Molecular genetics of the *E. coli gus* operon:

Medical and evolutionary implications for glucuronide and xenobiotic metabolism

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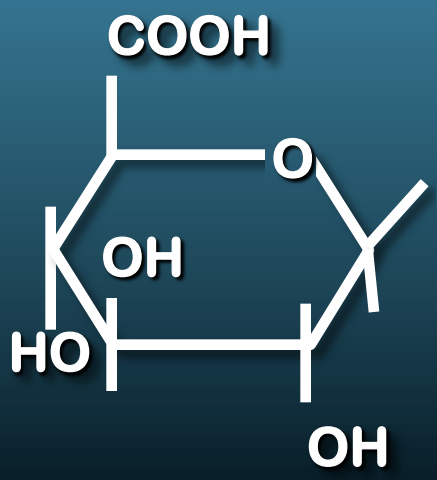
ß-Glucuronides are very diverse



ROH - aliphatic alcohols, amines, thiols

aromatic alcohols (phenols), amines, thiols

steroids

carbohydrates

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ß-glucuronides are a major conjugated form of most steroids, and are excreted into urine and bile

testosterone

pregnanediol

tetrahydrocortisone

androsterone

etiocholanolone

estriol

etc.

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ß-glucuronides are a major conjugated form of many important pharmaceuticals.

Glucuronidation mediated by UDP-glucuronyl transferases is often associated with the ‘detoxification’ and excretion of these compounds in body fluids.

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Gus R
glucuronide repressor

Encoded by *E. coli gus*R - 588 bp

196 aa

Two functional domains

Amino-terminal DNA binding domain

Carboxy-terminal glucuronide binding domain

Strongly binds many diverse glucuronides

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Gus A
ß-glucuronidase (E.C. 3.2.1.31)

- Encoded by *E. coli gus*A gene - 1809 bp
- 603 aa
- Very stable
- Efficiently hydrolyzes very diverse glucuronides
- Has been purified to homogeneity
- Broad pH optimum centered around neutrality
- Tolerates almost any amino- or carboxy-terminal fusion

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Gus B

ß-glucuronide permease (glucuronide transporter)

Encoded by *E. coli gus*B - 1371 bp

457 aa monomer

Proton symporter

Integral membrane protein

Purified to homogeneity

Transports very large and complex glucuronides, including steroid conjugates

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Gus C

ß-glucuronide outer-membrane transport facilitator

- Encoded by E. coli gusC - 1260 bp
- 420 aa precursor
- Outer membrane localized
- Conventional signal peptide-mediated localization
- Assists in substrate transport across outer membrane
- Not required for glucuronide permease activity in synthetic vesicles

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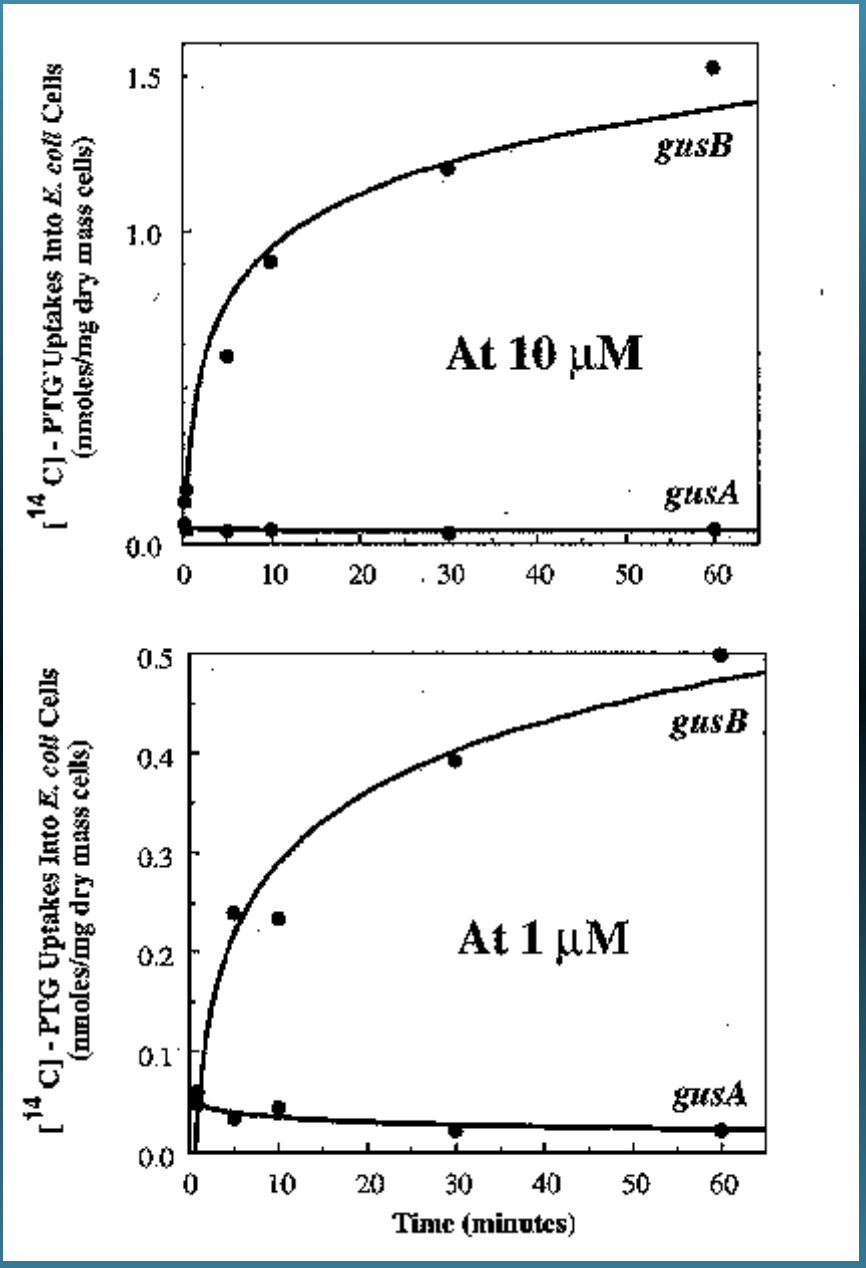

