



**Shaw** The Shaw Group Inc.

Real-Time PCR Detection and  
Monitoring of Perchlorate Reducing  
Organisms.



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# Perchlorate Uses

☞ **Perchlorate**       $\text{ClO}_4^-$

☞ **Propellant**

- Missiles
- Fireworks
- Nuclear Reactors

# Health Risk

## ☞ Thyroid

- Prevents synthesis of hormones

## ☞ Clean Up action level to reduce health risks

- 4 - 18ppb

# Bio-remediation



Perchlorate Reducing  
Bacteria

# Bacteria able to Reduce Perchlorate

- ☞ *Dechloromonas agitata*
- ☞ *Dechlorosoma suillum*
- ☞ *Dechloromonas aromatica*
- ☞ *Azoarcus strain LT-1*
- ☞ *Ideonella dechloratans*

# Chlorite Dismutase

## ➤ Enzyme responsible for Dissimilatory Perchlorate Reduction identified

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gaacctggtcggcatgacgaaggacttgaactacatcacaaaagataaatcgccaaatct  
caacgccggctctgactggcggcacctatagggacgcaacaccccgftacgccttcgtgat  
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```

Bender et al. 2002 Sequencing and Transcriptional Analysis of the Chlorite Dismutase Gene of *Dechloromonas agitata* and its Use as a Metabolic Probe. *Appl. Environ. Microbiol.*

68:4820-4826



# What DNA analysis can answer

☞ Are they there?

☞ Where are they?

☞ How many?

# Real-Time PCR ?

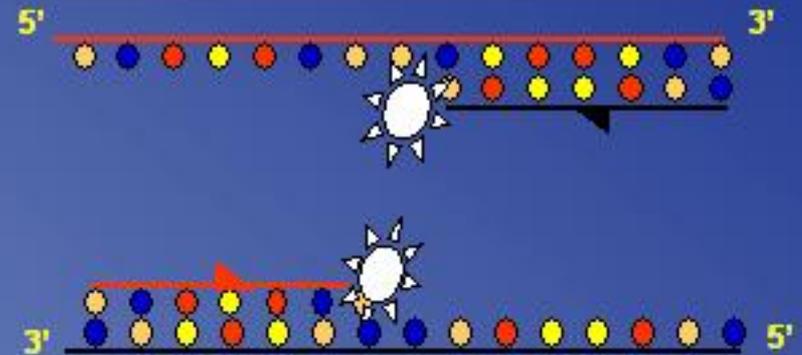
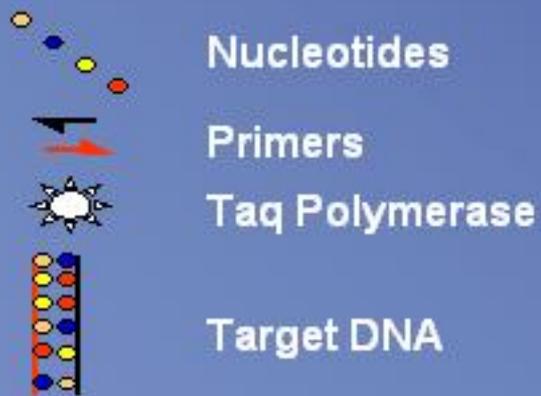
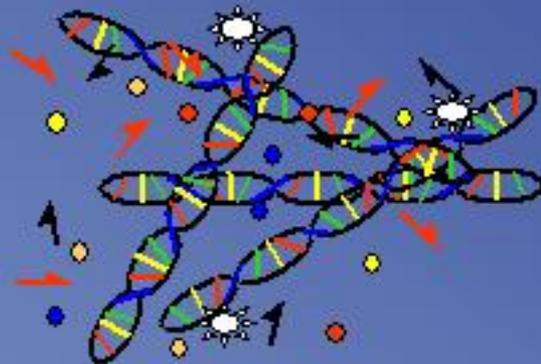
- ☞ PCR - Polymerase Chain Reaction
  - Replication of a DNA sequence
  - Very specific
  
- ☞ Real Time
  - Portable equipment
  - Can be operated in the field with minimal support
  - Reaction is monitored on a computer



