

# DIGGING THAT BIT DEEPER: A MOLECULAR APPROACH TO CONTAMINATED GROUNDWATER

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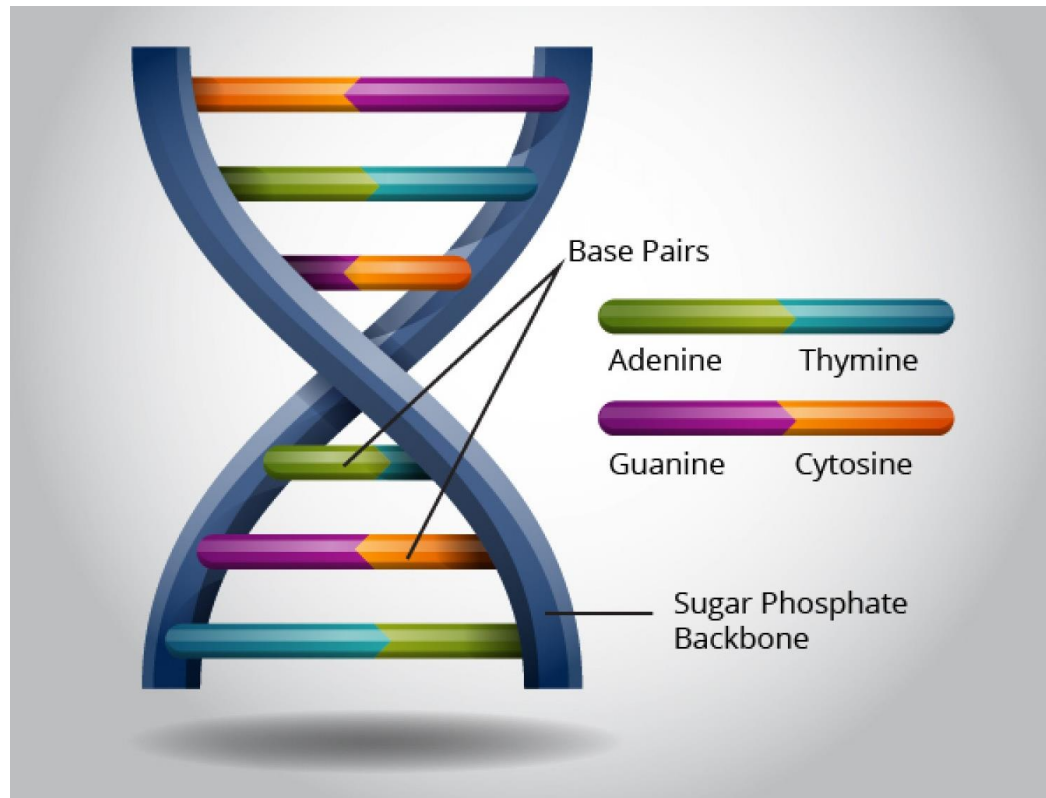
# ONCE UPON A TIME...