At very low concentrations of substrate, the GusB still actively accumulates glucuronides

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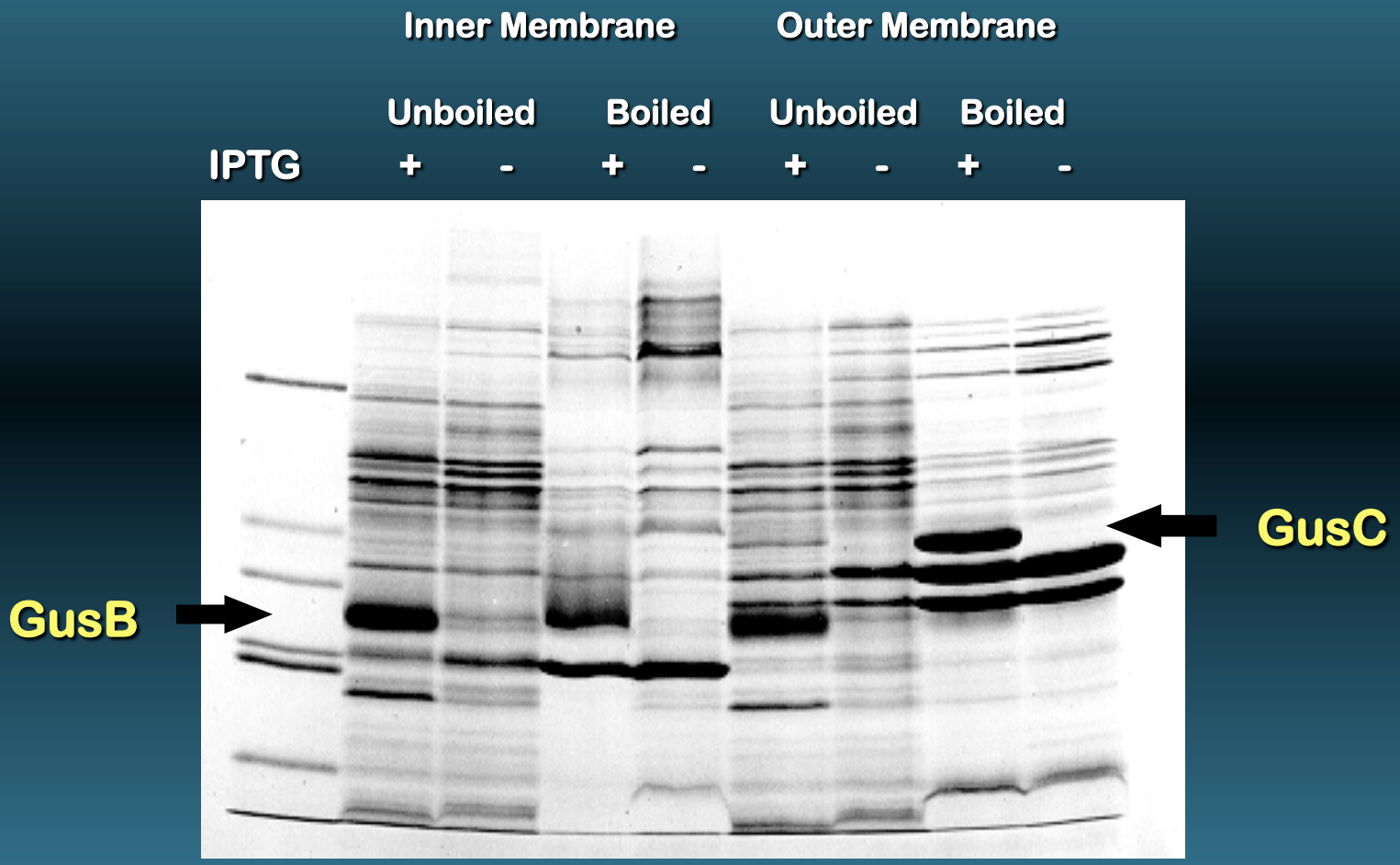
GusB and GusC are localized in the inner and outer membranes of *E. coli*



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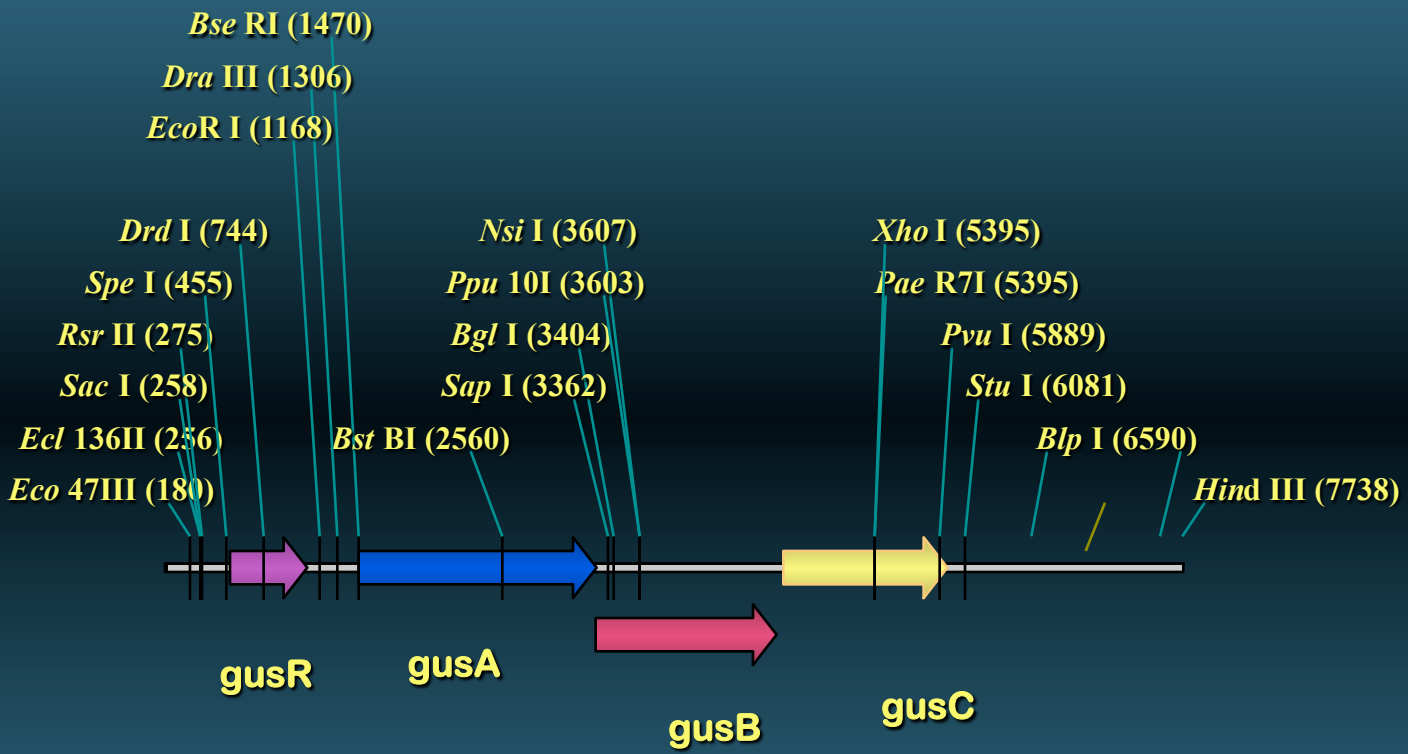
gusRABC operon of E. coli



