Bacterias para el medio ambiente: de la Bioremediación a la Biología Sintética

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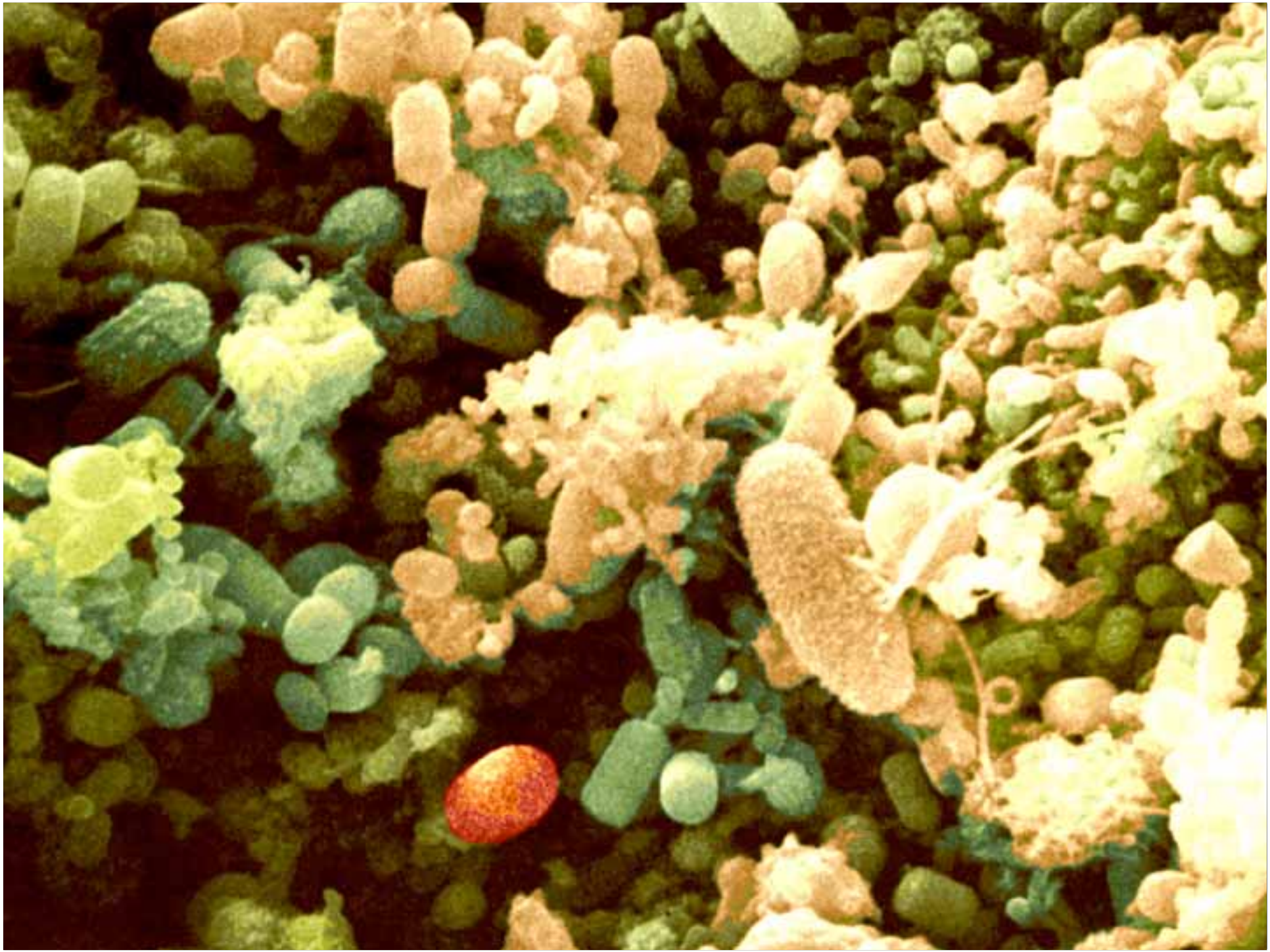
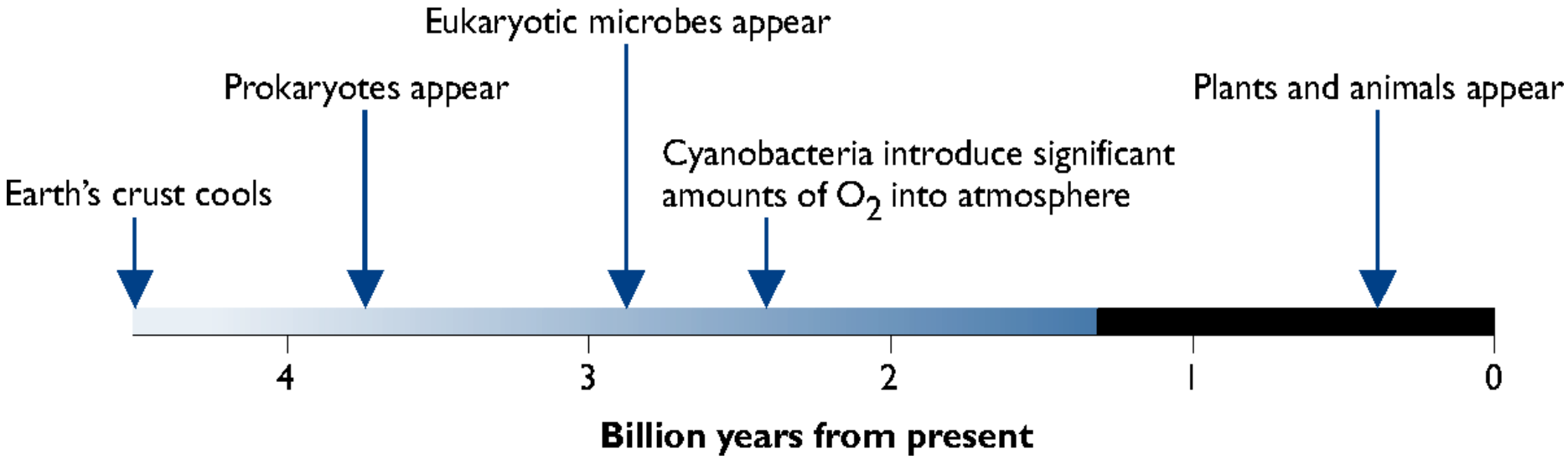
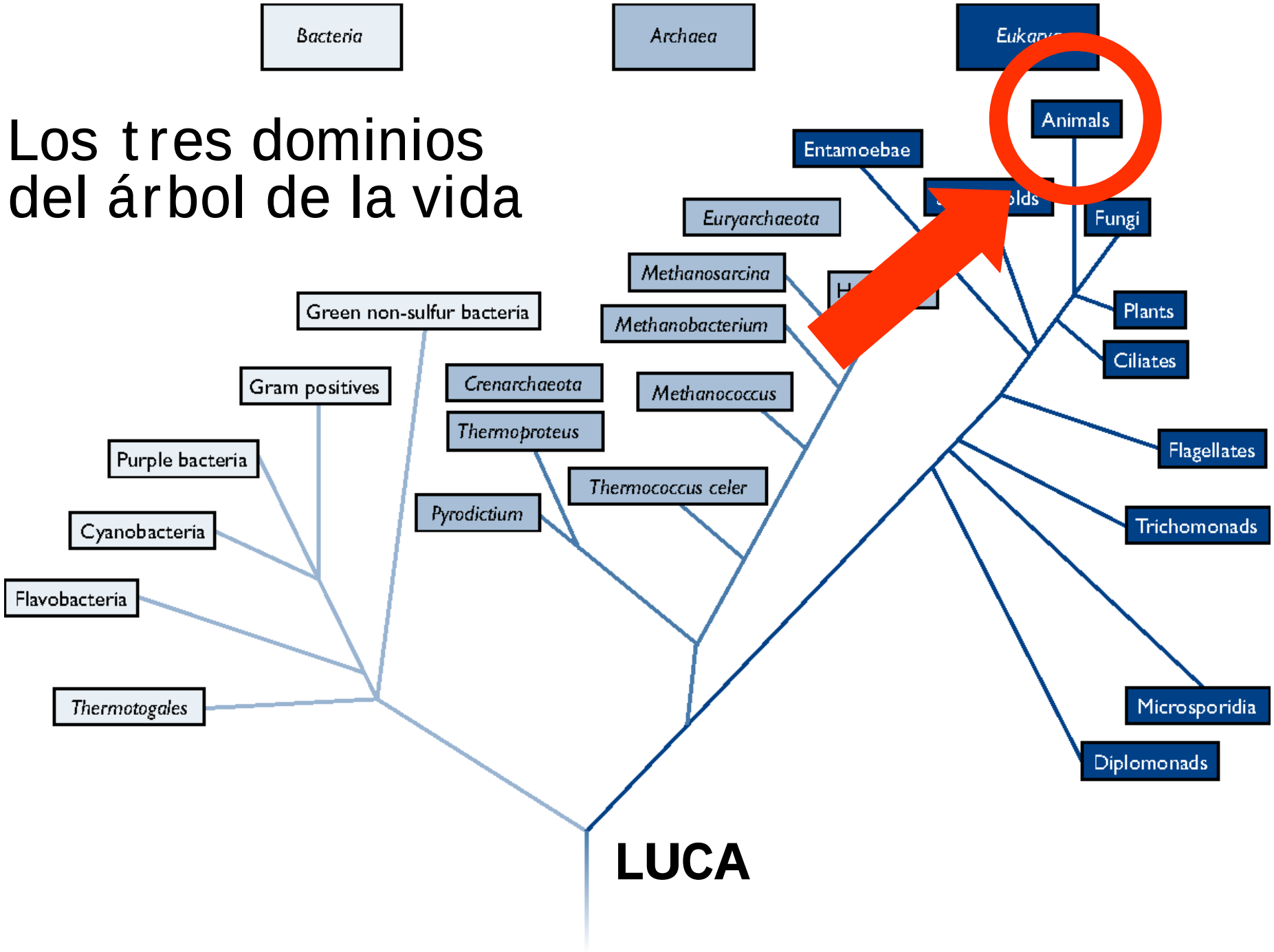
Madrid (Spain)

La Cloaca Maxima (Roma, s. V adC)




Eukaryotic microbes appear
Prokaryotes appear
Plants and animals appear
Cyanobacteria introduce significant amounts of O_2 into atmosphere
Earth's crust cools
4
3
2
1
0
Billion years from present

Los tres dominios del árbol de la vida



Microbial properties of environmental interest

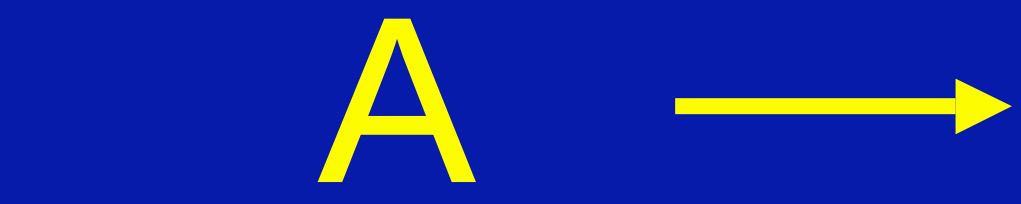
- **Biodegradative pathways for recalcitrant compounds**
  - mineralization of toxic pollutants
  - removal or organic sulphur from soil
  - biosensors
  - green chemistry
- **Tolerance to heavy ions**
  - volatilization
  - bioavailability/toxicity assays
  - bioaccumulation:removal of toxic ions
  - biotransformation and re-speciation
- **Surfactant production: bioremediation of oil spills**

BIO-REMEDIACION

A

Tóxico

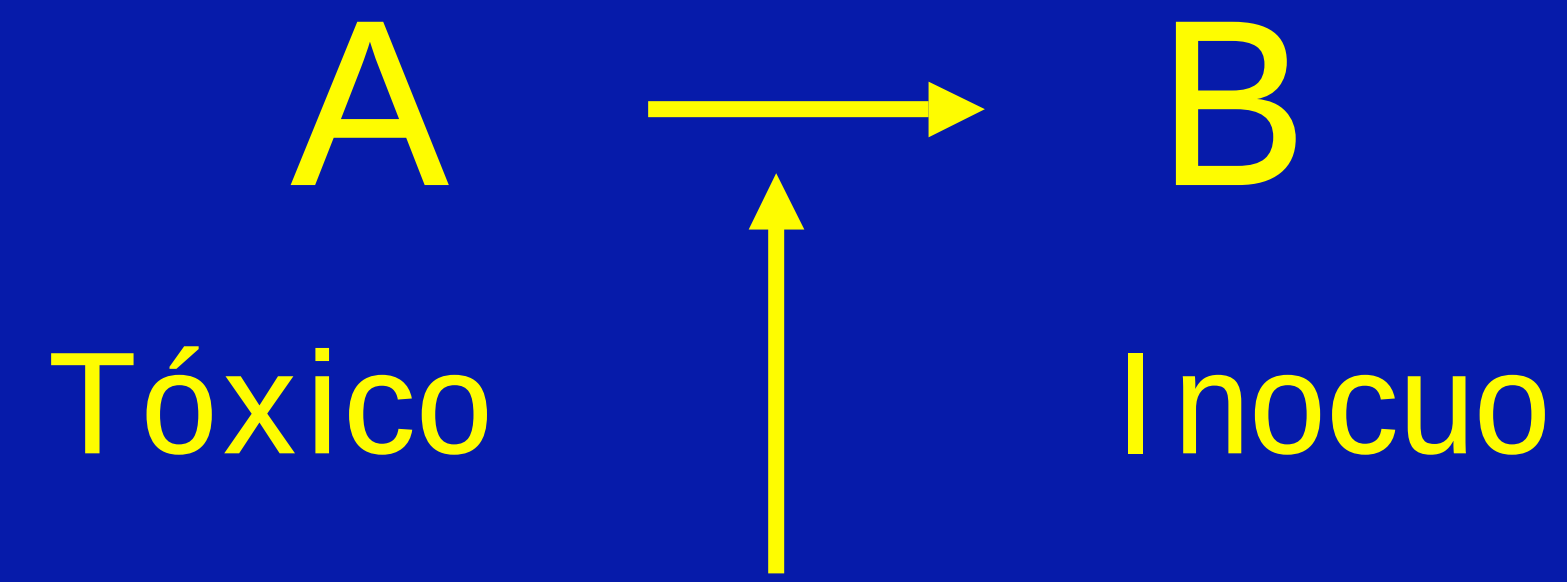
BIO-REMEDIACION



Tóxico

Inocuo

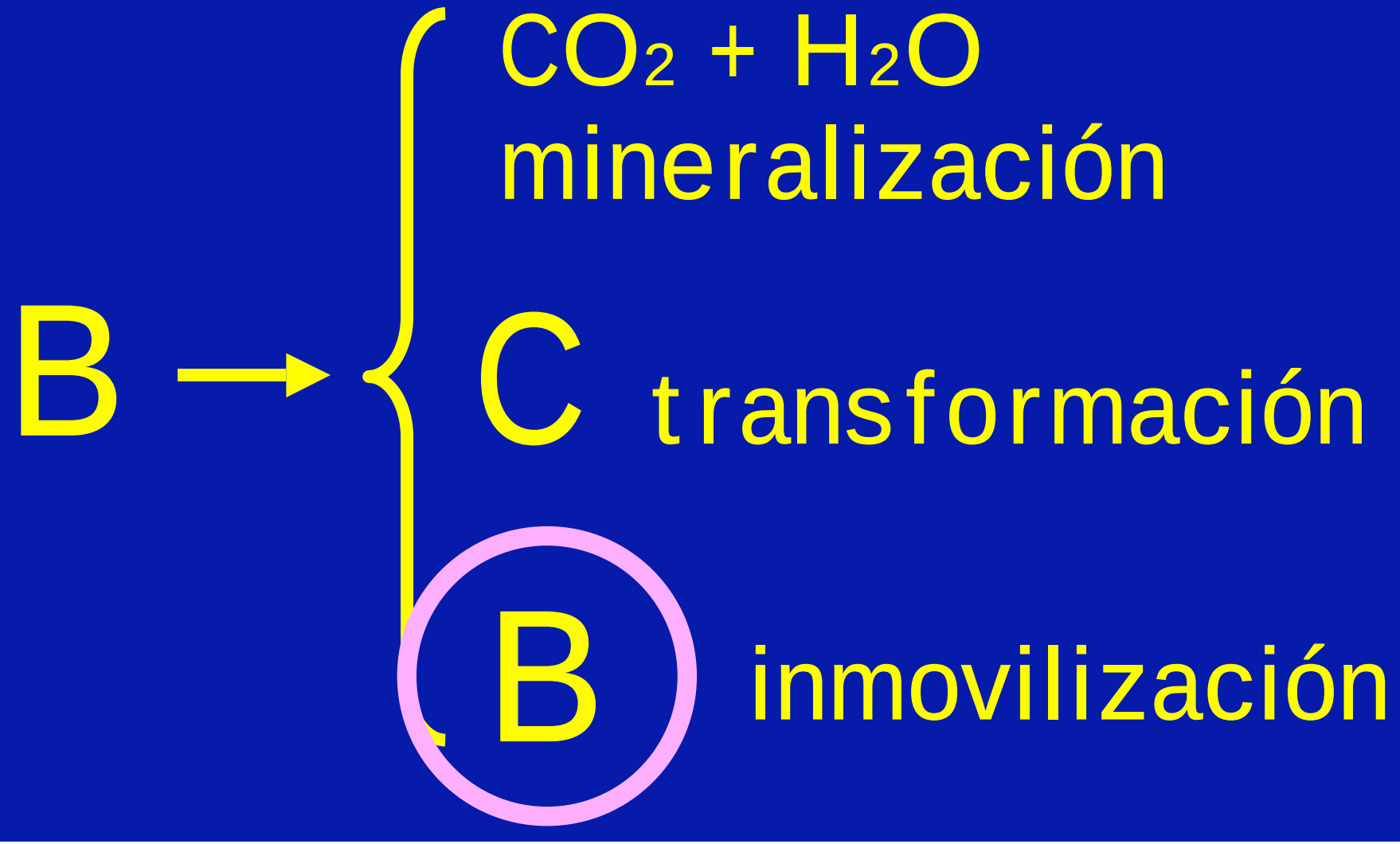
BIO-REMEDIACION



B

Inocuo

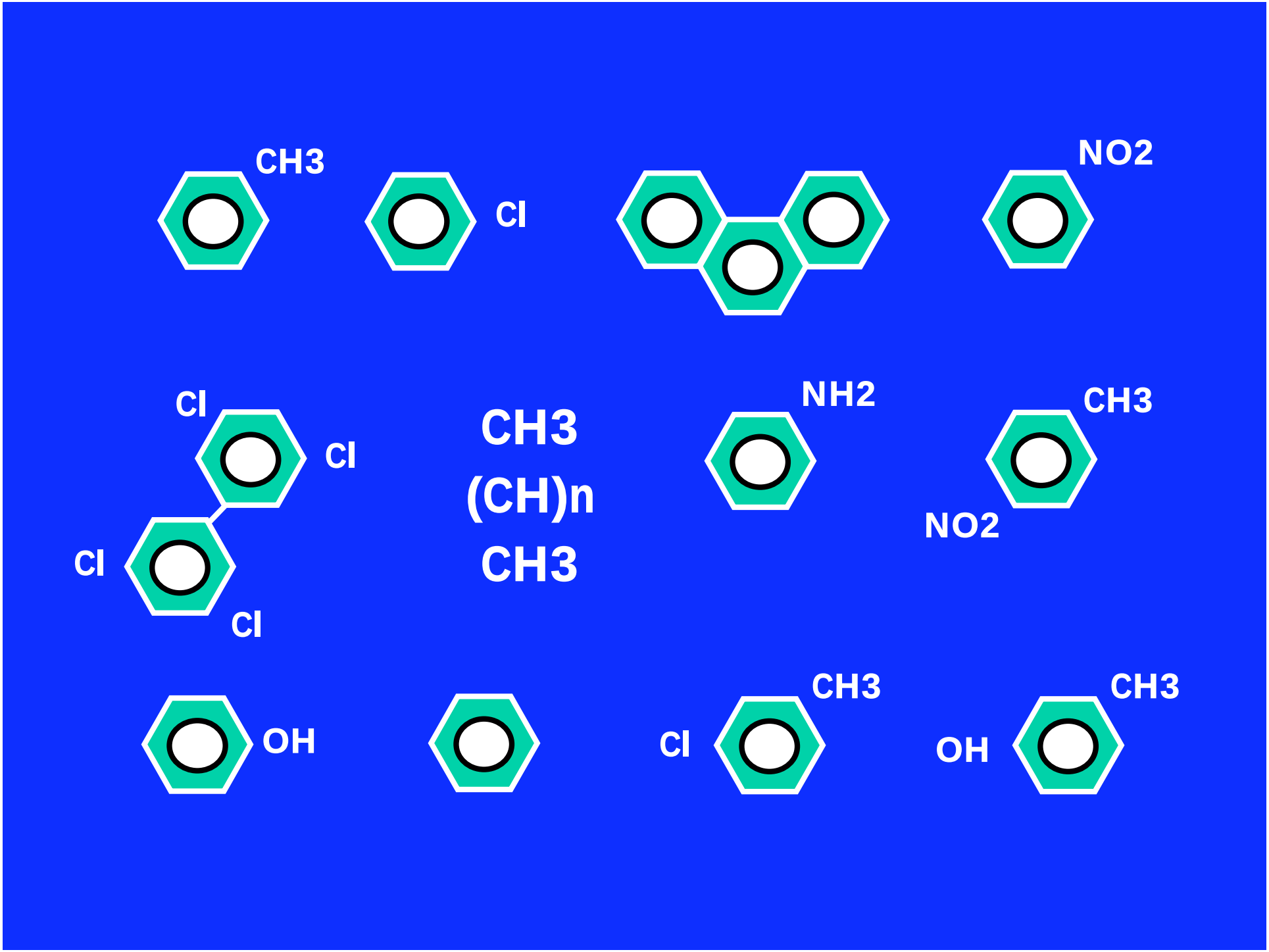
BIO-REMEDIACION



Strategies in Bioremediation

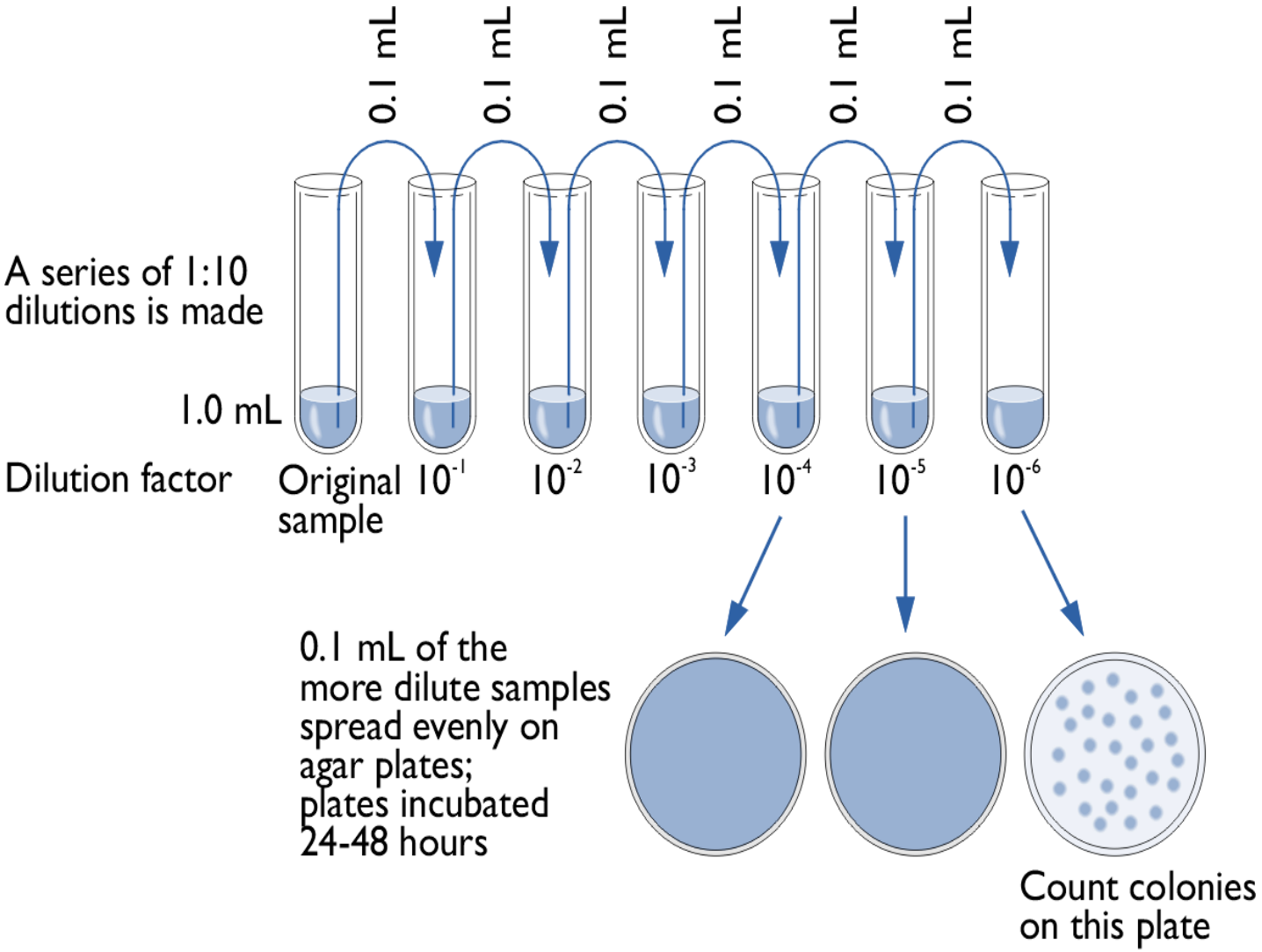
- Natural attenuation
- Bioaugmentation
  (incl. genetic engineer.)
- Biostimulation
  (Eco-engineering)

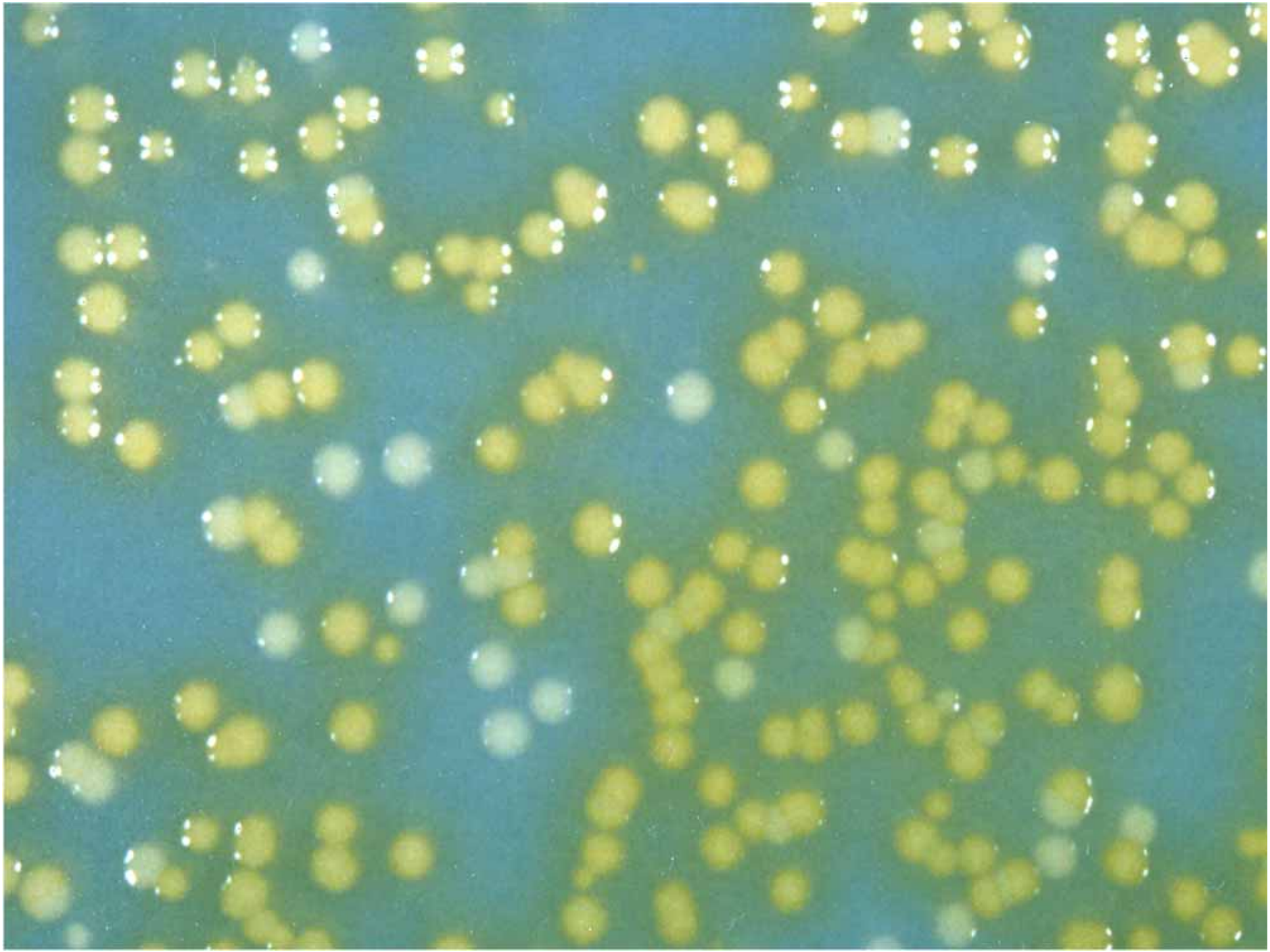

Our experimental system...


