

*From Life Science to  
Environmental Science:  
Successes and Challenges*

*Teresa Lettieri*

*European Commission Joint Research Centre;  
Institute for Environment and Sustainability*



<http://ies.jrc.ec.europa.eu/>

<http://www.jrc.ec.europa.eu/>

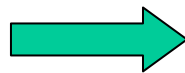
# The onset of Molecular Biology

✓ **Cloning**



Insertion of piece of DNA  
into a plasmid (vector) to  
propagate into bacteria

✓ **Sequencing  
technique**



How to “read” the DNA sequence  
GATTACA

✓ **Expression of  
recombinant protein**



Insulin was the first therapeutic  
recombinant protein

# First successes in Gene Cloning

*Proc. Natl. Acad. Sci. USA*  
Vol. 76, No. 1, pp. 106–110, January 1979  
Biochemistry

  
Proceedings of the National Academy of Sciences of the United States of America [www.pnas.org](http://www.pnas.org)

## Expression in *Escherichia coli* of chemically synthesized genes for human insulin

(plasmid construction/*lac* operon/fused proteins/radioimmunoassay/peptide purification)

DAVID V. GOEDEL\*†, DENNIS G. KLEID\*, FRANCISCO BOLIVAR\*, HERBERT L. HEYNEKER\*, DANIEL G. YANSURA\*, ROBERTO CREA\*‡, TADAAKI HIROSE‡, ADAM KRASZEWSKI‡, KEIICHI ITAKURA‡, AND ARTHUR D. RIGGS†‡

\*Division of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; and †Division of Biology, City of Hope National Medical Center, Duarte, California 91010

*Nature Vol. 294 24/31 December 1981*

## cdNA sequences of human glucose 6-phosphate dehydrogenase cloned in pBR322

M. G. Persico, D. Toniolo, C. Nobile, M. D'Urso & L. Luzzatto\*

International Institute of Genetics and Biophysics, CNR, Napoli, Italy





## Recombinant insulin

- Insulin was isolated in 1922
- For 60 years, cattle and pigs were the sources of insulin.
- In 1978, Genentech and City of Hope National Medical Center produced human insulin using recombinant DNA technology.
- ElyLilly licensed recombinant insulin from Genentech and developed it.
- In 1982, ElyLilly's Humulin became the first genetically engineered drug approved by the FDA.

From: C&EN SPECIAL ISSUE, The Top Pharmaceuticals That Changed The World, Vol. 83, Issue 25 (6/20/05)

# First successes in Gene Cloning

*Proc. Natl. Acad. Sci. USA*  
Vol. 76, No. 1, pp. 106–110, January 1979  
Biochemistry

PNAS

Proceedings of the National Academy of Sciences of the United States of America [www.pnas.org](http://www.pnas.org)

## Expression in *Escherichia coli* of chemically synthesized genes for human insulin

(plasmid construction/*lac* operon/fused proteins/radioimmunoassay/peptide purification)

DAVID V. GOEDEL\*†, DENNIS G. KLEID\*, FRANCISCO BOLIVAR\*, HERBERT L. HEYNEKER\*, DANIEL G. YANSURA\*, ROBERTO CREA\*‡, TADAAKI HIROSE‡, ADAM KRASZEWSKI‡, KEIICHI ITAKURA‡, AND ARTHUR D. RIGGS†‡

\*Division of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; and †Division of Biology, City of Hope National Medical Center, Duarte, California 91010

*Nature Vol. 294 24/31 December 1981*

## cdNA sequences of human glucose 6-phosphate dehydrogenase cloned in pBR322

M. G. Persico, D. Toniolo, C. Nobile, M. D'Urso & L. Luzzatto\*

International Institute of Genetics and Biophysics, CNR, Napoli, Italy



# Glucose 6-phosphate dehydrogenase (G6PD) enzyme

G6PD' s role in red blood cells is to provide molecules enable to protect against oxidative stress generated by several oxidant agents

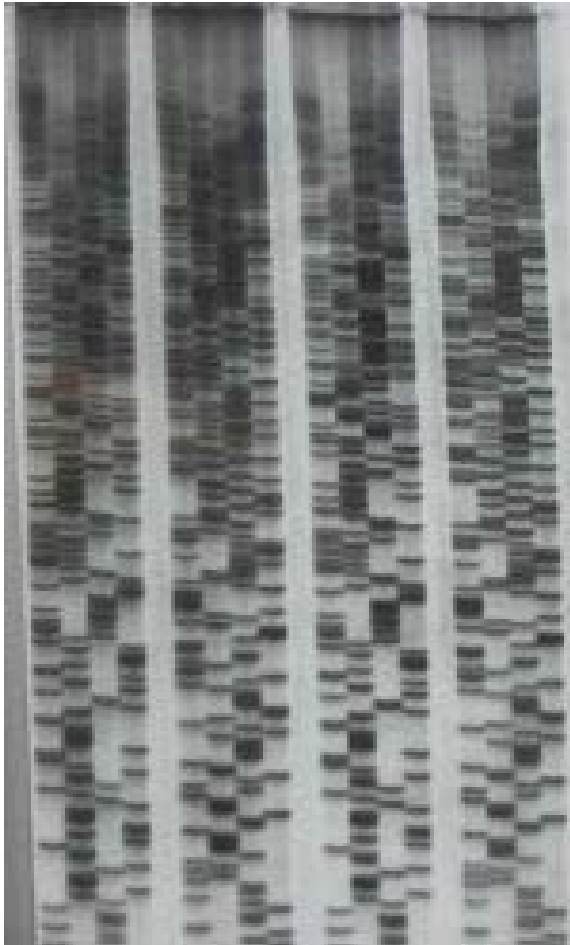
- G6PD deficiency is the most common human enzyme defect, present in more than 400 million people worldwide

