Designer microbes via synthetic biology

Dr. Nick Coleman

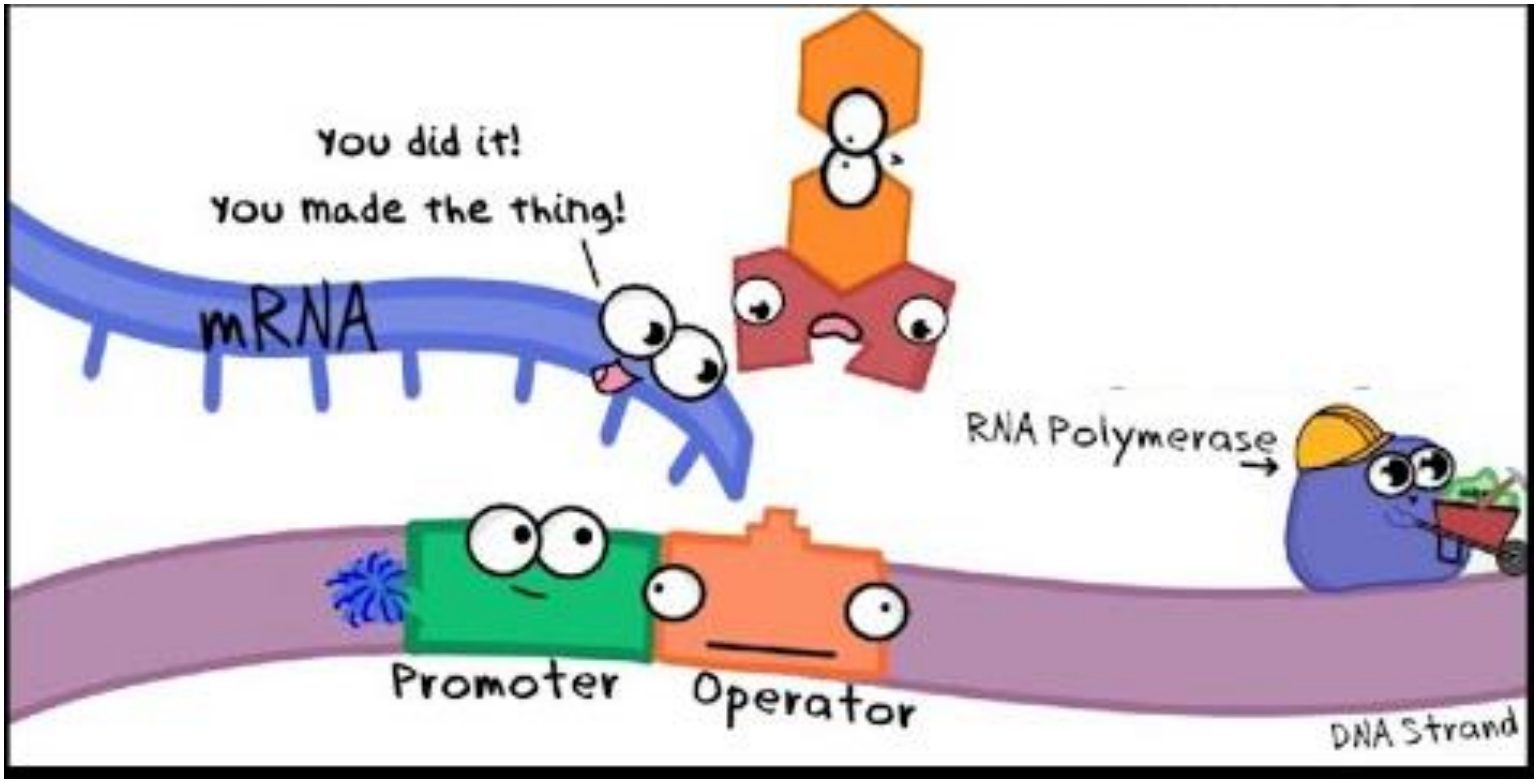
Learning objectives

Students should be able to :

- **Explain the importance of bacteria for biotechnology**
- **Define these terms: operon, regulator, repressor, activator, operator, promoter, terminator, inducible**
- **Describe how gene regulation works in bacteria, using the example of the lac operon**
- **Define and explain what synthetic biology means**
- **Describe how a biosensor can be built from standard biological parts, using a specific example**