CH3
Cl
NO2
Cl
Cl
Cl
CH3
(CH)n
CH3
NH2
CH3
NO2
OH
CH3
Cl
CH3
OH

Enrichment cultures

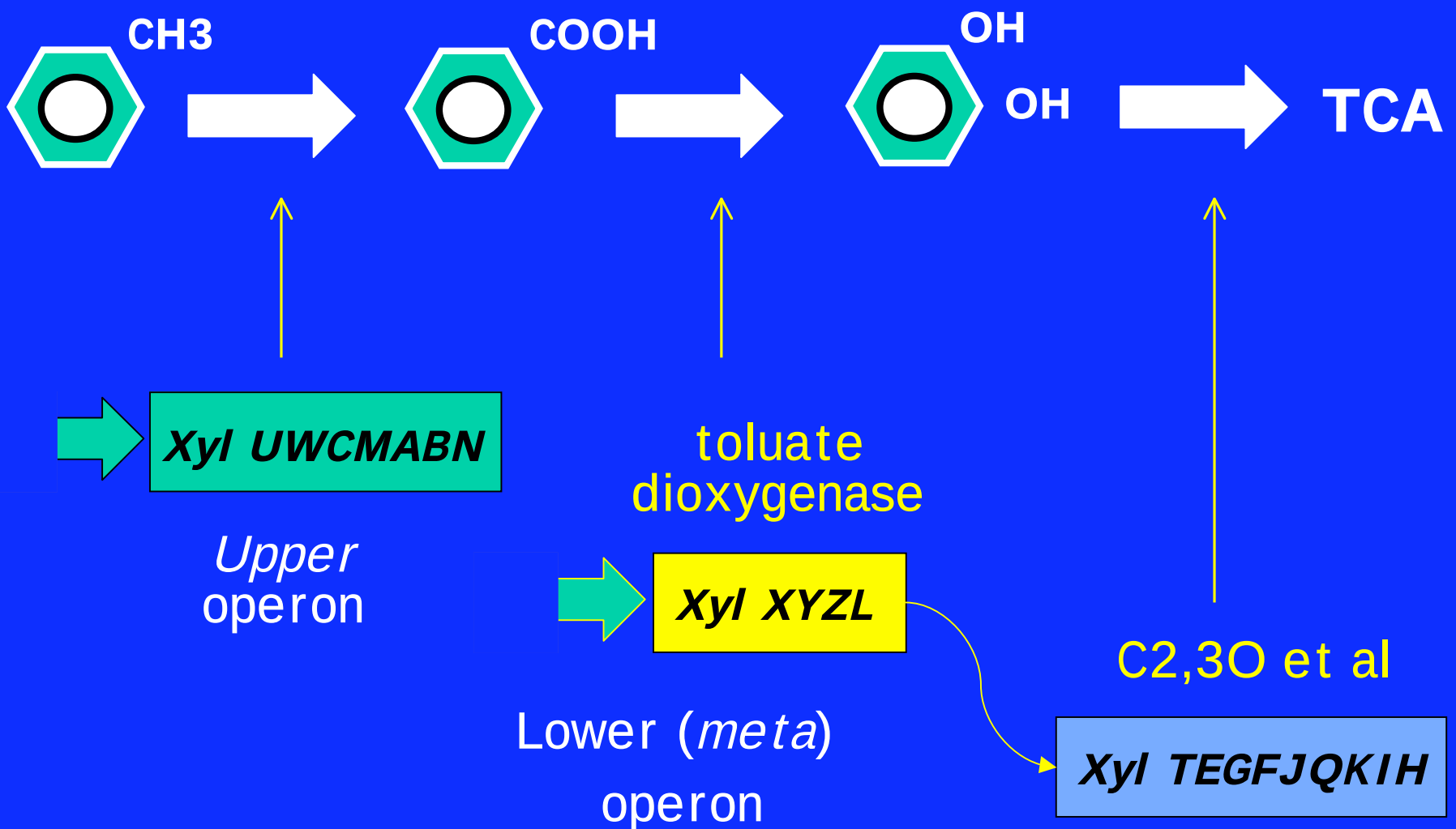
Accessing the diversity of soil microbes

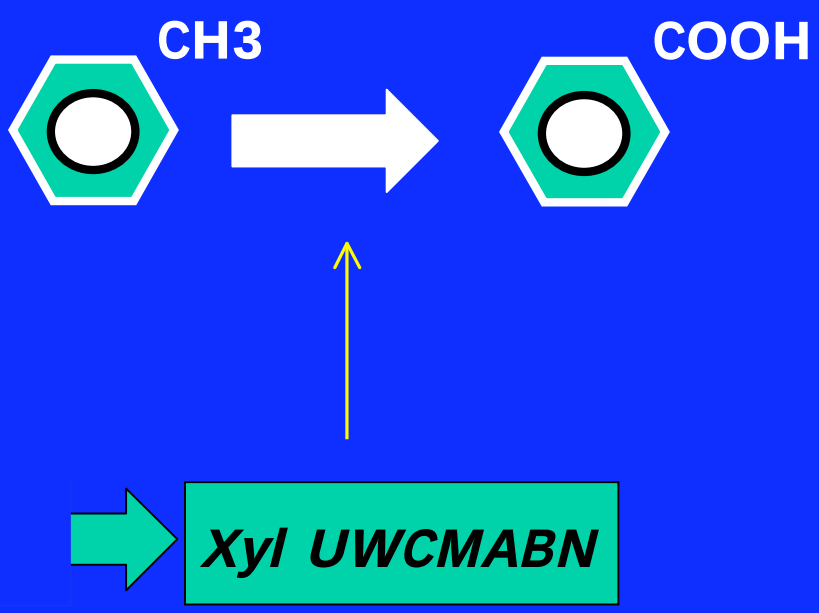




Cepa (Plásmido)	Contaminante
P. putida mt-2 (pWW0)	Tolueno
Pseudomonas sp, CF600 (pVI150)	Fenol
Burkholderia sp. RP007	Fenantreno
P. putida TMB	Trimetilbenceno
P. putida NAH (NAH7)	Naftaleno
Acinetobacter sp. ADP1	Aril esteres
R. eutropha (pJP4)	2,4 D
Pseudomonas sp. (pP51)	Triclorobenceno
P. putida UCC22 (pTDN1)	Anilina
P. oleovorans (pOCT)	n-alcanos
Burkholderia cepacia LB400	Bifenilo, PCBs
Pseudomonas sp. VLB120	Estireno

El sistema TOL de *Pseudomonas putida*

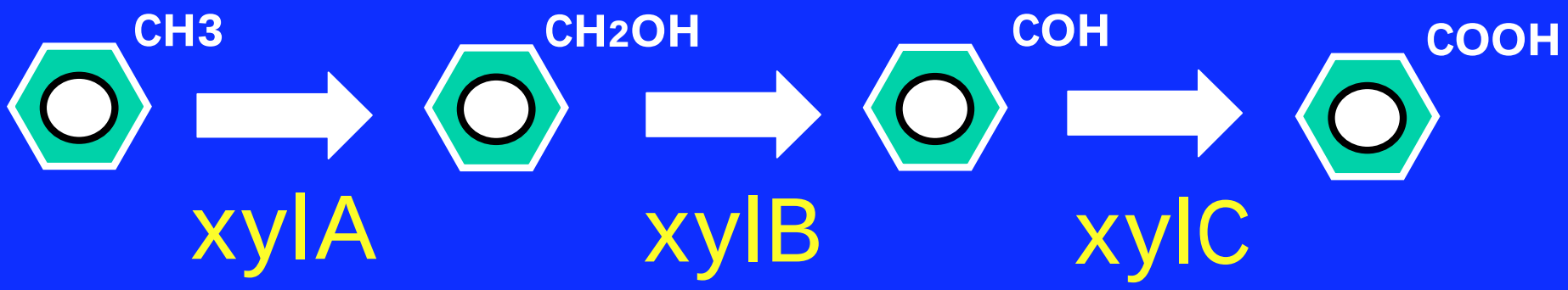




Xyl UWCMABN

Upper
operon

De la biodegradación a la química verde



De la biodegradación a la química verde

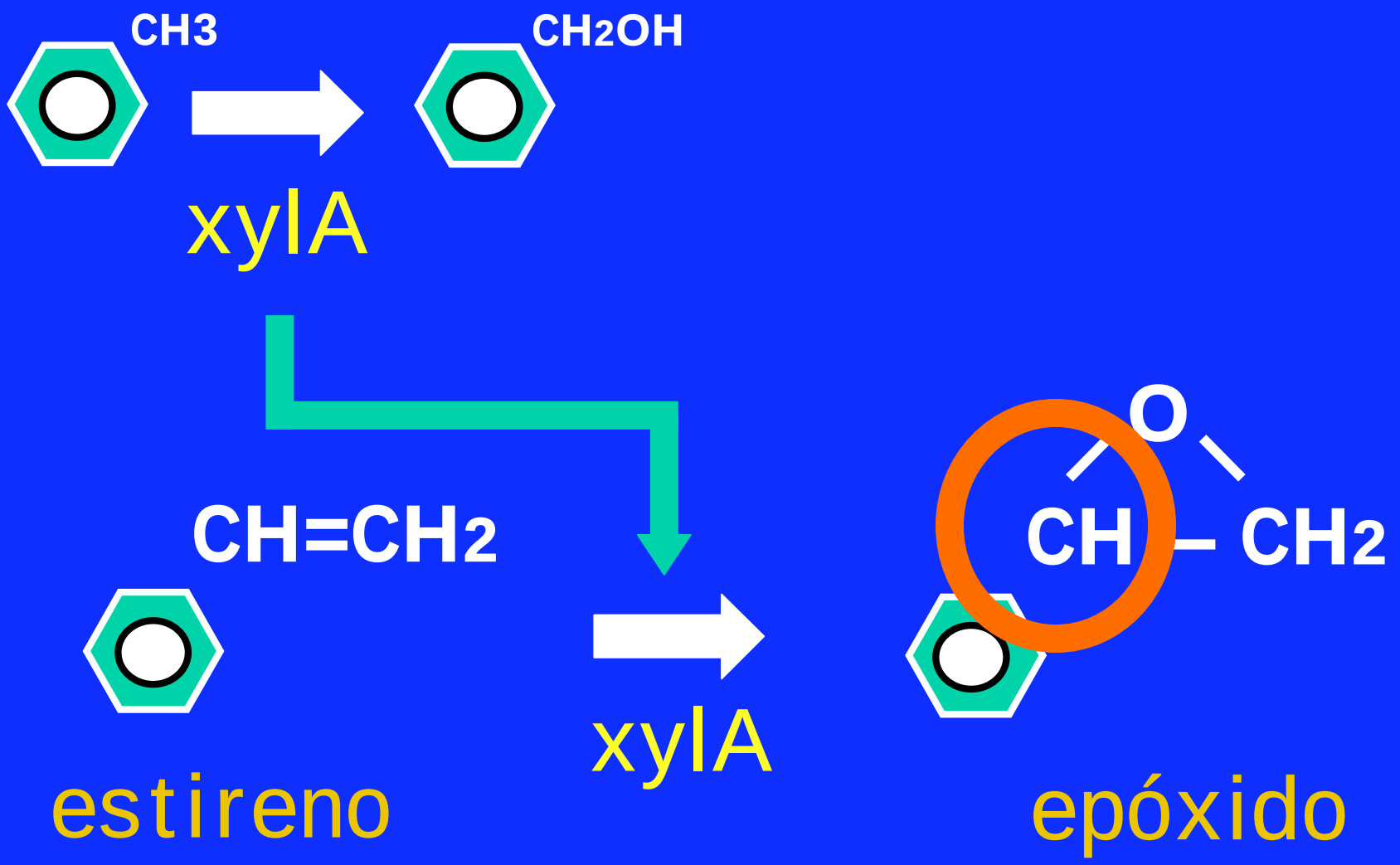


Table 5. **Comparison of outputs**

Chemical process = 100

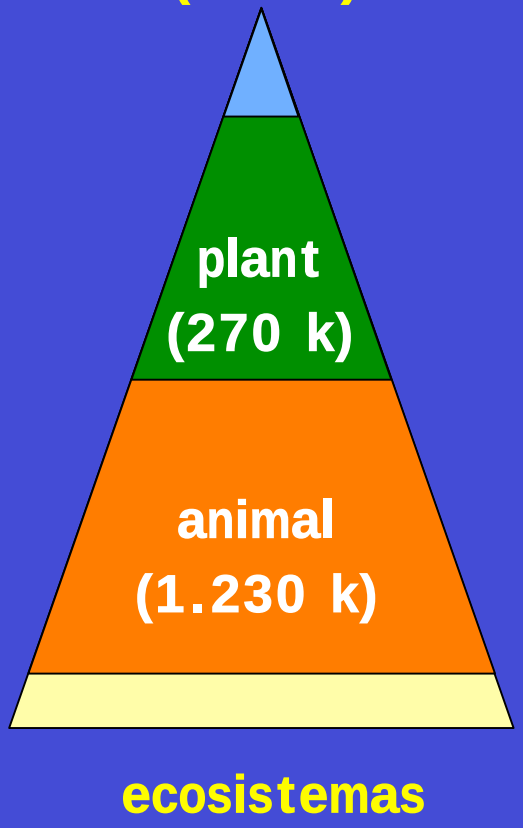
	Chemical process	Biological process
Material for incineration	100	0.7
Wastewater	100	90
Solvents, class 1	100	0
Solvents, class 3	100	2.5
Zinc disposal	100	0

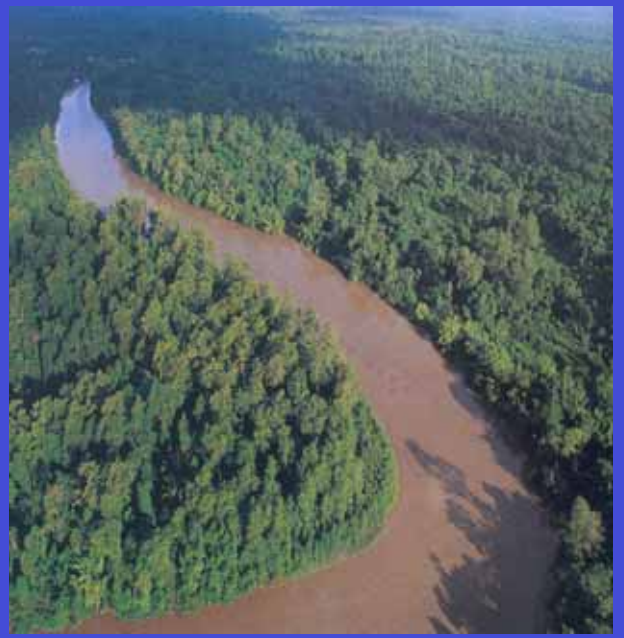
The Application of Biotechnology to Industrial Sustainability

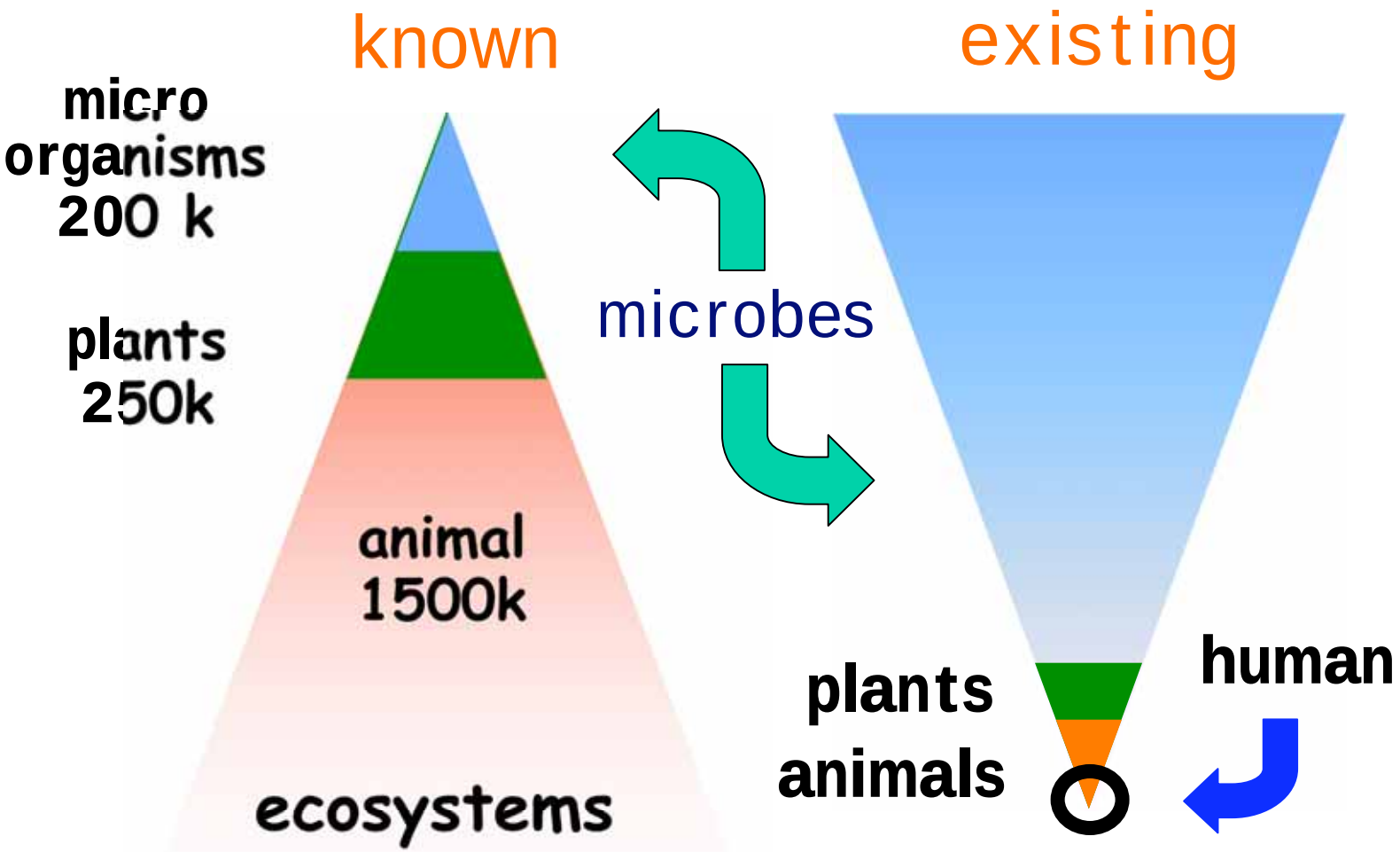
SUSTAINABLE DEVELOPMENT

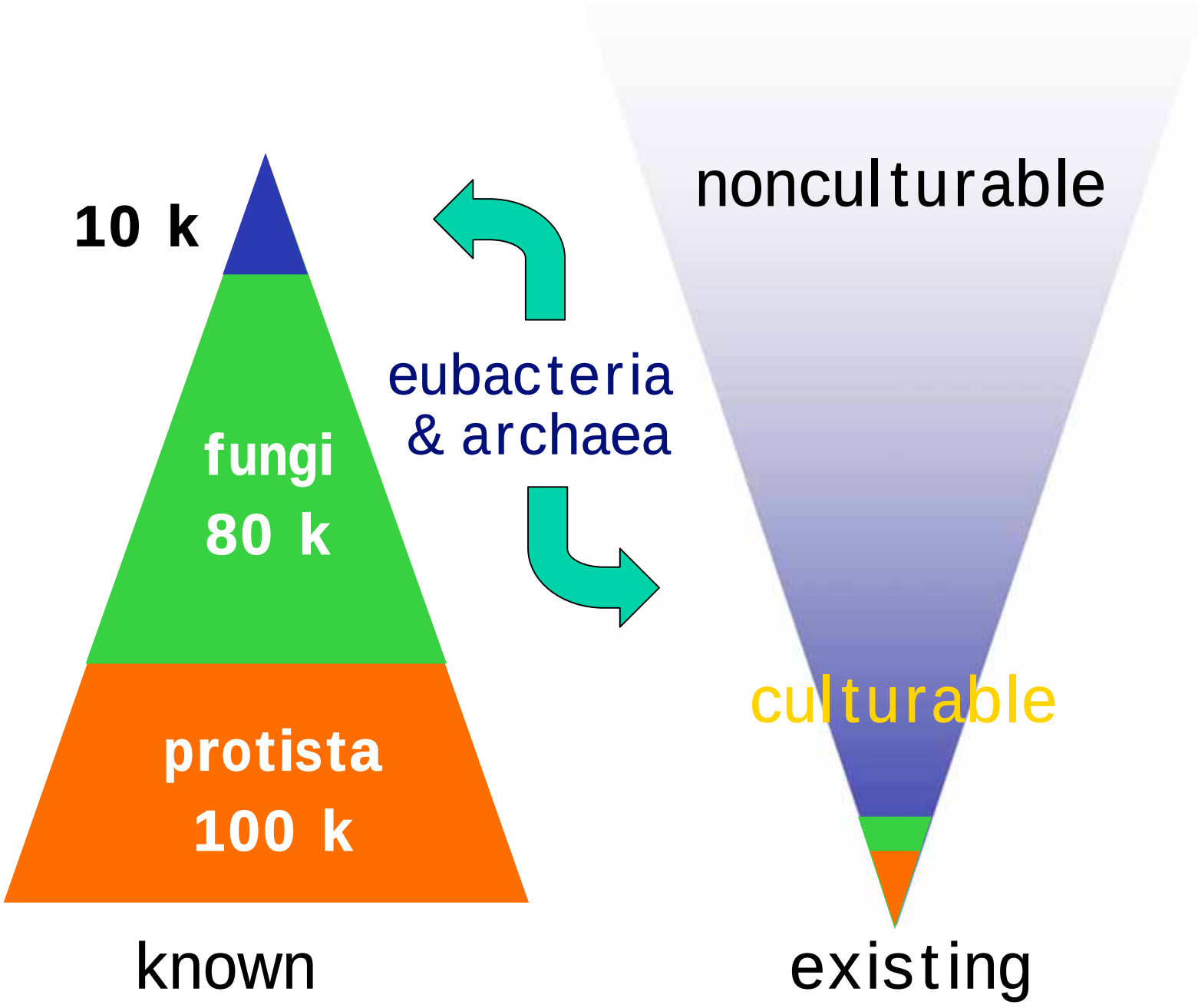
OECD

Diversidad conocida



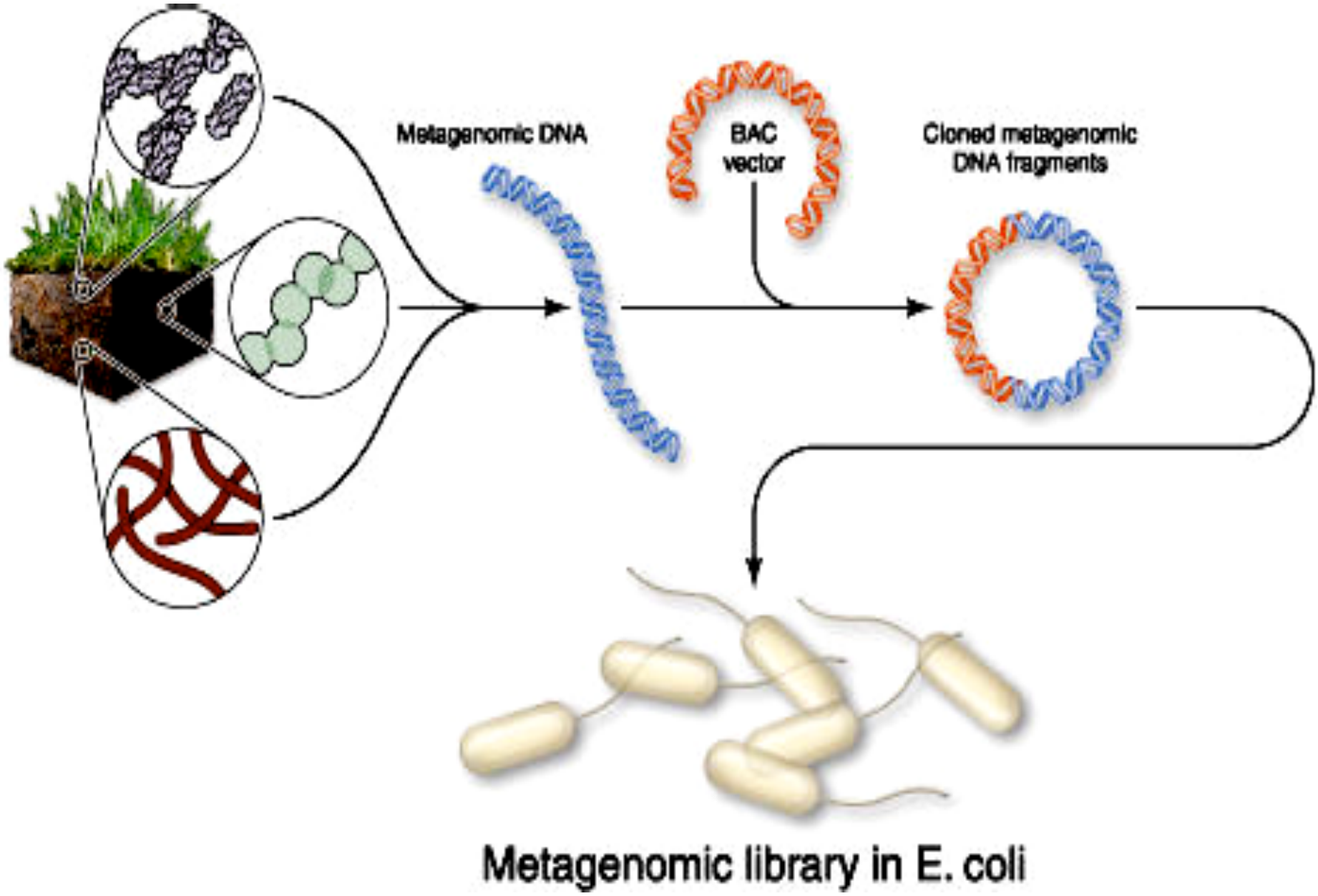



known
existing
micro organisms 200 k
plants 250k
animal 1500k
ecosystems
microbes
plants
animals
human