→ Hemolysis induced by drug (induced by drug)

→ Favism: Hemolytic anemia induced by ingesting fava beans

→ Hereditary non spherocytic hemolytic anemia

# Sequencing method

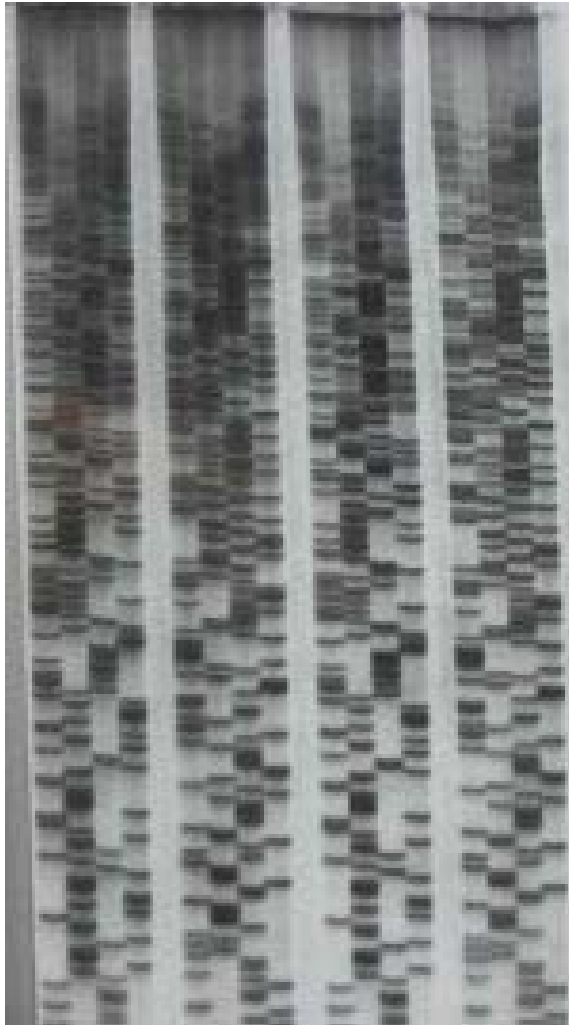


CATG

CATG

Big challenge at early '80s:  
sequencing G6PD from several  
individuals to identify the mutations

# The automation of sequencing



CATG CATG

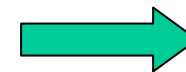


1 capillary



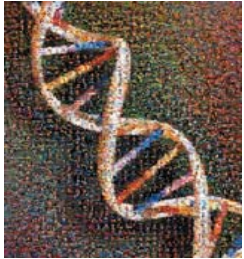
96 samples

96 capillaries

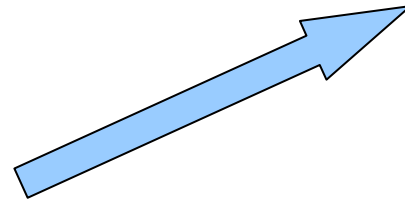


96 samples  
at the same time!!

# Genome Sequencing



Nature, 15 February 2001

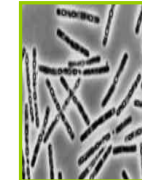
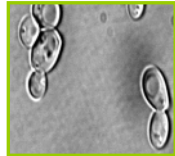
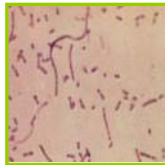
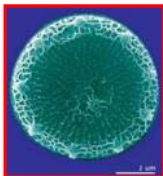
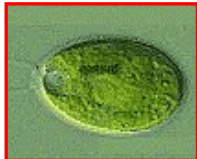


*Haemophilus influenzae*  
The first organism to be  
Sequenced (1995)



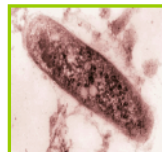
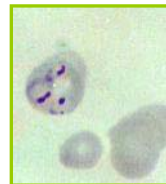
Completed in 2003

# Some considerations



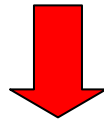
✓ *At beginning the sequencing was concentrated on pathogens or species relevant for biomedical field*