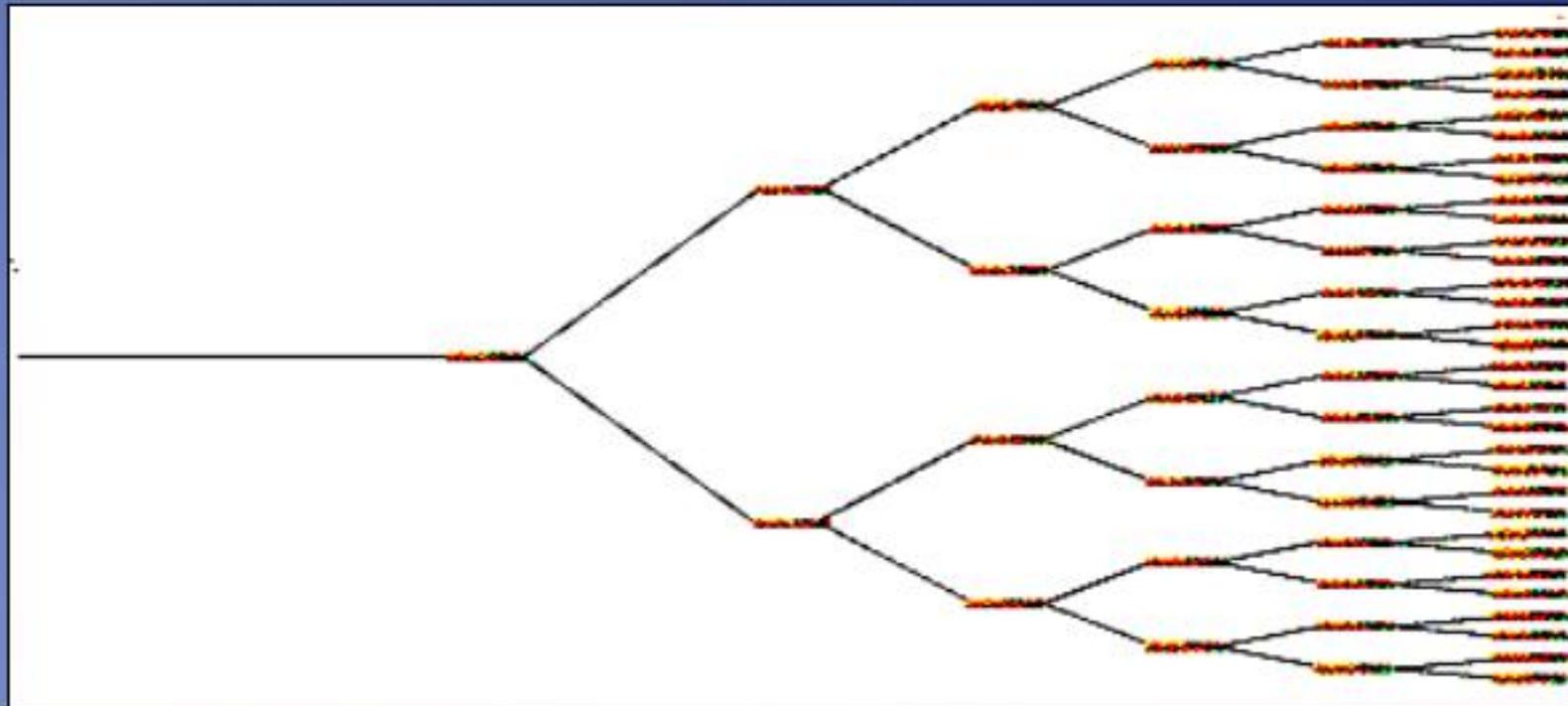
# PCR: Amplification DNA

- ☞ PCR (Polymerase Chain Reaction)
- ☞ Amplifying a specific sequence of DNA or RNA so that it can be detected.
- ☞ Detect DNA or RNA in a sample
  - Organism
  - Enzyme (cld- chorite dismutase)

