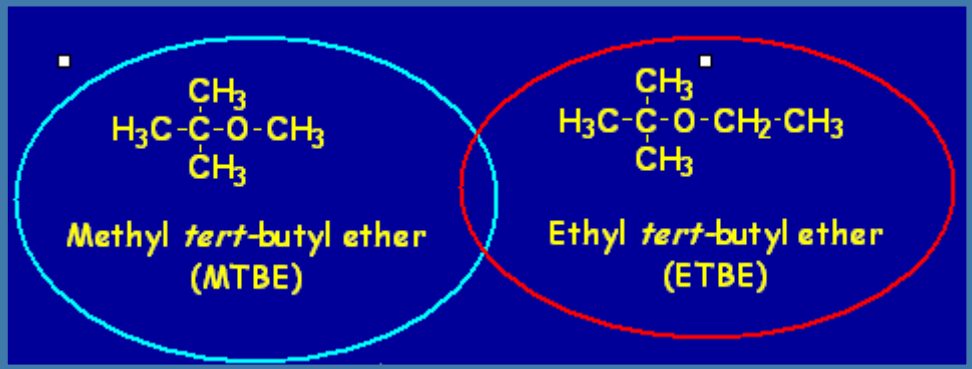
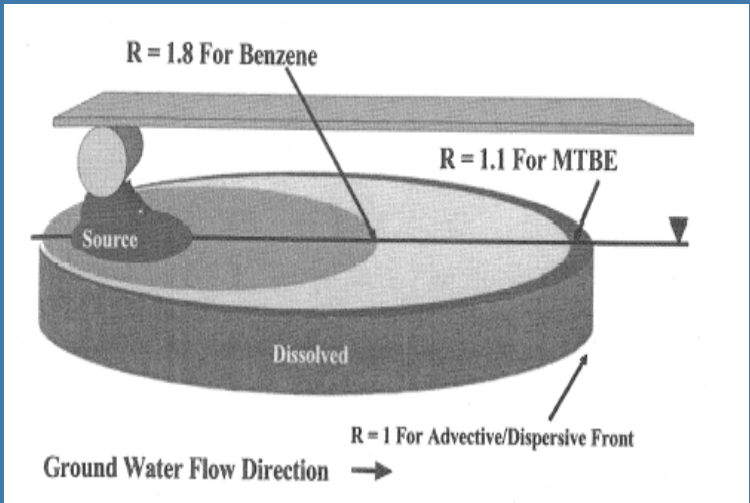
New tools of detection for the management of groundwater polluted by ether-fuels



Françoise FAYOLLE-GUICHARD

Problems encountered with the use of MTBE/ETBE-supplemented gasoline

- Extent of MTBE (ETBE) plumes when an aquifer is polluted by MTBE/ETBE-supplemented gasoline (Wilson, 2003) :



- High solubility (40 g/L for MTBE and 10 g/L for ETBE)
- Low adsorption on organic matter
- Recalcitrance to biodegradation

Questions posed to people involved in depollution of such sites

-How to estimate the potential for natural attenuation (if any) to both minimize and potentialize the actions that have to be undertaken for depollution ?

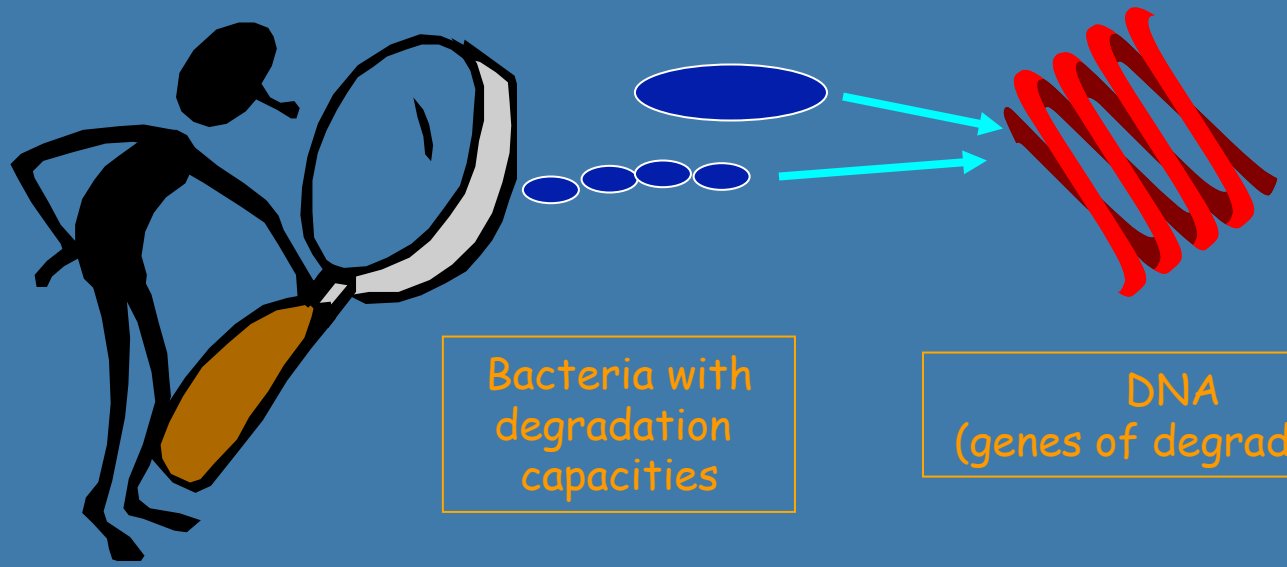
-How to monitore efficiently and at lower costs the ether-fuels concentrations in such large polluted areas ?

-How to clean the site if no biodegradation capacities have been detected ?