✓ *Many other organisms relevant in environment field were sequenced*



# DNA Microarray Technology

✓ *High density spatial synthesis on chip*

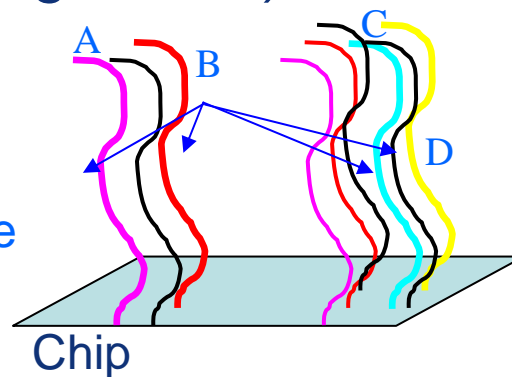


✓ *Thousands of genes or the whole genome on the chip*



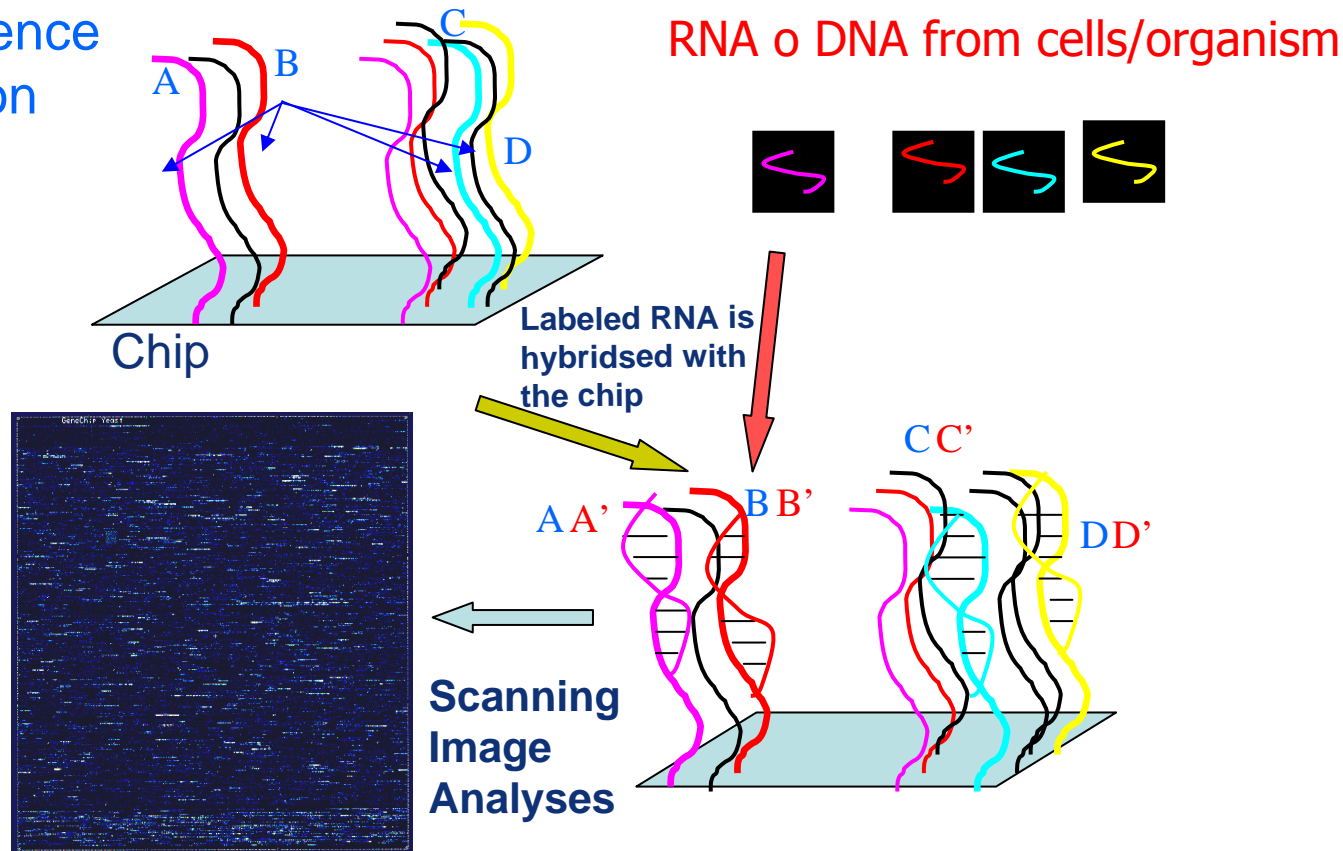
✓ *Possibility to analyze the expression level of several genes (or the whole genome) at the same time*