- Bacteria were observed!
- Revolutionary scientific discoveries

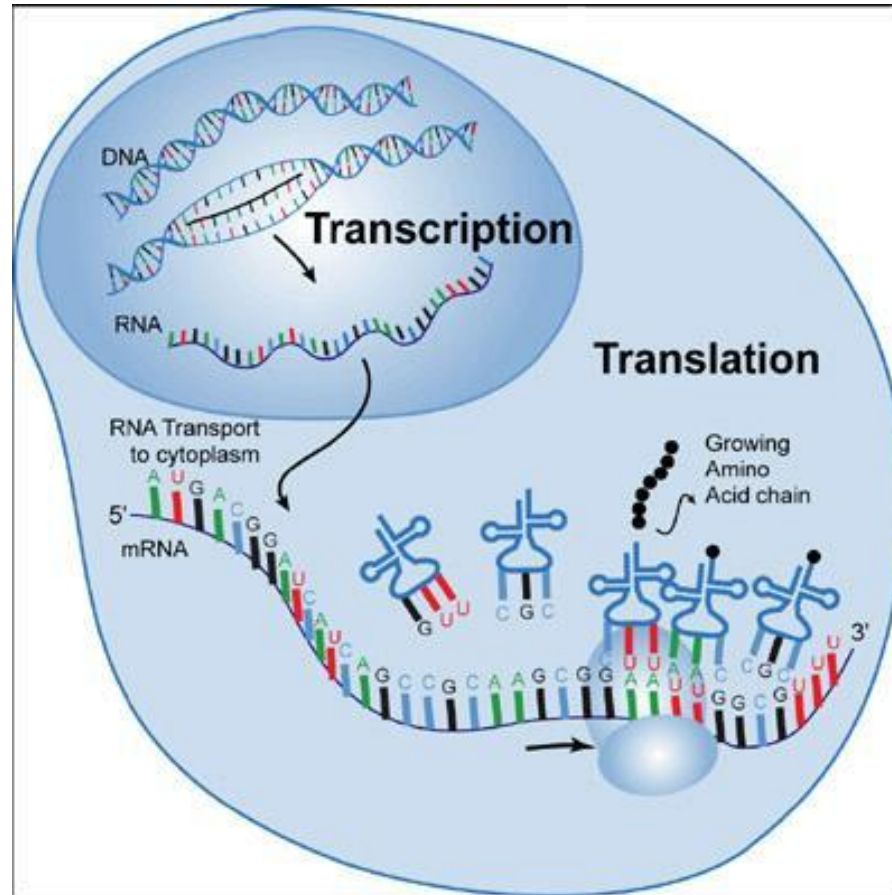


- Great, but...
  - Majority of bacteria do not grow in isolation
  - Variance in behavior when cultured in lab vs natural environment
- Molecular techniques can overcome these limitations

# DNA (DEOXYRIBONUCLEIC ACID)



# A BIT FURTHER...



# ALL IN SEQUENCE

## DNA sequencing

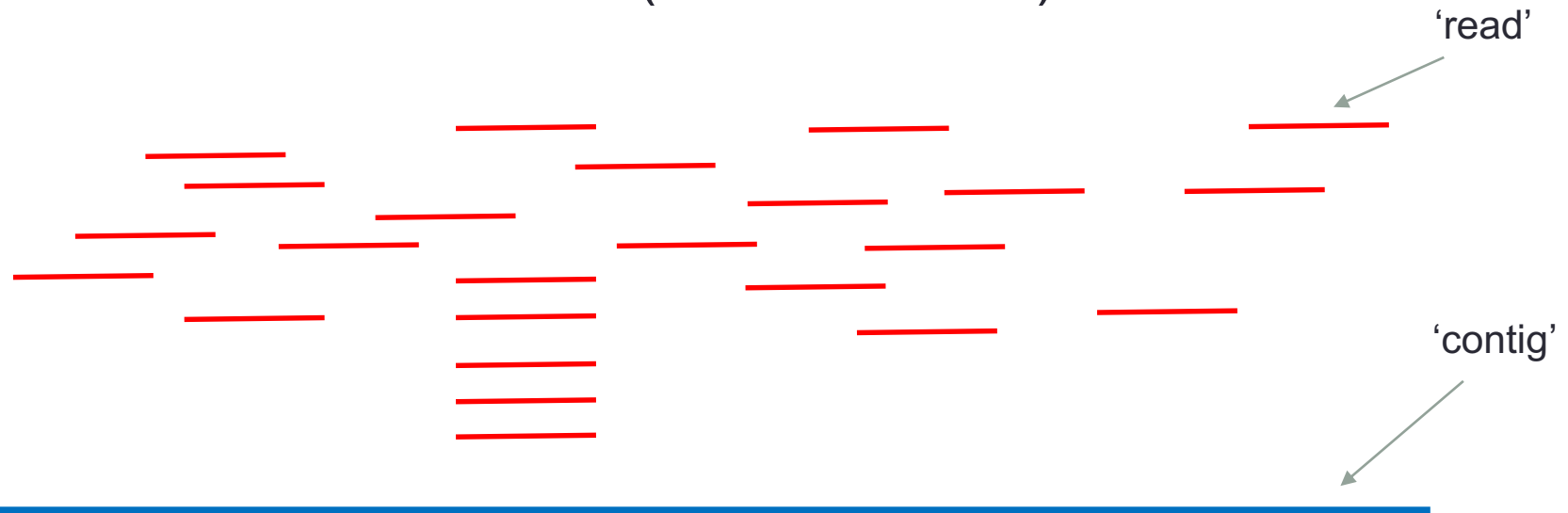
- Next Generation Sequencing (NGS) Platforms
  - Illumina (sr)
  - Ion Torrent (sr)
  - PacBio (lr)
  - Minlon (lr)



\* sr = short reads, lr = long reads

# DATA OVERLOAD!

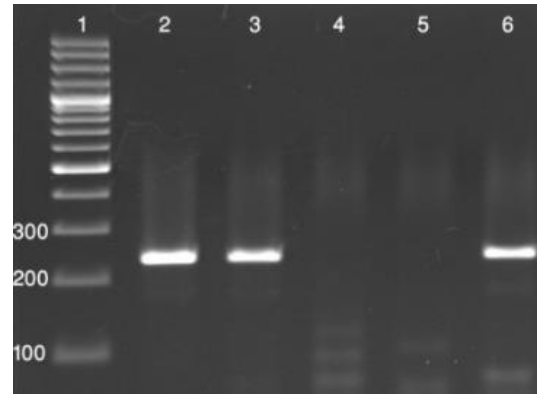
- Assemble all that data! (Bioinformatics)