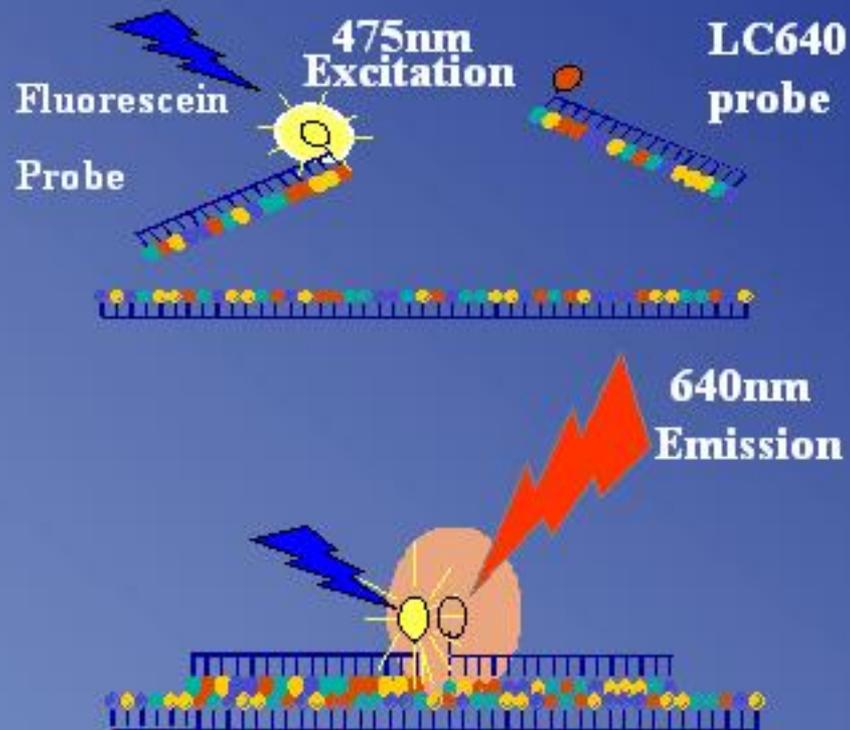
# Primers and Extension



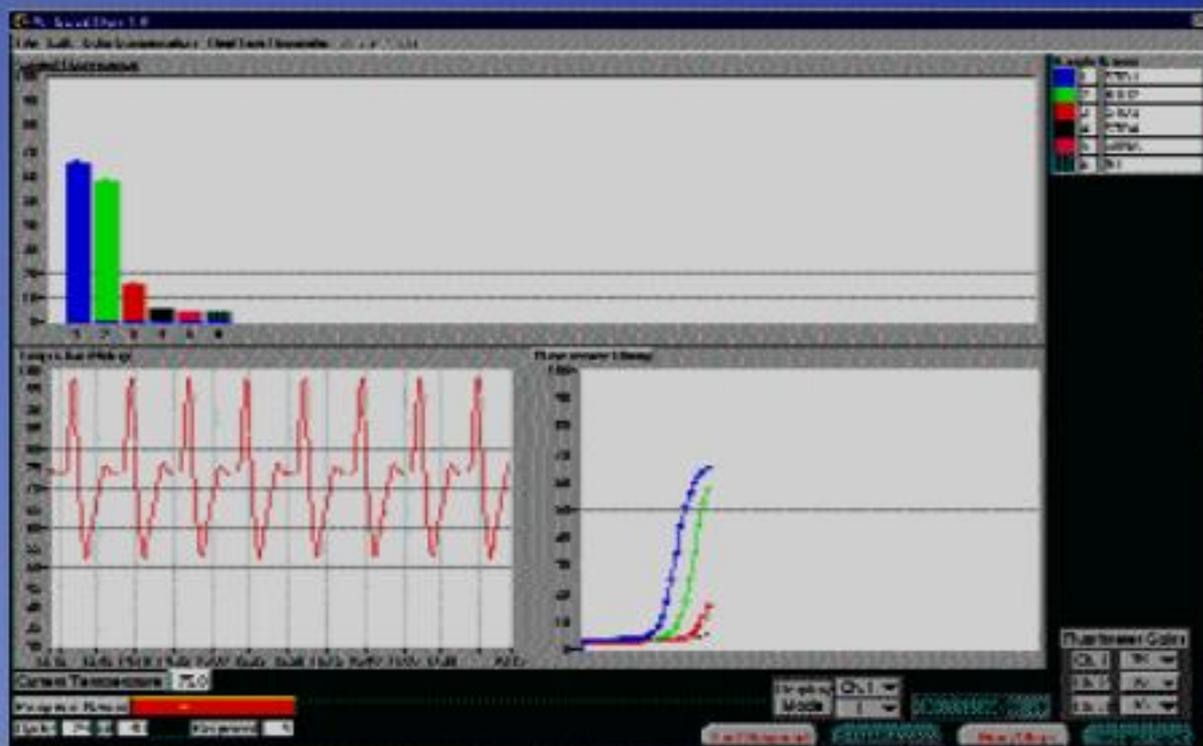
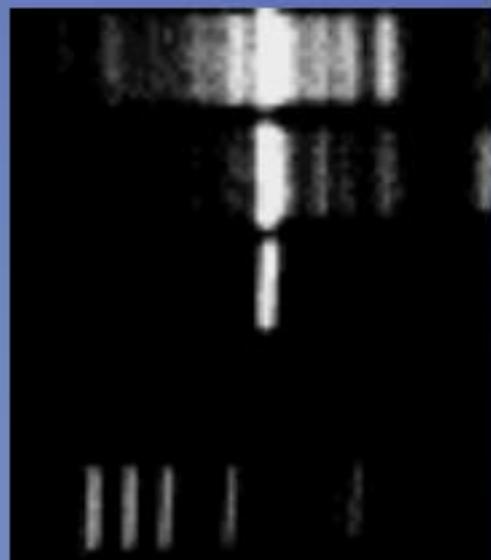
# Amplification of Target DNA