Part of each  
gene sequence  
is spotted on  
the chip



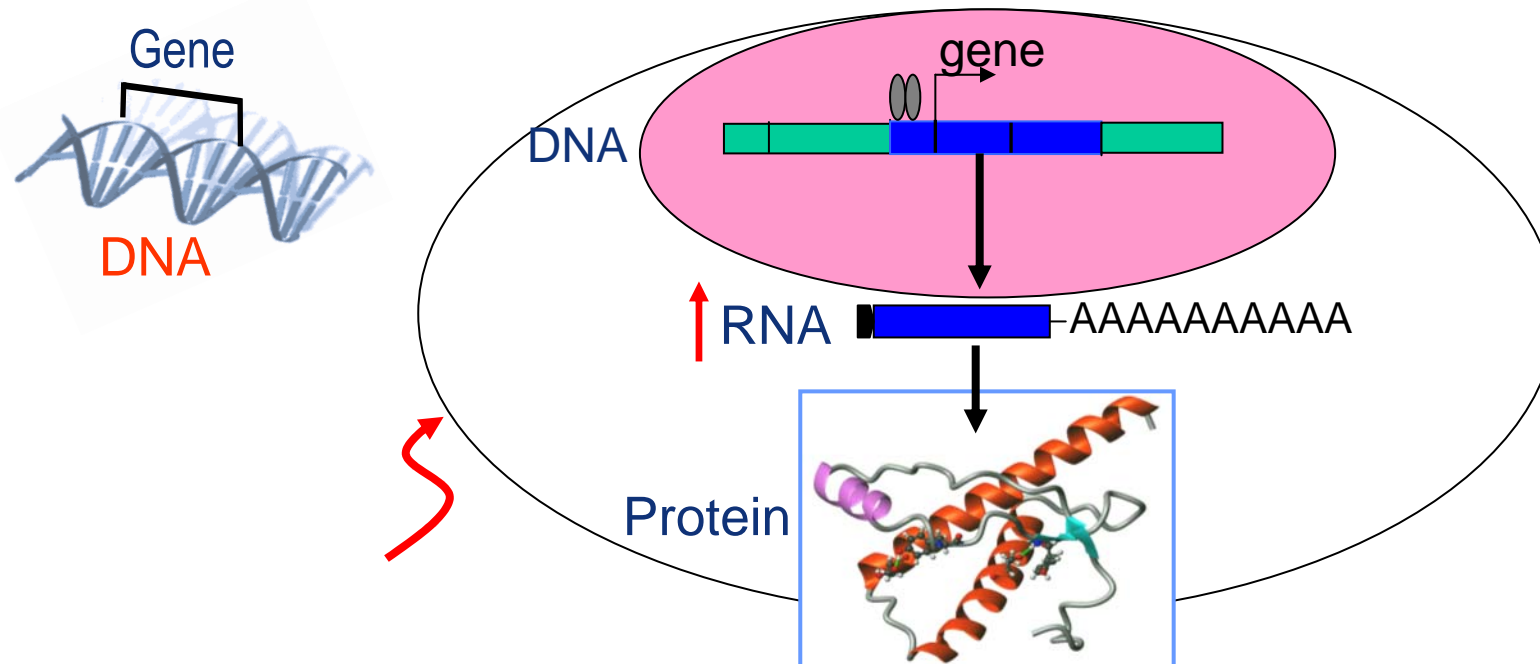
# DNA Microarray

Part of each  
gene sequence  
is spotted on  
the chip



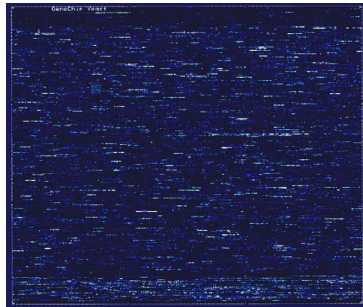
The fluorescence signal is directly proportional to the abundance of RNA


# Inside the cell/ organisms

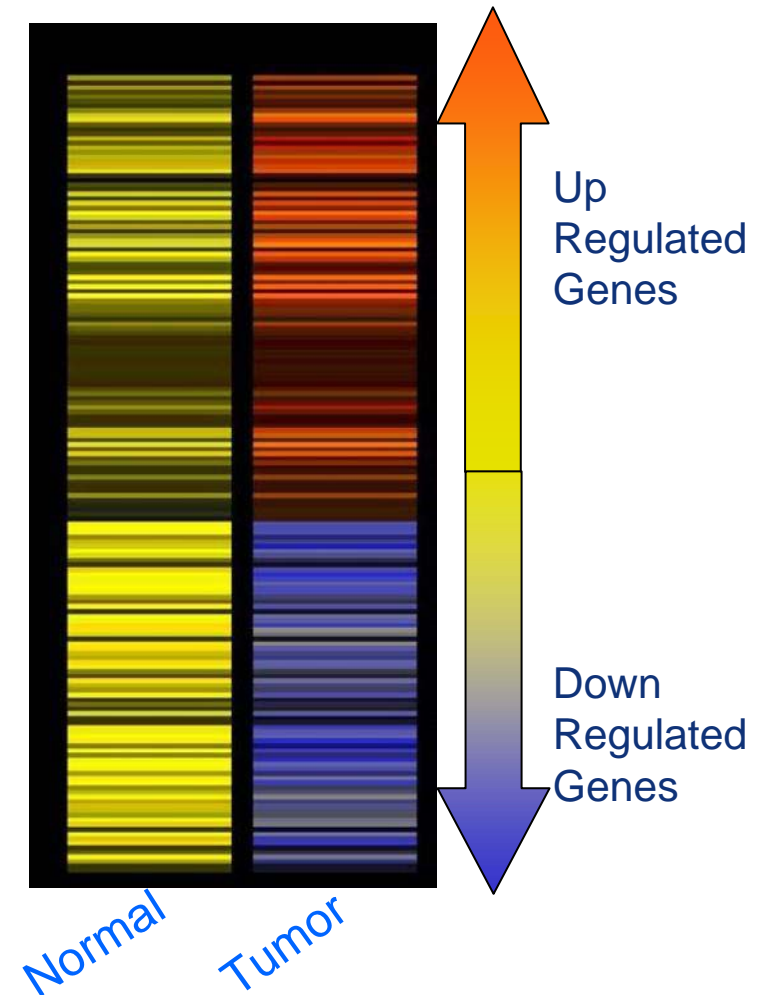


**Stressors can induce changes at expression level such an increase  $\uparrow$  or decrease  $\downarrow$  of RNA)**  
(drug, chemical pollutants, UV lights)