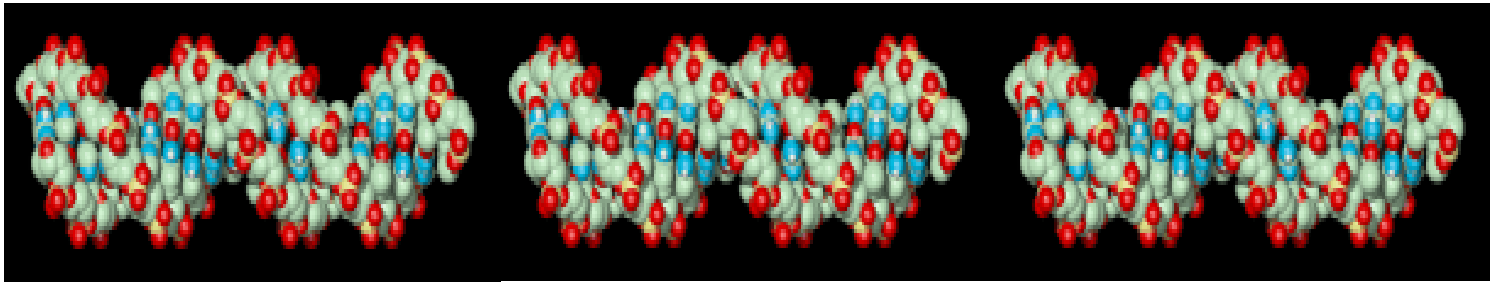
Part 1.

Bacterial genetics and gene regulation



Assumed knowledge

- Basic structure and functions of DNA, RNA, protein
- "Central dogma" of mol. biol: DNA → RNA → protein
- How transcription works – RNA polymerase, mRNA
- How translation works – ribosomes, proteins
- Use of plasmids to clone and express foreign genes



Importance of microbiology to biotechnology

- **Bacteria** are uniquely useful for biotechnology – why?

  –rapid growth on simple substrates

  –large population sizes in small spaces

  –small haploid genomes

  –no introns

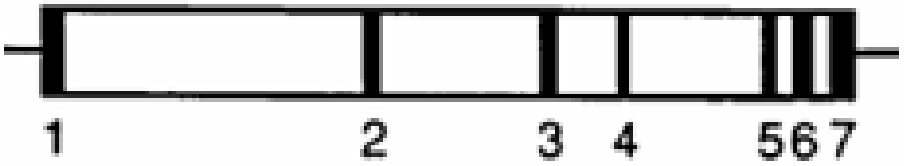
  } Convenient model system

  –source of DNA-modifying enyzmes

  –mobile genetic elements

  } Source of useful tools

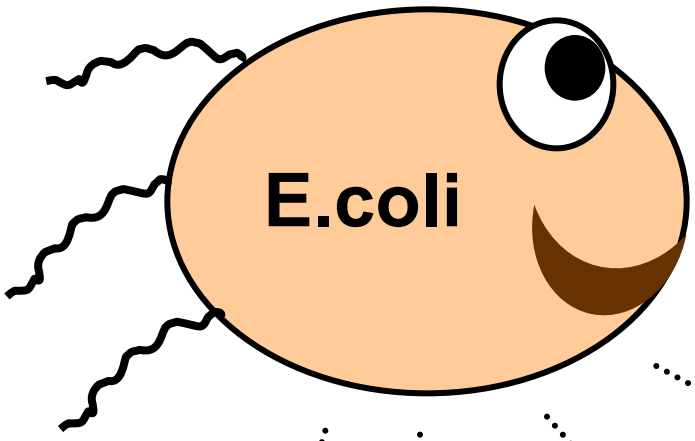
E.coli α-galactosidase gene
1 kb size, no introns



Human α-galactosidase gene
10 kb size, 7 exons, 6 introns

ref 2.