- Draft genome assembly
- Gene identification & characterisation

# OTHER MOLECULAR METHODS

- PCR
- qPCR
- SIP



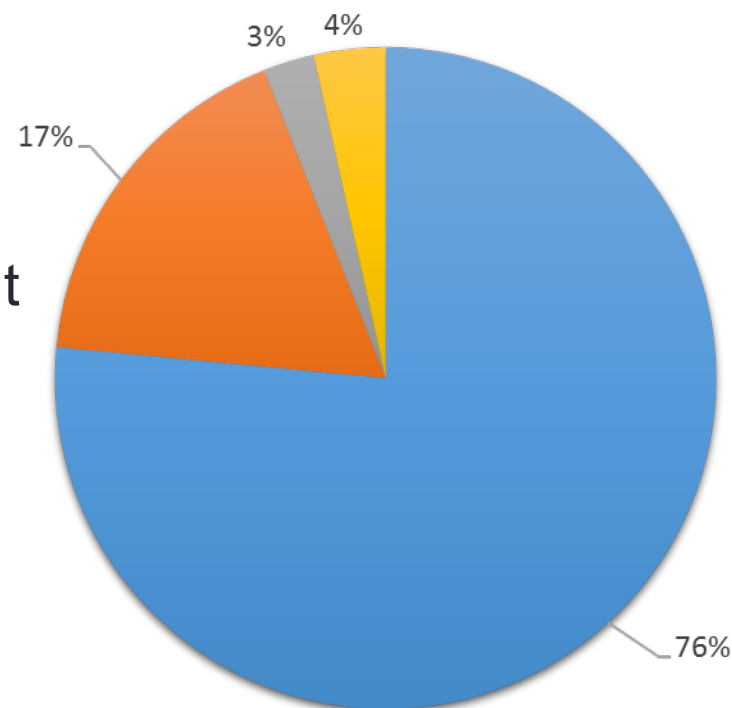
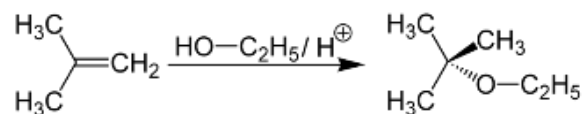
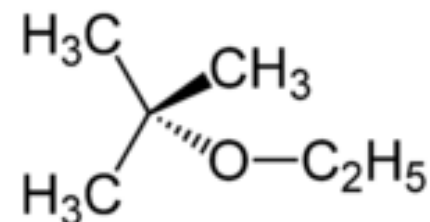
- Transcriptomics (RNA)
- RT-qPCR



# ETBE

Ethyl tert-butyl ether (ETBE)

- Petrol additive
- Produced from bioethanol
- Annual market demand increase
- High solubility in water
- Safety concerns
- Slow to break down in environment



■ Europe
 ■ Asia
 ■ North America
 ■ Rest of the world



# WHAT'S ALREADY KNOWN?

- Many different organisms isolated have the ability to grow using ETBE as a sole carbon source (ETBE → Biomass & CO<sub>2</sub>):
  - *Betaproteobacteria* sp. IFP 2047
- Syntrophic interactions shown to be important for complete removal of primary substrate and intermediate compounds, e.g.
  - *Rhodococcus* sp. ETBE → TBA
  - *Bradyrhizobium* sp. TBA → Biomass & CO<sub>2</sub>
- Some organisms can use different GEOs (MTBE, ETBE & TAME), albeit at different rates
  - *Achromobacter xylosoxidans* MCM2/2/1
- Limitations of laboratory culturing, as discussed on previous slides

# APPLICATION OF MOLECULAR METHODS TO ETBE

- Identified ETBE-degradative encoding genes – *ethRABCD*
  - *EthR* – Transcriptional regulator for the AraC/XylS family
  - *EthA* – Ferredoxin reductase
  - *EthB* – Cytochrome monooxygenase P-450
  - *EthC* – Ferredoxin
  - *EthD* – Protein of unknown function
- High sequence similarity of the *eth* genes between strains
- Useful as a ‘molecular marker’, i.e. is the gene present (indicating ETBE degradation potential)
- Limited evidence of alternative genes – are there more yet to be identified?
- DNA sequence can reveal *who* is present and the potential mechanisms (*how*), whereas RNA sequencing can detail both

# RNA STUDIES

- Lack of information highlighting the mechanisms how other contaminants have an effect on ETBE degradation. BTX have been shown to reduce ETBE degradation (Gunasekaran et al. 2013)
- Example for MTBE – Benzene delayed and ethylbenzene inhibited the expression of known degradative genes for MTBE and TBA (Joshi et al. 2016)
- These studies can highlight not only *who* is actively responsible for ETBE degradation, but also *how*. Additionally, if any other contaminants affect these



# SUMMARY

- Molecular approach can be applied to groundwater contamination to gain a greater understanding of *in situ* and *ex situ* degradation processes
- These techniques are also complementary to ‘traditional’ methods
- Allows for the development of bioremediation strategies

# Thank You

Any questions?

## Acknowledgements

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concaawe

ENVIRONMENTAL SCIENCE FOR THE EUROPEAN REFINING INDUSTRY



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