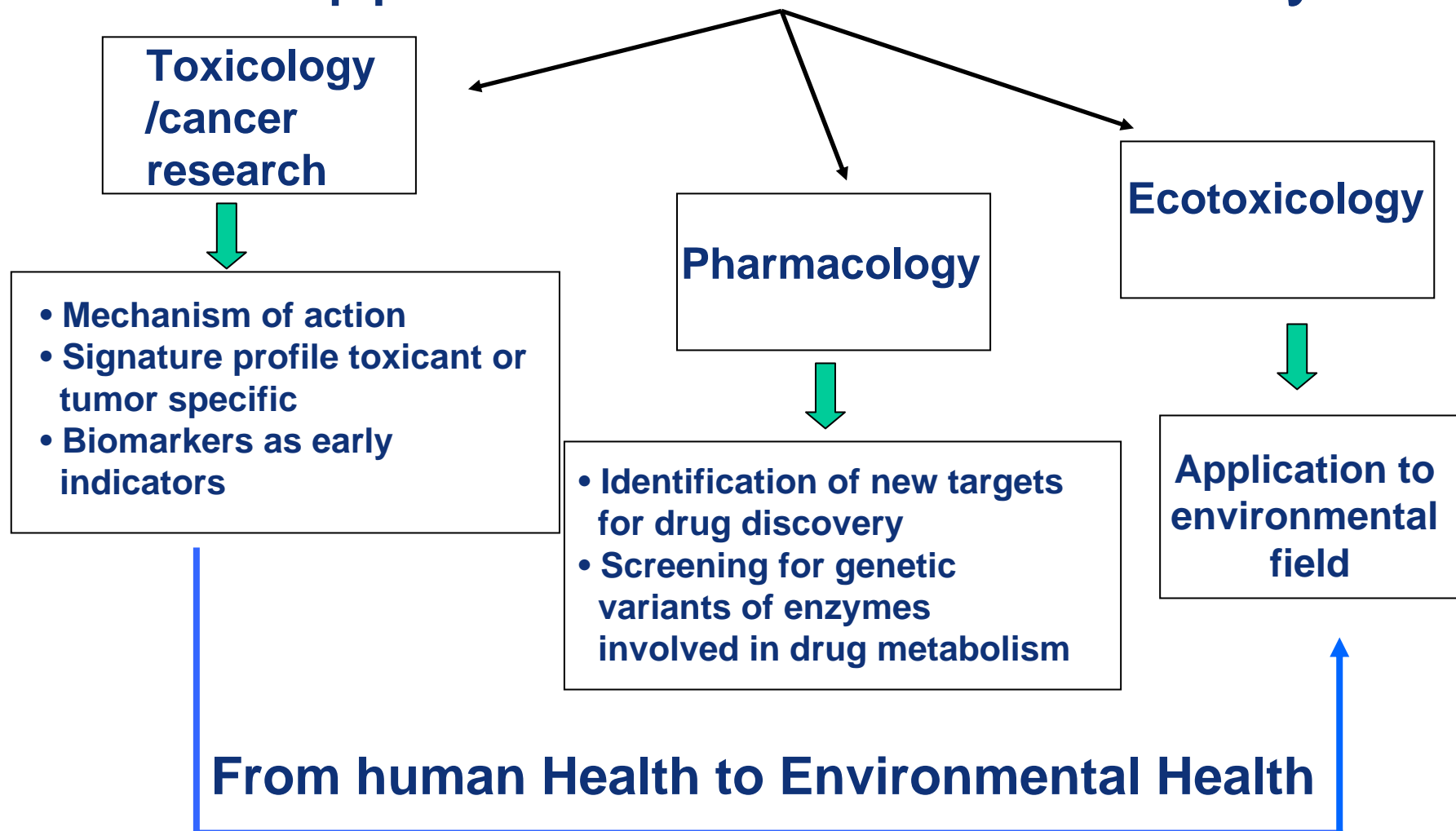
# Gene Expression Profile



- ✓ Gene profile of tumor cell compared to normal cells  Markers
- ✓ Identify new targets for drug development



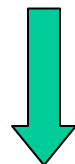
# Application of DNA Microarray



# DNA Microarray to Environmental sciences

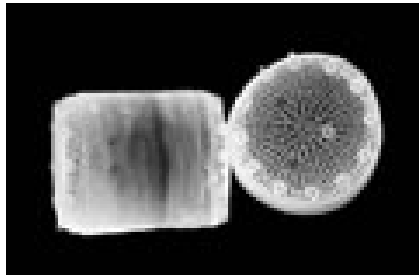
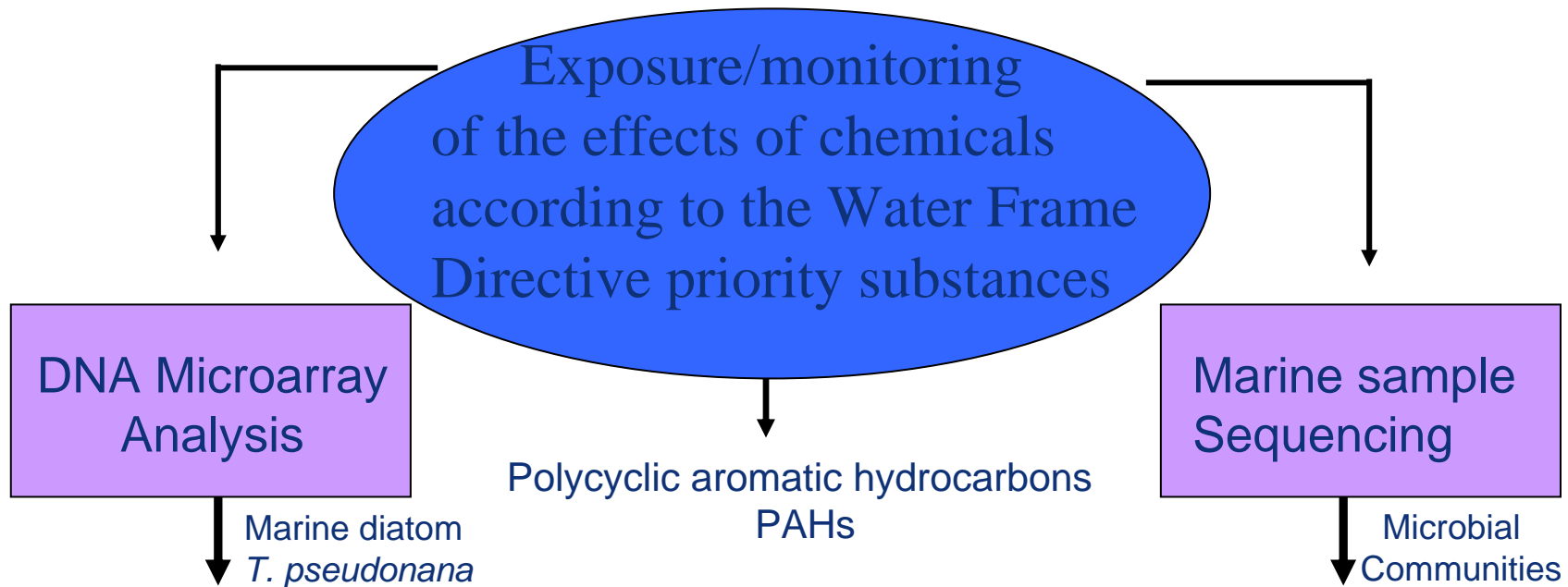