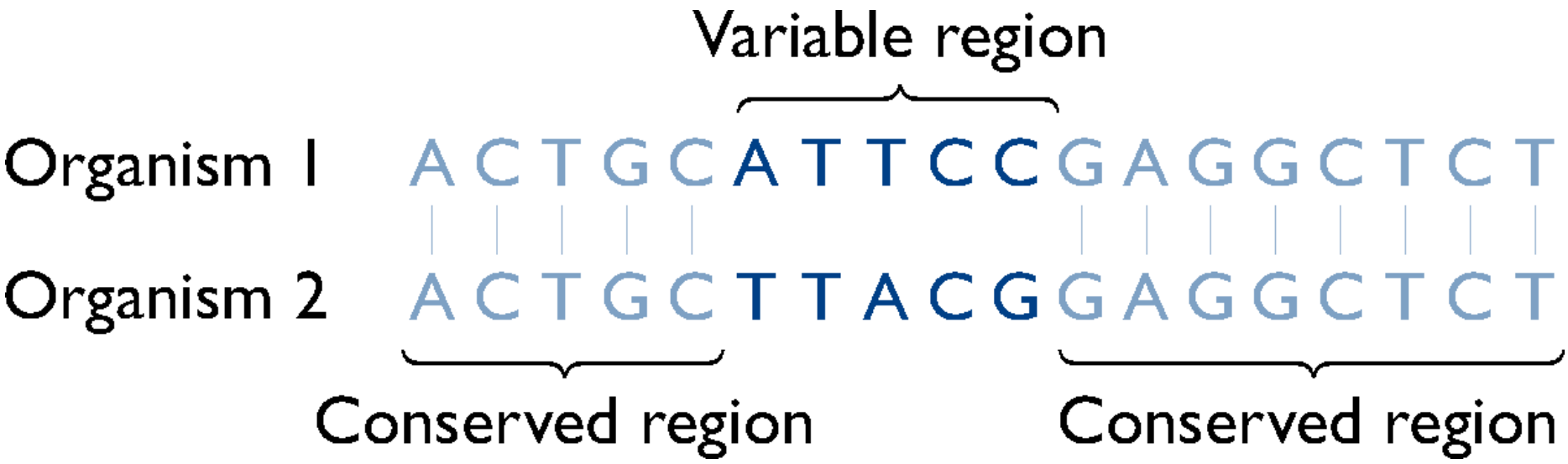

10 k
fungi
80 k
protista
100 k
known
eubacteria
& archaea
nonculturable
culturable
existing

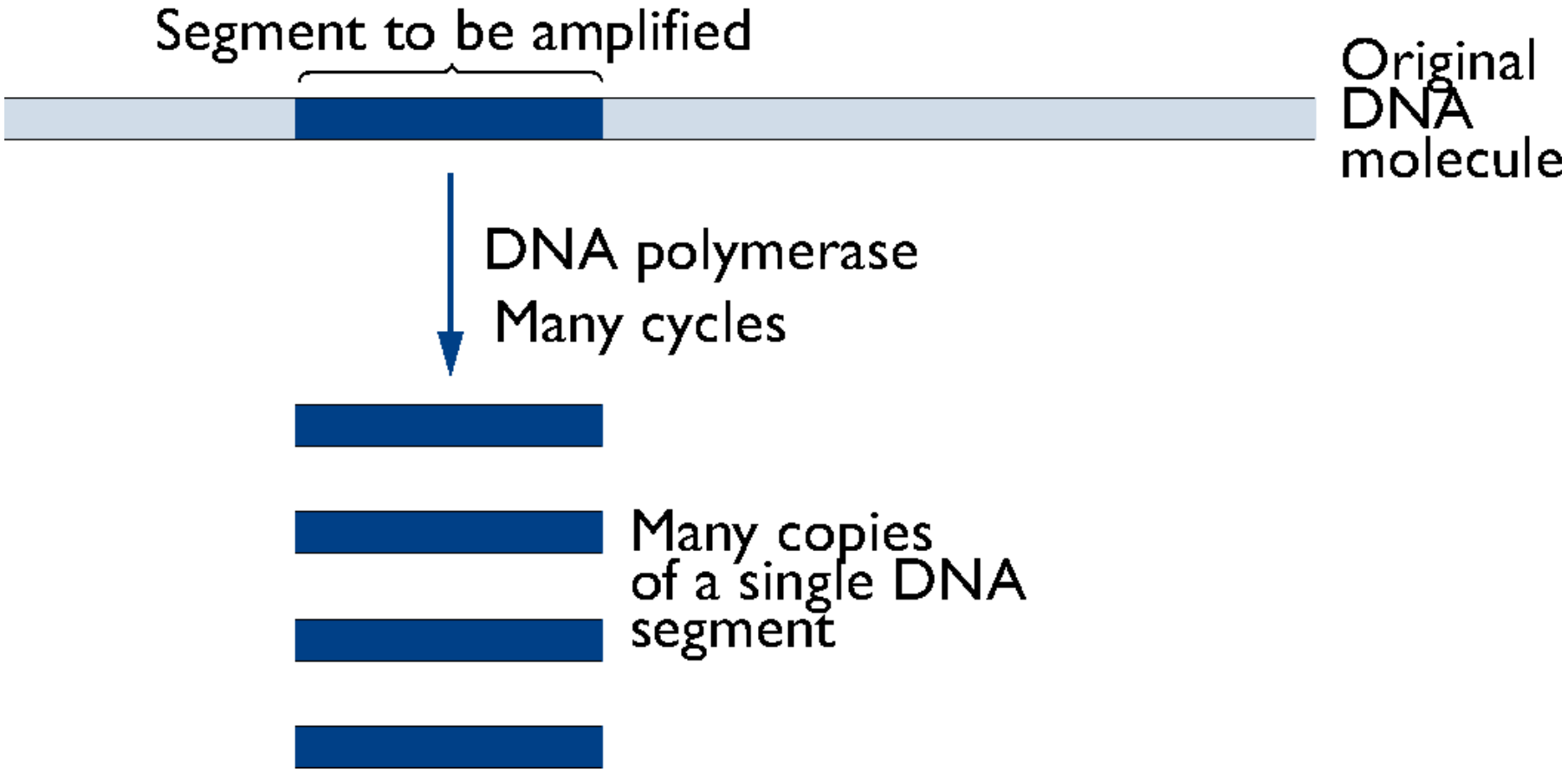
Exploring new worlds…

Metagenomics

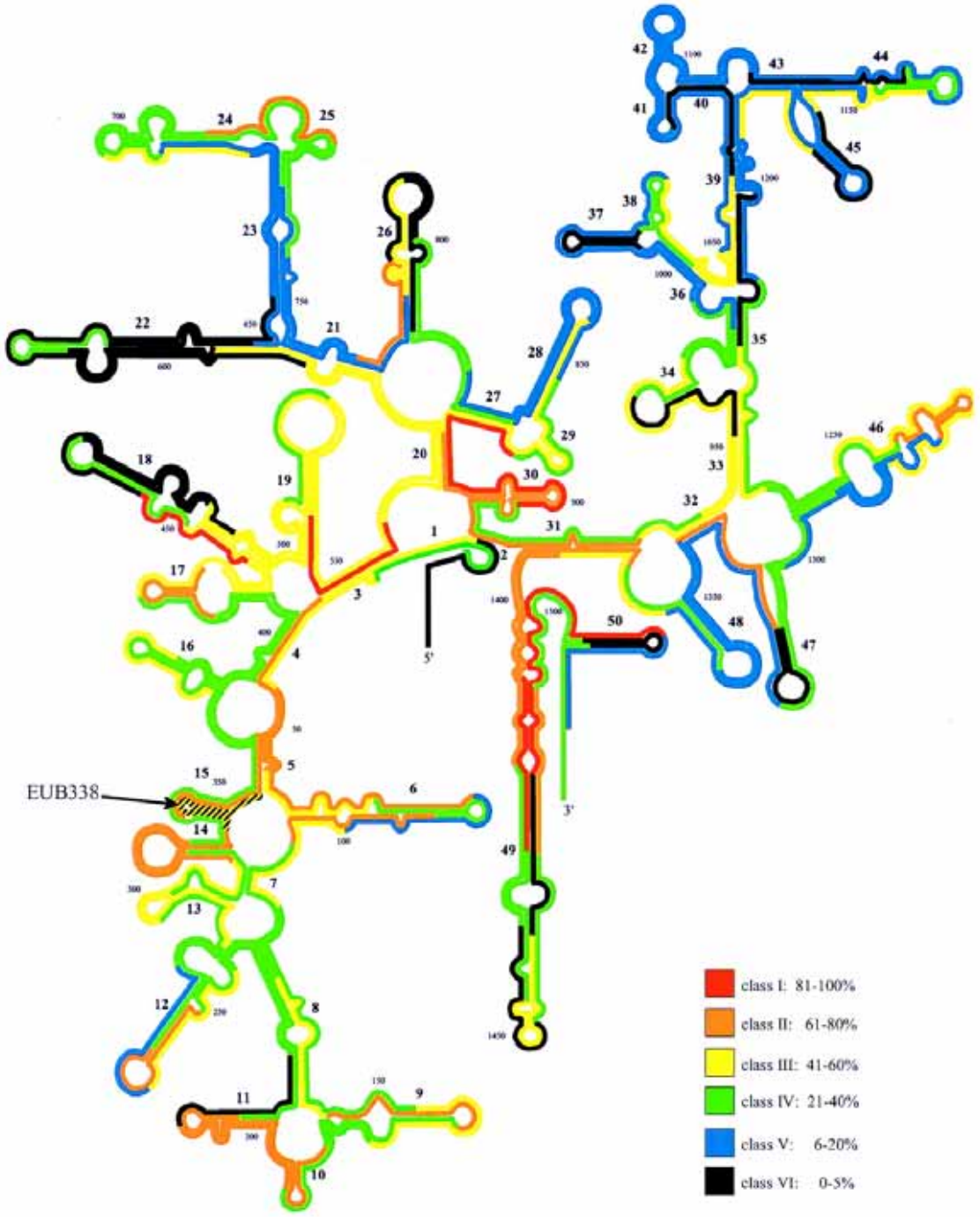



04


Variable region
Organism 1
A C T G C A T T C C G A G G C T C T
Organism 2
A C T G C T T A C G G A G G C T C T
Conserved region
Conserved region

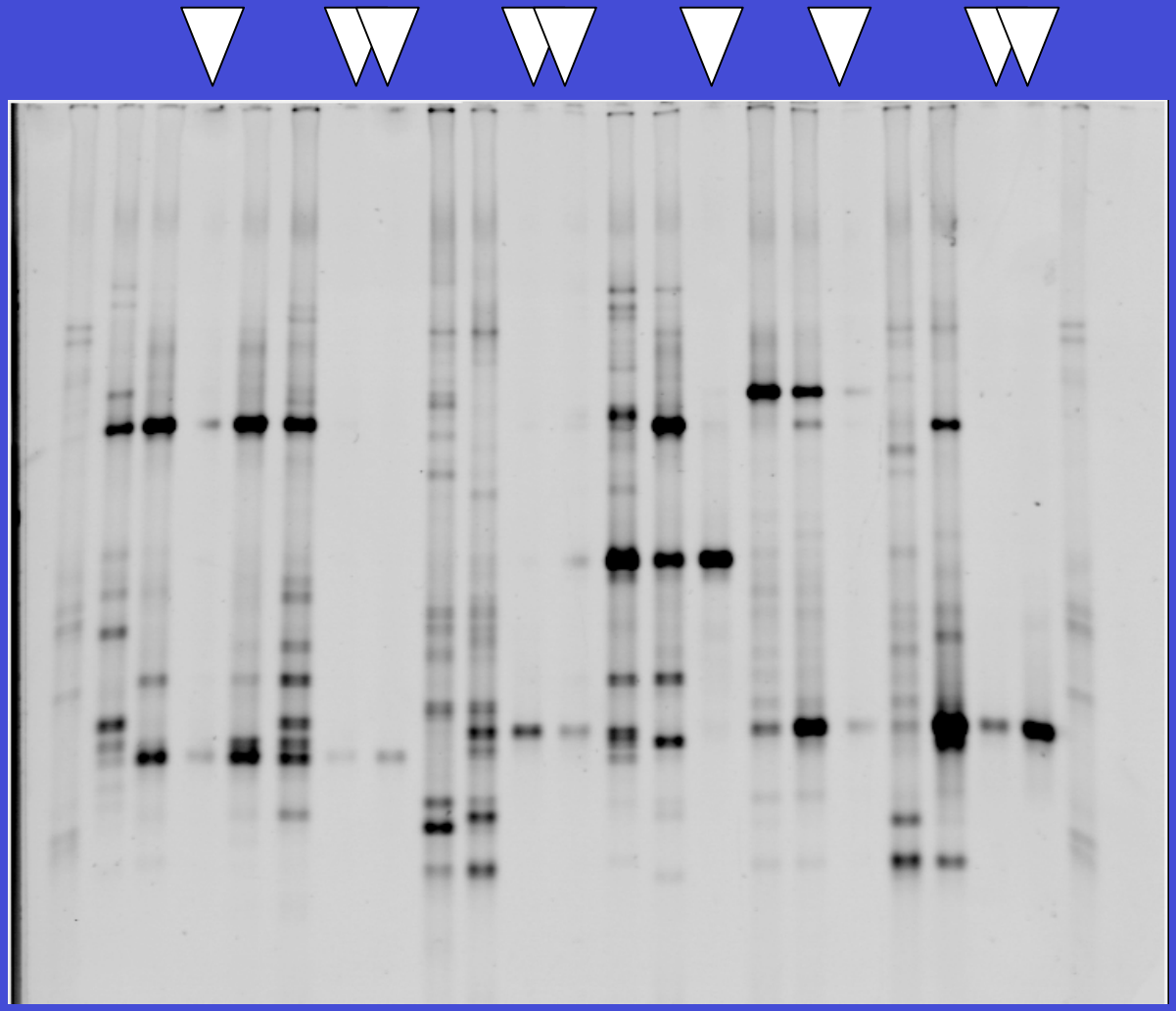

Segment to be amplified
Original DNA molecule
DNA polymerase
Many cycles
Many copies of a single DNA segment

16S RNA

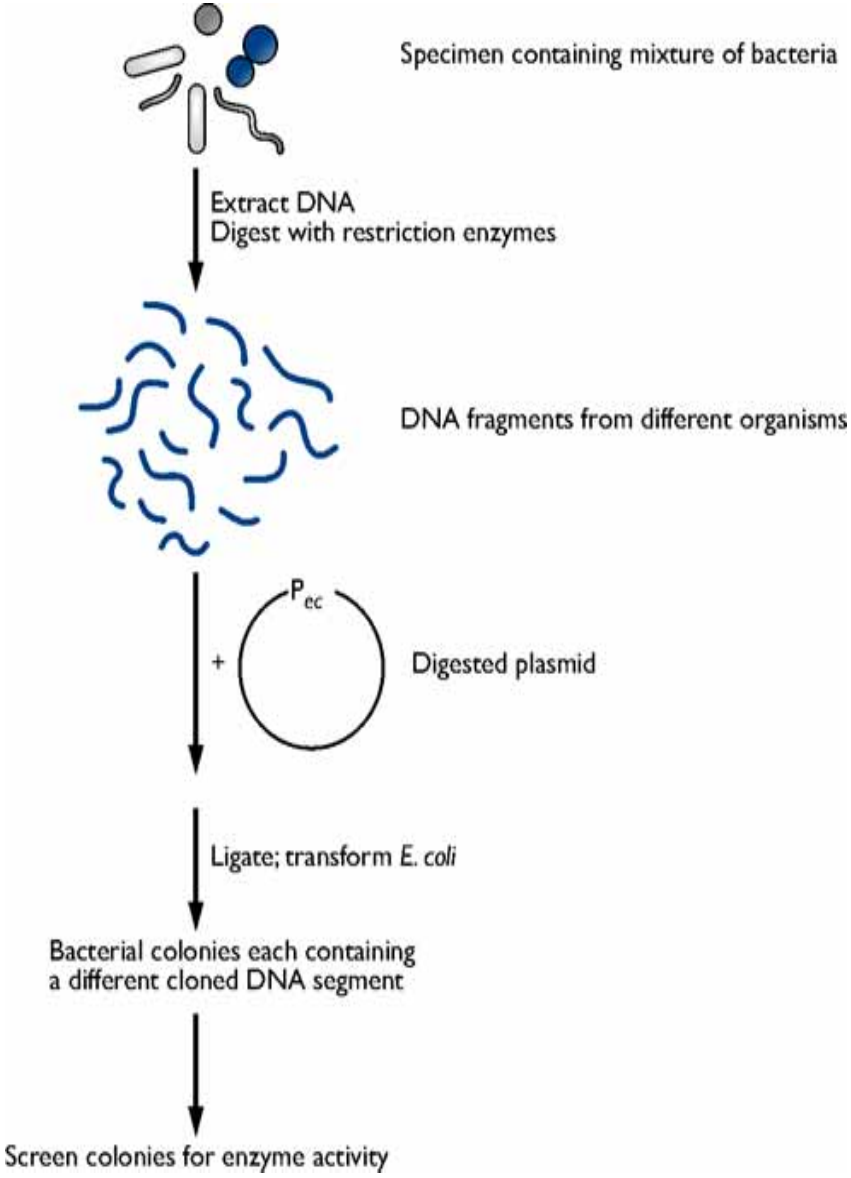

EUB338
class I: 81-100%
class II: 61-80%
class III: 41-60%
class IV: 21-40%
class V: 6-20%
class VI: 0-5%

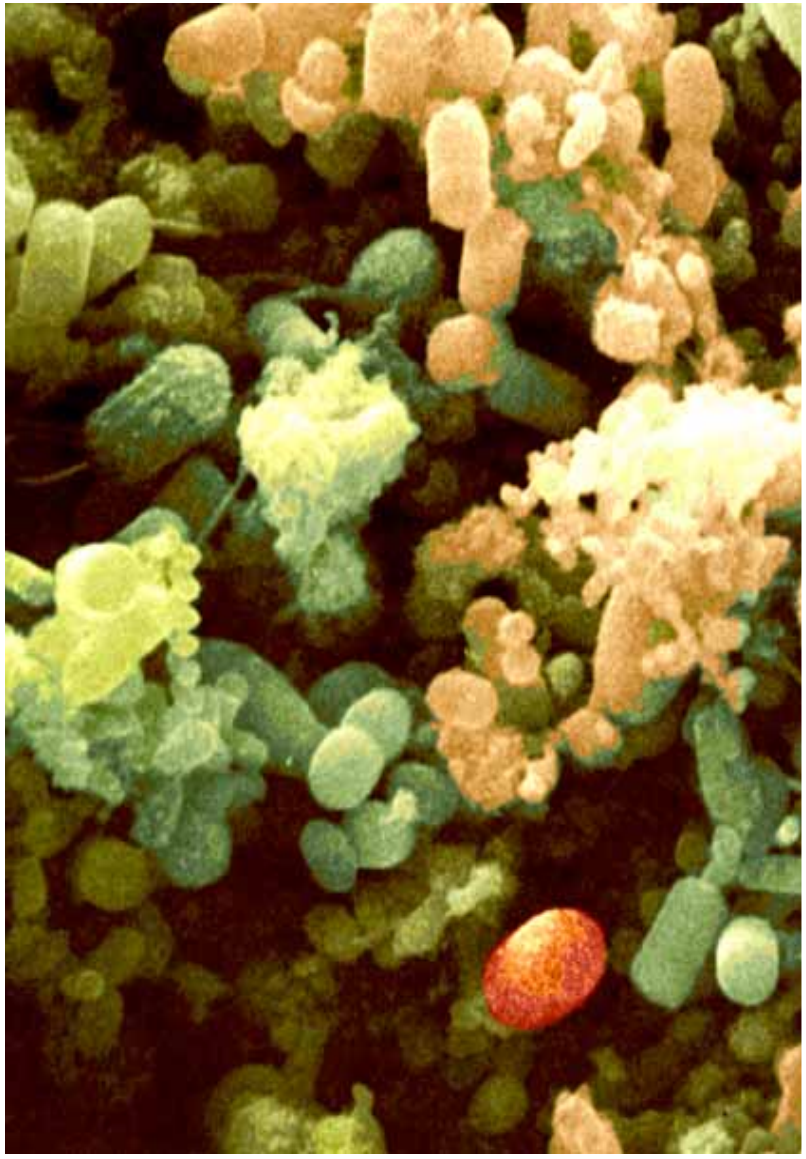
DGGE analysis of 16 s RNAs of lindane-polluted soils

enriched



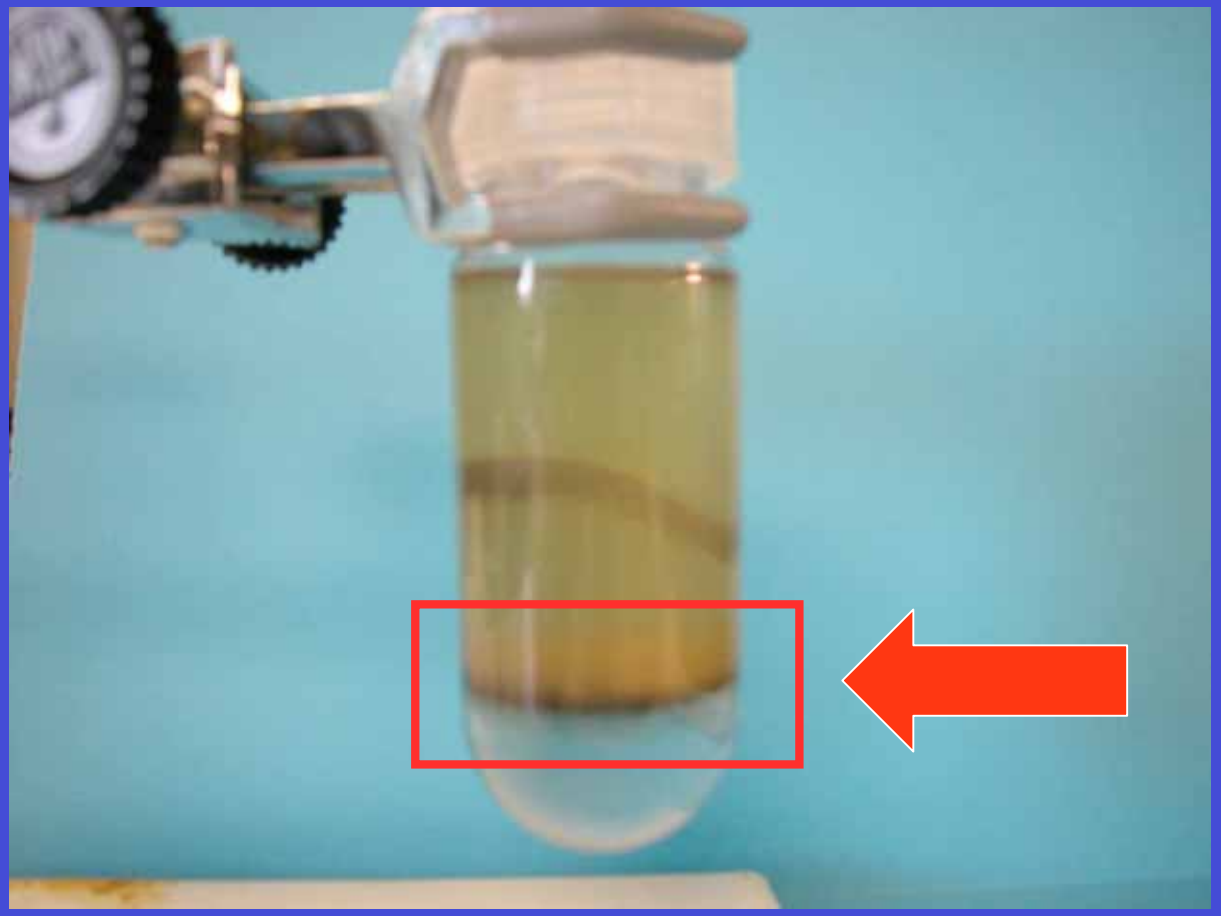
Acceso al metagenoma microbiano



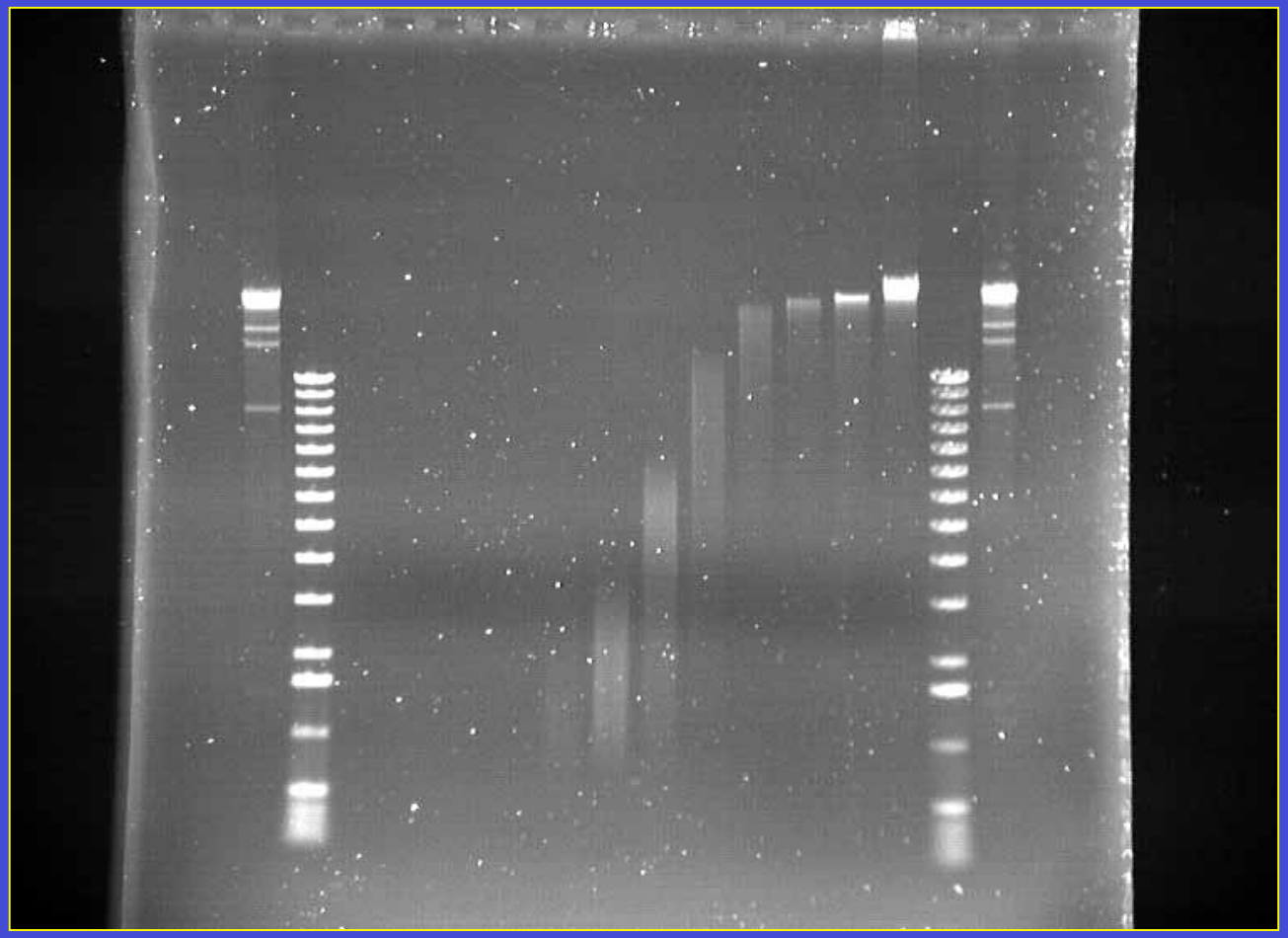



S.F

Extraction of intact bacterial fraction from soil



Fractionation of metagenomic soil DNA for cloning in lambda vectors



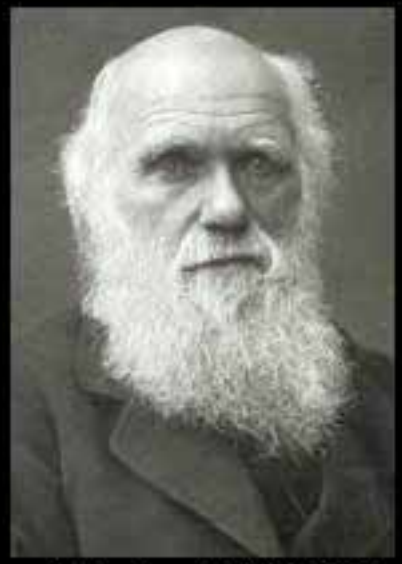
Pero… ¿podemos mejorar a la Naturaleza?