ECOGUSRABC

7742 bp

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Gus Operon of *E. coli*

gusR	gusA	gusB	gusC
587 bp	1809 bp	1371 bp	1260 bp
repressor	ß-glucuronidase	permease	outer-membrane facilitator

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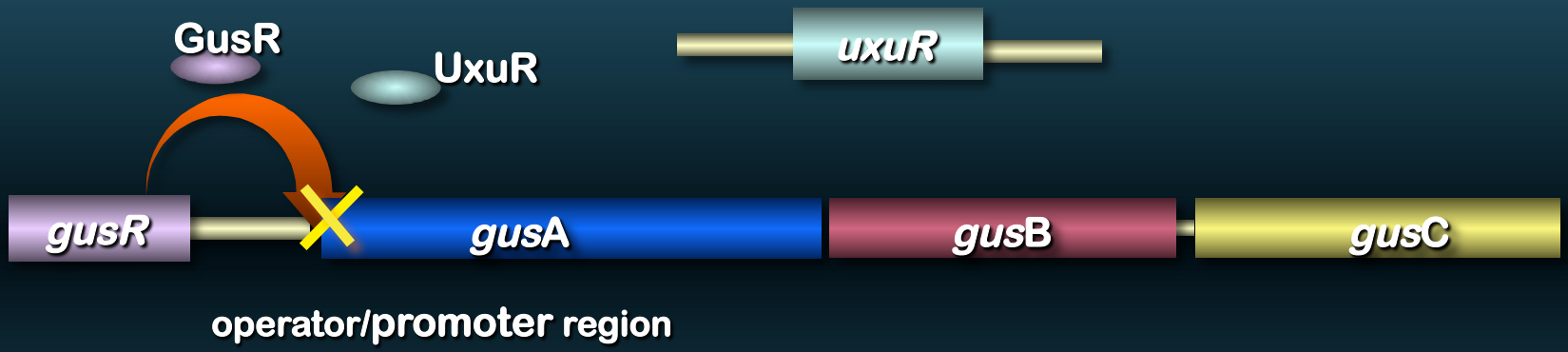
Gus Operon regulation

gus operon transcription known to be negatively regulated by 2 repressor proteins: GusR and UxuR



Operon is induced in the presence of glucuronides, found in high abundance in vertebrate urine, bile and sweat.

GusR binds glucuronides specifically,UxuR binds metabolic end products.

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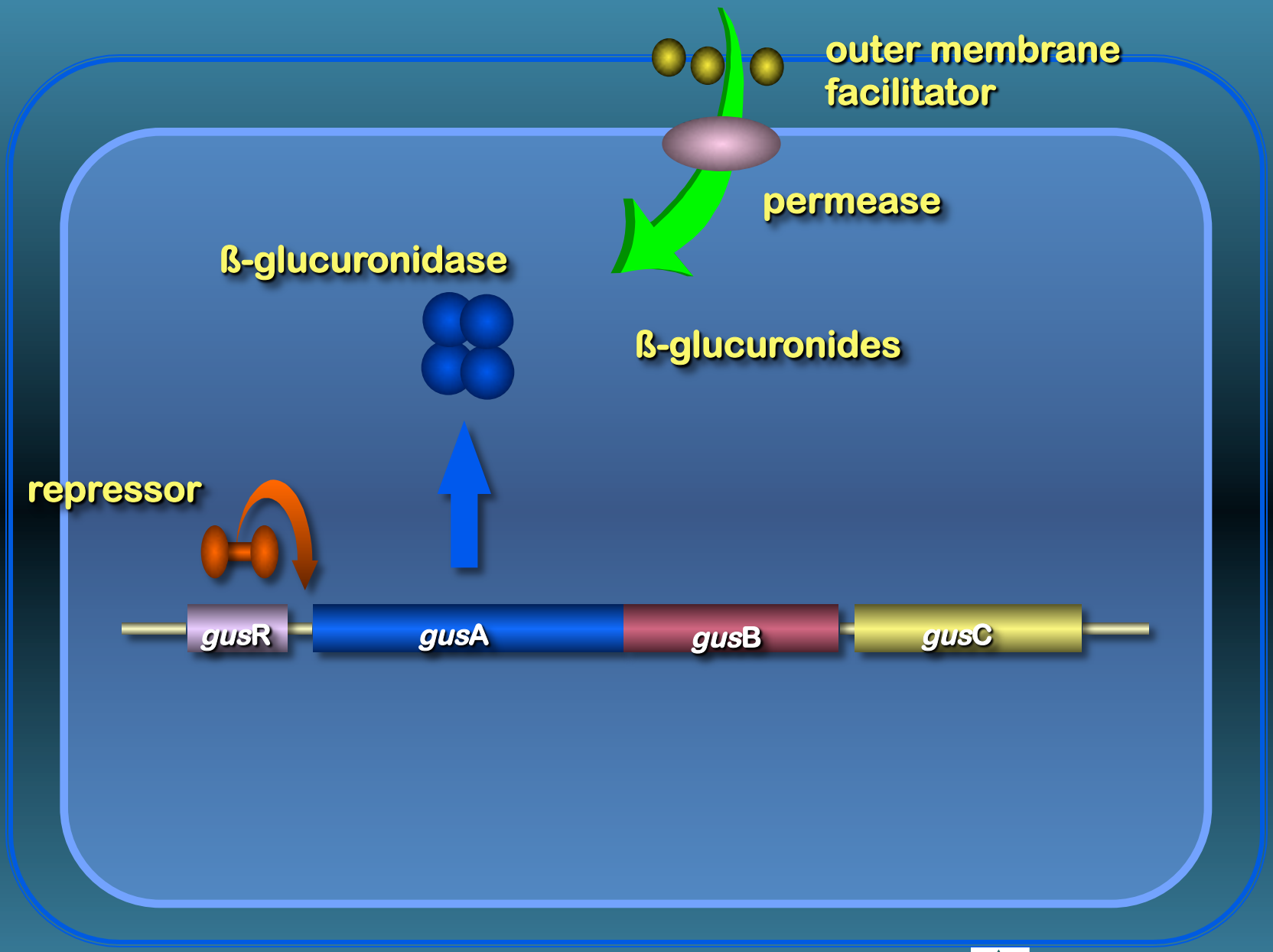
outer membrane facilitator
permease
ß-glucuronidase
ß-glucuronides
repressor
gusR
gusA
gusB
gusC