Exposed to anthropogenic pollutants  
such as pesticides, polyhydrocarbons,  
pharmaceutical compounds



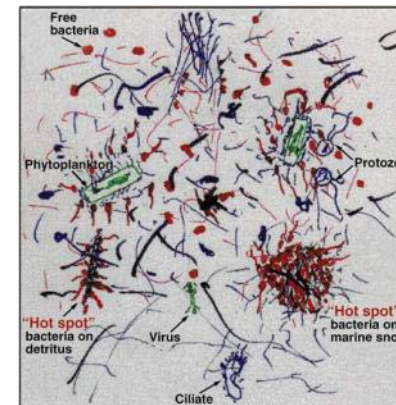
All components of the ecosystems  
(fishes, mammals, algae, bacteria, viruses)

# Ecogenomics at JRC

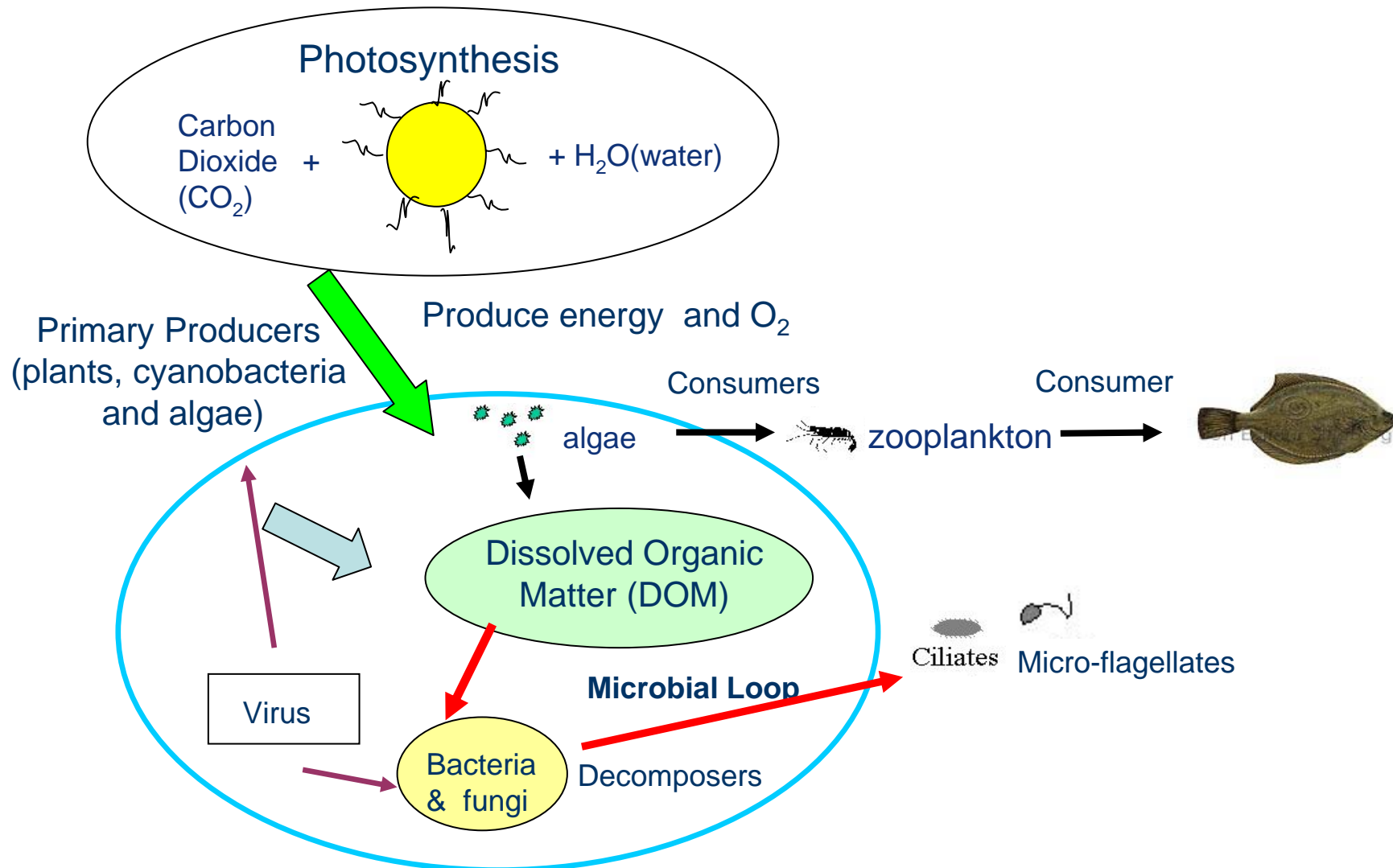


M.Hildebrand (UC, San Diego)

## Why?

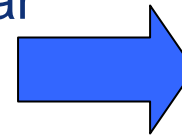


# Ecosystem's Drivers



## *Thalassiosira pseudonana*

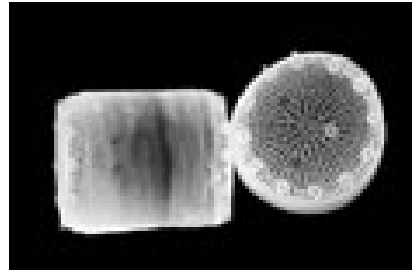
- Diatoms are responsible for 20-25% of the global carbon cycle  
(Armbrust et al, Science 2004)
- *T. pseudonana* is a single cell marine algae and is ubiquitously distributed in water worldwide
- Its genome has been sequenced making it a good model for identifying molecular biomarkers