# Probes and Fluorescence



# Real Time PCR



# Running an Analysis – Sample Collection

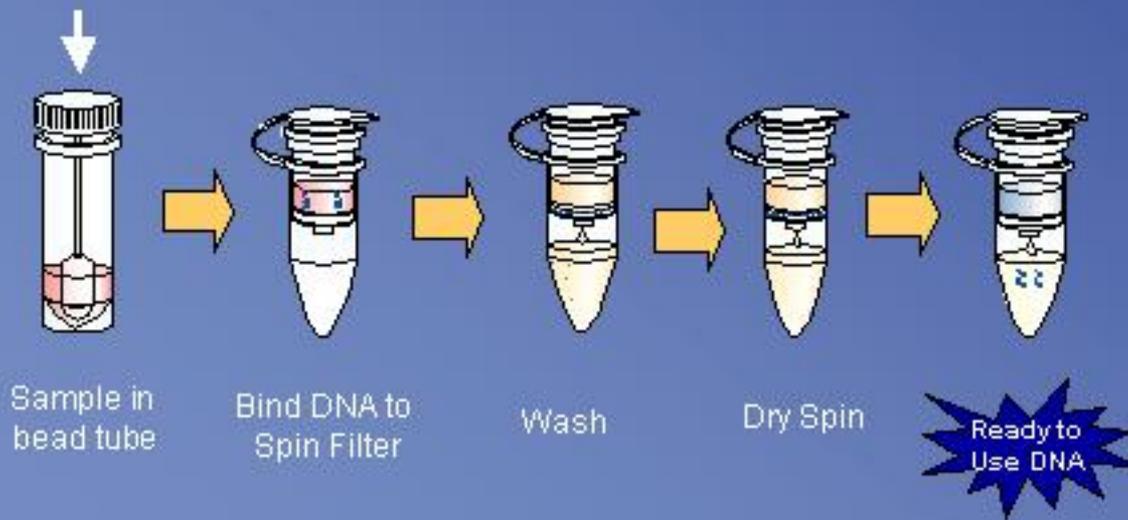
- ✦ **Collect sample**
  - **Ground water: 1 L glass bottle, no head space**
    - **filter and then isolate DNA**
  - **Soil: no headspace**
    - **Isolate DNA**