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The Hologenome

- The evolutionarily selected unit is not a ‘single’ organism, but the suite of organisms that comprise a ‘performance unit’.

- This unit comprises the contribution of many, sometimes thousands, of individual genomes, in varying combinations and numbers.

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The Hologenome

DNA preparations of virtually all multicellular eukaryotic samples will inevitably comprise a hologenetic sample of the 'scaffold' genome (e.g. a plant or animal) as well as the numerous commensal or symbiotic organisms that contribute to the fitness of the complex.

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Enterohepatic circulation of glucuronides

- *E. coli gus* operon is responsible for the uptake and cleavage of numerous glucuronides in the intestinal tract.
- Reabsorption of the released aglycone can then occur in the intestine.
- Lifetimes and efficacies of endogenous steroids or exogenous pharmaceuticals is thus dramatically affected by their metabolism by the gus operon.

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The Hologenome

- **The extracted DNA of a plant or metazoan will actually represent the suite of genomes that, together, contribute to the selected unit.**

- **Thus, DNA analysis of plants or metazoa is not confounded by epi - or endophytic organisms, rather enriched.**

- **However, our methodology for analysis and understanding of these combinations needs to be seriously re-evaluated and developed.**

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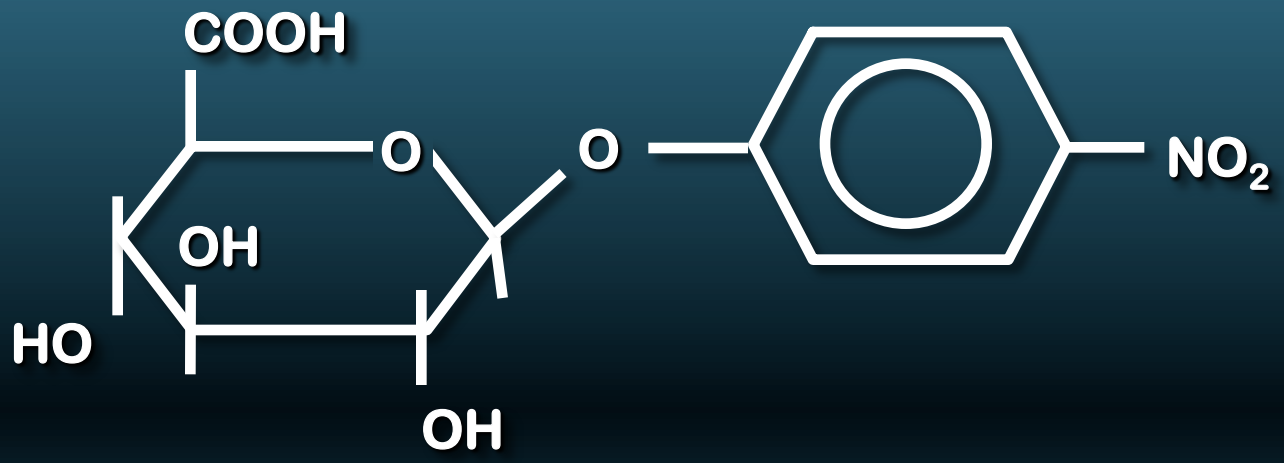
p-nitrophenyl-ß-D-glucuronide



+ GUS

Glucuronic acid

p-Nitrophenol

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The Hologenome

- 'Hologenetics' is the science of genetics addressing itself to the combinatorial possibilities of differing interacting populations.

- Manipulation of the hologenome can occur by 'conventional' genetic means, or by adjusting relative population structures.

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Ecotherapeutics: the implications of hologenomics

- This approach lends itself to ecological therapeutics, or Ecotherapeutics in which microbial endo- or exo-symbiont populations are adjusted to maximize performance of the whole system.