DNA microarray developed at JRC  
and manufactured by Agilent

## Cytotoxic effects

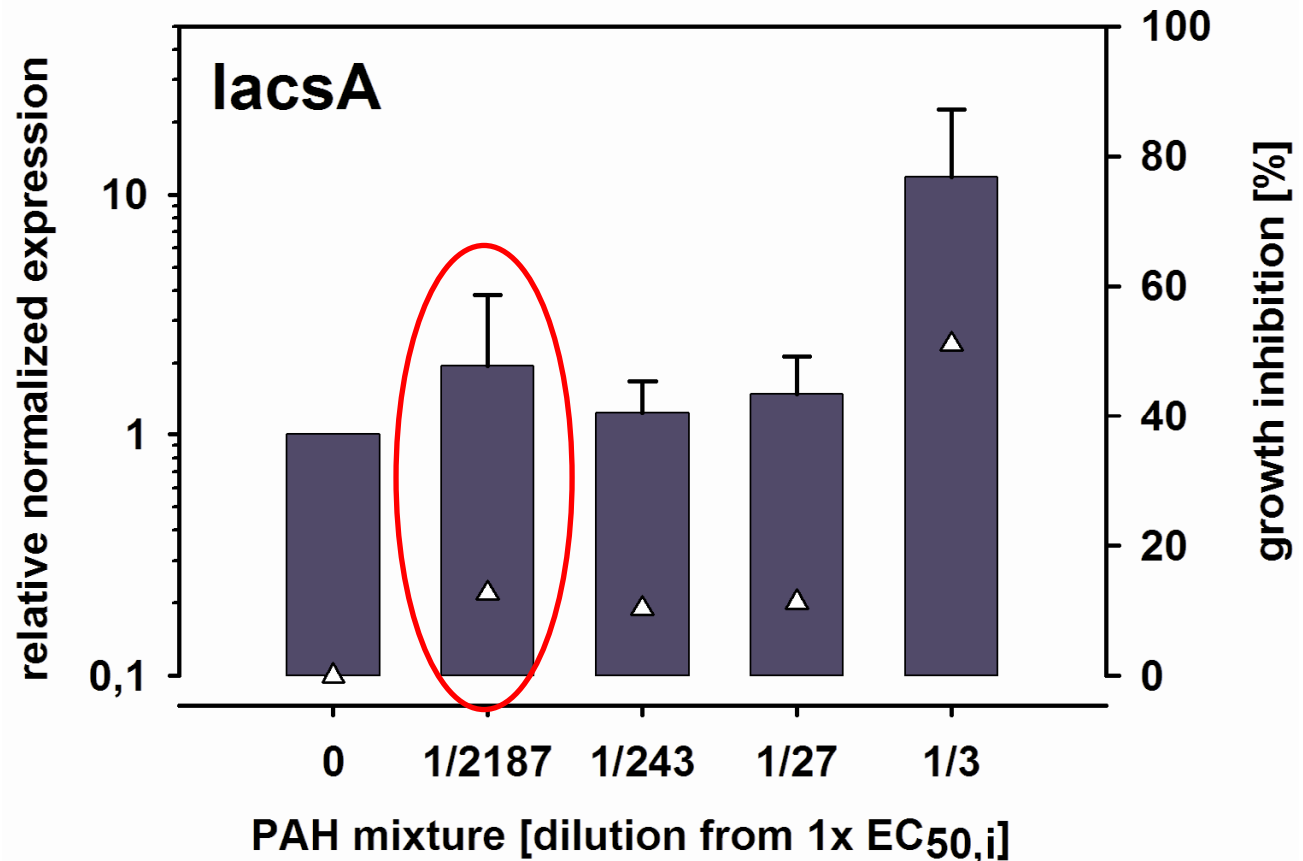
Dose-response curves  
 → Effect concentrations