¡Si! pero utilizando sus mismos mecanismos

2ª Generación

3ª Generación

1ª Generación



Charles Darwin (1809-1882)
Padre de la Teoría Evolutiva

4ª Generación

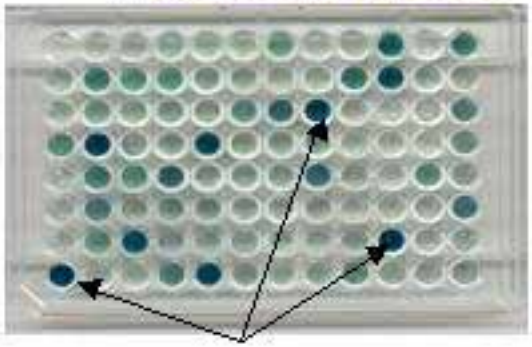
Evolución natural — Miles de millones de años

Evolución dirigida — Semanas o meses

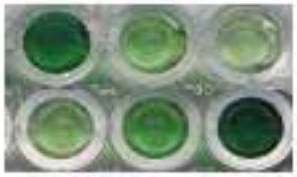
ESCALA TEMPORAL

Diferentes métodos colorimétricos para evolucionar distintas propiedades de la enzima lacasa

Plato de 96 pocillos



"Ganadores" de la generación



Nivel de expresión en levadura

Transformación de xenobióticos

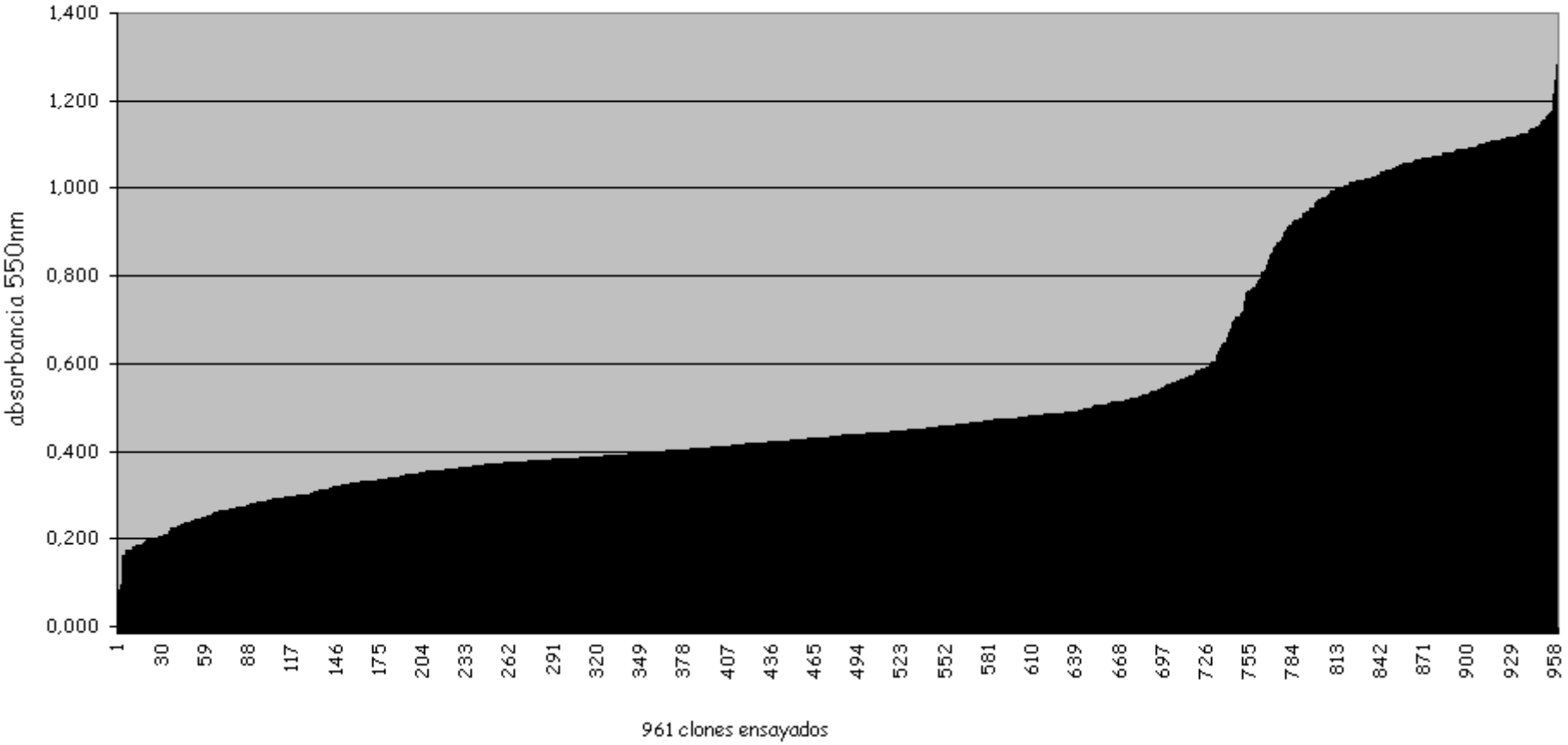
Degradación de la lignina (blanqueado del papel)

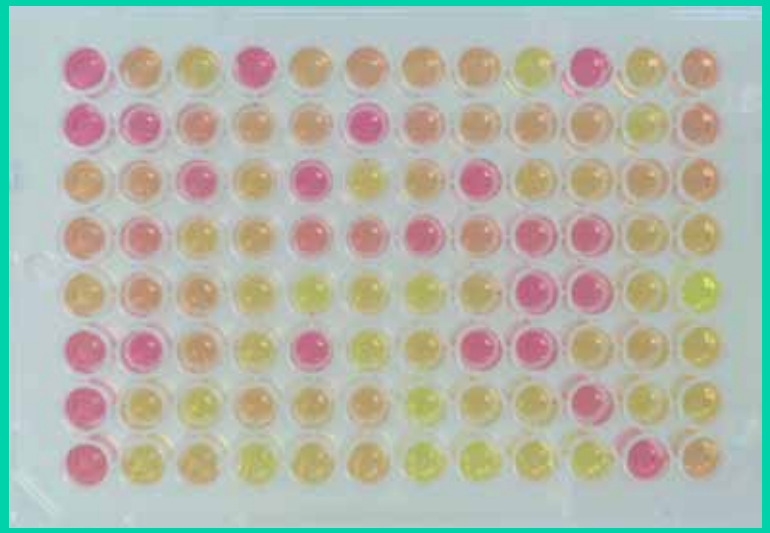
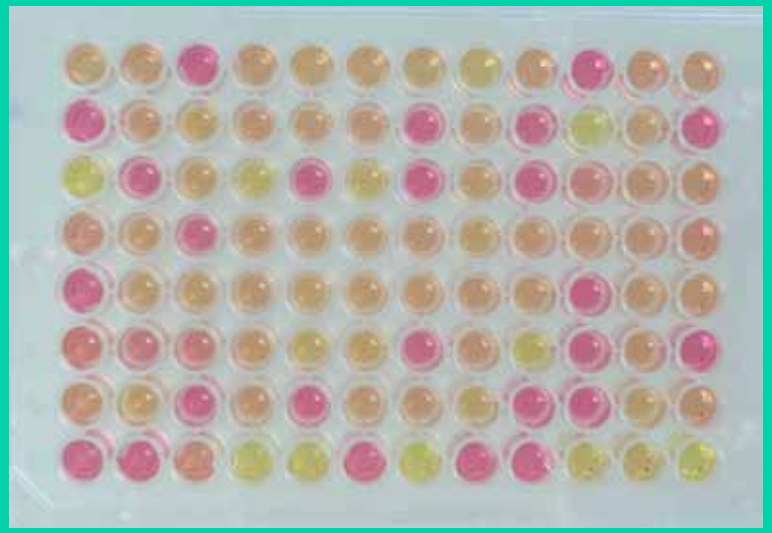
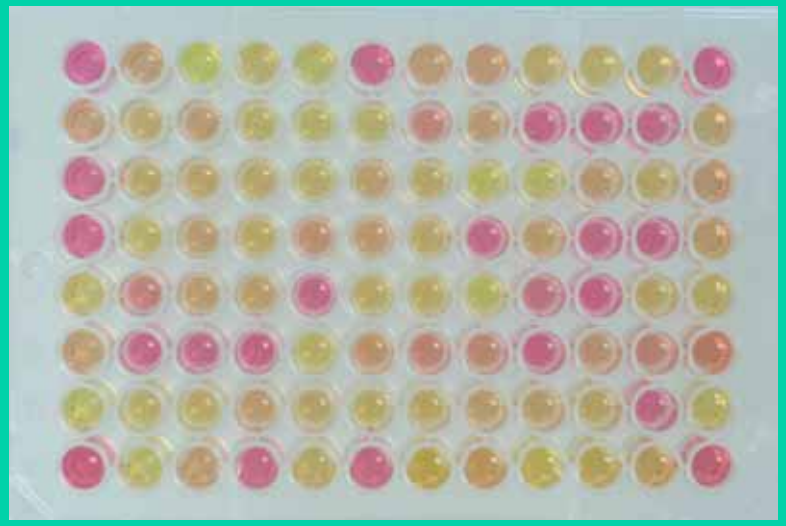
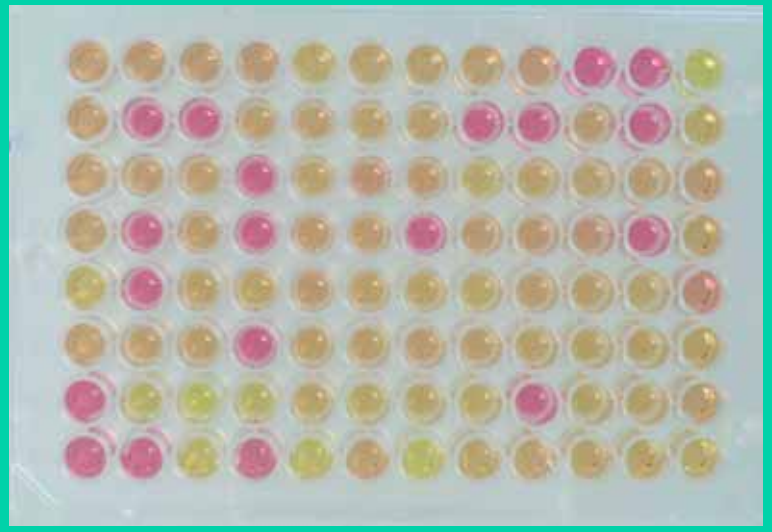
Evolución de rutas metabólicas: Búsqueda de carotenoides en fase sólida

Colonias con diferentes colores, contienen distintos carotenoides. Para su análisis se emplean procesadores digitales

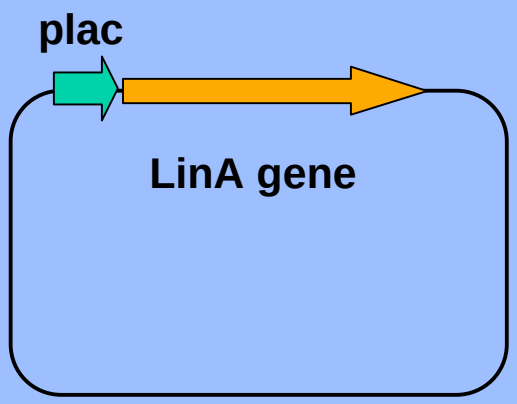
Carotenoides identificados

Declorination activity of linA-like clones



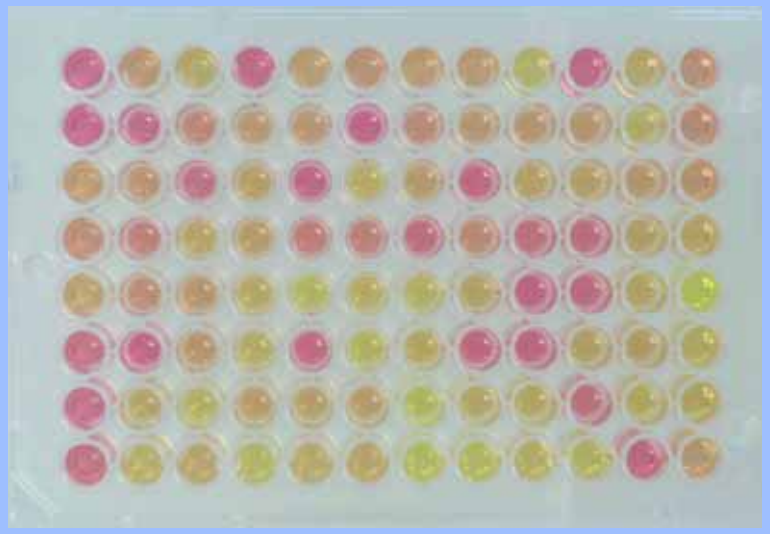





DIRECTED EVOLUTION OF Lindane Dechlorinase (LinA)



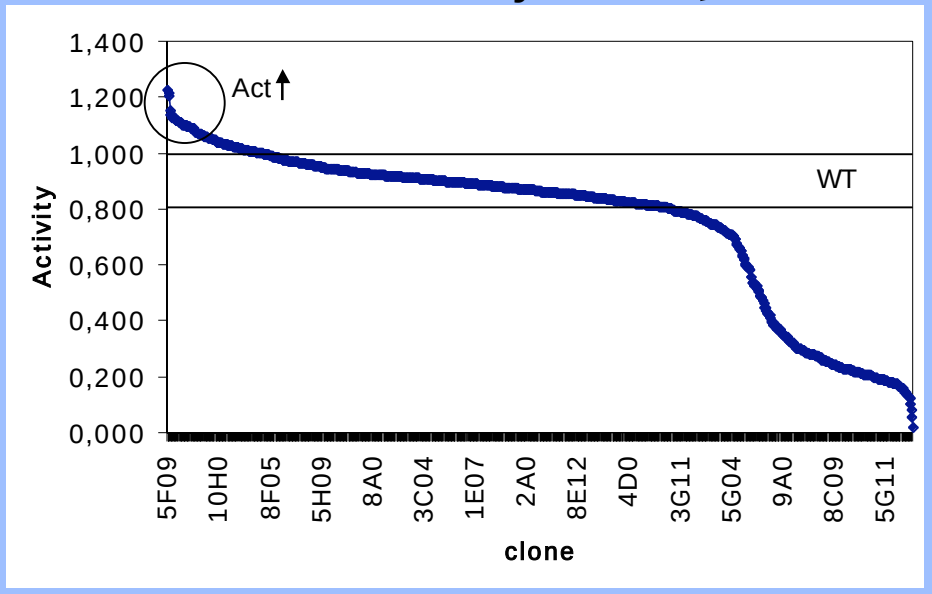
Mutagenic PCR

Screen Activity assay

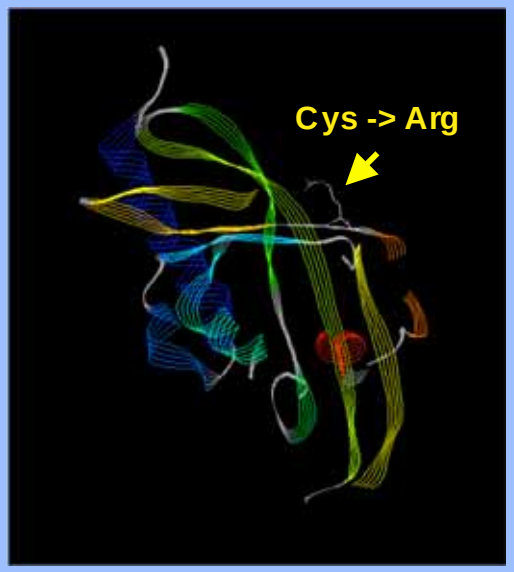


Next round

Data Analysis



Study of Improved Variants



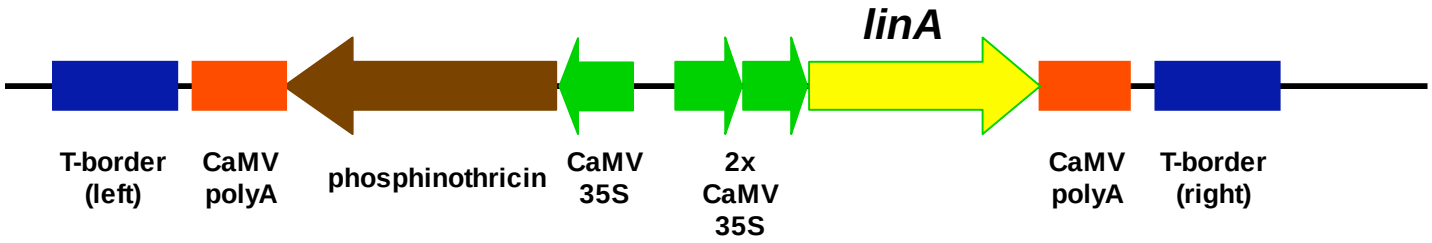
Reintroducing *linA-in the biodegradation network with* transgenic plants

José Eduardo González-Pastor, Carolina González de Figueras y Víctor de Lorenzo

Centro de Astrobiología (INTA-CSIC) (Madrid)
Centro Nacional de Biotecnología (Madrid)

pCAMBIA3500, a binary vector to transform *Arabidopsis thaliana*

- Replication origins for *Escherichia coli* and for *Agrobacterium tumefaciens*
- T-DNA from *Agrobacterium* (DNA that is excised and integrated in the plant) was engineered to contain:
  - a 35S promoter from cauliflower mosaic virus (CaMV35S) driving a gene encoding resistance to phosphinothricin (herbicide) for selection of the plant transformants
  - Two CaMV35S promoters driving expression of the transgenes, and a CaMV polyA (modified by Carlos Alonso-CNB)



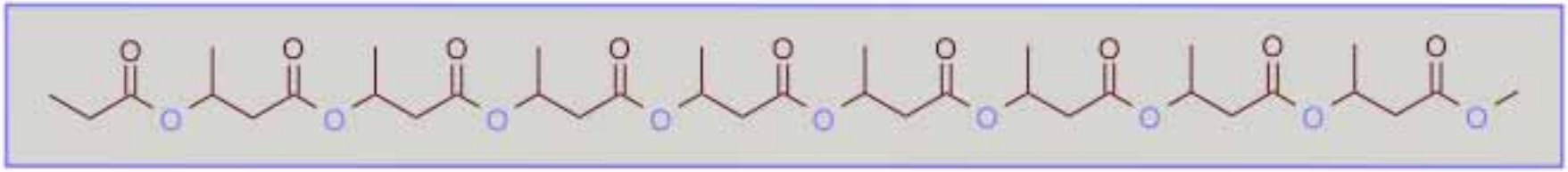
linA-transgenic *Arabidopsis thaliana* growing in the presence of lindane (10 mM)

Polyhydroxyalkanoates - PHA

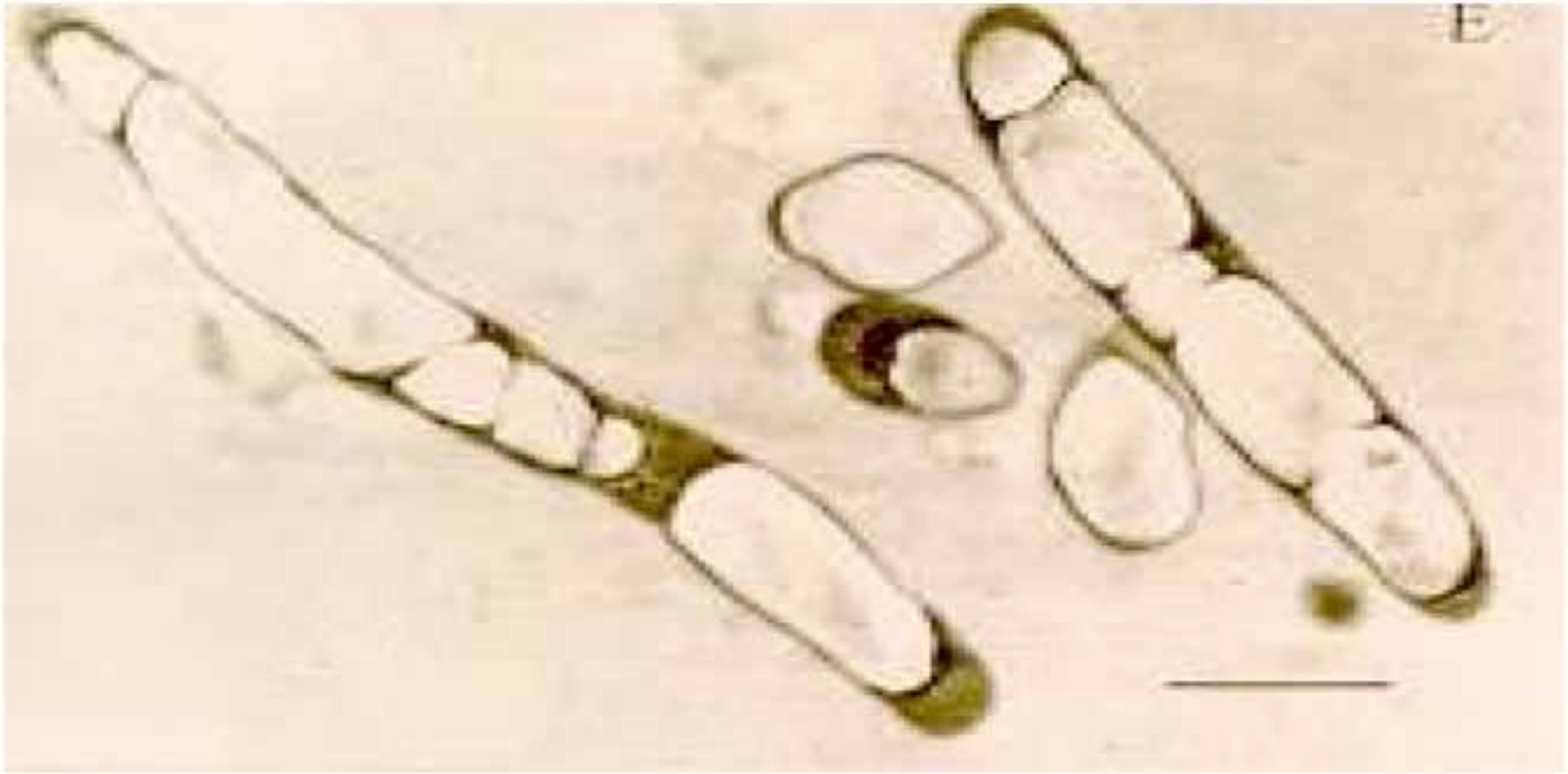
Bacterial storage compounds

for carbon and energy

Most well-known example: Poly(3-hydroxybutyrate)

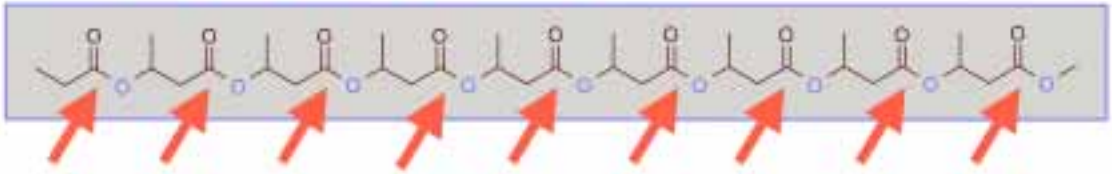


Cells of *Ralstonia eutropha* (TEM)



Properties of PHAs

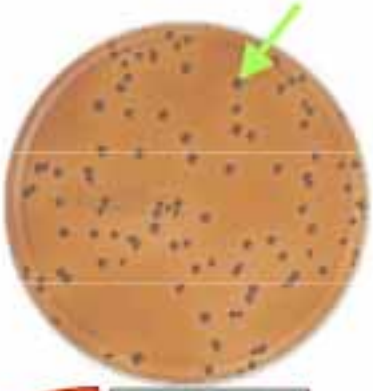
- Thermoplastic and/or elastomeric
- Biodegradable



- Often available from renewable resources
- Insoluble in water
- High degree of polymerization
- Enantiomerically pure
- Non toxic – biocompatible - piezoelectric

Shampoo bottles made from PET or Poly(3HB-*co*-3HV)

From colonies towards products



Colony

Laboratory Research

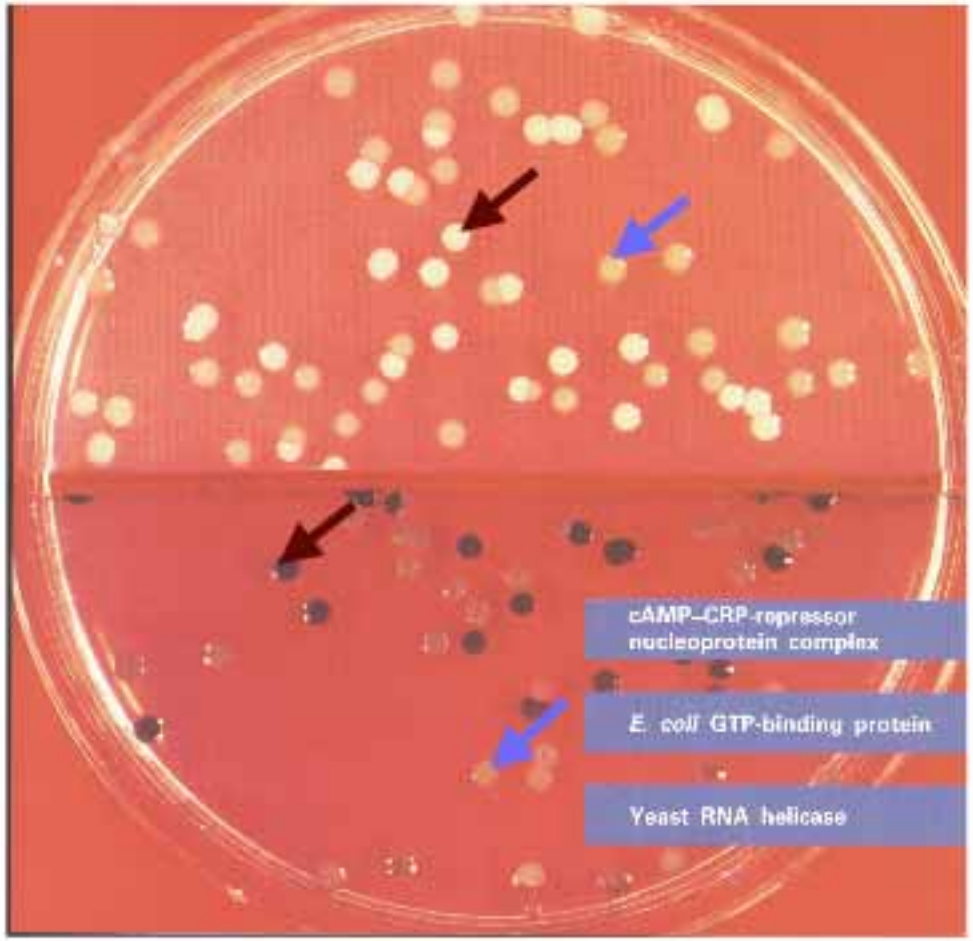
Pilot Plant

Industrial Plant

Transgenic Plants

Product

Wild type and PHA negative mutants of *Ralstonia eutropha*



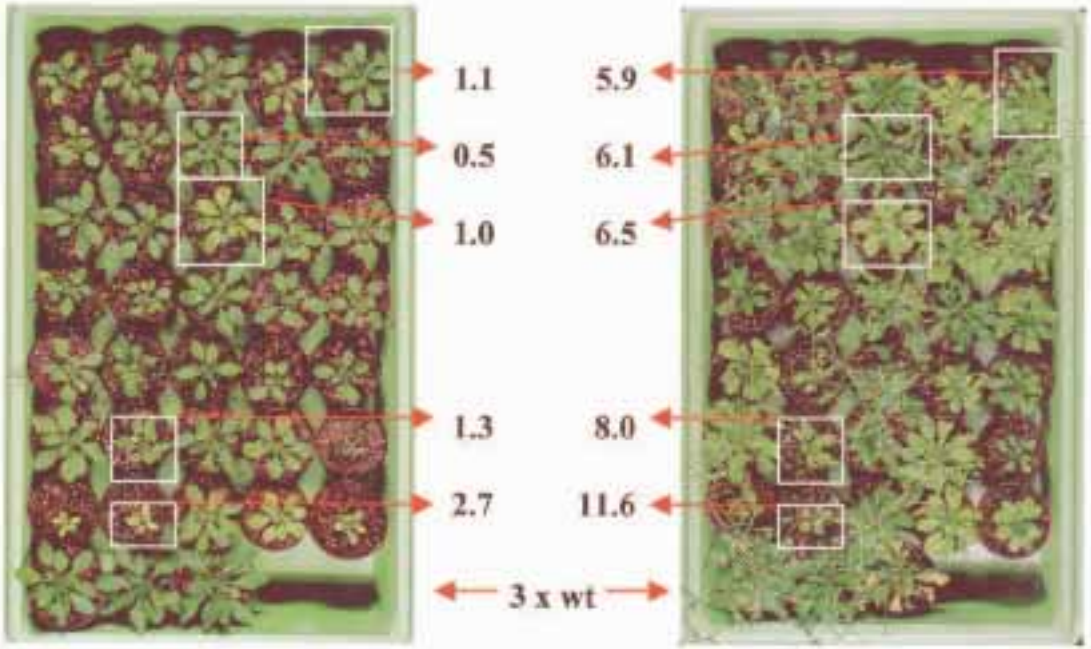
Phenotype: PHA positive
Genotype: wild type