Interest to study strains with MTBE/ETBE biodegradation potential

Xenobiotic compound

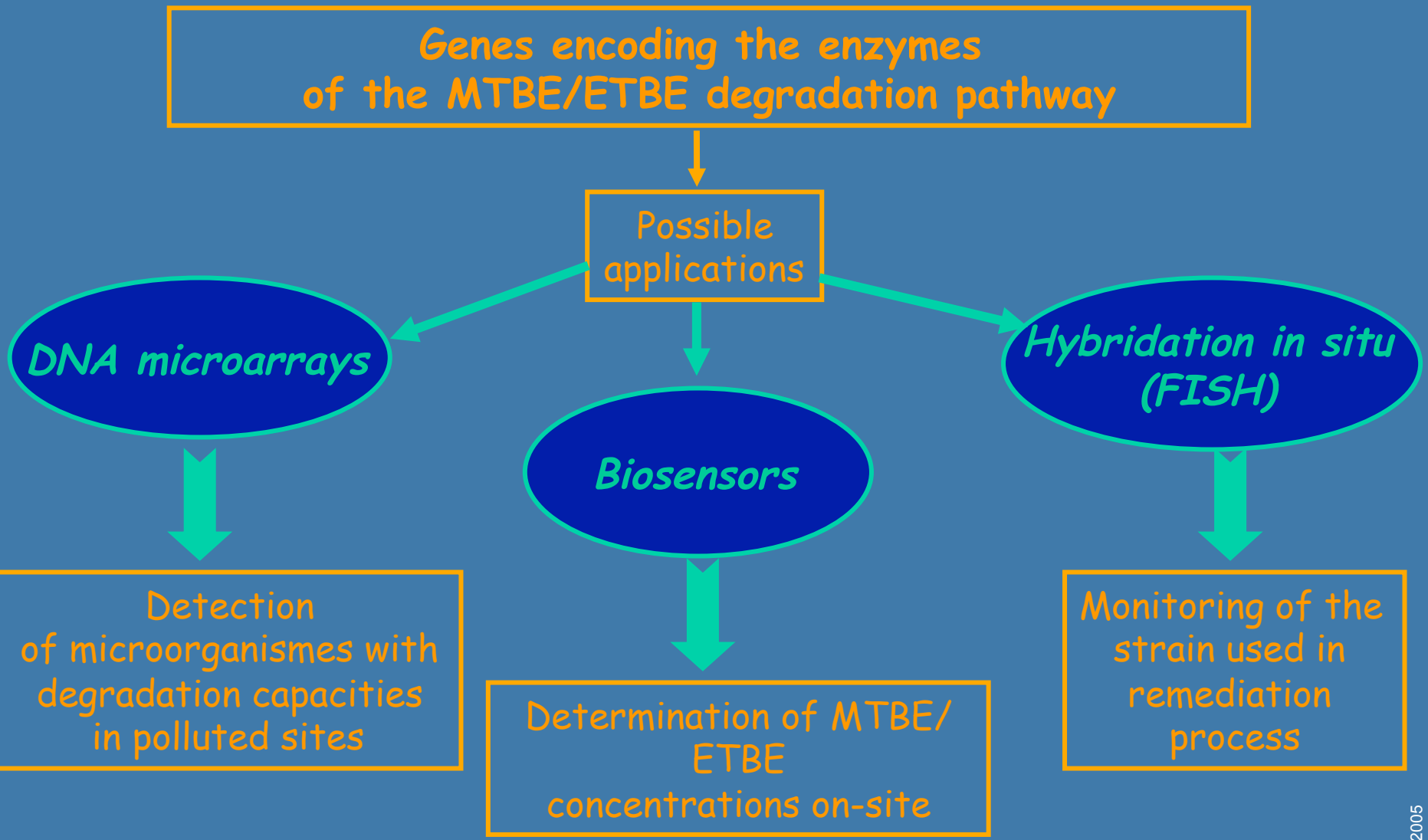
Poor biodegradation= persistence in polluted environment



Bacteria with degradation capacities

DNA (genes of degradation)

Possible applications: new tools for environmental management



First question:

How to estimate the potential for natural attenuation?

IFP with Biotechnology Research Institute, Montréal

(Environmental Microbiology Group,

head: Dr Charles W. GREER)

Usual tools to estimate the potential for natural attenuation

-Classical respirometry tests:

* Production of CO_2
* O_2 consumption

} Not very useful in such a case

-Monitoring MTBE or ETBE residual concentrations during degradation tests using microflora present in the contaminated site:

→ Capacity of biodegradation present *in situ* ?

→ Estimation of the production of degradation intermediates (TBF, TBA)

} -requires to have the technical tools for such analyses
-time-consuming

Biodegradability of ether-fuels by various microflora

Origin of microflorae	Biodegradation of MTBE (%)	Biodegradation of ETBE (%)	Biodegradation of TBA (%)	Biodegradation of TAME (%)
Activated sludge A	40	100	100	100
Activated sludge V	31	100	100	84
Activated sludge F	0	8	100	0
Soil M	37	100	100	0
Soil B	0	5	100	5
Soil G	4	0	100	0
Soil N	0	0	100	0