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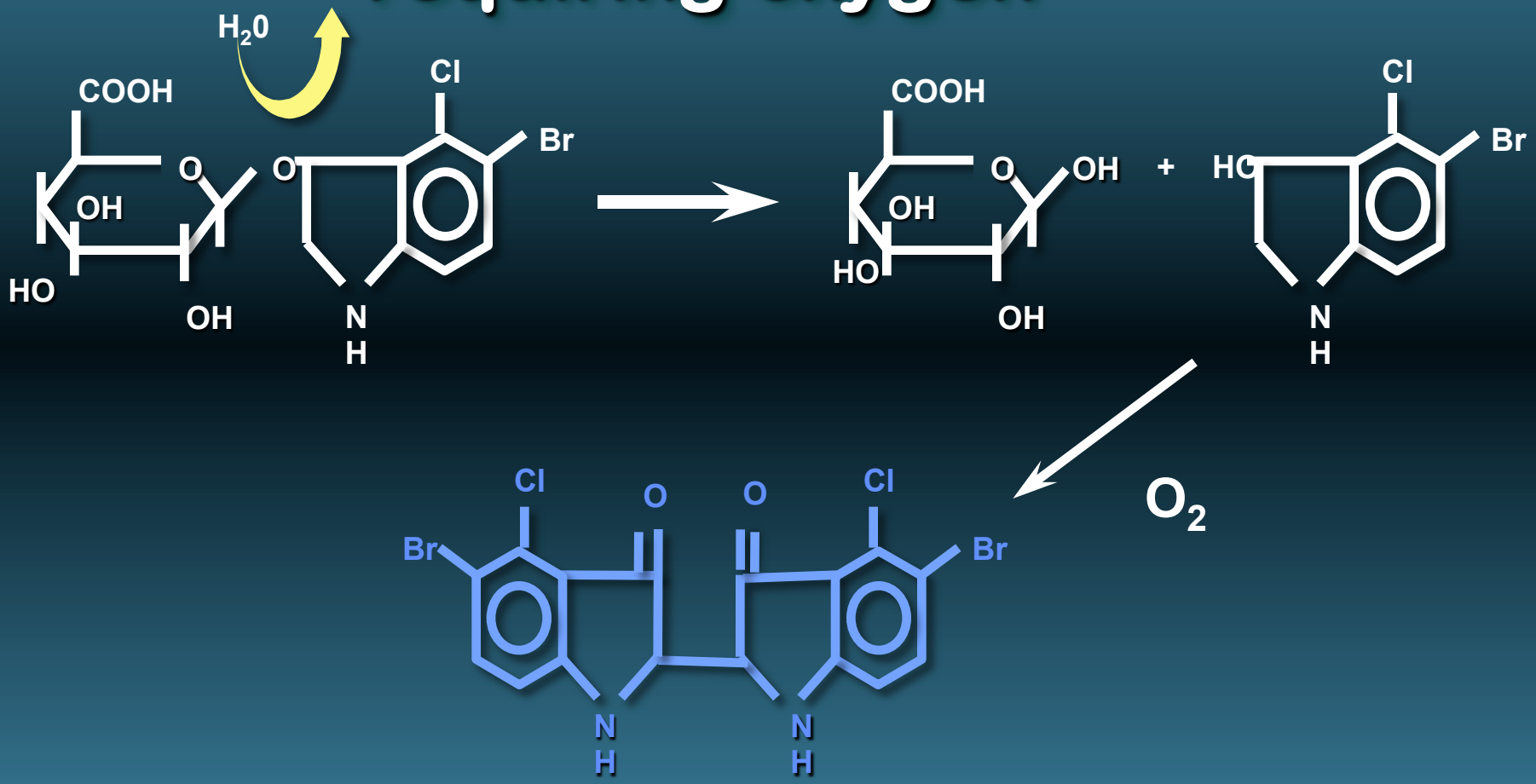
Production of the blue precipitate from X-glcA is a two-step reaction requiring oxygen



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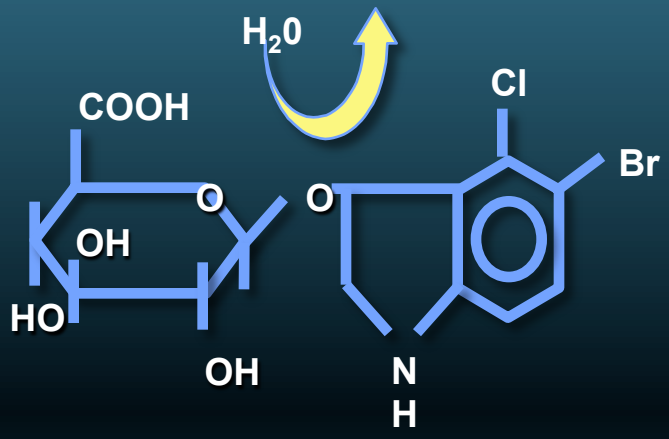
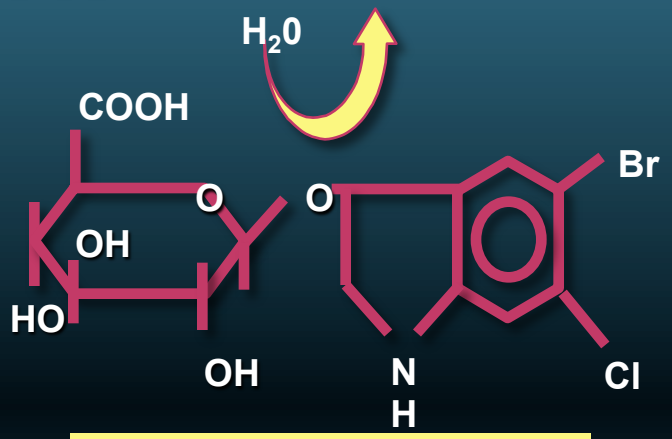
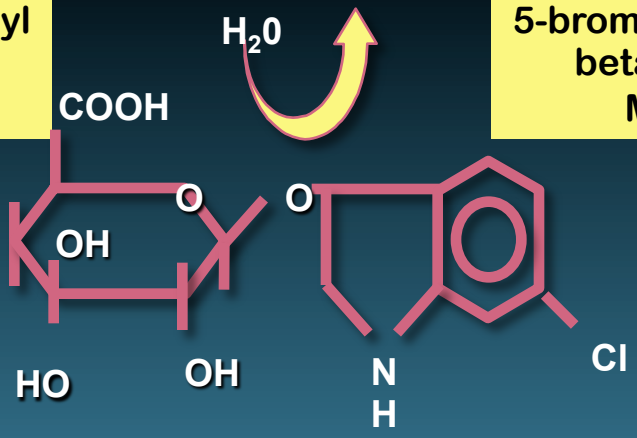
Differently- halogenated substrates give rise to differently-coloured reaction products