Phenotype: PHA negative
Genotype: *phaC*::Tn*5*

Phenotype: PHA leaky
Genotypes: *phaL*::Tn*5*
phaP*::Tn*5
phaH*::Tn*5
phaI*::Tn*5

Transgenic plants producing PHAs

Establishment of the bacterial PHA biosynthesis pathways in plants may allow cheap production of bulk PHAs



Arabidopsis thaliana

Brassica napus

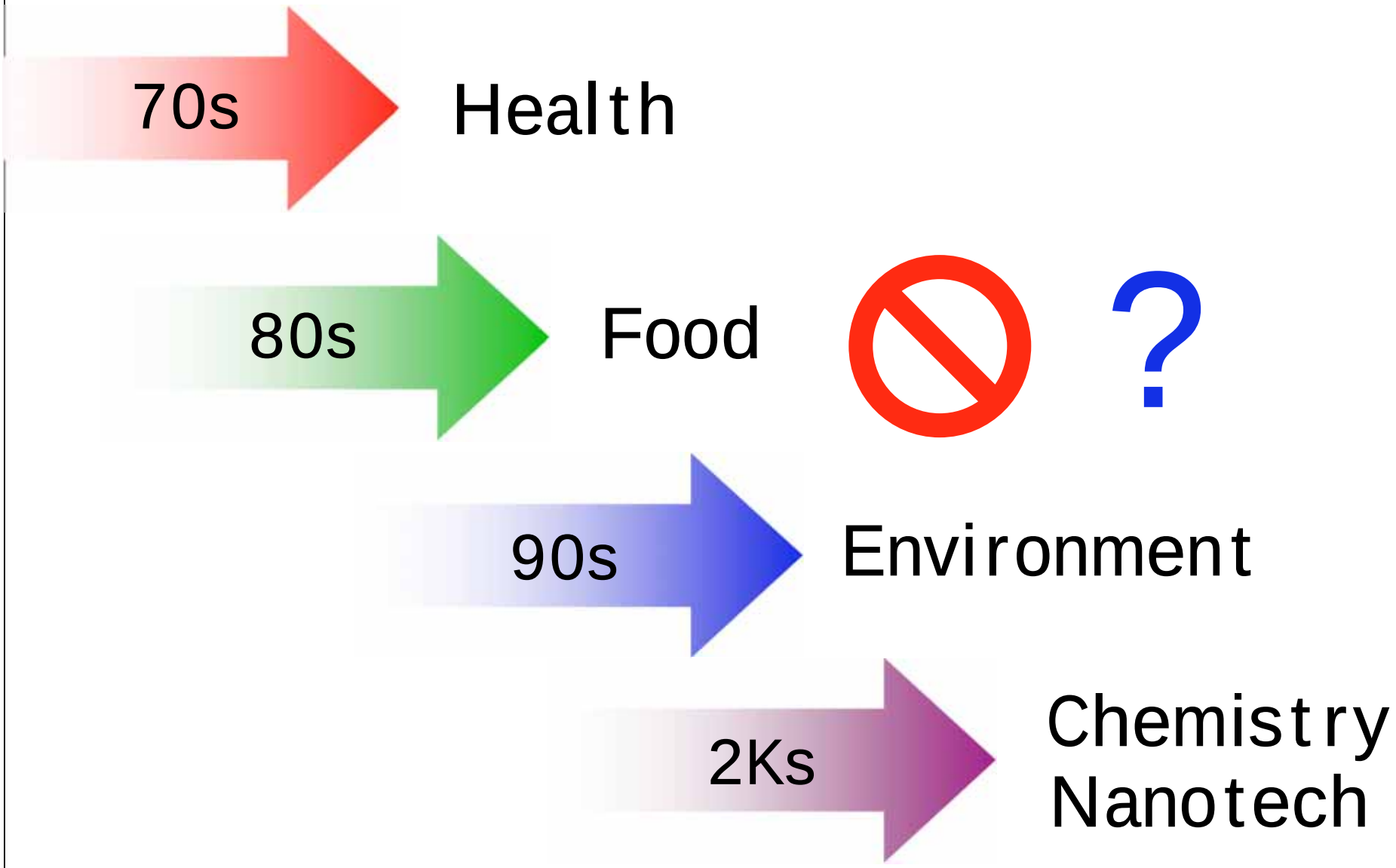
Gossypium hirsutum

Nicotiana tabacum

Solanum tuberosum

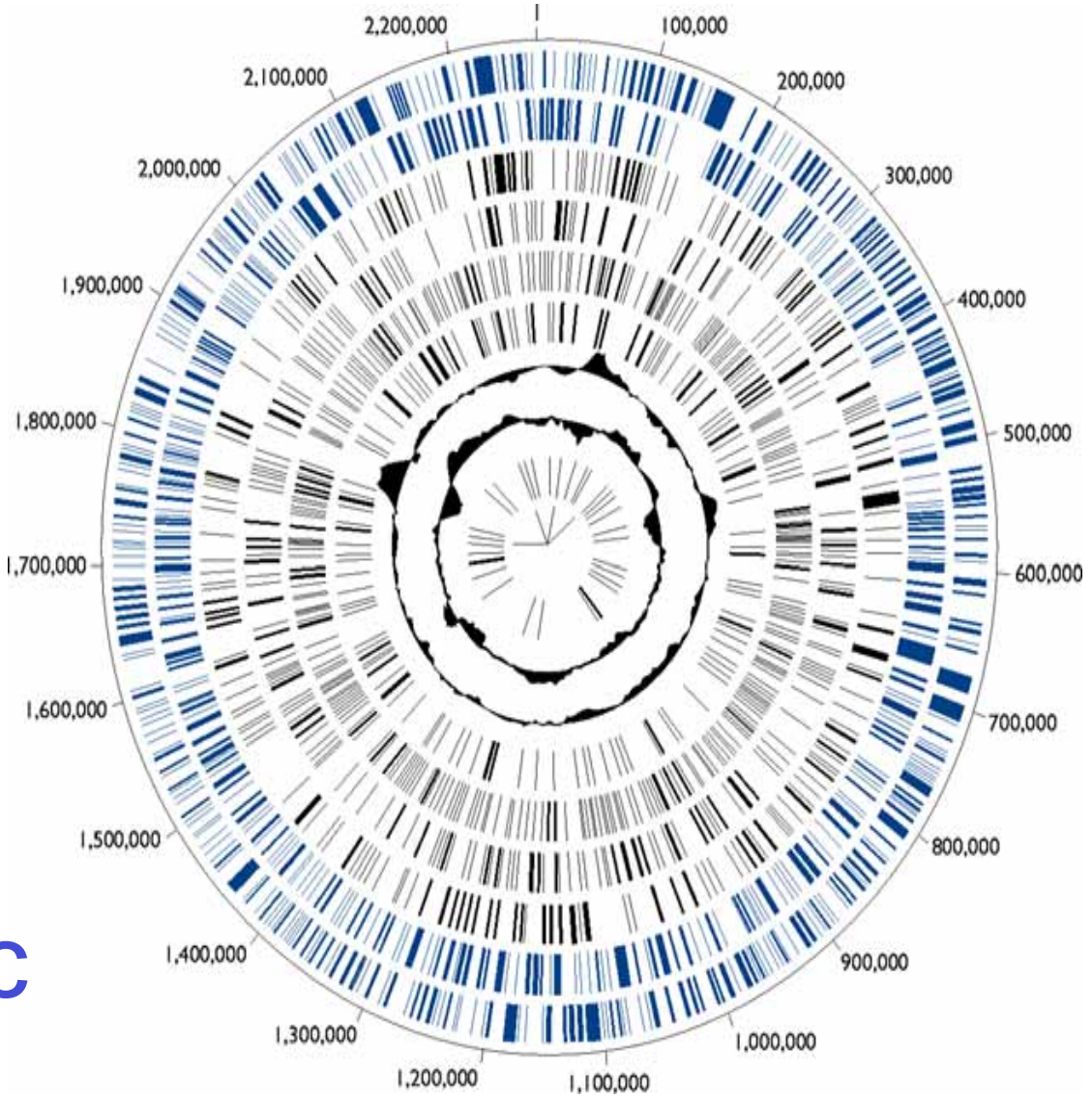
Zea mays

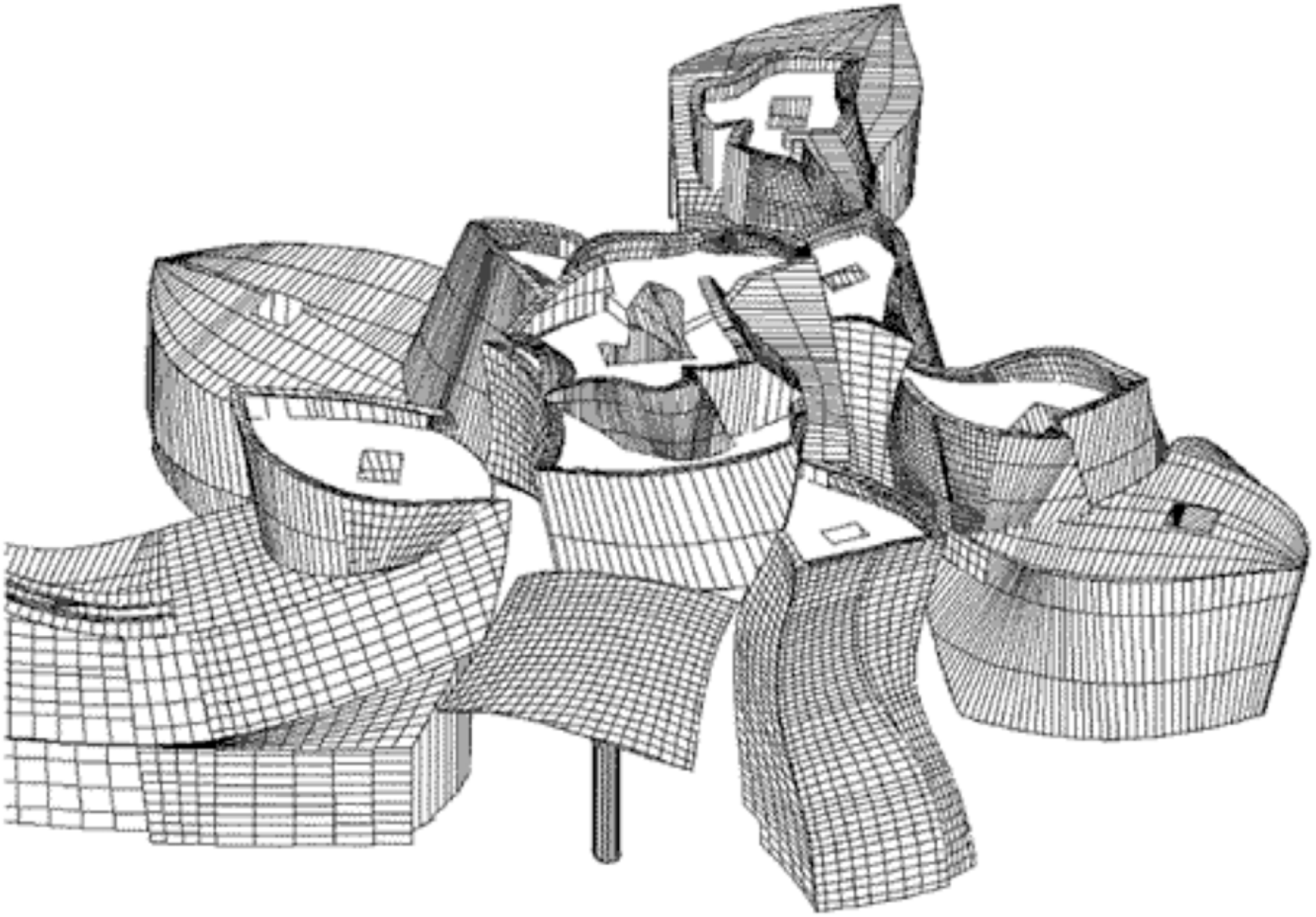
Widening of Biotechnologies




Biología Sintética
Rediseñar los sistemas vivos

The *big leap forward*: from Genetic Engineering to Synthetic Biology

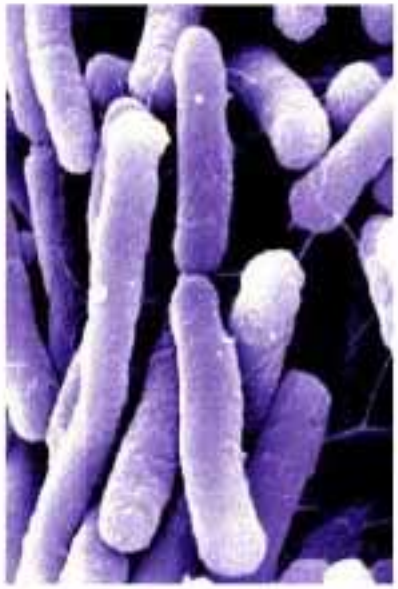




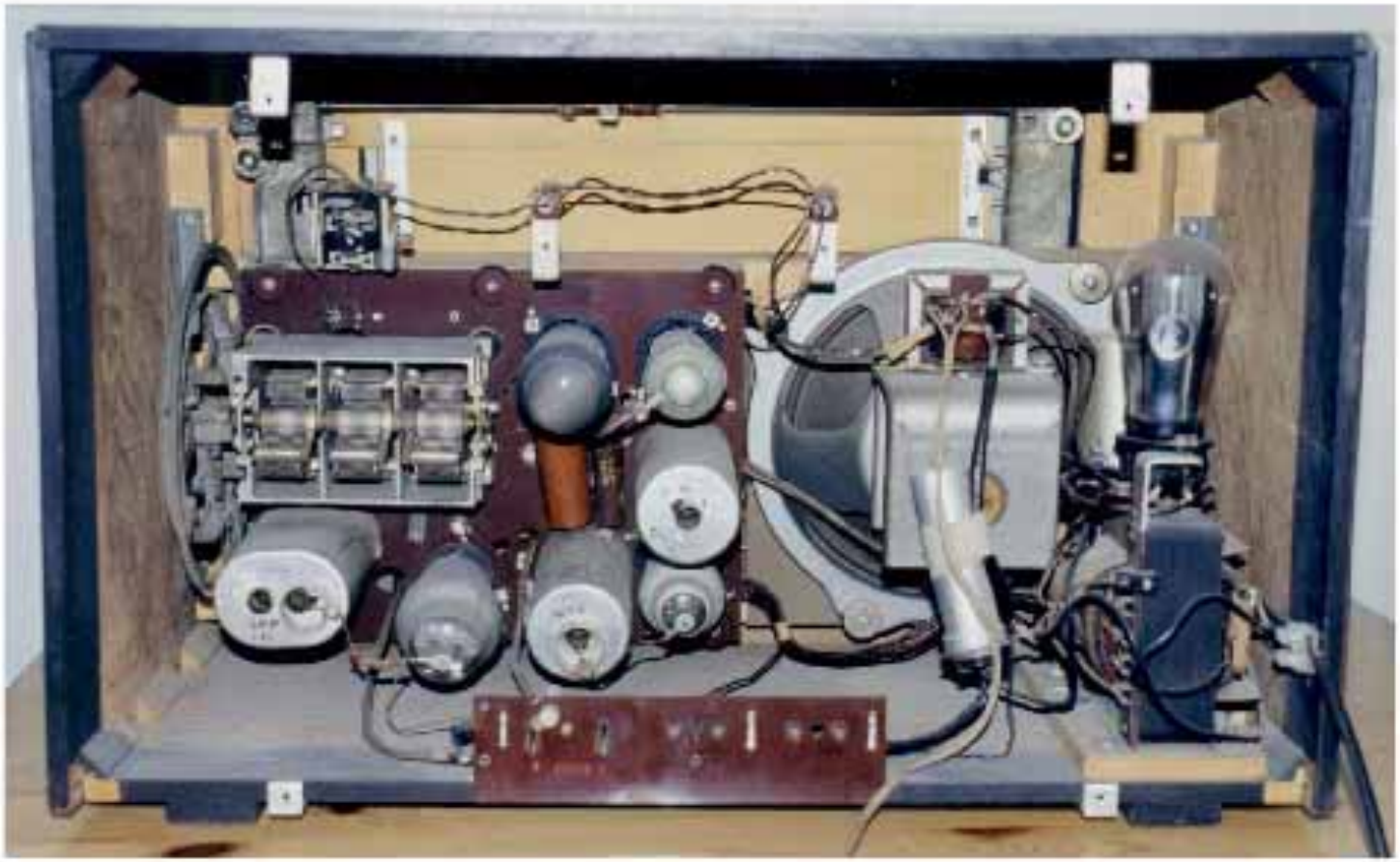
Synthetic Biology

syn•the•sis *n.* 1.a. the combination of separate elements to form a coherent whole.

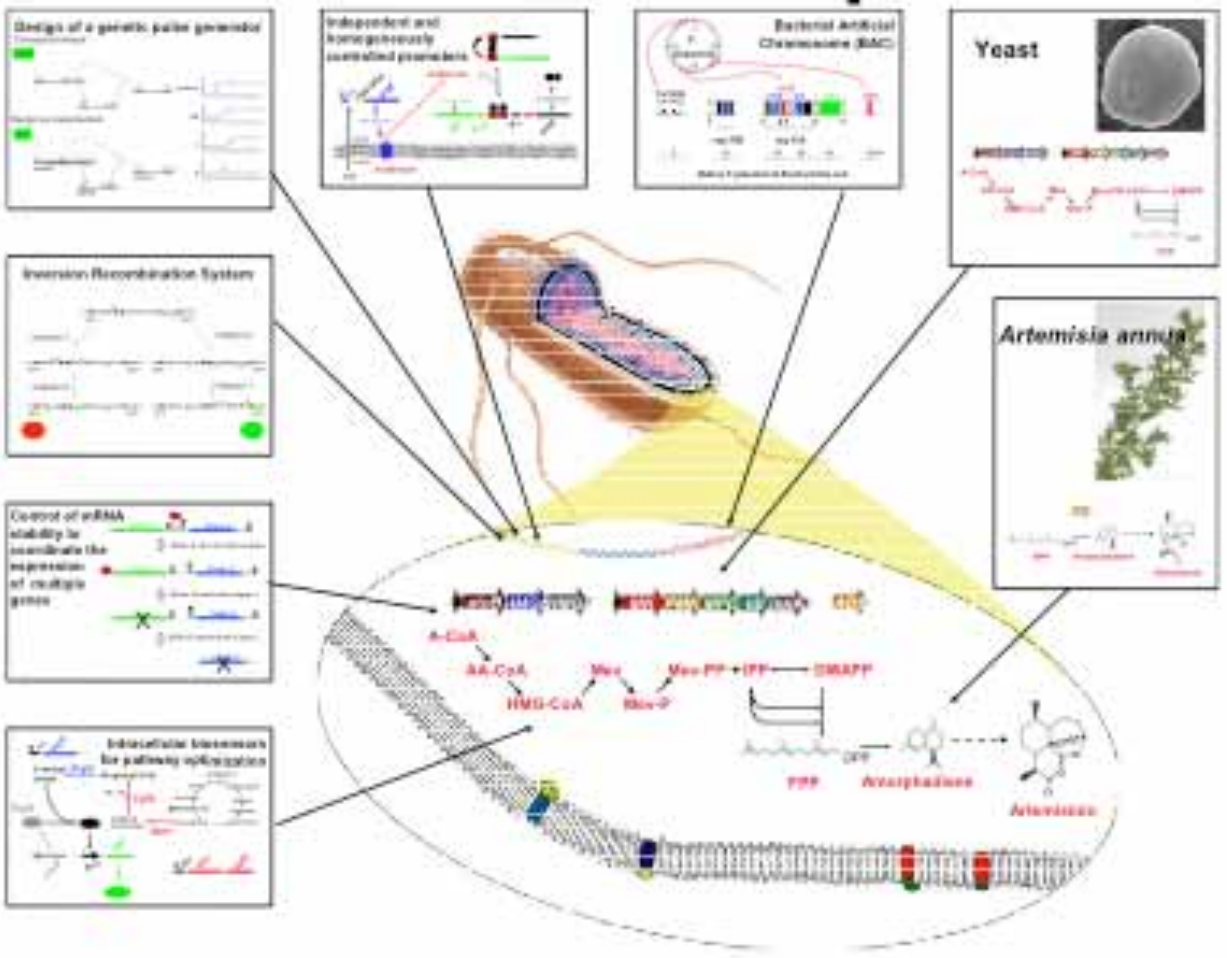
- Synthetic biology seeks to understand and design biological systems and their components to address a host of problems that cannot be solved using naturally-occurring entities
- Enormous potential benefits to medicine, environmental remediation and renewable energy
- Need the ability to write a 'blueprint'