## Effects on gene expression at mRNA level

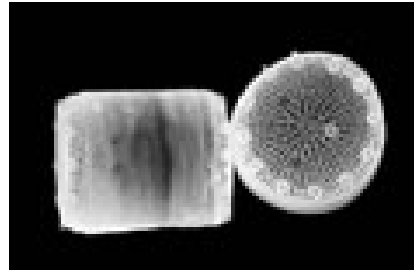
Fatty acid metabolism

*lacsA*: Long chain acyl-coA synthetase is involved in fatty acid metabolism



## Cytotoxic effects

Dose-response curves  
 → Effect concentrations



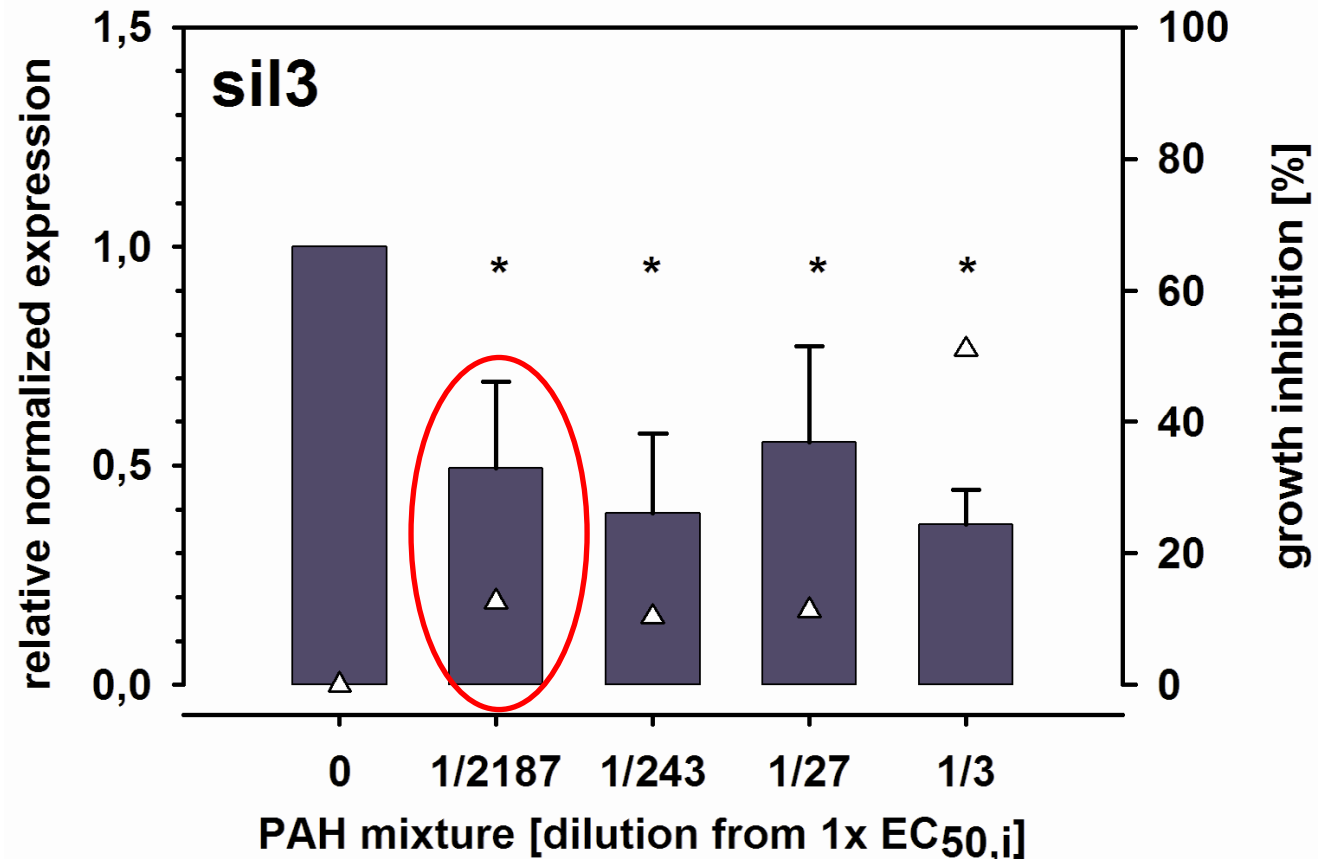
## Effects on gene expression at mRNA level

Silica shell formation

*Sil3*: silaffin precursor 3  
 is involved in silica shell  
 formation



Silaffin transporter is  
 down regulated

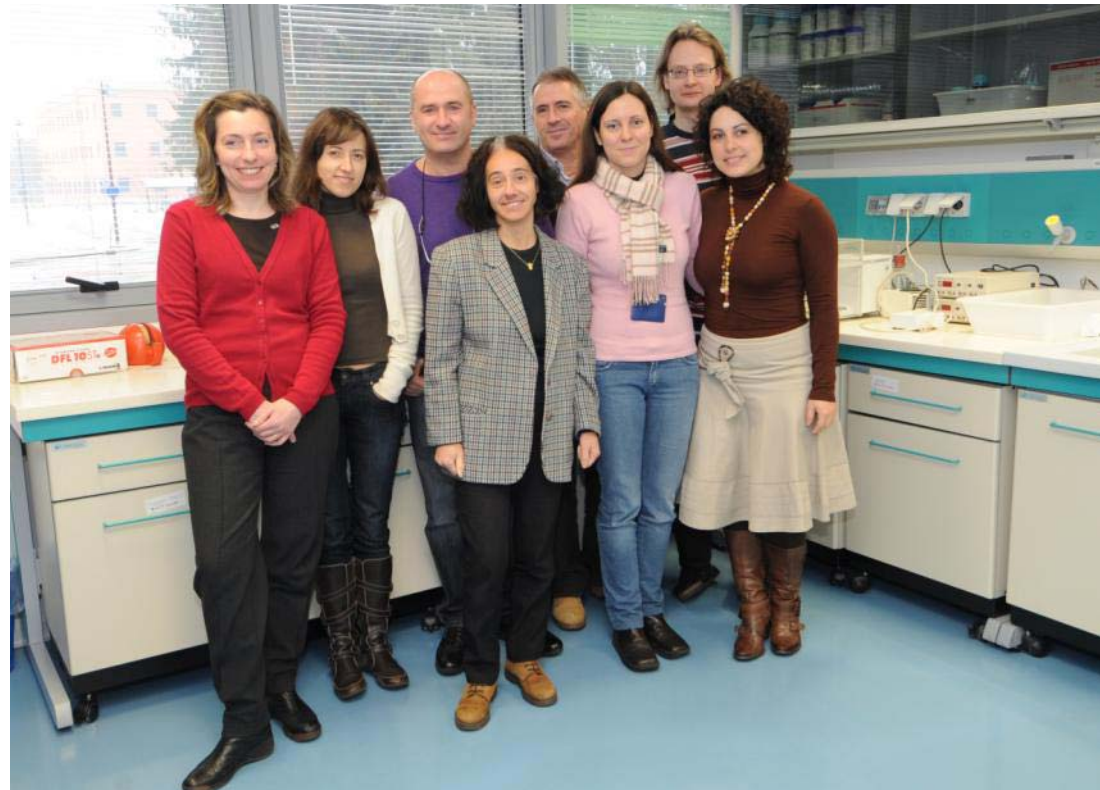


# Conclusion / Remarks

- ❖ Molecular Biology has been extremely successful in helping us to understand how genomic information is translated into biology
- ❖ The applications to environmental sciences is at an earlier stage due to the more complex problems and the many variables in a real ecosystem
- ❖ However the advanced genomic technologies are providing new tools to determine the composition of e.g. microbial communities; to understand their interactions and then to understand how they respond to the global change

# The Future will be....

## Molecular Environmental Biology



**Thank you for your attention**