# Running an Analysis – DNA Extraction

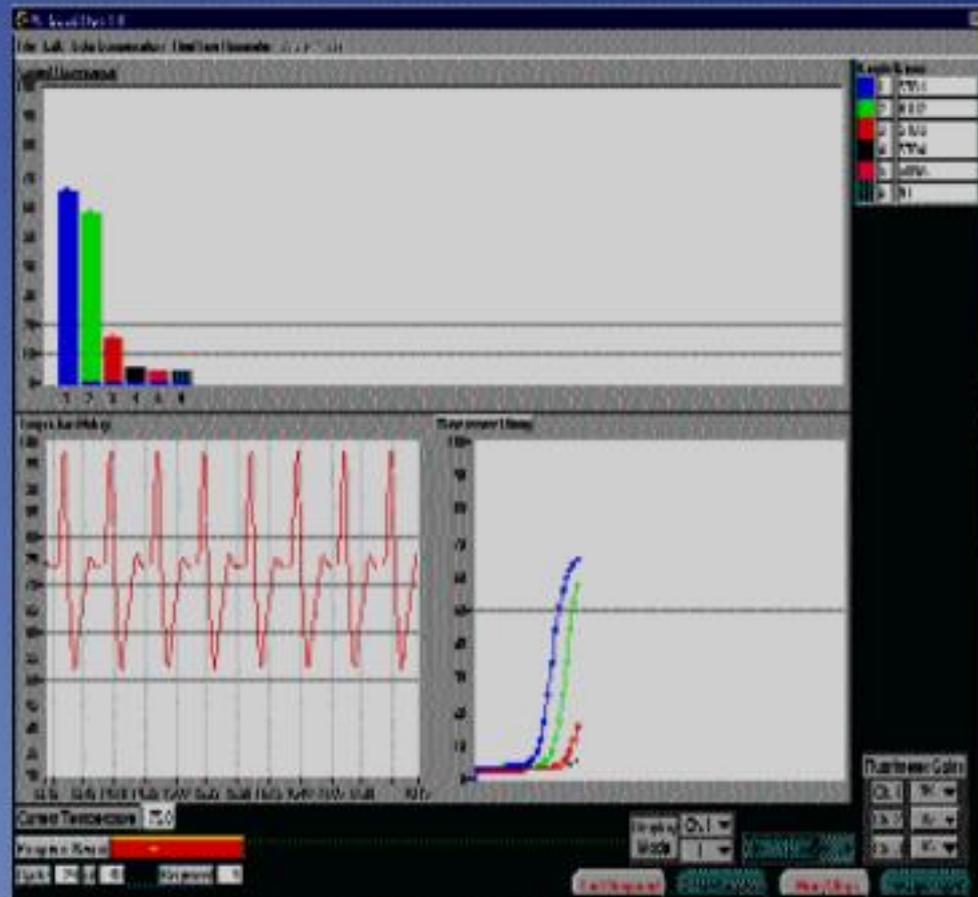
## ✦ Isolate DNA:

- G.W.
- Soil
- ~45 min.



