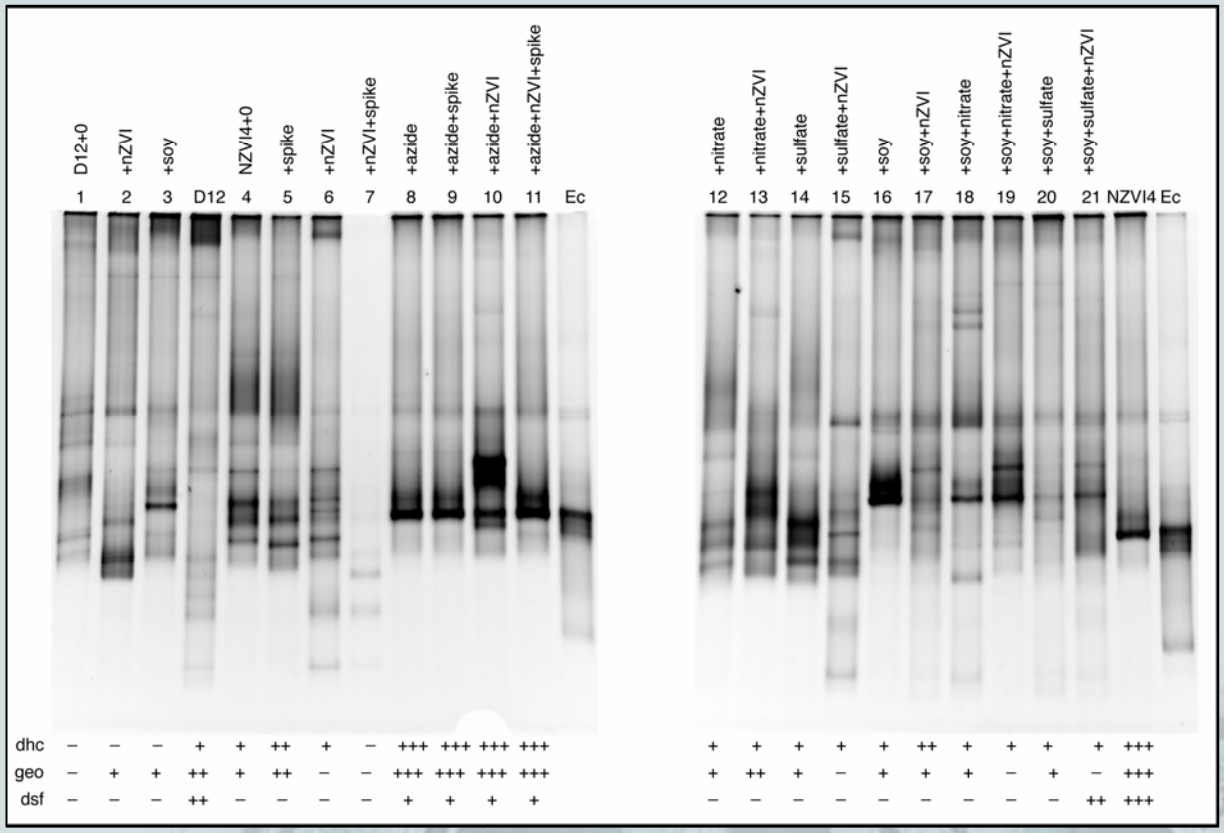
Biotreatability Study





- N&P alone causes big changes
- Soy protein has a short term effect on the bacterial populations
- nZVI abrogates some of the nutrient effects

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Other Uses - DGGE

- Bacterial ID – sequencing of fingerprints for definitive ID
- Monitoring of microbes, invertebrates, plants, etc.
- Baseline studies of soil
- Indoor air quality

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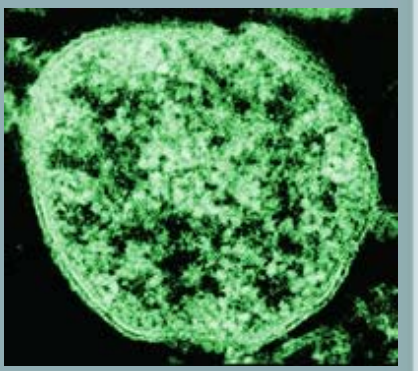
Other Uses - PCR



- Microbial ID
  - *Dehalococcoides* and other hydrocarbon degraders
  - *Legionella*
  - *Cryptosporidium*
- Biological Activity ID
- Culture Maintenance
- Plant ID
- Source Tracking
  - *Enterococcus*
  - *Bacteriodes*
- Animal Tracking