Principle of DNA microarrays

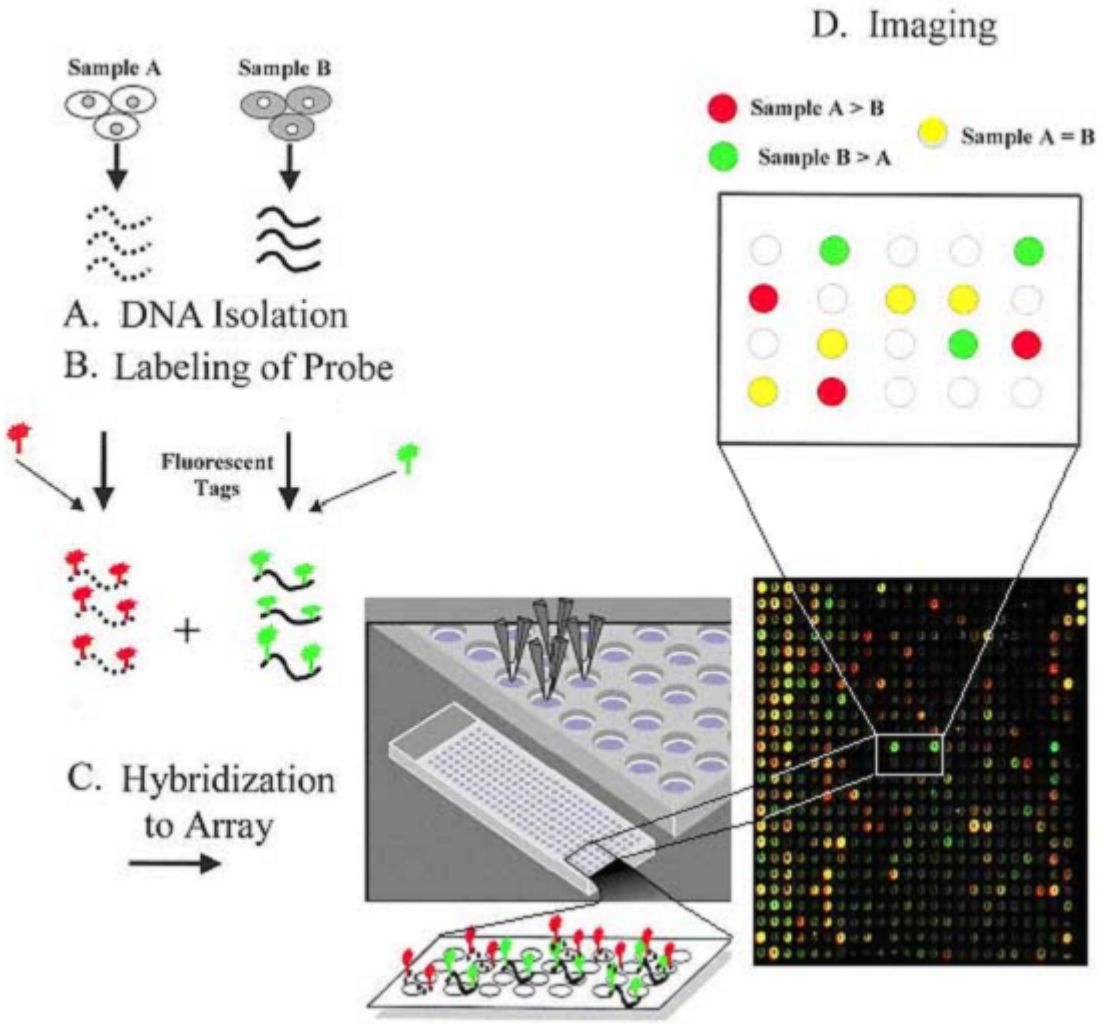
It is based on the following facts:

- DNA is a double-strand molecule,

- DNA sequences on each strand are complementary,

- the strands can easily be separated (DNA denaturation),

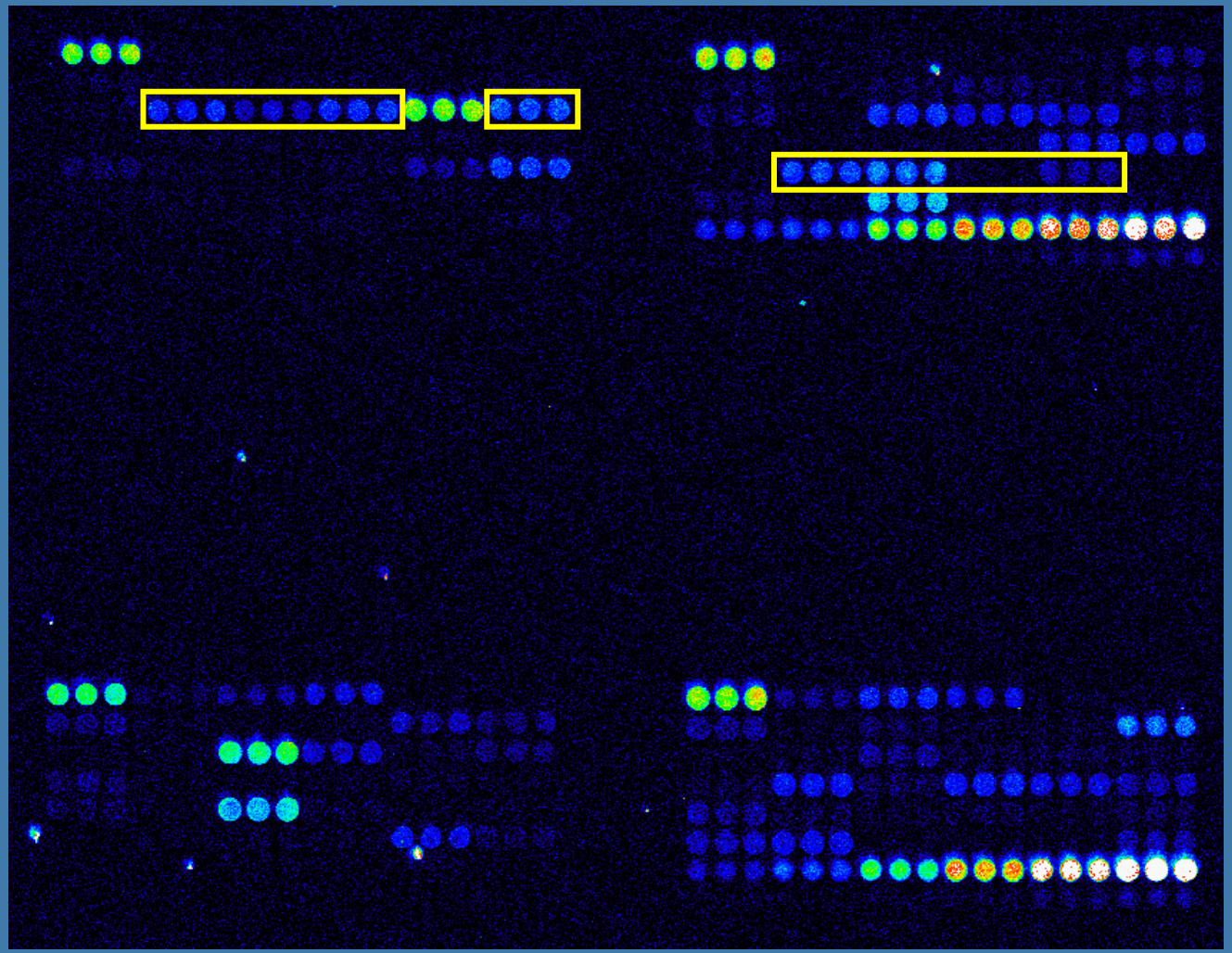
- each strand can then hybridized with a DNA sequence wich is complementary,