5-bromo-4-chloro-3-indolyl beta-D-glucuronide
X-glcA



5-bromo-6-chloro-3-indolyl beta-D-glucuronide
Magenta-glcA



6-chloro-3-indolyl beta-D-glucuronide
Salmon-glcA

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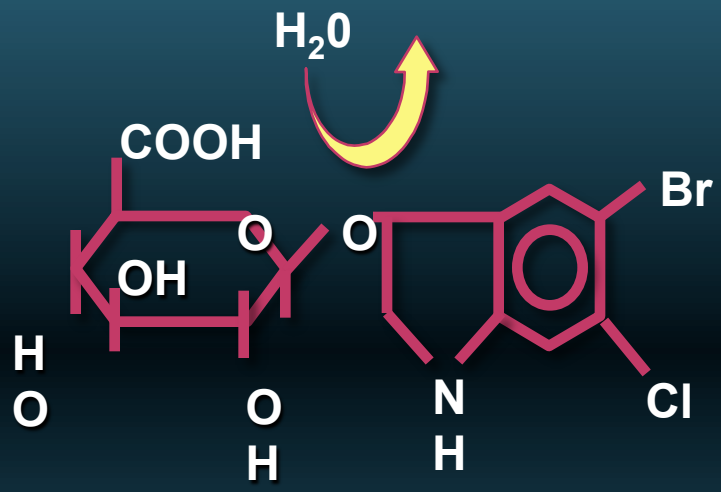
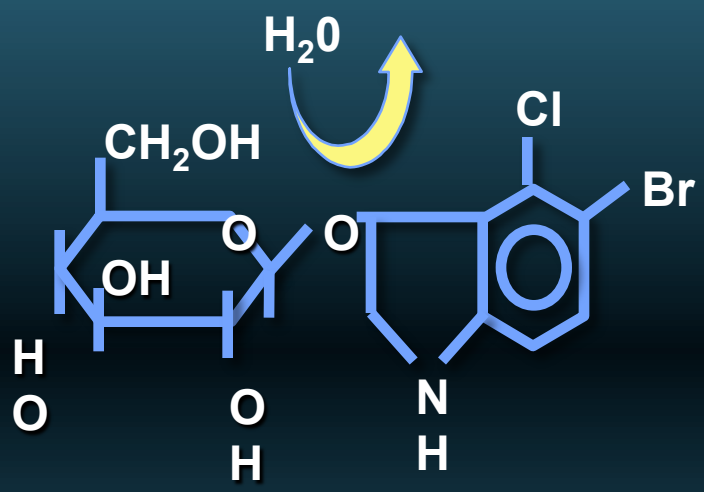
celB and *gusA* can be assayed together



5-bromo-6-chloro-3-indolyl beta-D-glucuronide
Magenta-glcA

GUS



5-bromo-4-chloro-3-indolyl beta-D-galactoside
X-gal

CELB

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Adriana Parra - CIAT

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Antoon Akkermans - Wageningen, The Netherlands
Richard Jefferson - CAMBIA
Mark Peoples - CSIRO, Canberra, Australia
Doug Beck - CIAT

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The *celB* marker gene

- ***celB*** **comes from the thermophilic bacterium *Pyrococcus furiosus which can grow at 100°C***
- **it encodes a beta-glucosidae with beta-galactosidase activity**
- **the enzyme is stable at high temperature, enabling inactivation of background activity by heat treatment**

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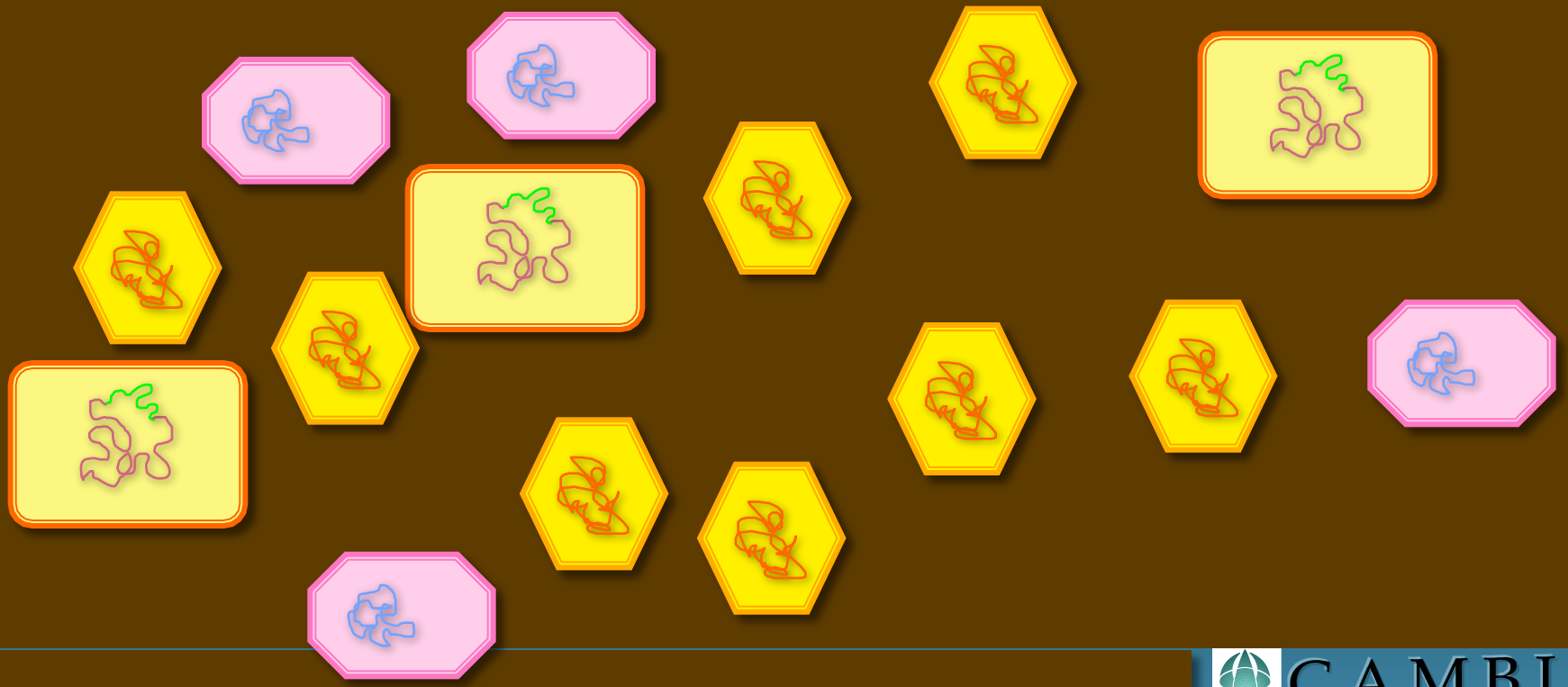
Nucleic Acid Techniques