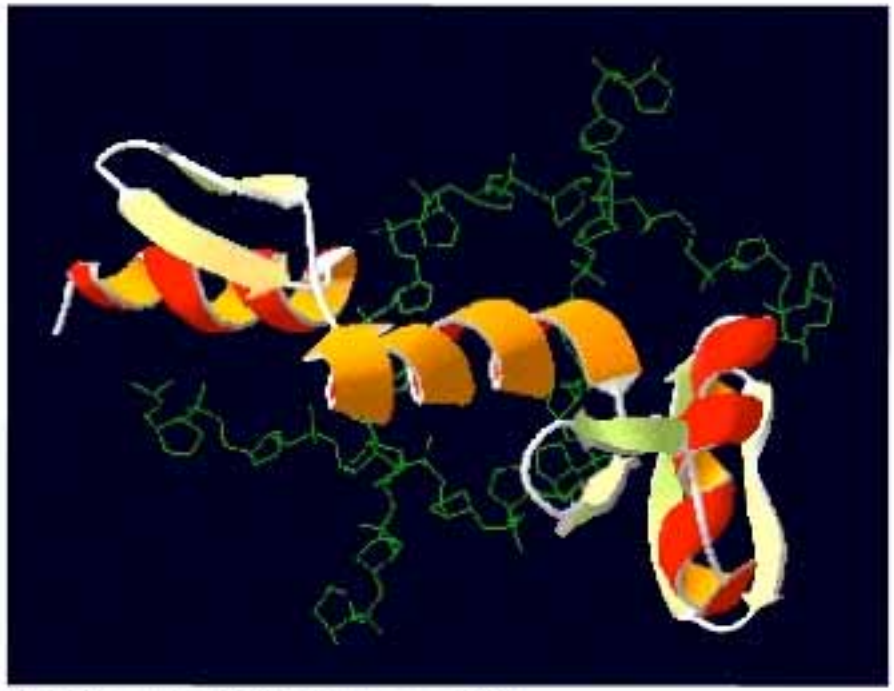
Building a radio with parts



Building a cell with well characterized parts

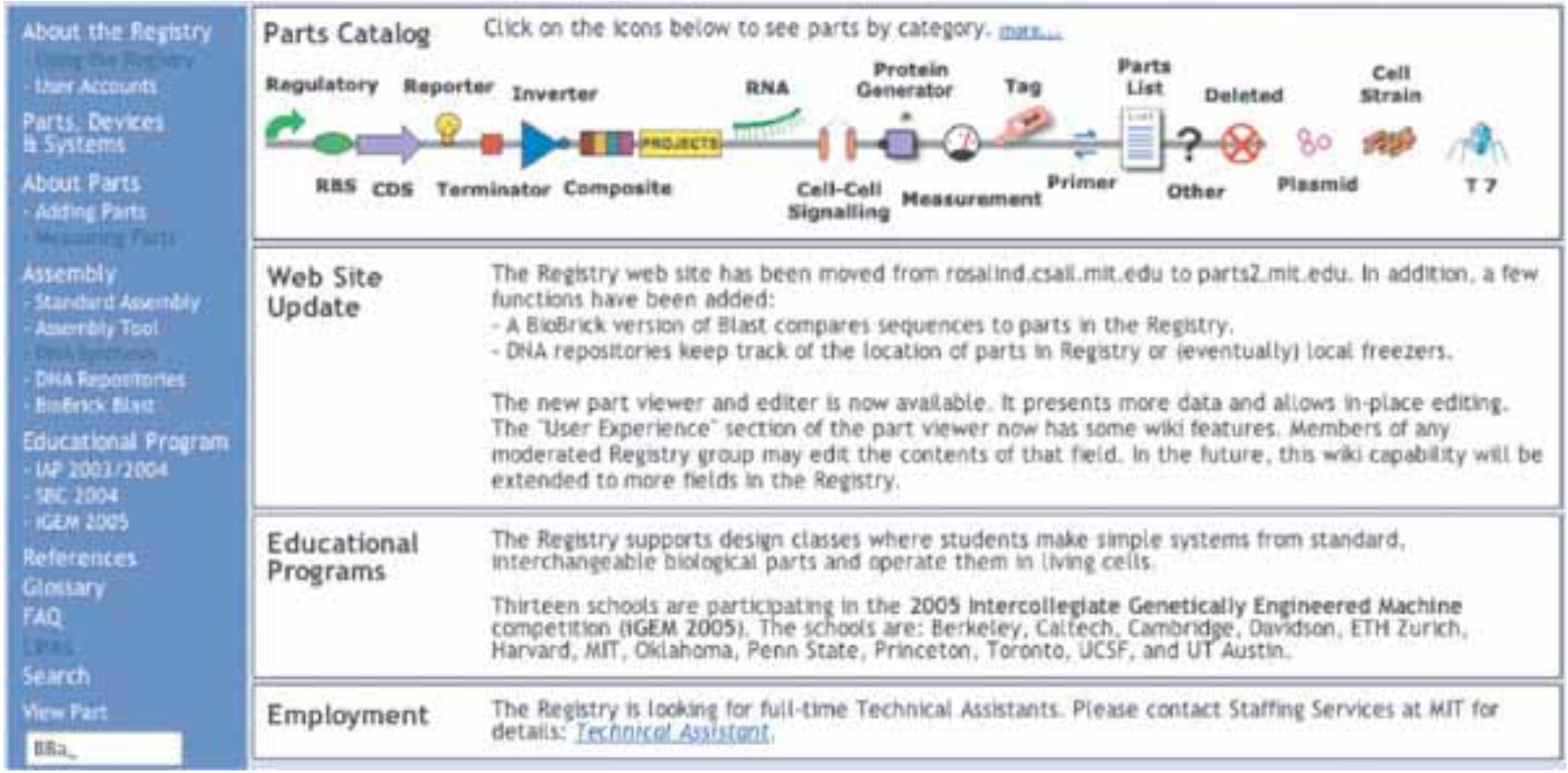


Parts



Zif268, Paveltich & Pabo c. 1991

Catalog of parts



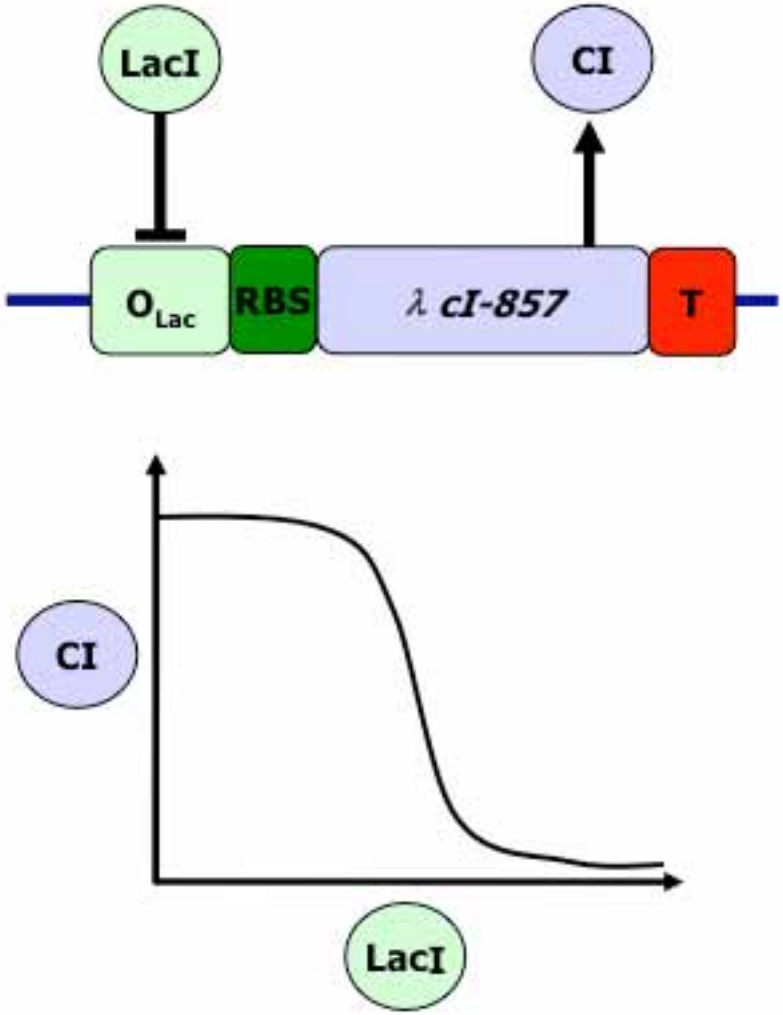
Abstraction Hierarchy

Systems

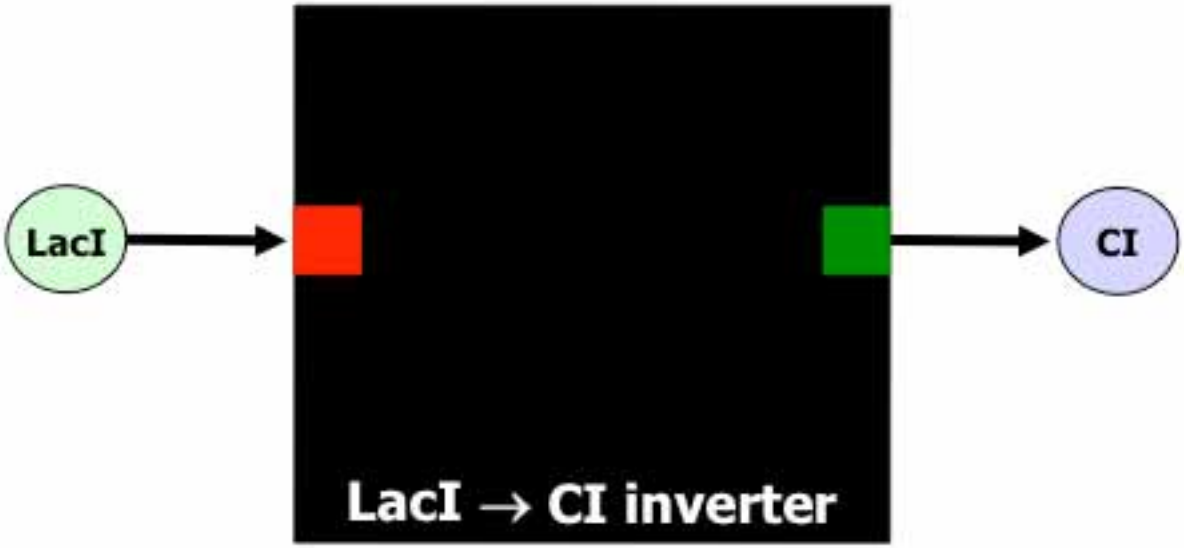
Devices

Parts

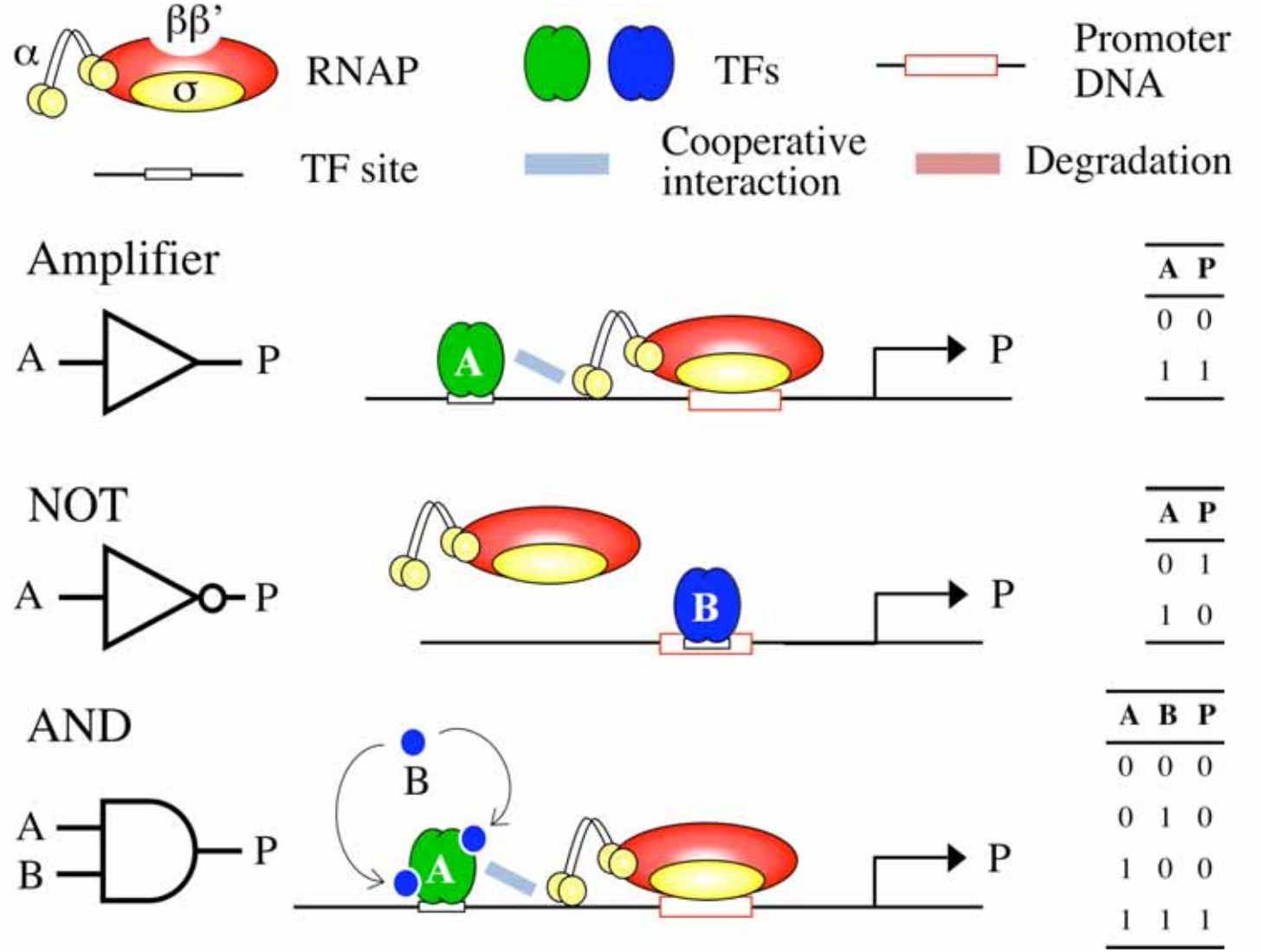
Devices



Devices

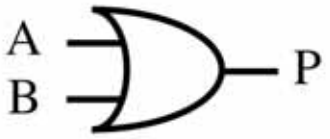


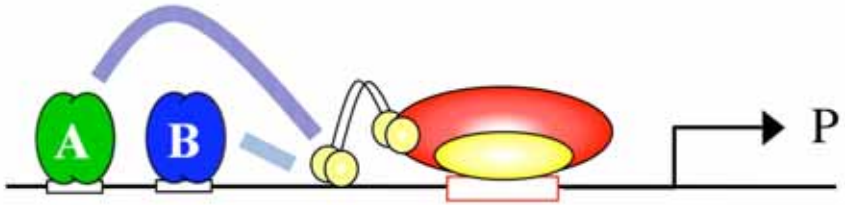
Bacterial promoters as logic gates (I)



Bacterial promoters as logic gates (II)

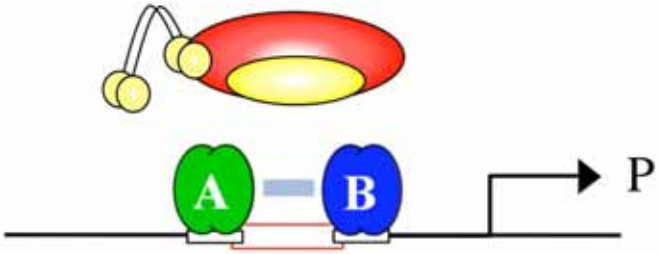
OR





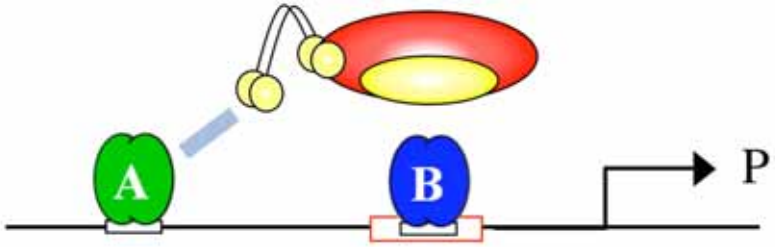
A	B	P
0	0	0
0	1	1
1	0	1
1	1	1

NAND



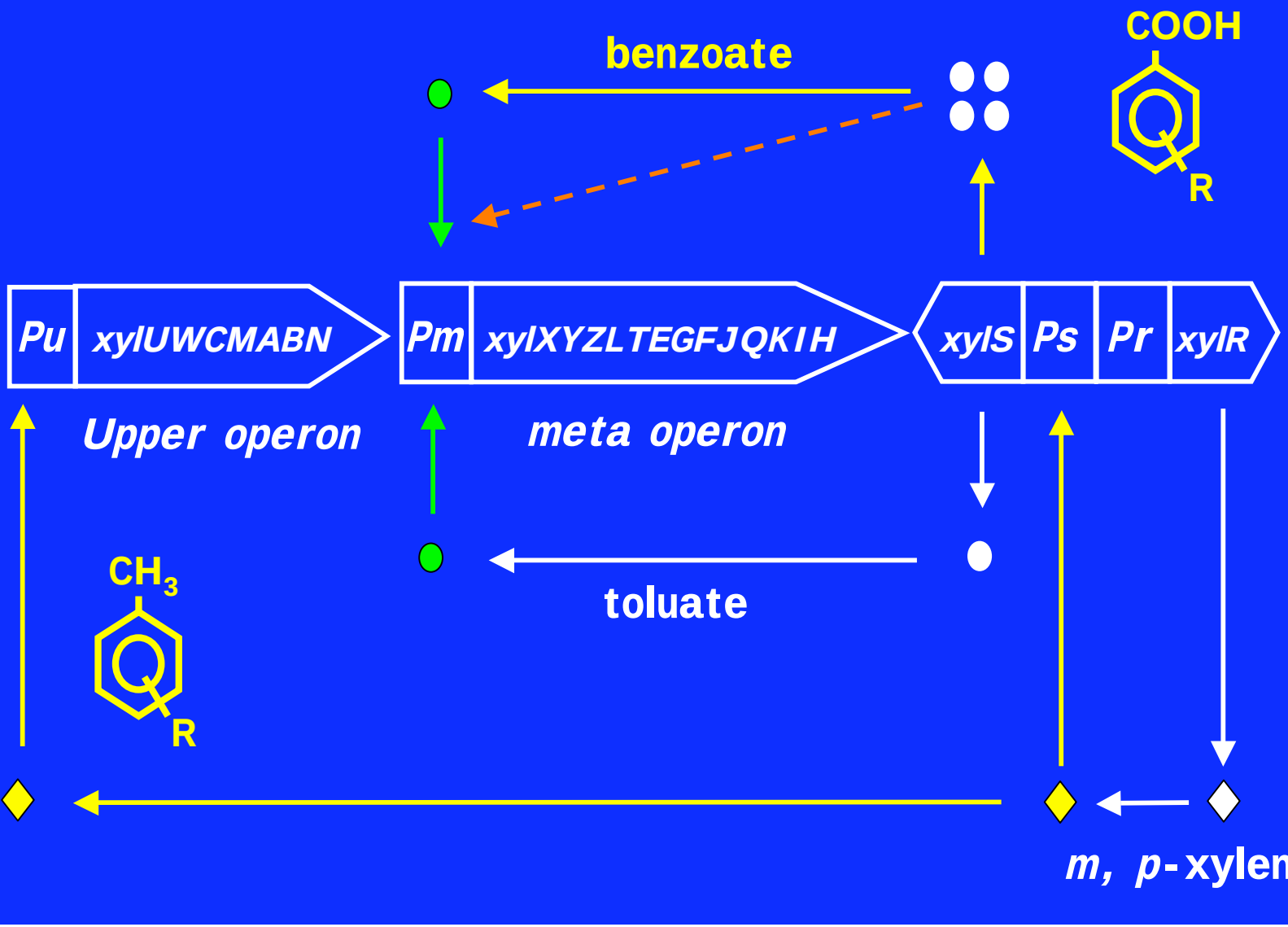
A	B	P
0	0	1
0	1	1
1	0	1
1	1	0

ANDN

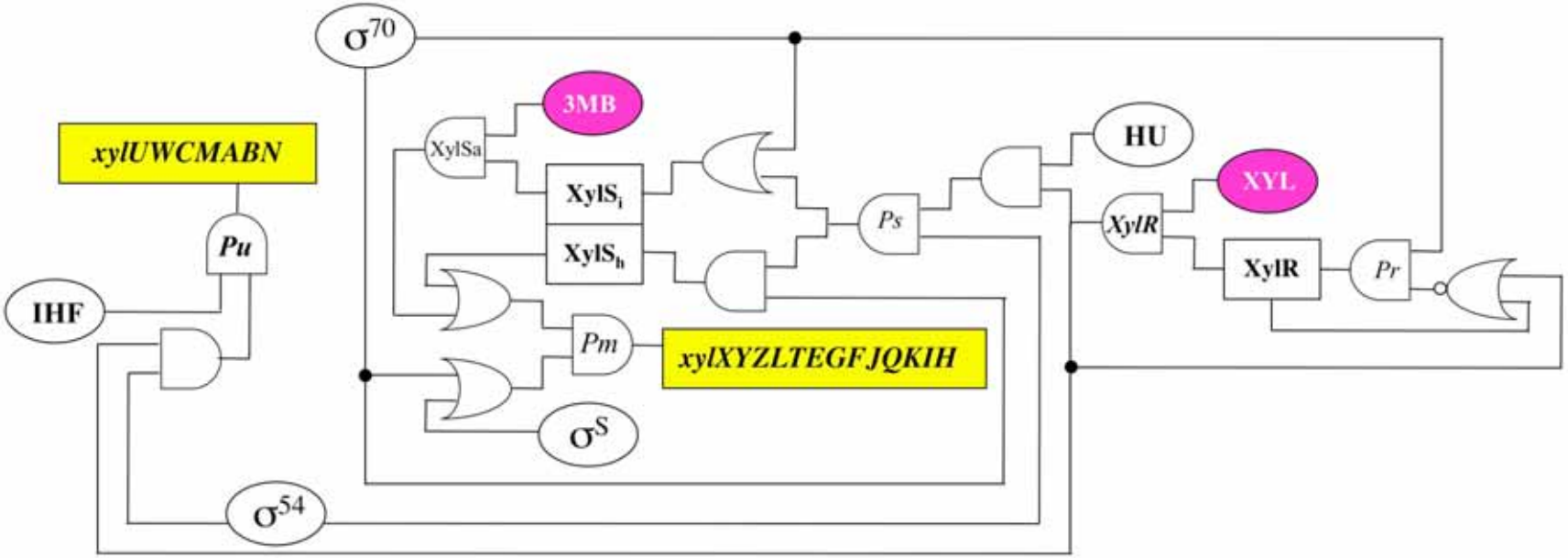


A	B	P
0	0	0
0	1	0
1	0	1
1	1	0

The regulation of the TOL system



Regulation of the TOL system as a whole of logic gates

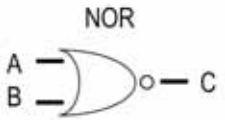


input

output

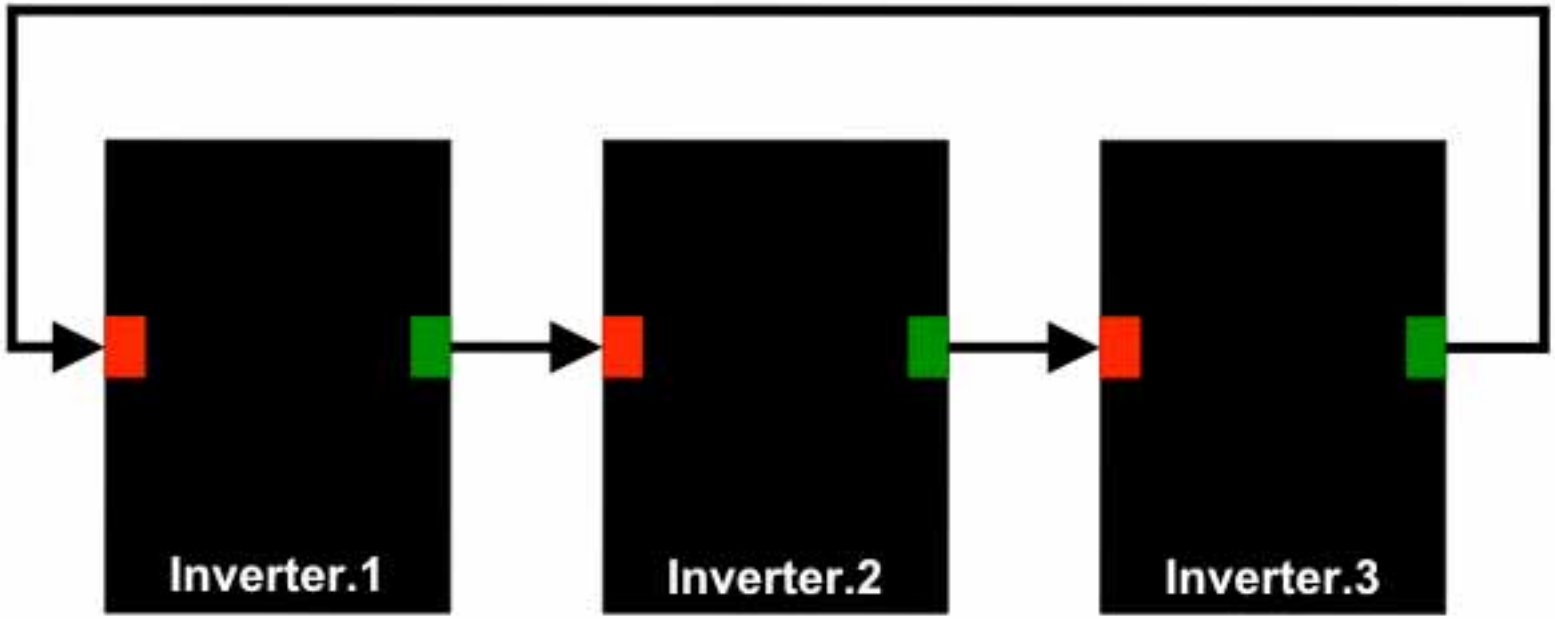
A	B	C
0	0	0
1	0	0
0	1	0
1	1	1

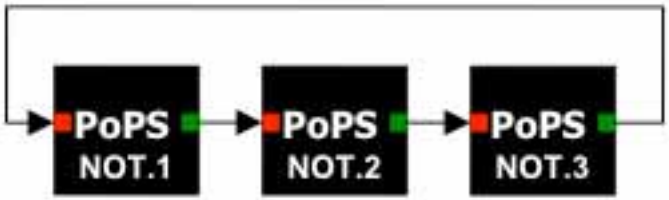
A	B	C
0	0	0
1	0	1
0	1	1
1	1	1



A	B	C
0	0	1
1	0	0
0	1	0
1	1	0

Systems





Systems

'Can I have three inverters?'

'Here's a set of PDP inverters, 1→N, that each send and receive via a fungible signal carrier, PoPS.

Devices

'I need a few DNA binding proteins.'

'Here's a set of DNA binding proteins, 1→N, that each recognize a unique cognate DNA site, choose any.'

Zif268, Paveltich & Pabo c. 1991

Parts

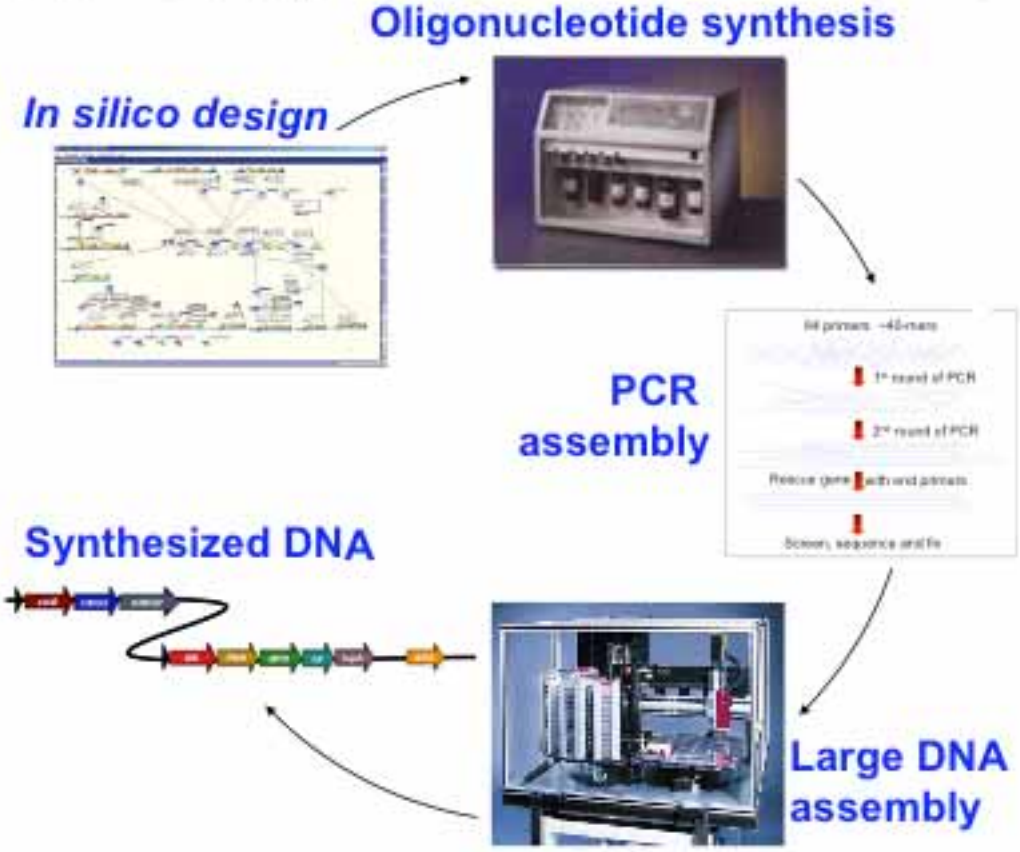
'Get me this DNA.'

'Here's your DNA.'

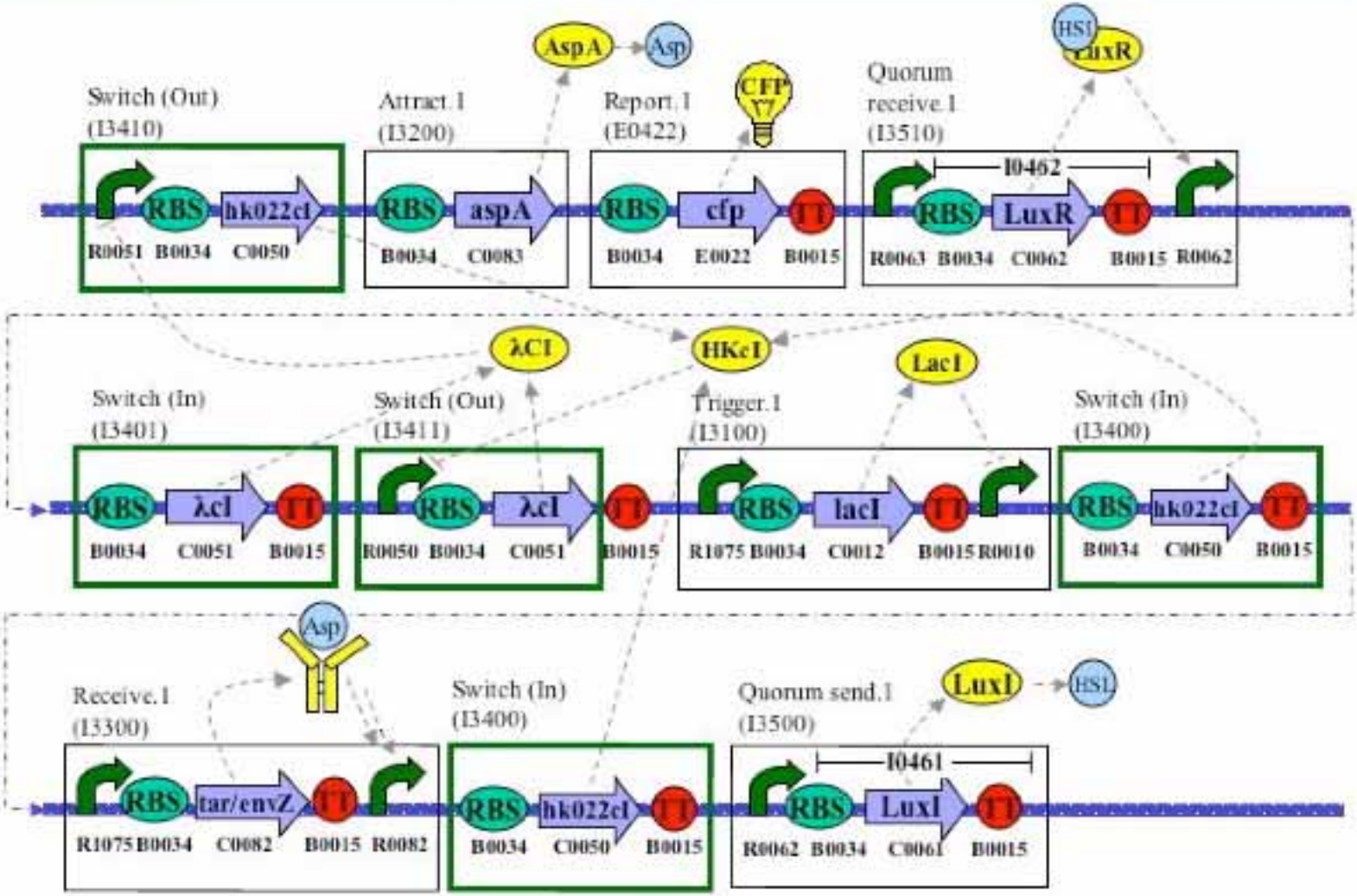
TAATACGACTCACTATAGGGAGA

DNA

Future approach: build *de novo*



DNA Layout

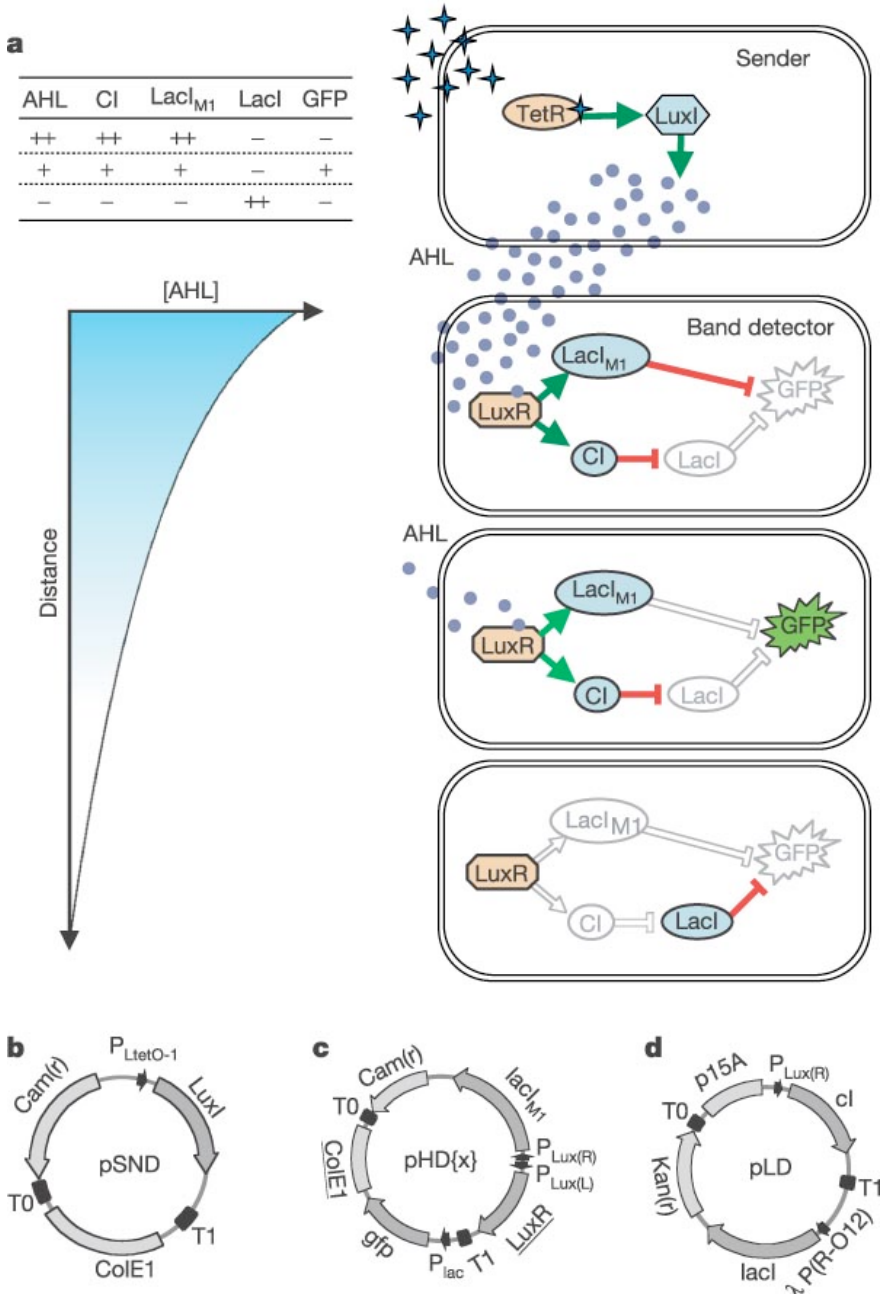

AspA
Asp
HSL
LuxR
CFP
Switch (Out)
(I3410)
Attract.1
(I3200)
Report.1
(E0422)
Quorum
receive.1
(I3510)
I0462
RBS
hk022cI
R0051 B0034 C0050
RBS
aspA
B0034 C0083
RBS
cfp
TT
B0034 E0022 B0015
RBS
LuxR
TT
R0063 B0034 C0062 B0015 R0062
λCI
HKeI
LacI
Switch (In)
(I3401)
Switch (Out)
(I3411)
Trigger.1
(I3100)
Switch (In)
(I3400)
RBS
λcI
TT
B0034 C0051 B0015
RBS
λcI
TT
R0050 B0034 C0051 B0015
RBS
lacI
TT
R1075 B0034 C0012 B0015 R0010
RBS
hk022cI
TT
B0034 C0050 B0015
Asp
LuxI
HSL
Receive.1
(I3300)
Switch (In)
(I3400)
Quorum send.1
(I3500)
I0461
RBS
tar/envZ
TT
R1075 B0034 C0082 B0015 R0082
RBS
hk022cI
TT
B0034 C0050 B0015
RBS
LuxI
TT
R0062 B0034 C0061 B0015