DNA microarrays:detection of alkane degradation genes

Biotechnology Research Institute (BRI), Canada
Group of Charles GREER

Soil DNA Extract Hybridized on a Catabolic Microarray : Soil contaminated with petroleum hydrocarbons

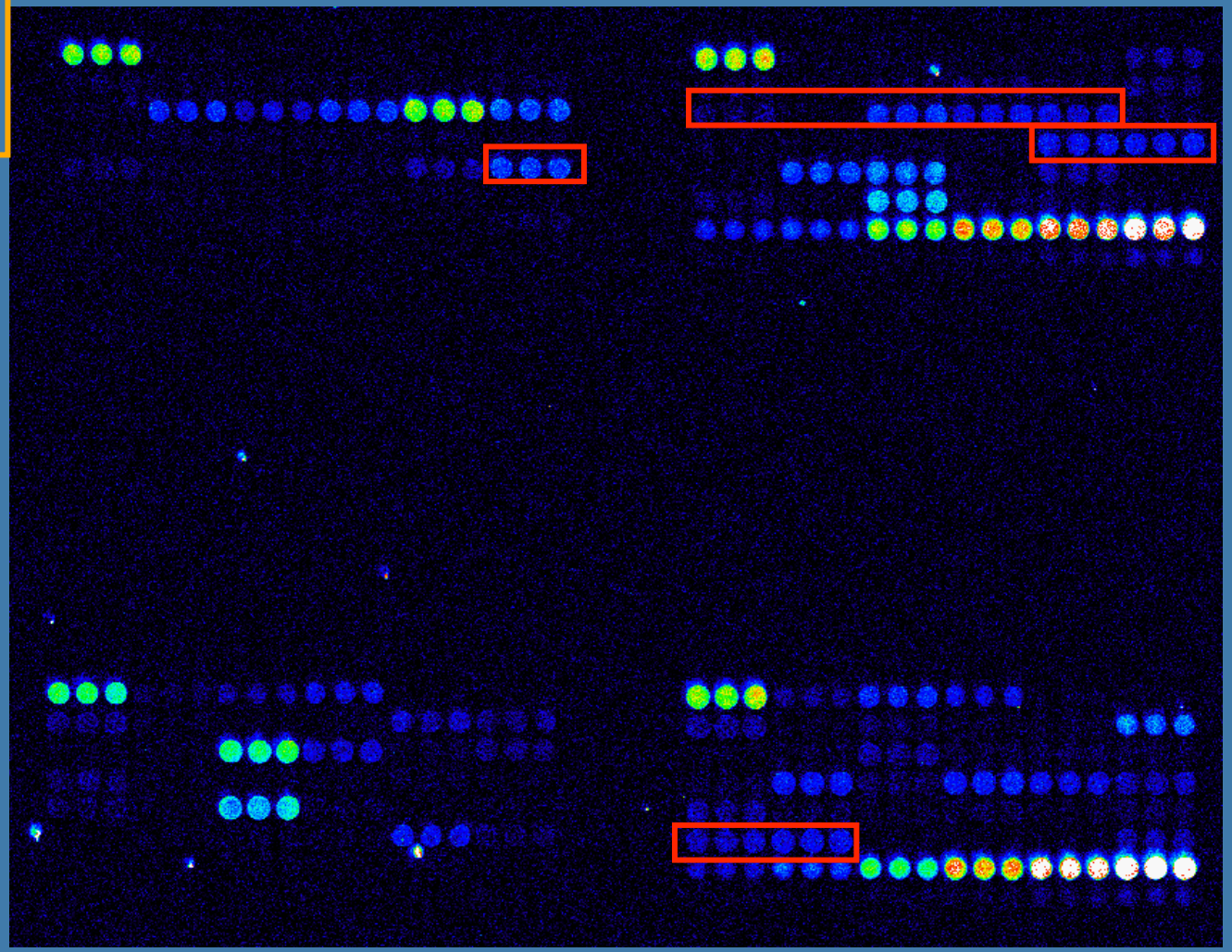


Alkanes degradationg enes

DNA microarrays:detection of naphtalene degradation genes

Biotechnology Research Institute (BRI), Canada
Group of Charles GREER

Soil DNA Extract Hybridized on a Catabolic Microarray : Soil contaminated with petroleum hydrocarbons