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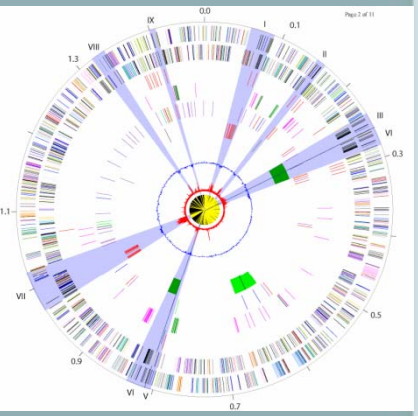
New Products



- Quantitative PCR
  - Species ID and Quantitation
- Quantitative RT-PCR
  - Gene activity measurements
  - Isolation of RNA

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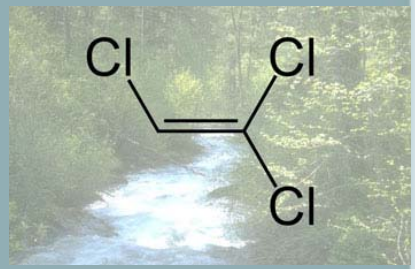
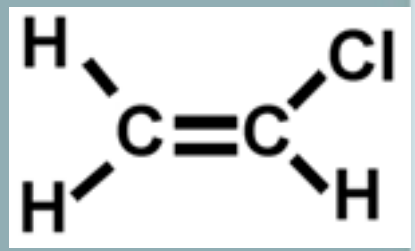
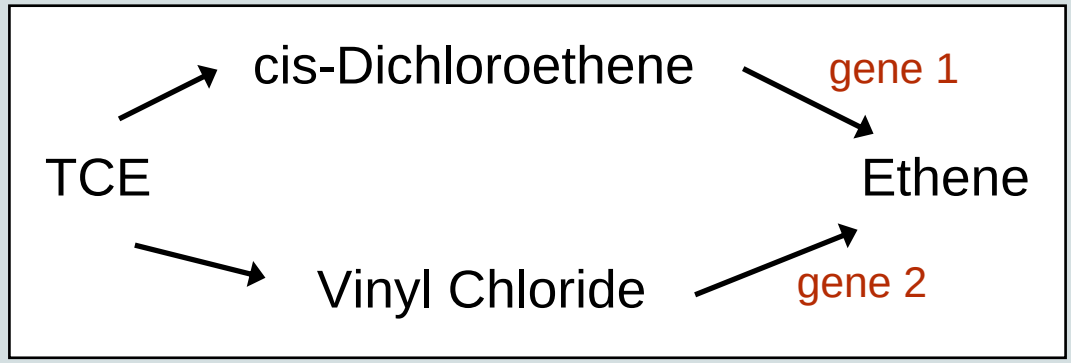
TCE Bioremediation RT-PCR







- PCR
  - Detects *Dehalococcoides* spp.
  - Detects gene 1 & 2 in the genome
- PCR indicates if the bacteria with the correct genes are present but not if the bacteria are alive and degrading TCE
- RT-PCR
  - Detects the expression of gene 1 & 2
- RT-PCR indicates that TCE is already being degraded to ethene

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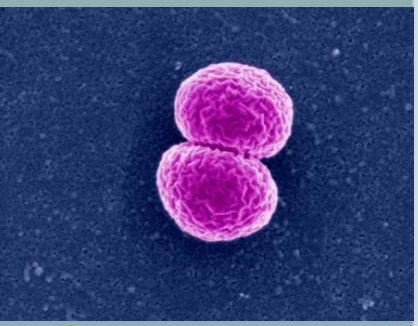
Summary



- DGGE/PCR
  - Used for monitoring soil and water
  - Superior to culturing – speed and sensitivity
  - More complete picture of in situ populations

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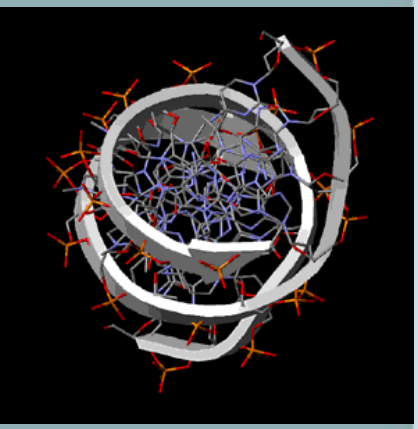
Questions?



- Contact information
  - Annemarie Douglas
  - (403) 253-7121
  - Annemarie_Douglas@golder.com

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