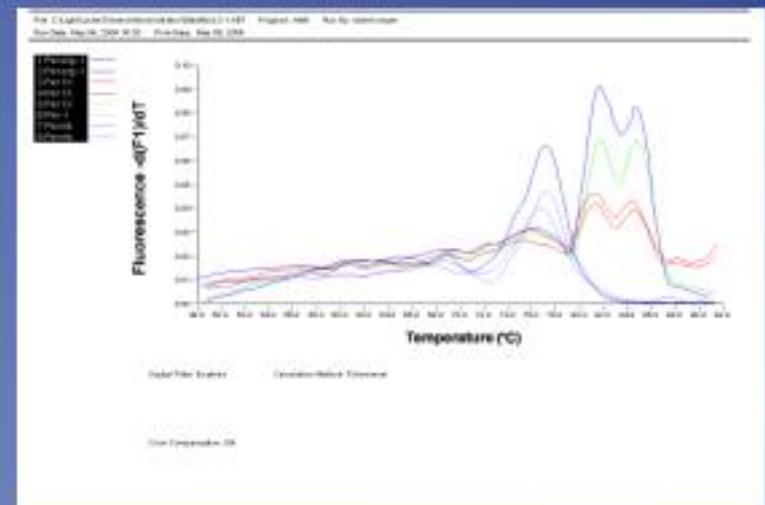
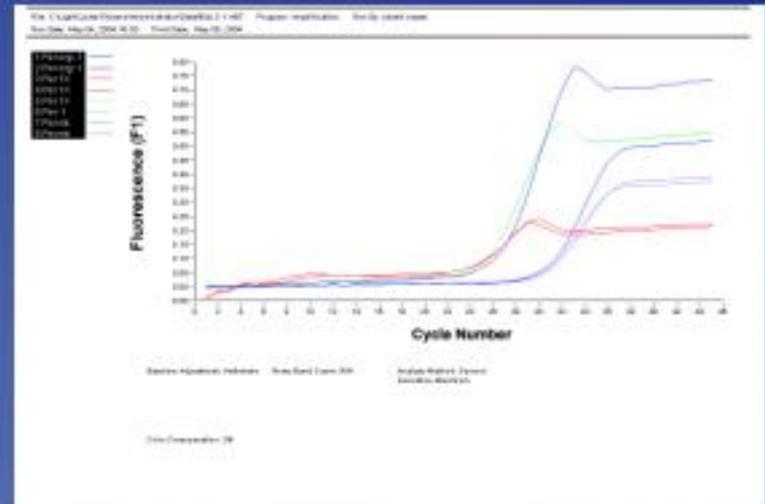
# Running the Analysis - PCR

- ☞ **Load Samples**
  - ~10 min
- ☞ **Start the Instrument and allow the PCR replication to occur**
  - ~40 min
- ☞ **Interpret data**
  - ~15 min



# PCR of Perchlorate Reducing Bacteria

- Utilizes the primers and probes specific for the chlorate dismutase enzyme
  - Provides specificity while targeting perchlorate-reducing bacteria
- Allows real-time determination on the number of perchlorate-reducing bacteria present in the soil or groundwater



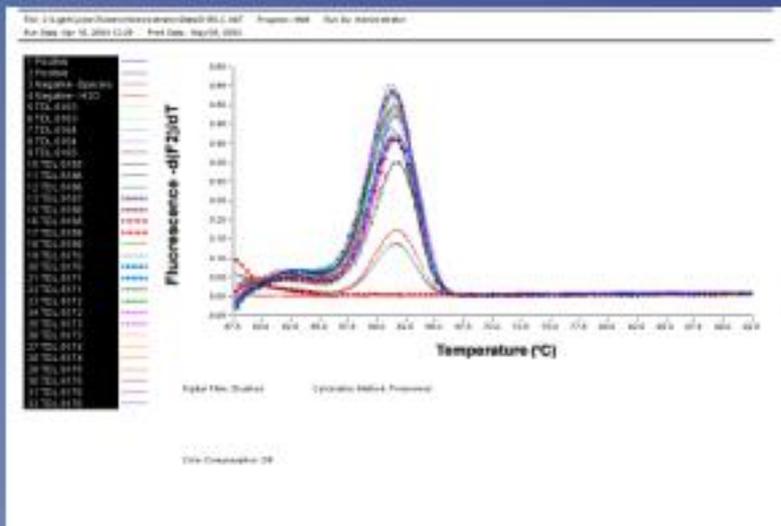
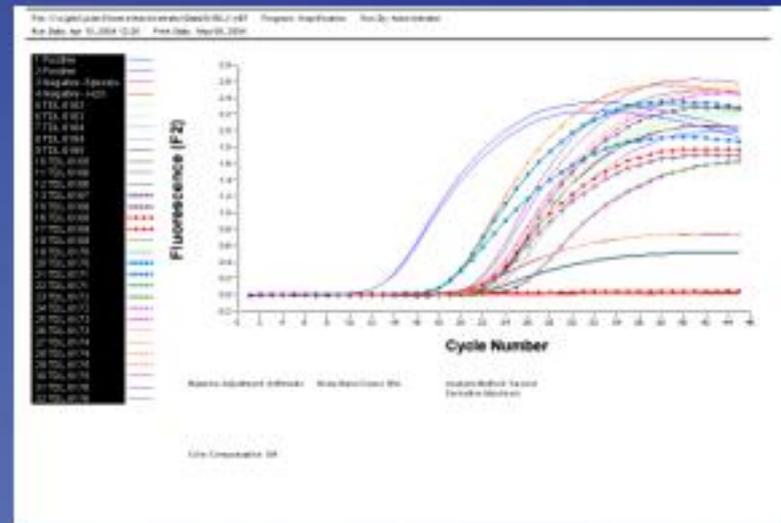
# Uses for Monitoring Microorganisms