- bacteria are identified by specific DNA or RNA sequences



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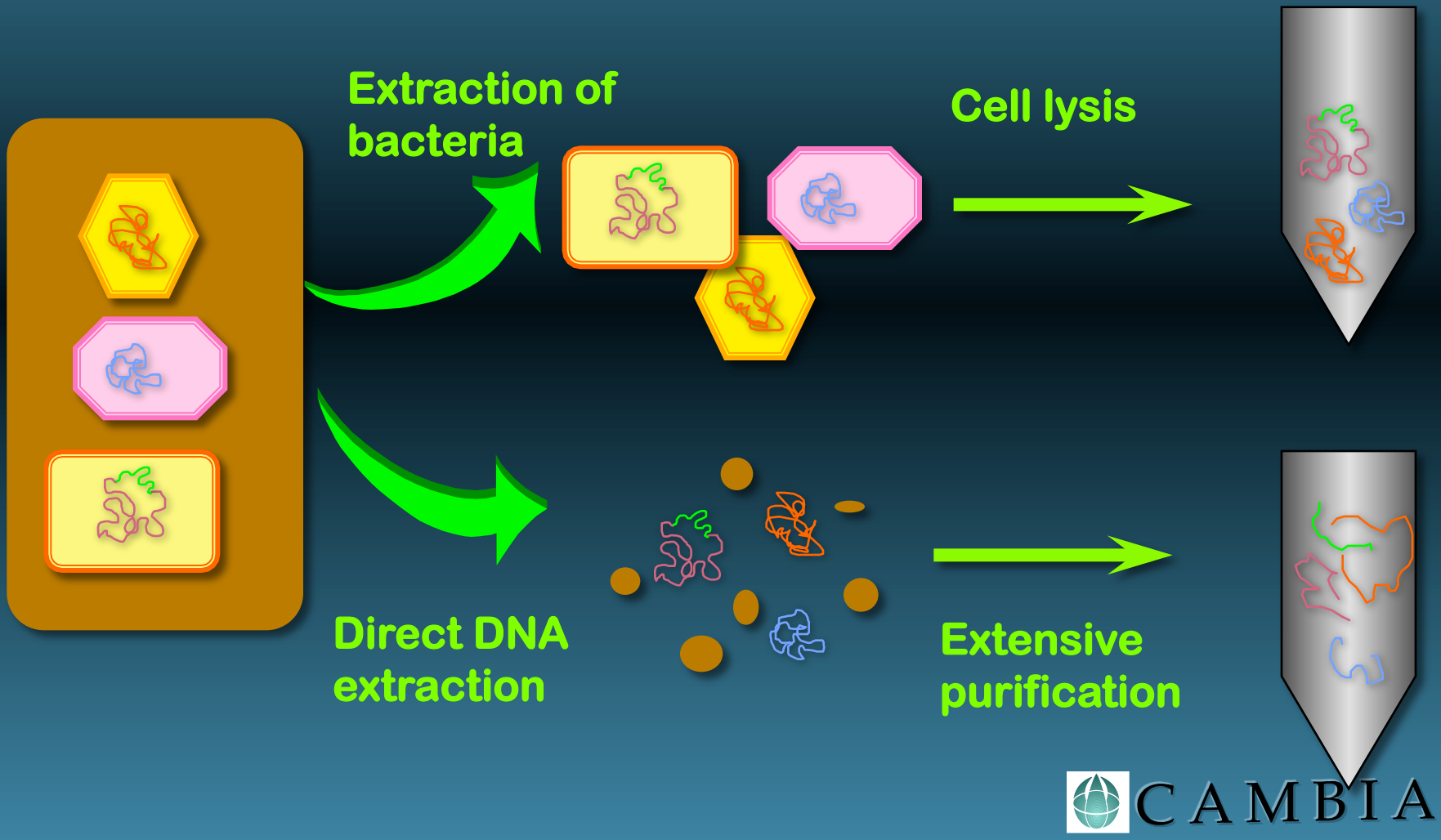
Nucleic Acid Techniques

- DNA isolation



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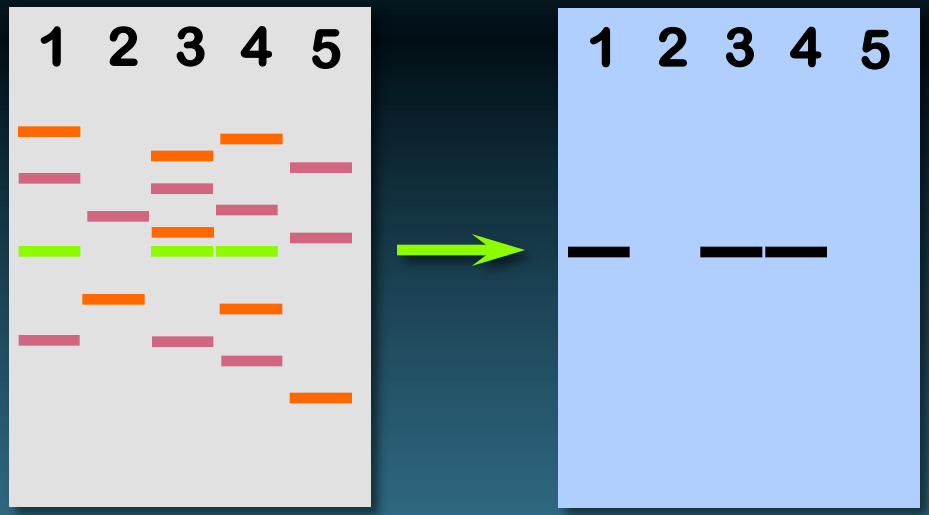
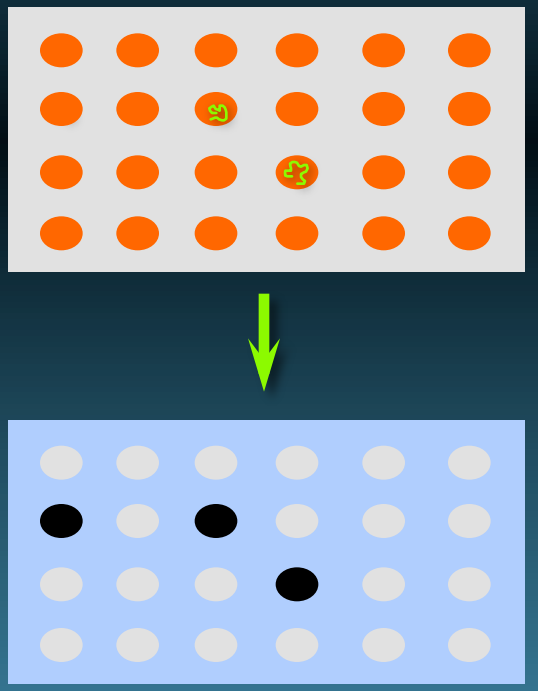
Nucleic Acid Techniques