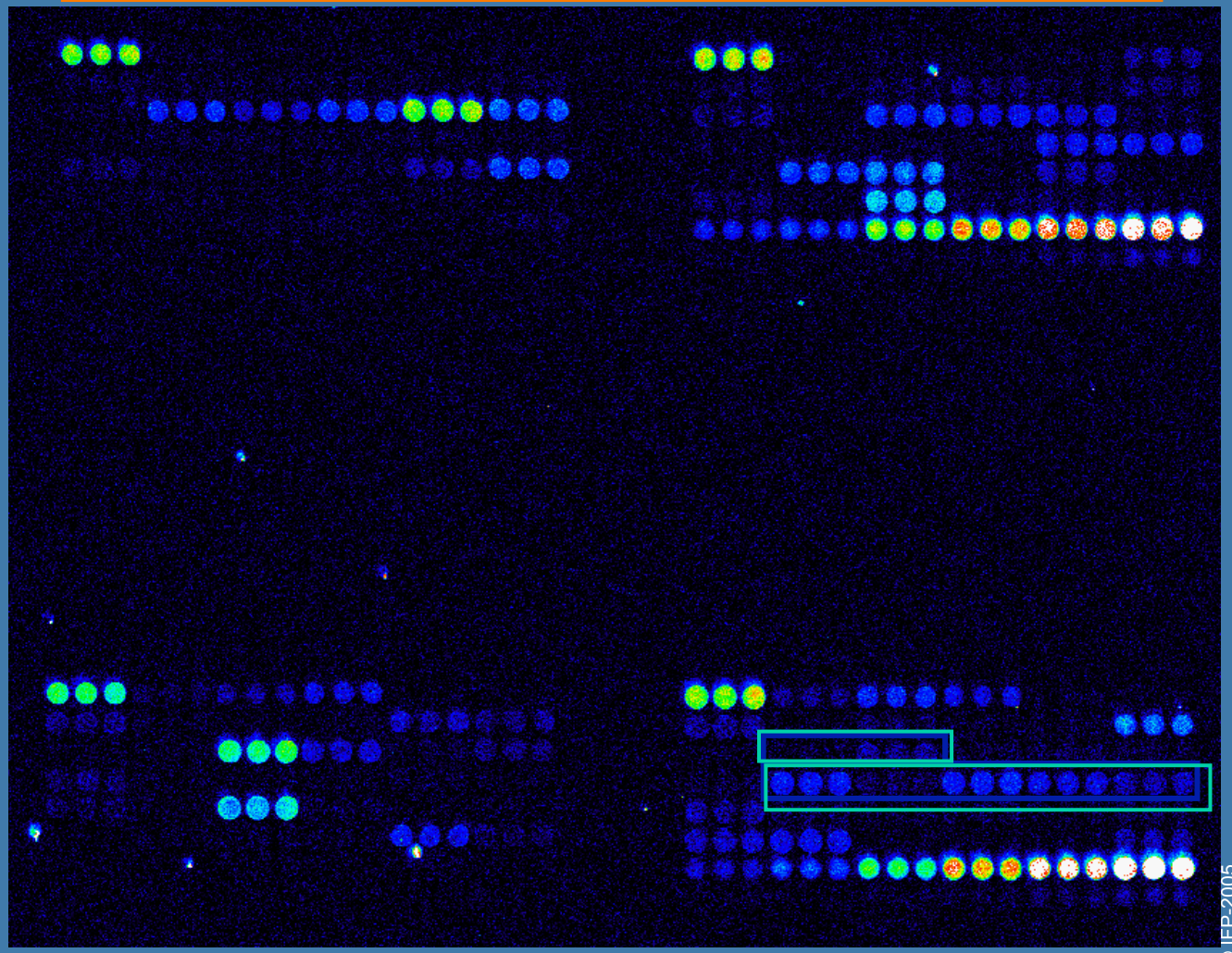


Alkanes degradationg enes

DNA microarrays:detection of monoaromatics degradation genes

Biotechnology Research Institute (BRI), Canada
Group of Charles GREER

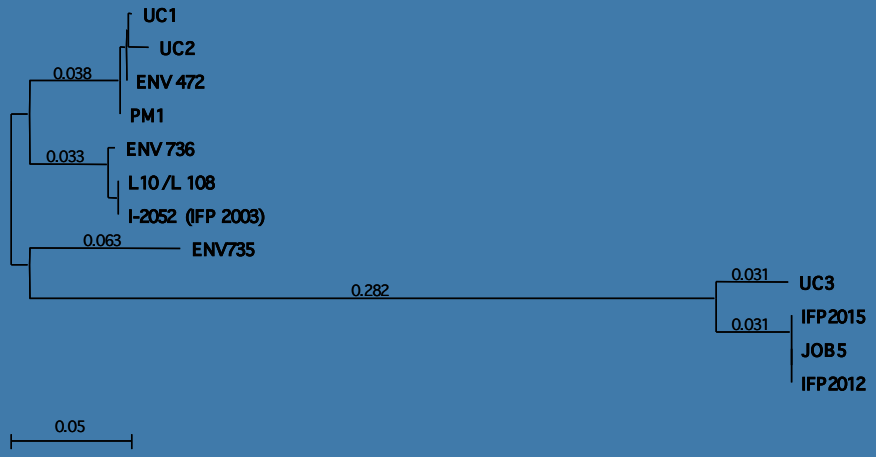
Soil DNA Extract Hybridized on a Catabolic Microarray : Soil contaminated with petroleum hydrocarbons



Monoaromatic compounds degradation genes

Ecology of MTBE biodegradation

- Isolation of strains with degradation capacities and their phylogenetic characterization:



Sequences of the 16S rDNA for detection of strains belonging to the different groups of the phylogenetic tree of MTBE-degraders

- Characterization of the enzymes involved in the degradation pathway Isolation and cloning of the corresponding genes:

eth genes

alk genes

mpd genes

Microarrays for detection of MTBE/ ETBE degradation capacities

Several genes characterized

Validation step

-determination of limits for detecting the interesting genes in samples containing complex DNA mixture.

-determination in parallel of:

* the MTBE/ETBE/TBA degradation capacities on samples from sites contaminated with ether fuels
* the presence of the genes of interest in these samples.

Second question:

How to monitore ether-fuels concentrations in polluted sites ?

IFP with Center for Environmental Biotechnology, Knoxville (USA)

(Head: Pr Gary SAYLER)