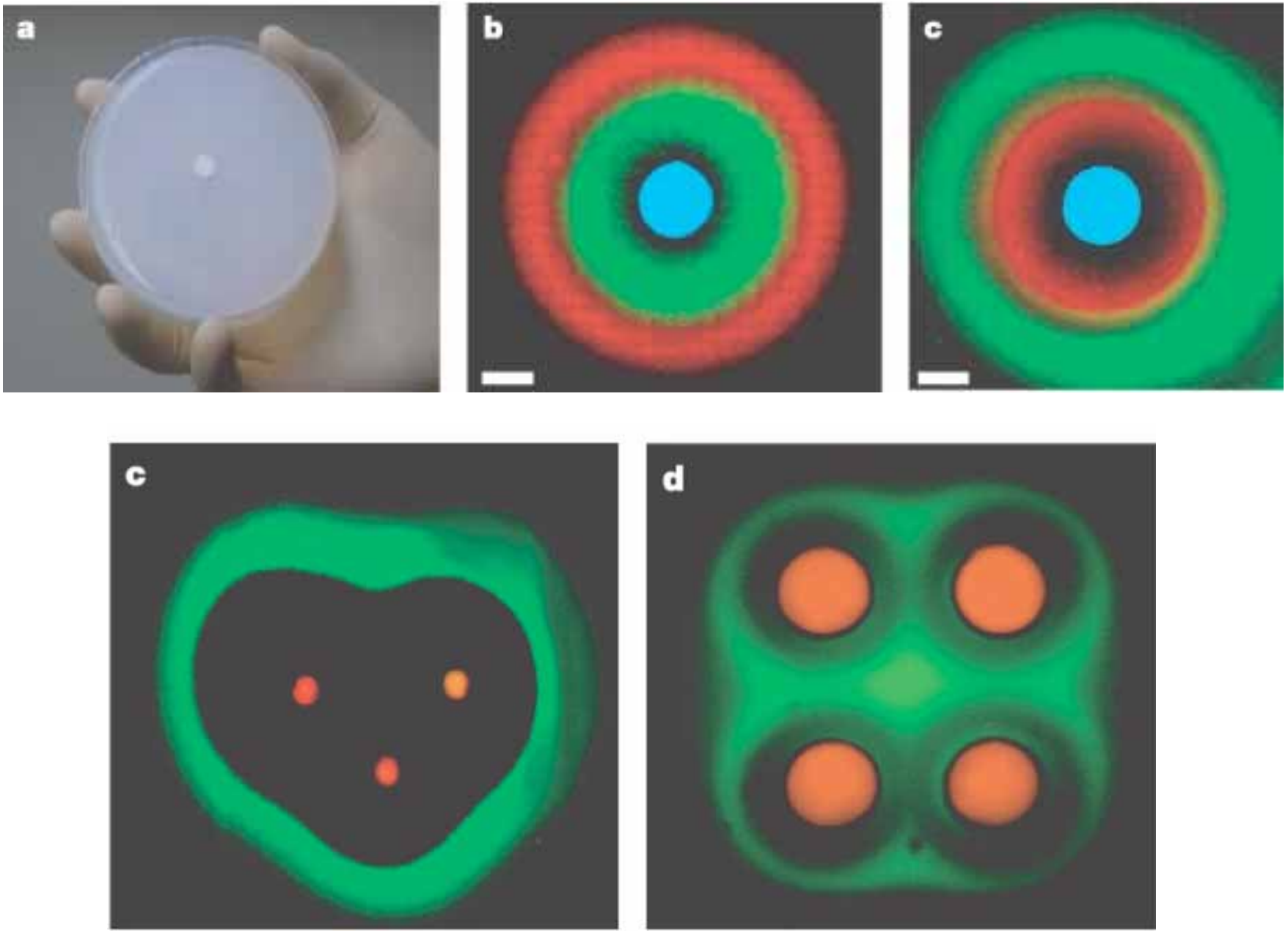
What can be done with all this?

Engineering developmental patterns in bacterial layers by designing genetic circuits with Quorum-Sensing components

R. Weiss

Programming an artificial genetic circuit with parts of the bacterial Quorum Sensing system



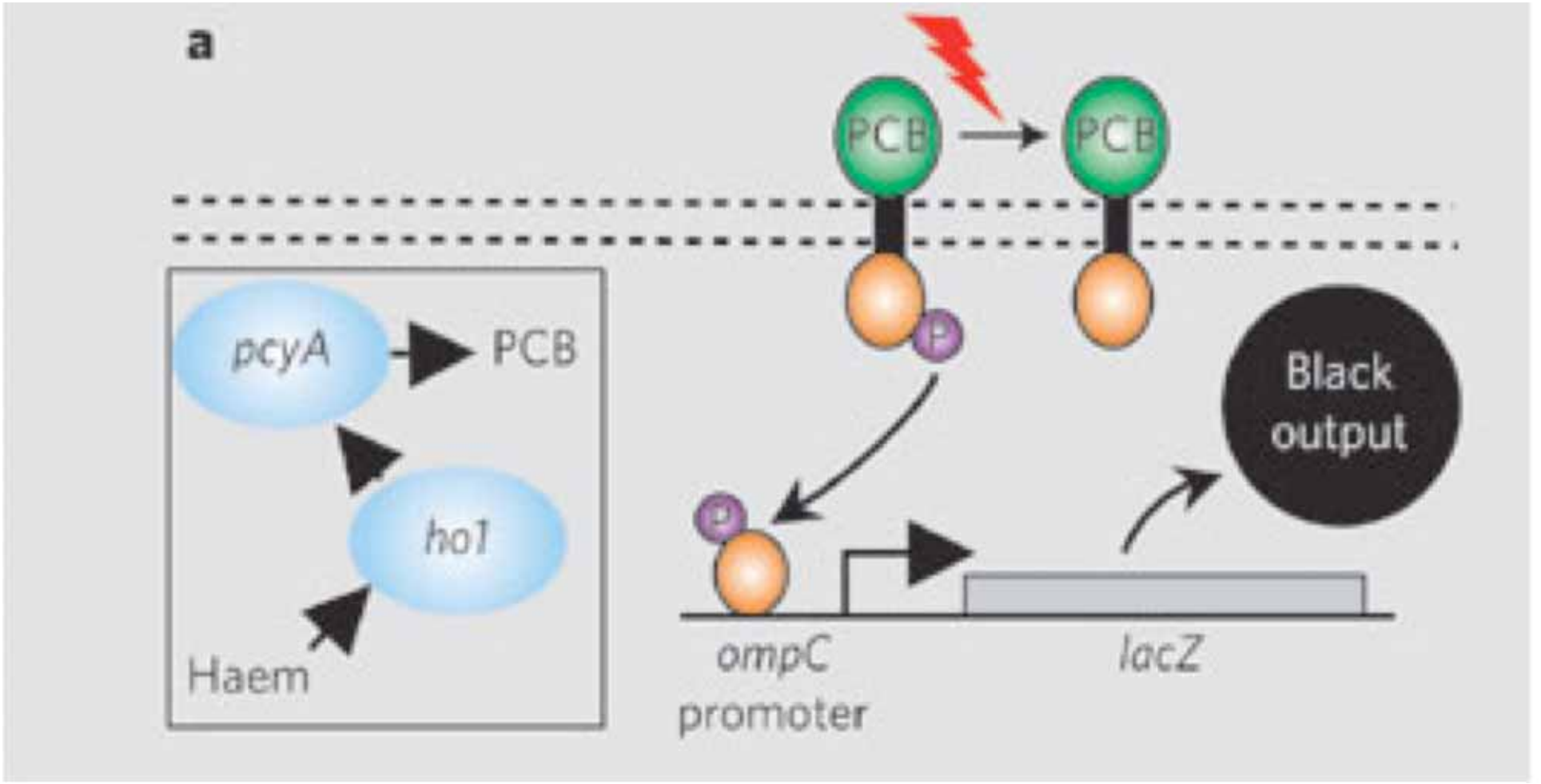

a
b
c
c
d

Designing *E. coli* strains with Light-responsive properties

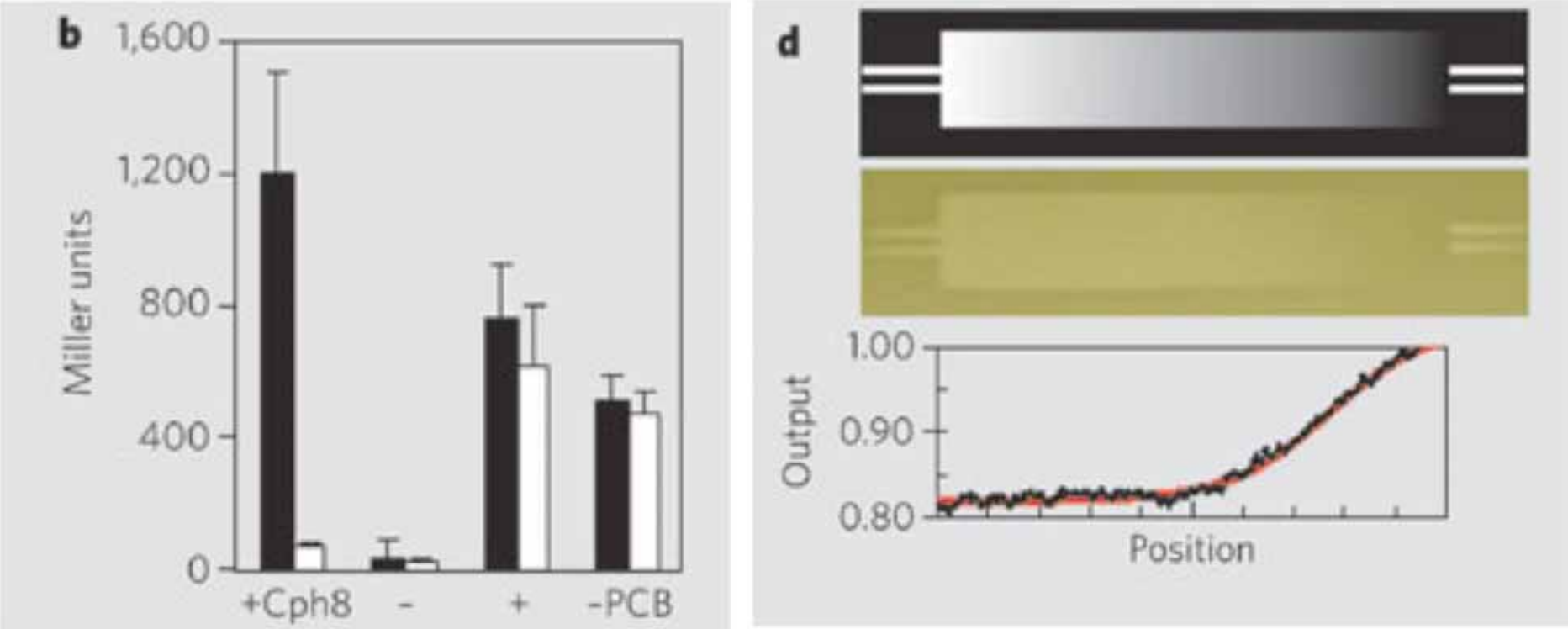
Voigt

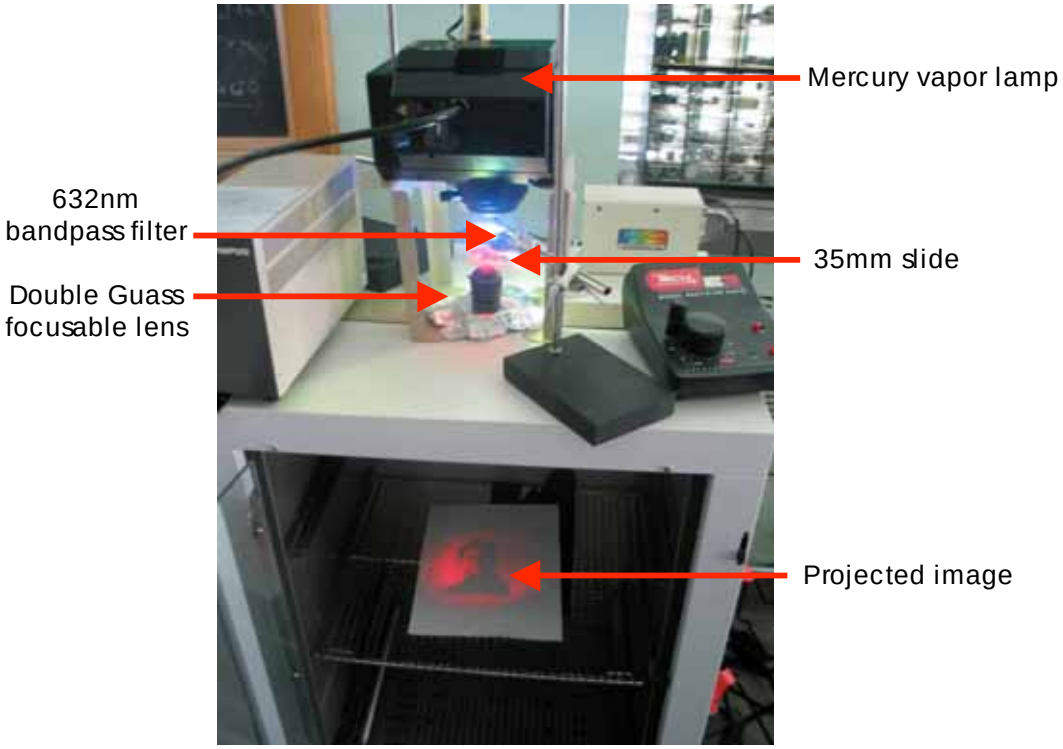
Chimaeric light receptor

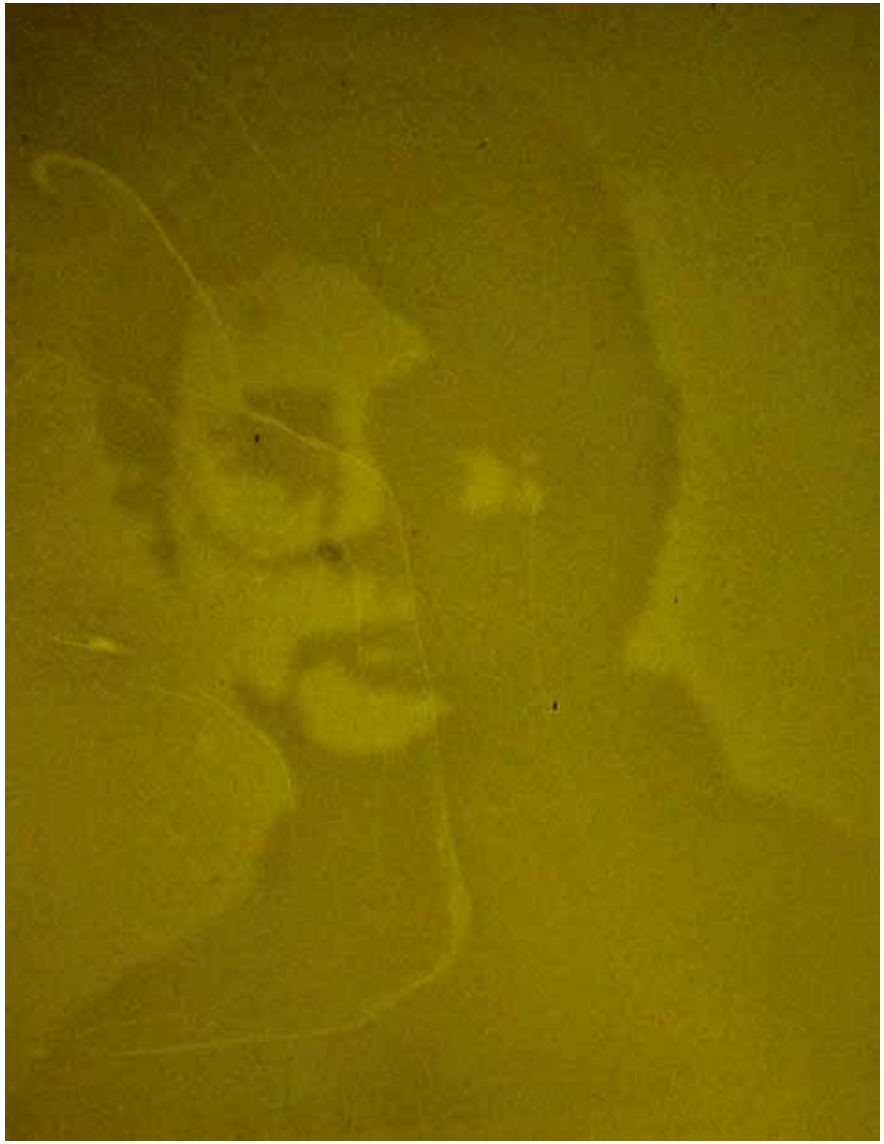
- Phytochrome Cph1 from *Synechocystis*
- EnvZ
- Phycocyanobilin (PCB) *ho1, pcyA*



Light-dependent *lacZ* expression




Mercury vapor lamp
632nm
bandpass filter
35mm slide
Double Guass
focusable lens
Projected image



Only games?

nature Vol 440|13 April 2006|doi:10.1038/nature04640

LETTERS

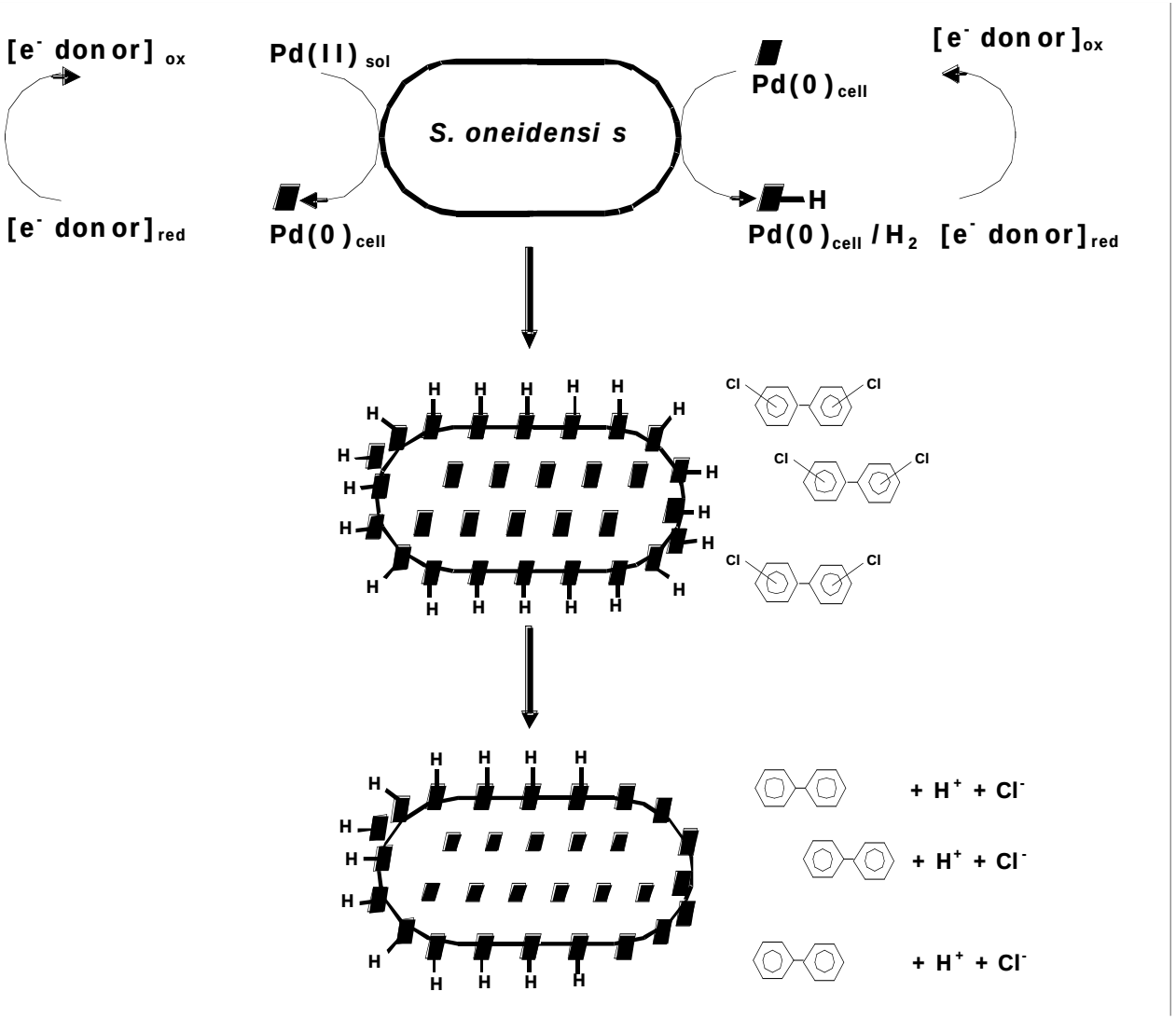
Production of the antimalarial drug precursor artemisinic acid in engineered yeast

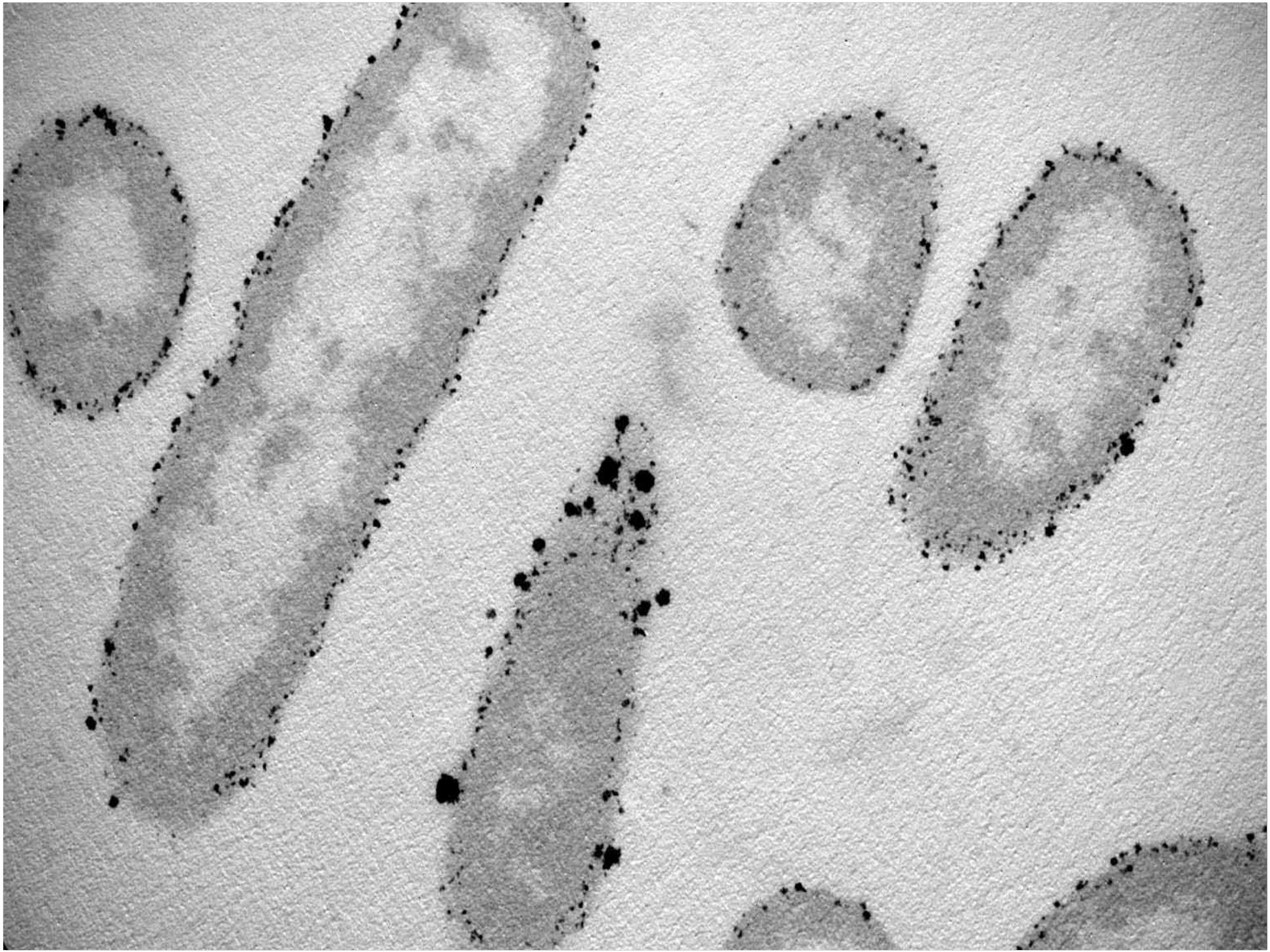
Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually[1]. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*[2,3]. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing[4,5]. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers[6]. Although total synthesis of artemisinin is difficult and costly[7], the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin[8,9]. Here we report the engineering of *Saccharomyces cerevisiae* to produce high titres (up to 100 mg l^{-1}) of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase, and a novel cytochrome P450 monooxygenase (*CYP71AV1*) from *A. annua* that performs a three-step oxidation