- DNA detection

Southern hybridization



Dot blot hybridization



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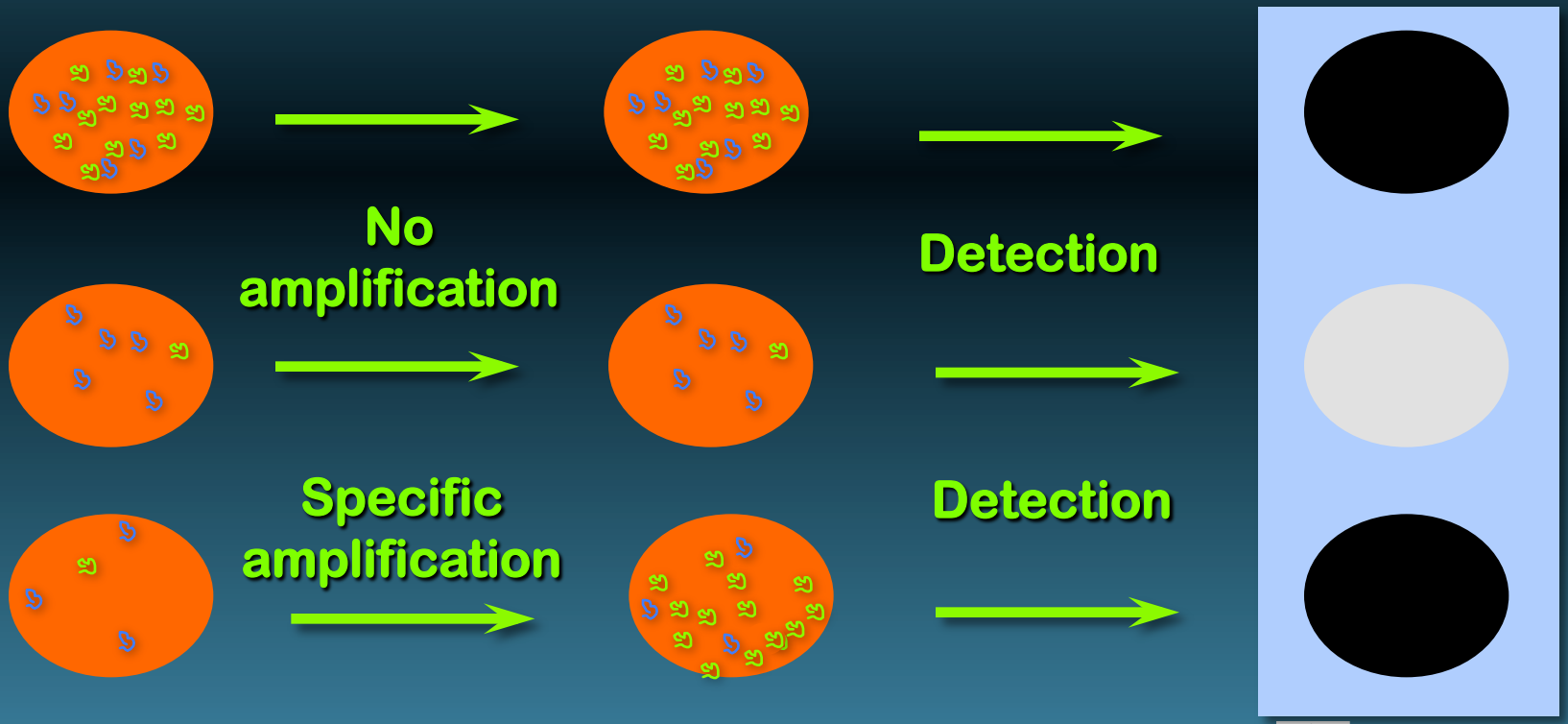
Nucleic Acid Techniques

- Enhancement of sensitivity through DNA amplification using PCR (Polymerase Chain Reaction)



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Nucleic Acid Techniques

Potential sensitivity of methods:

- **Without amplification:**

  10^3-10^4 cells per gram soil

- **With PCR amplification:**

  1 cell per gram soil

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Nucleic Acid Techniques

Types of probes:

- species specific sequences
- ribosomal RNA genes
- traits e.g. *nod* genes

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Nucleic acid probes

- no need for culture
- specific probes needed
- technically difficult
- expensive

Marker genes

- culture required
- general methods
- technically simple
- inexpensive

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Nucleic acid probes

- organisms die
- no effect on ecology
- difficult to quantify
- good for population surveys

Marker genes

- used on living organisms
- possible effect on ecology
- quantitative
- good for tracking specific strains

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Nucleic Acid Techniques

The future:

- improved DNA extraction and purification procedures
- development of new probes
- automation of technology
- development of quantitative methods

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Marker Genes

The future:

- development of new marker genes
- construction of diverse gene cassettes
- extension to non-culturable organisms
- quantitation without plating
- extensive testing of effects on ecology

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