- **MNA**
- **Enhanced Bioremediation**
- **Bioaugmentation**
- **Ex-Situ Reactors**
- **Laboratory Studies**
- **In-situ Pilot and Full-Scale Applications**

# MNA Evaluations

## ☞ Monitored Natural Attenuation

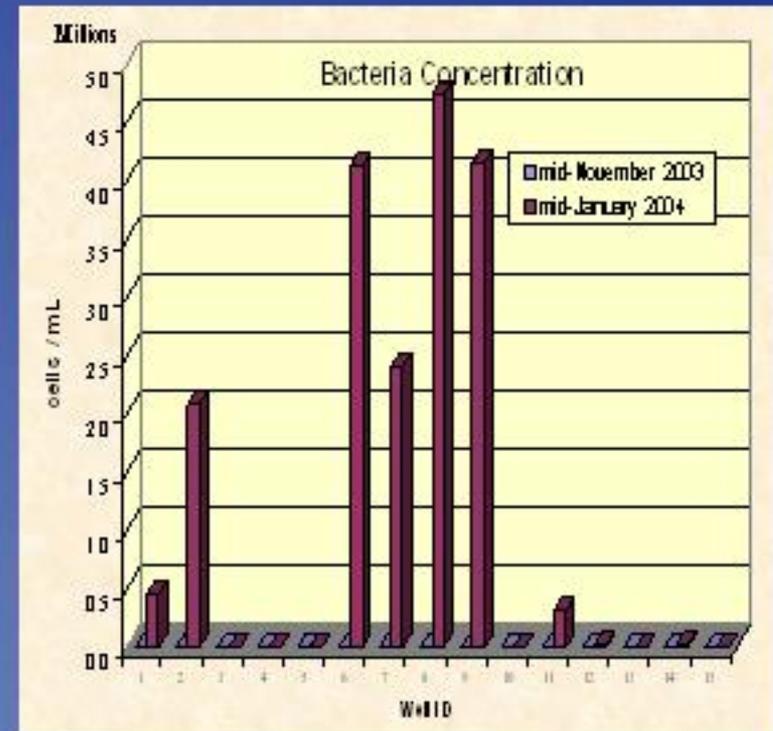
- Determine the existence of organisms that degrade a contaminant
- Determine the concentration of organisms that are present





# Bioaugmentation

- Addition of microorganisms to biodegrade contamination.
  - Collection of Organisms
  - Monitor the Growth in Lab
  - Monitor the dispersal Organisms after Injection
  - Monitor the growth of Organisms in-situ



# Ex-Situ Reactors

- ☞ **Monitor Bacteria Populations**
  - **Concentration**
  - **Gene expression**



# Laboratory Studies

## ☞ Treatability Studies

– Wetlands



– Soil Columns



# Conclusions

- ➔ **Real-time PCR monitoring of Perchlorate Reducing Bacteria can answer:**
  - Are they there? – Are they present to do the job
  - Where are they? - Are they in all the areas needed
  - How many? - Are they healthy and present in sufficient numbers? Does a carbon source or more bacteria needed to be added?

## Conclusions (con't)

- ➔ **Real-Time PCR monitoring is an important tool for Environmental Projects**
  - Aid in determining the Correct Course of Action
  - Aid in monitoring existing bioremediation projects