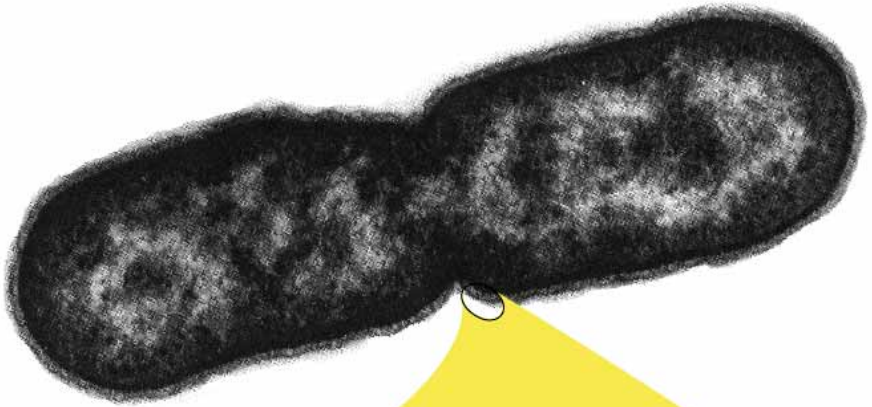
To increase FPP production in *S. cerevisiae*, the expression of several genes responsible for FPP synthesis was upregulated, and one gene responsible for FPP conversion to sterols was downregulated. All of these modifications to the host strain were made by chromosomal integration to ensure the genetic stability of the host strain. Overexpression of a truncated, soluble form of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*tHMGR*)[12] improved amorphadiene production approximately fivefold (Fig. 2, strain EYP208). Downregulation of *ERG9*, which encodes squalene synthase (the first step after FPP in the sterol biosynthetic pathway), using a methionine-repressible promoter (P_{MET3})[13] increased amorphadiene production an additional twofold (Fig. 2, strain EPY225). Although *upc2-1*, a semi-dominant mutant allele that enhances the activity of *UPC2* (a global transcription factor regulating the biosynthesis of sterols in *S. cerevisiae*)[14], had only a modest effect on amorphadiene production when overexpressed in the EPY208 background (Fig. 2, strain EPY210), the combination of downregulating *ERG9* and overexpressing *upc2-1* increased amorphadiene production to 105 mg l^{-1} (Fig. 2, strain EPY213). Integration of an additional copy of *tHMGR* into the chromosome further increased amorpha-

Dechlorinating PCBs with Bio/inorganic catalysts





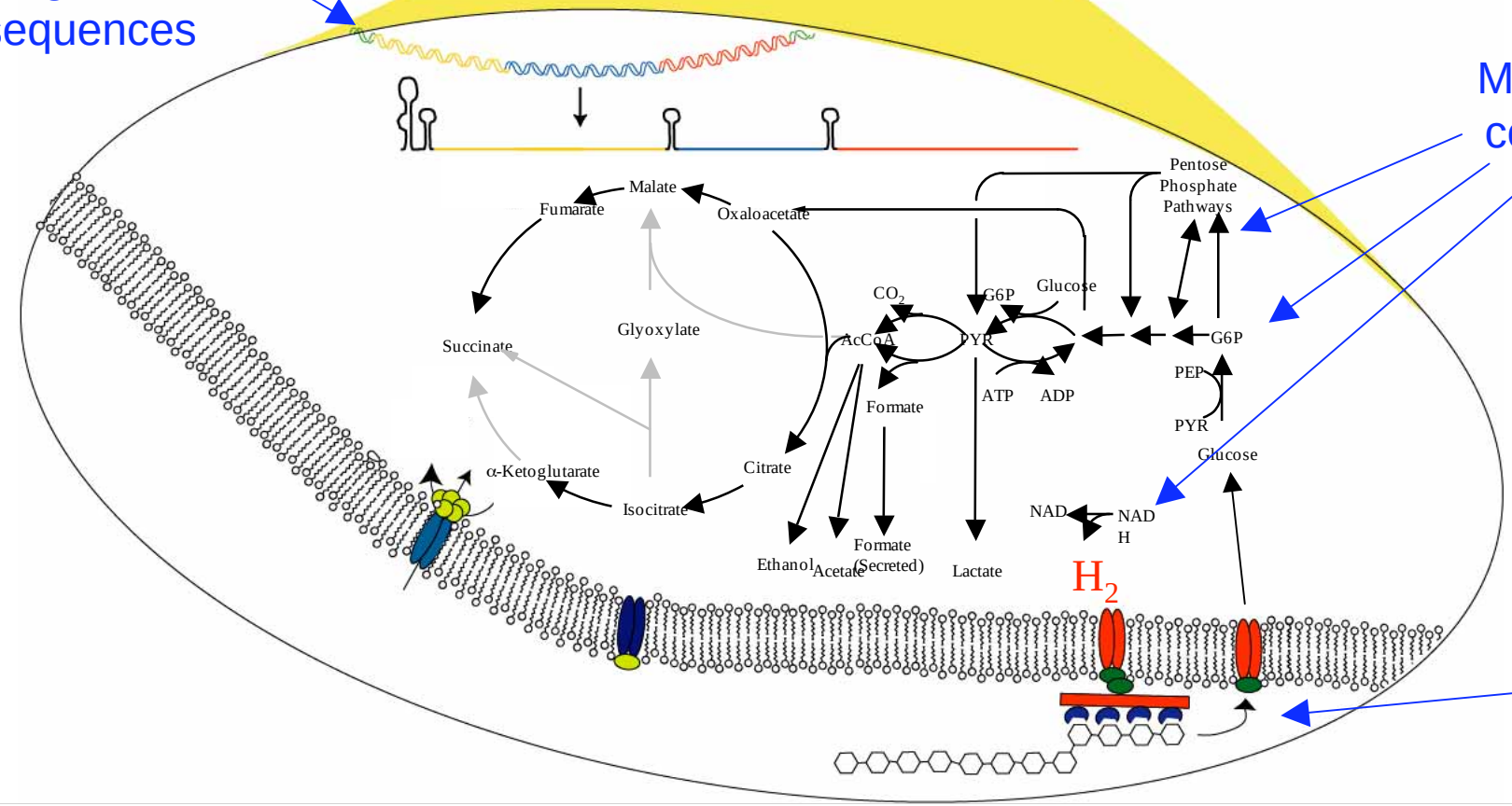
Building a better H_2 producer



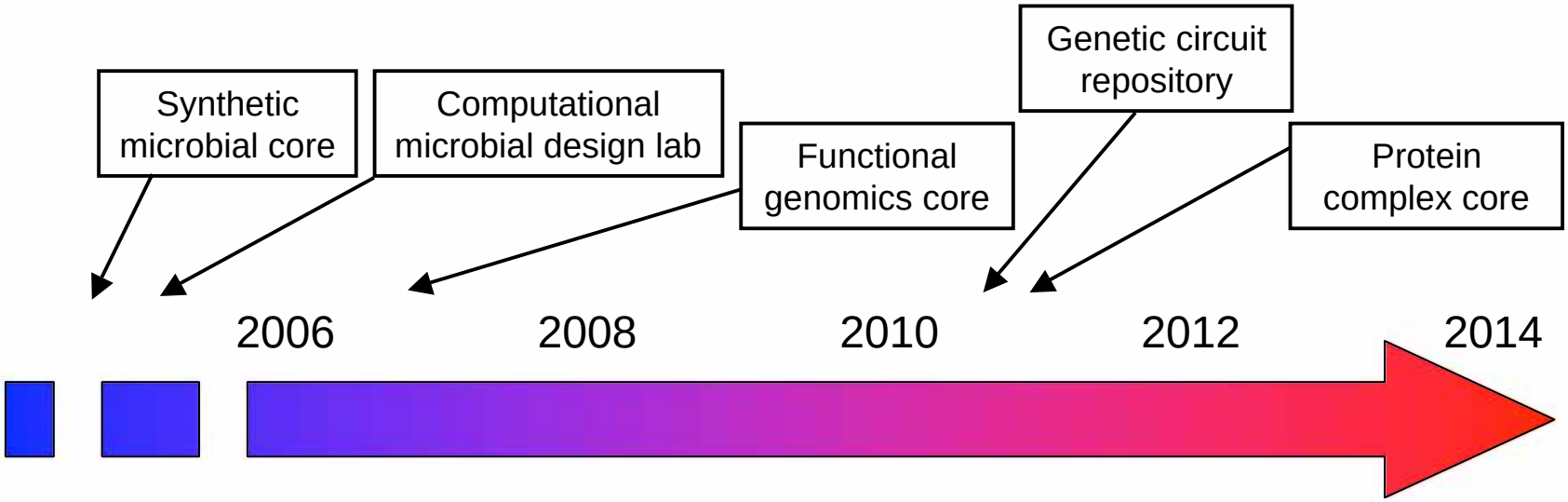
Building a new chromosome based on genome sequences

Maximizing conversion to H_2

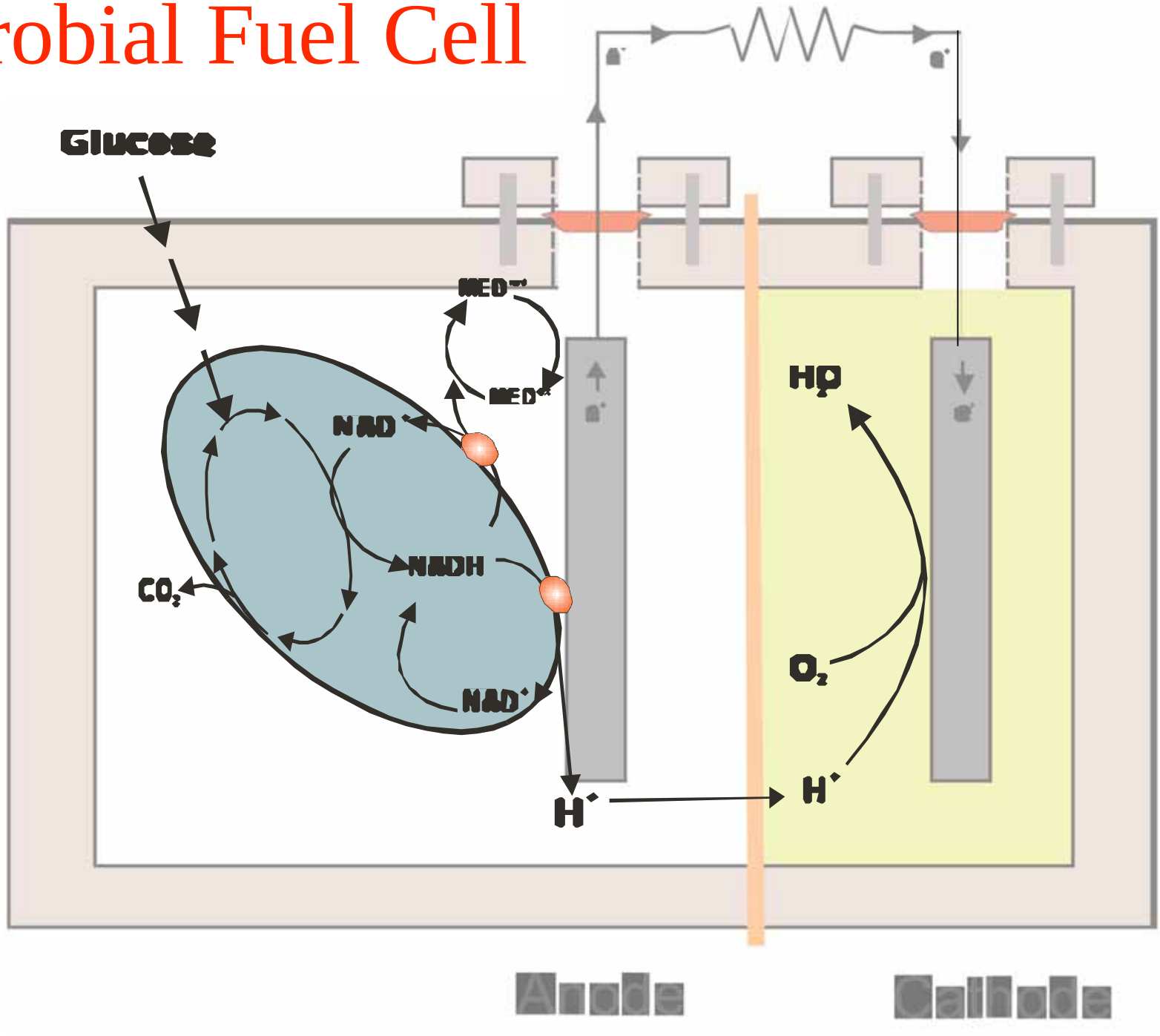
Maximizing renewable resource utilization



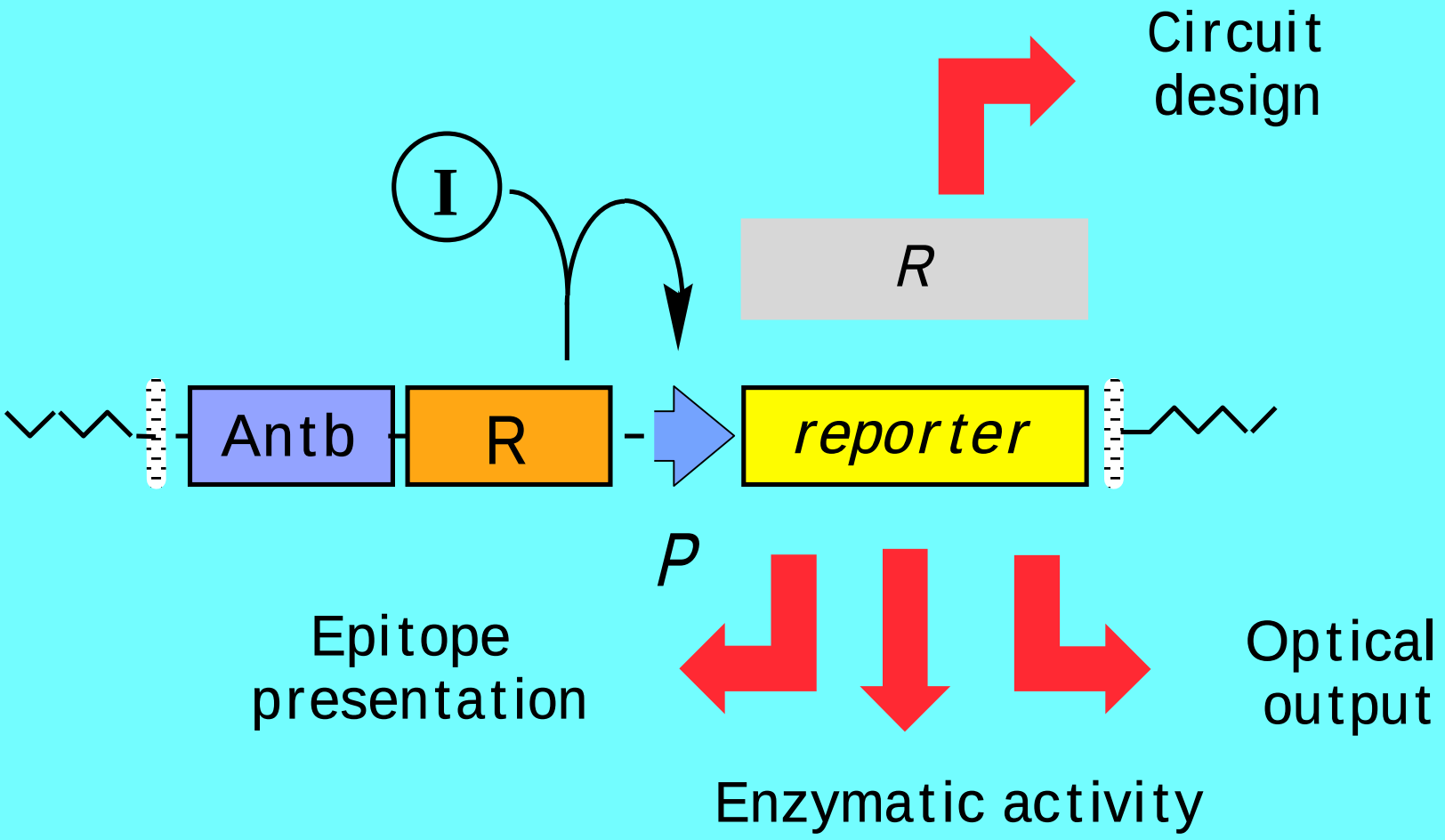
Towards a an H_2 super-producer (?)



Microbial Fuel Cell



Transcriptional regulators *à la carte?*




MINES
மி
தி
வெ
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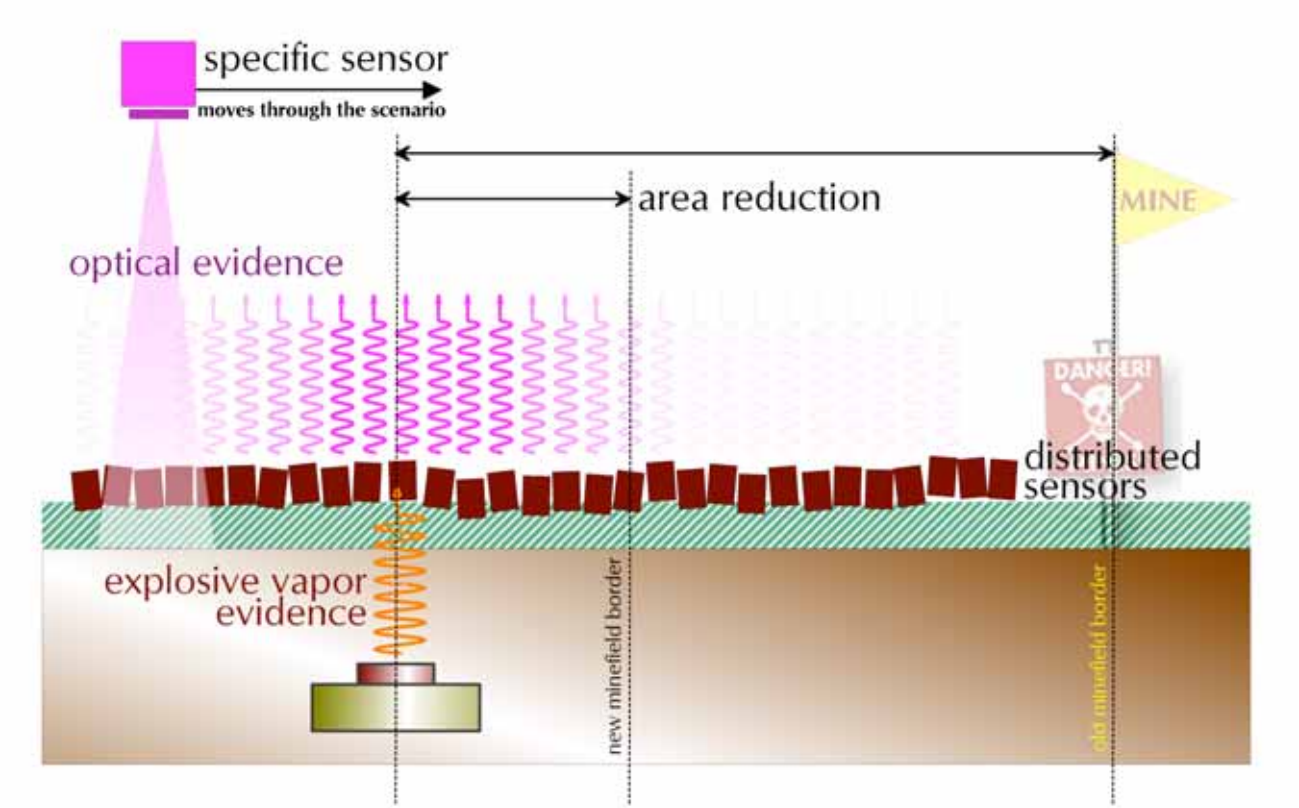
ම
බෝ
MINES

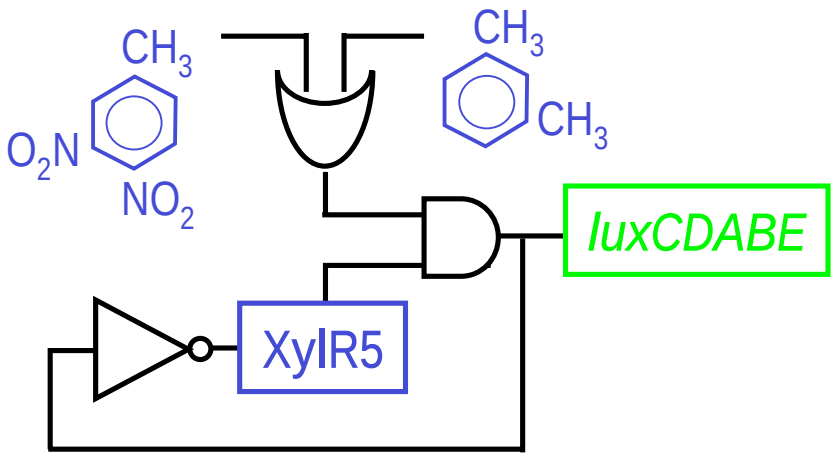
Area Reduction:

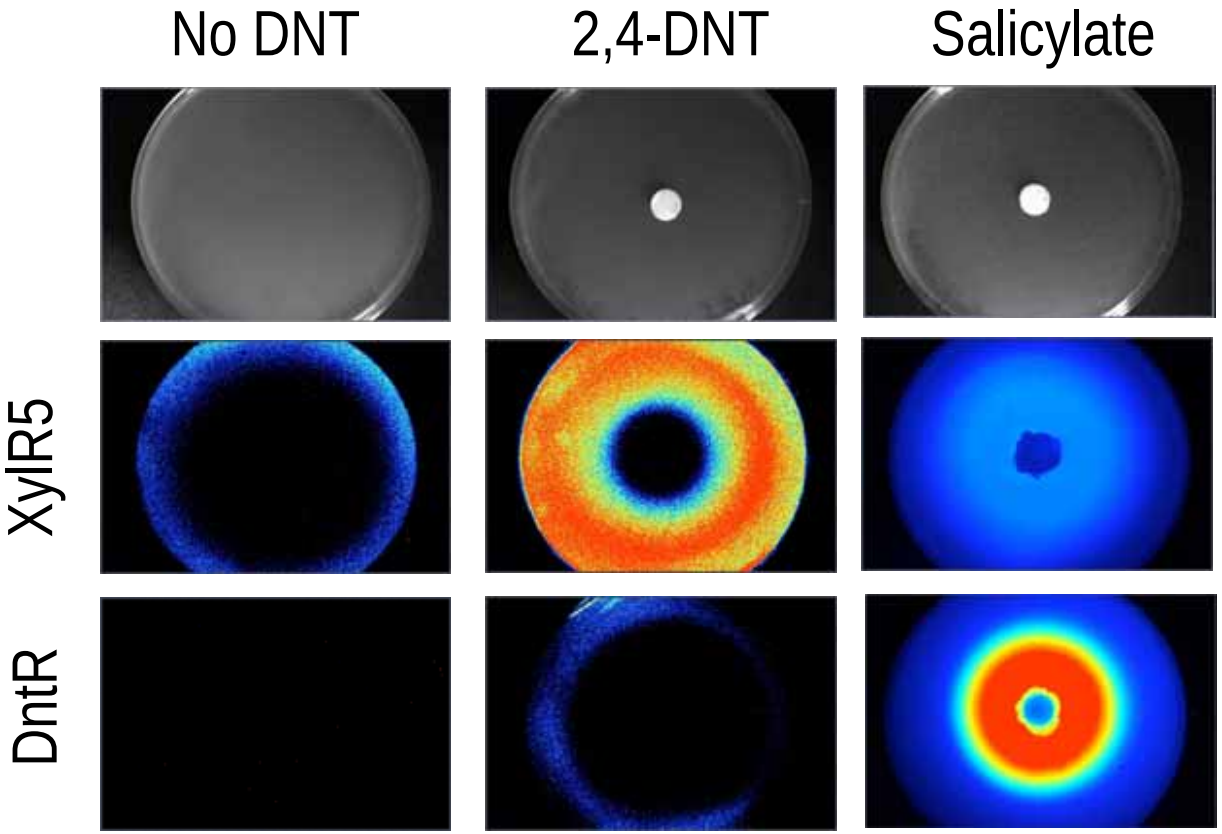
THE DISTRIBUTED & SPECIALIZED SENSOR



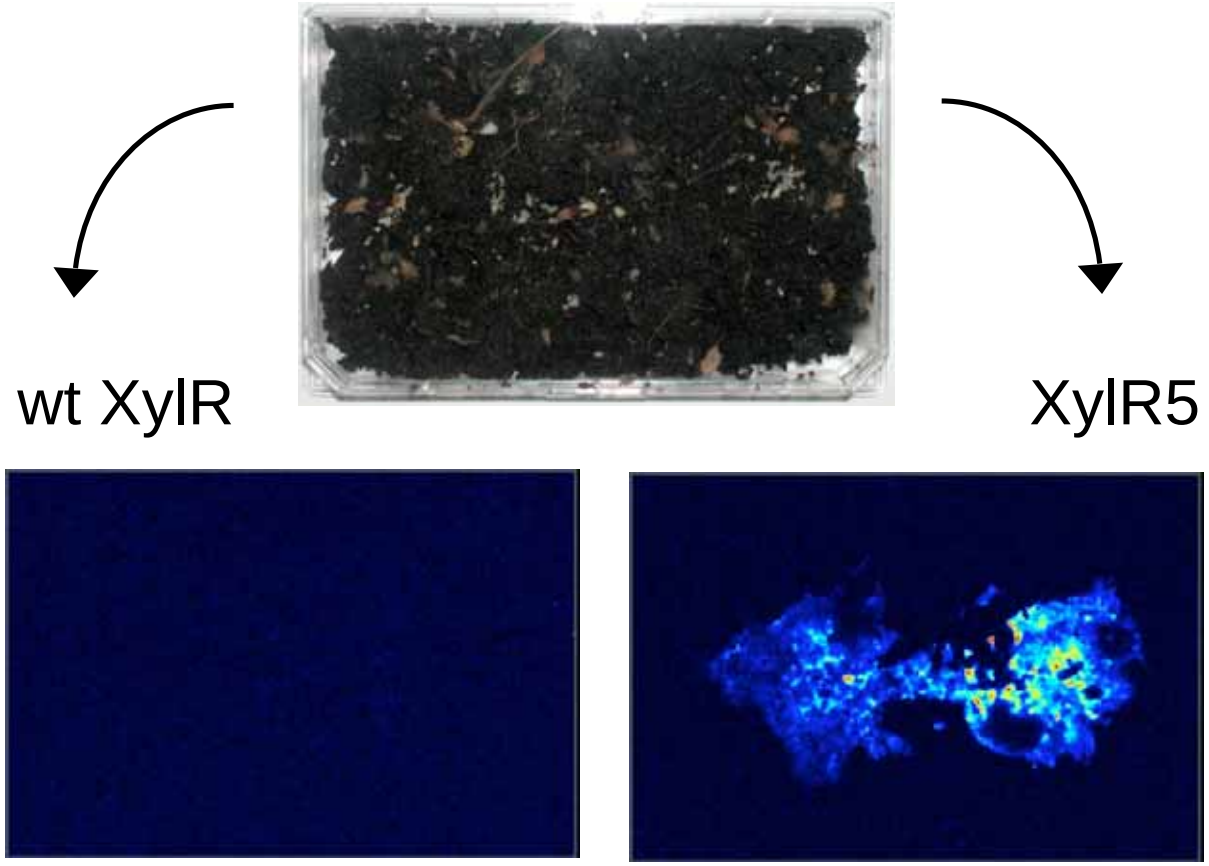
The MMDS provides a distributed & specialized sensor that detects explosive traces and reacts providing an enhanced EM evidence.

A second sensor (a mobile one) easily detects the new EM evidence, which is the translation and amplification of the original explosive one.


CH3
O2N
NO2
CH3
CH3
luxCDABE
XylR5


No DNT
2,4-DNT
Salicylate
XylR5
DntR

Spreading sensor bacteria on a soil microcosm



Revealing underground 2,4 DNT in a soil microcosm