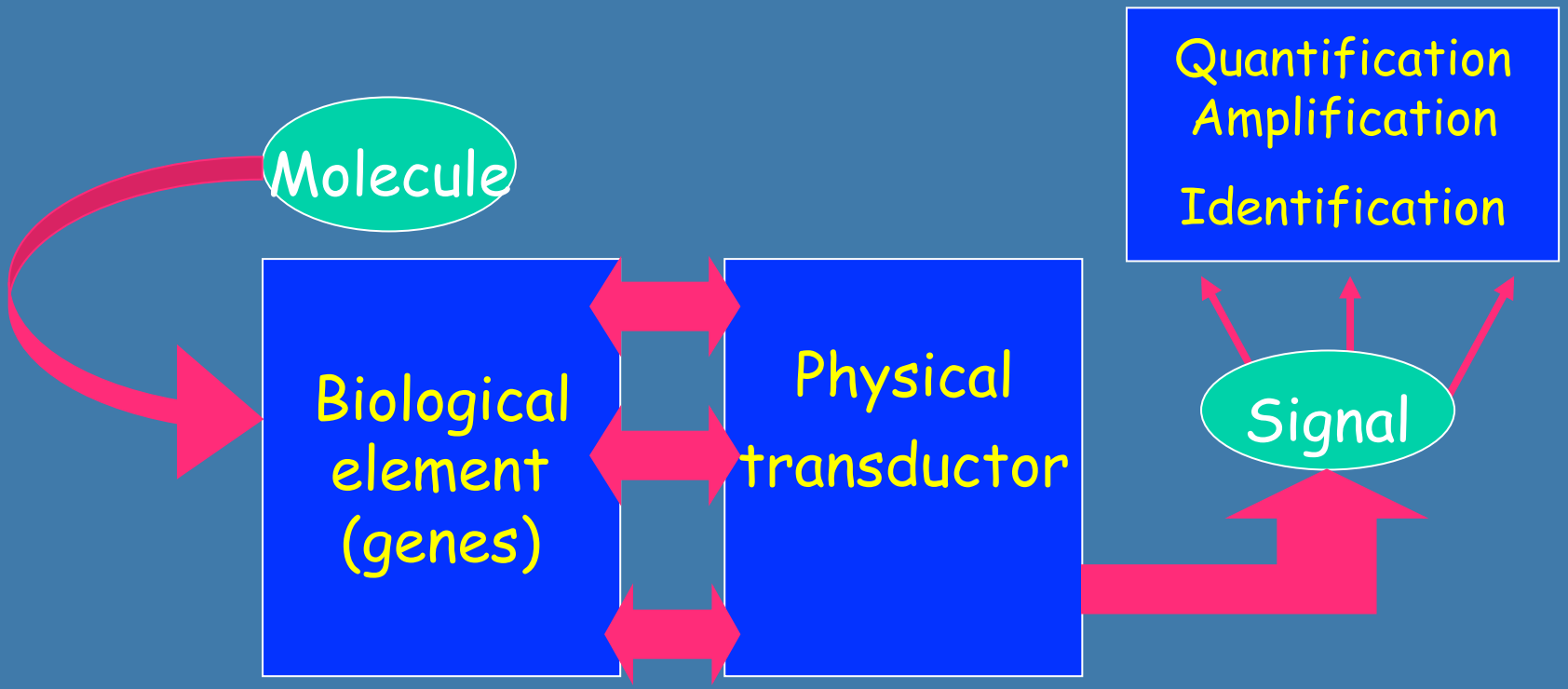
Usual tools to measure ether fuels concentrations

- Classical analytical method are very efficient (lower detection limit 0.02 µg/L):
  - GC/MS
  - GC/FID
  - Handling of numerous samples
  - Analysis is costly and time-consuming

A new detection tool allowing specific determination and reliable quantification of ETBE or MTBE would be usefull:

⇒ Bioluminescent biosensor

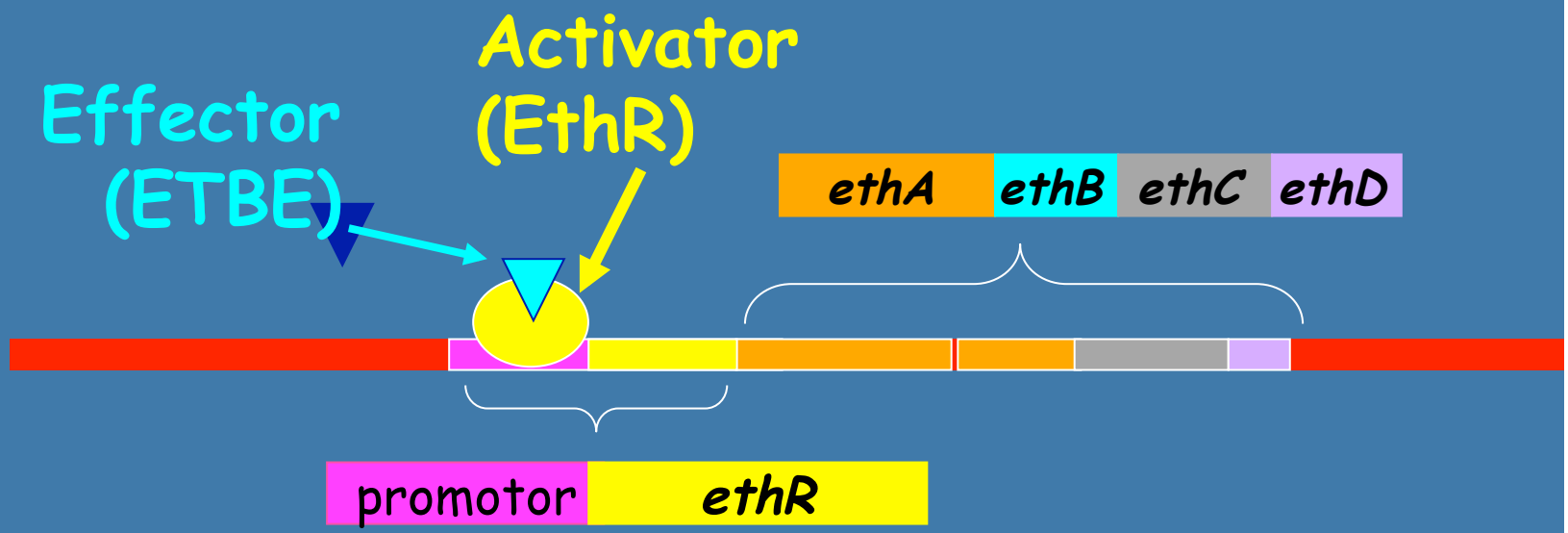
General principle of a biosensor



Elements required to built a bioluminescent biosensor

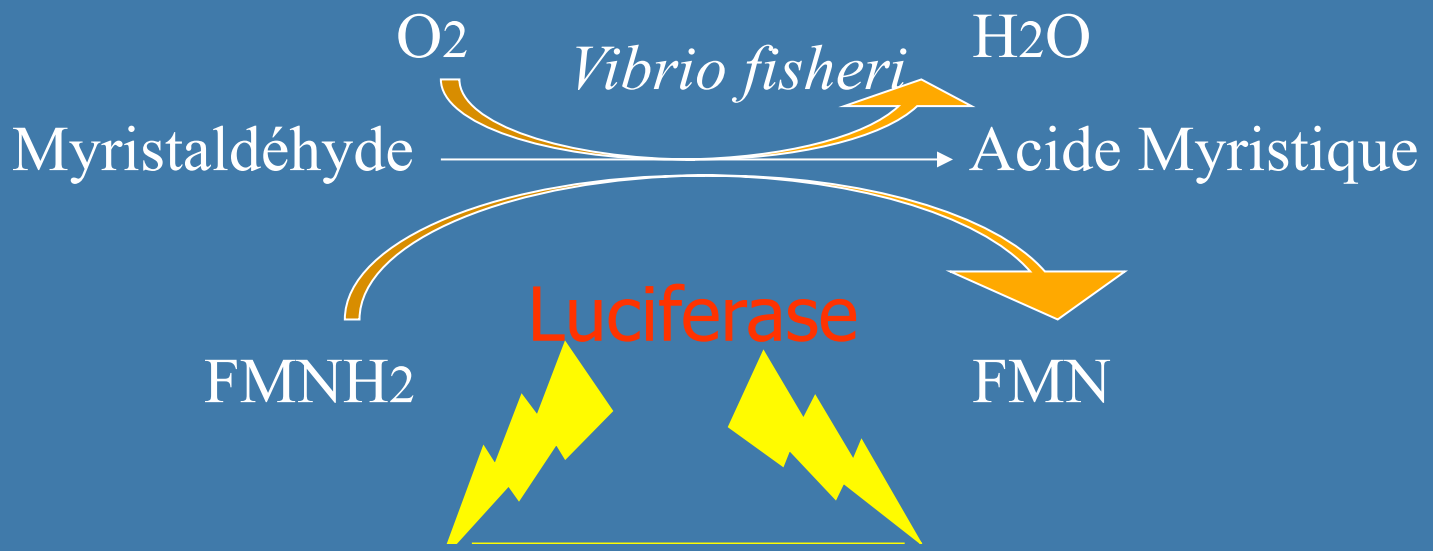
1

Genes for identification of ETBE:



Elements required to built a bioluminescent biosensor

2 Genes emitting light:



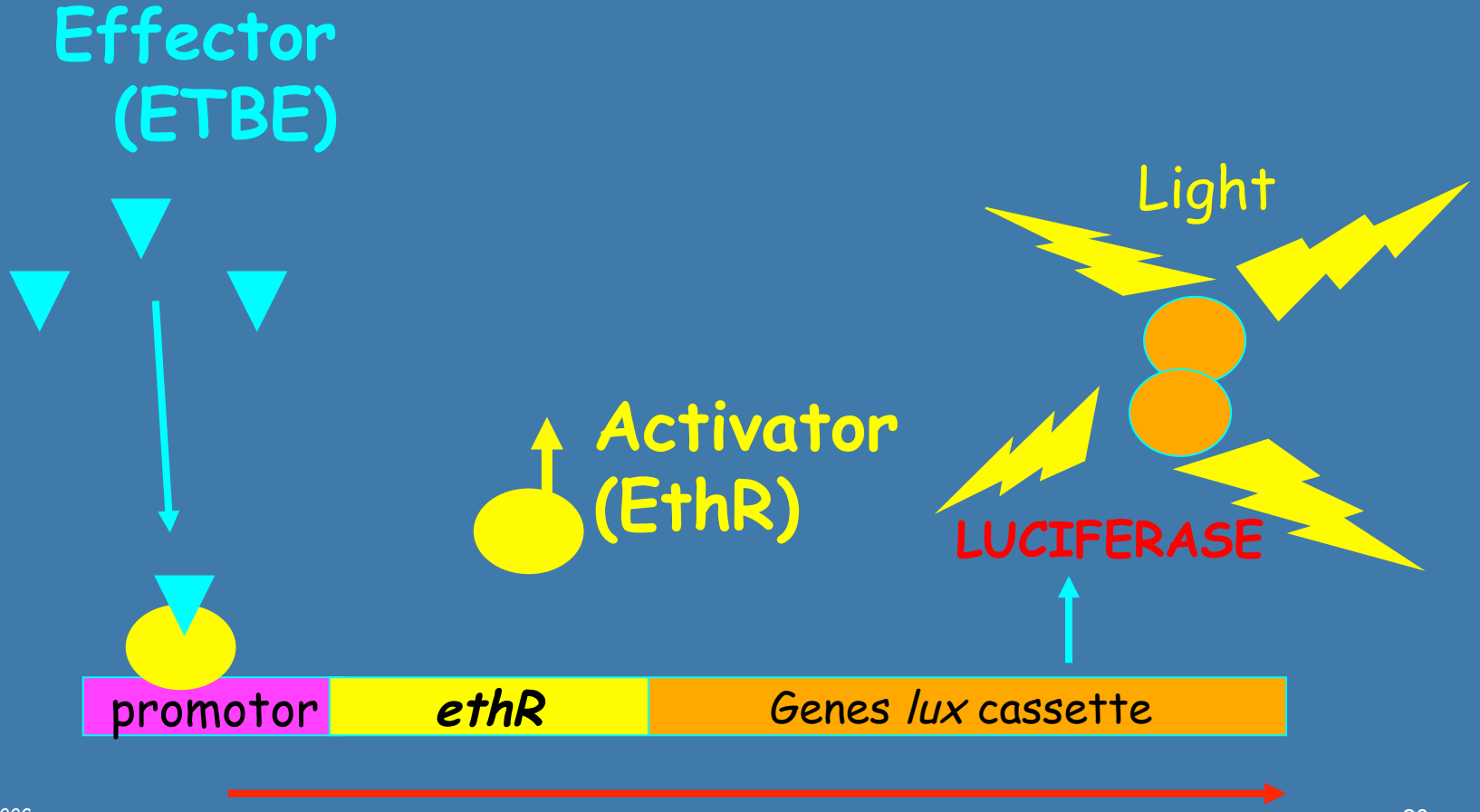
Genes of *V. fischeri* responsible for the synthesis of luciferase:

Cassette of *lux* genes = **cassette *lux* commercialy available**

The bioluminescent biosensor to detect ETBE

3

Transcriptional fusion of genes:



Third question:

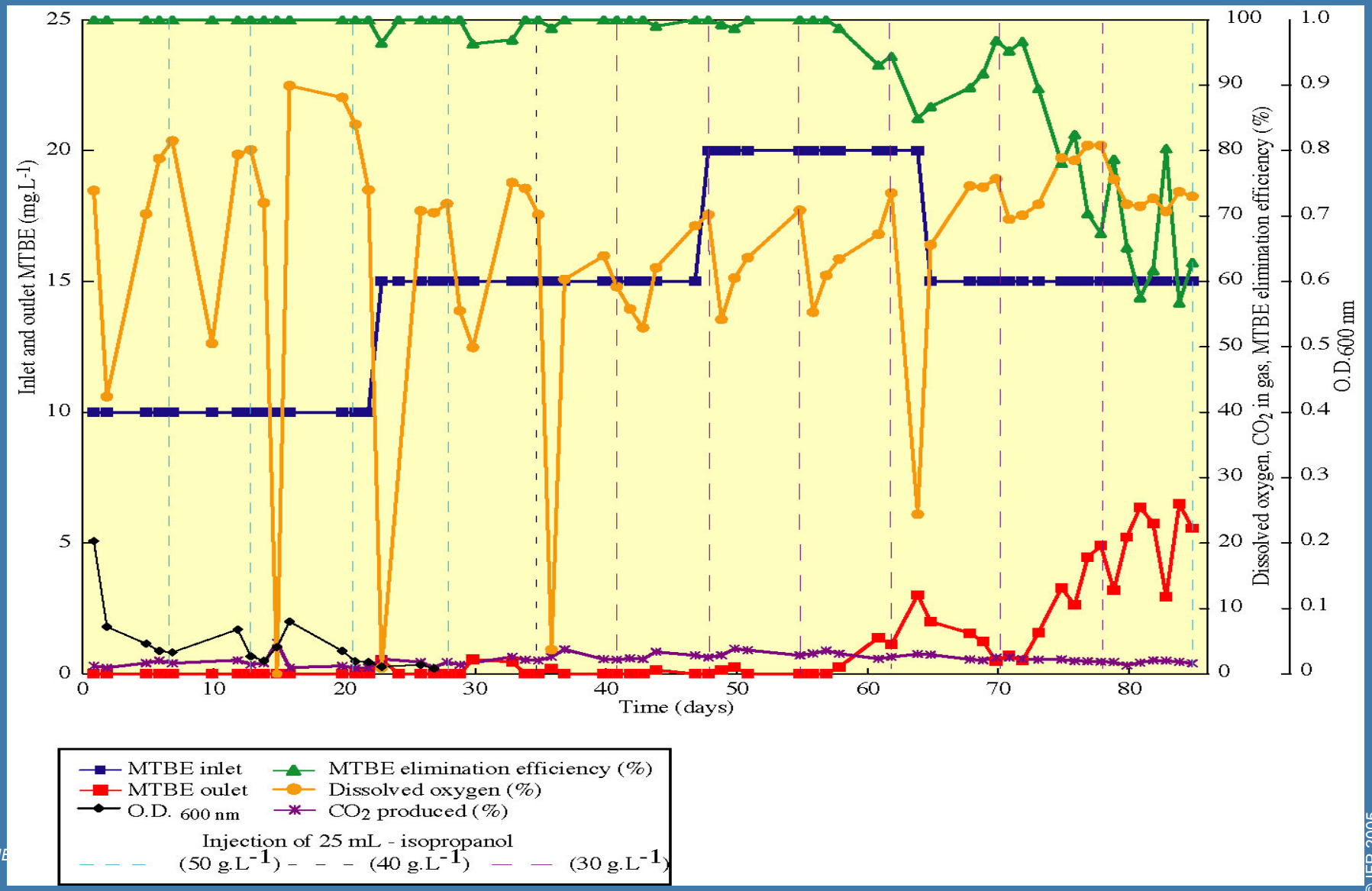
How to clean a MTBE-contaminated site ?

IFP with Biotechnology Research Institute, Montréal

(Group Environment Biotechnology,

head: Dr Serge Guiot)

Bioremediation process using *M. austroafricanum* IFP 2012: biofilter

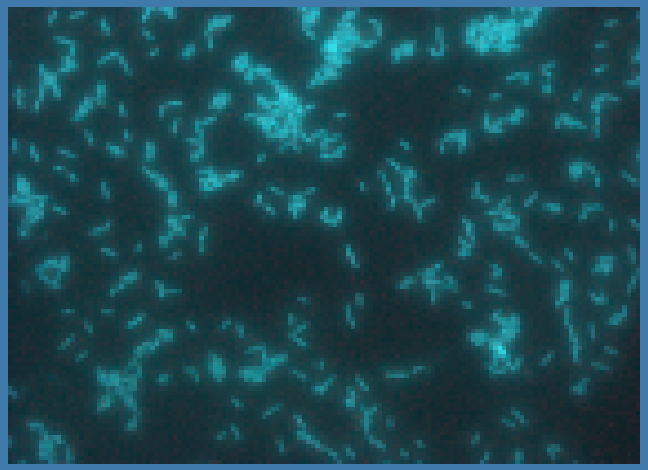


Monitoring *M. austroafricanum* IFP 2012 on the biofilter or in the site

Use of fluorescent in situ hybridization (FISH):

-labeling of probe (16S rDNA) with fluorescent tag

-specific for the detection of a given microorganism



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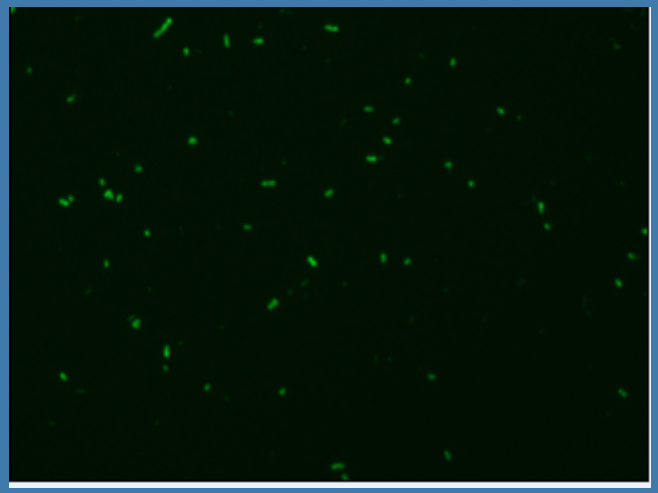

FISH

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Serge Guiot

-Center fior Environmental Biotechnology, Knoxville, USA :

Steven Ripp, Gary Sayler.