Bacteria and their mobile elements are an excellent source of useful genetic tools



Host for cloning + expressing foreign genes

Restriction enzyme EcoRI

Klenow fragment of DNA pol I

T4 phage DNA ligase

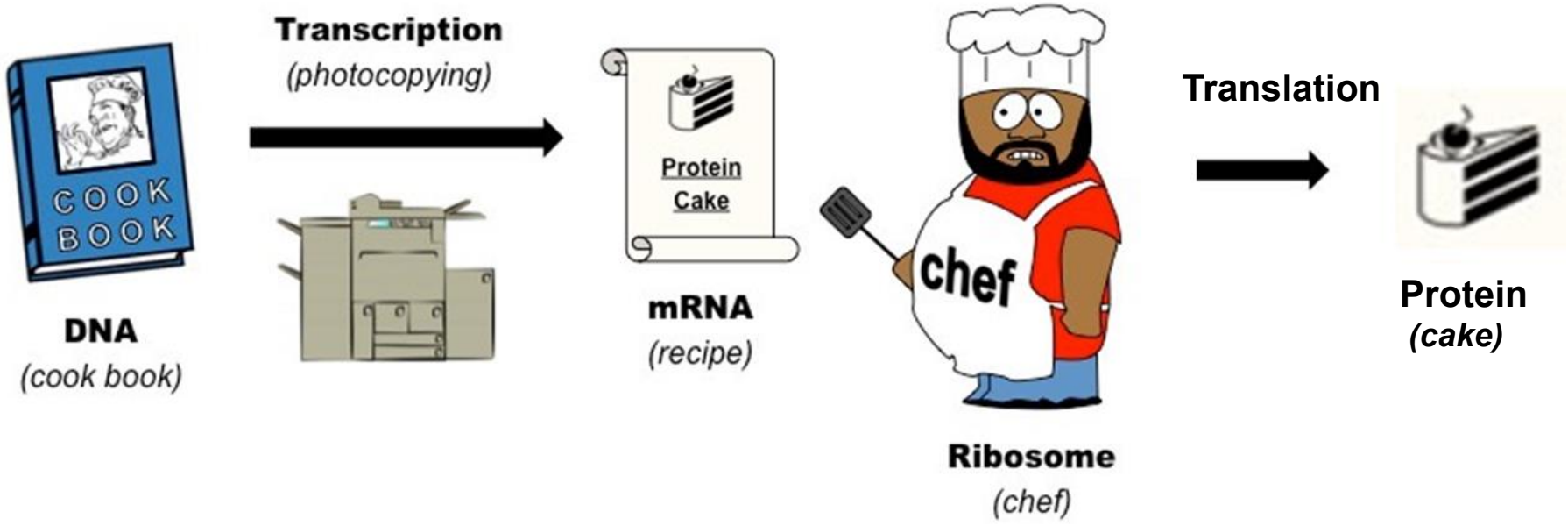
Cloning vectors from lambda phage

Cloning vectors from F plasmid

What is a prokaryote gene? How does it work?

- A gene is a length of DNA that codes for a protein. Typical length approx 500-1000 base pairs (bp)

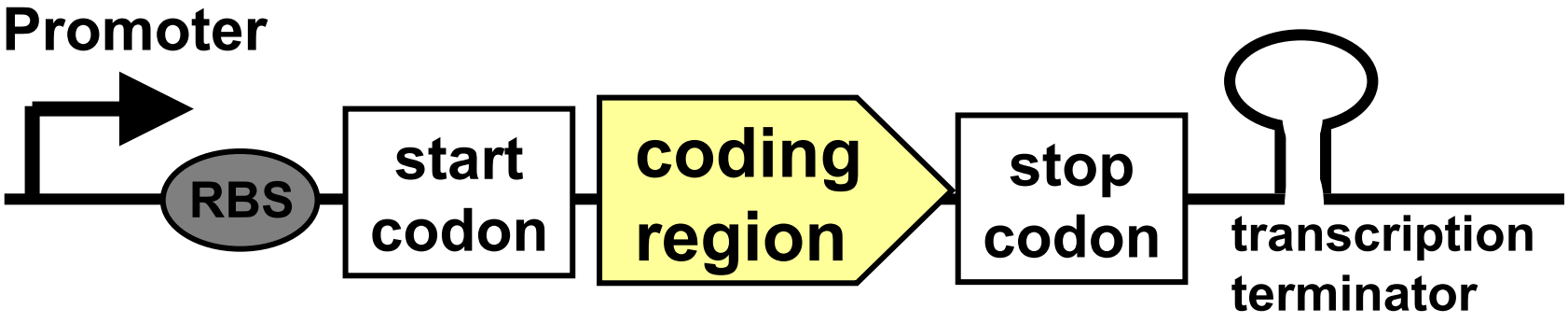
- Prokaryote genes do not usually have introns, but do require non-coding DNA sequences at 5' and 3' ends



What is a prokaryote gene? How does it work?

- Transcription of a gene into mRNA requires a promoter to bind RNA polymerase and a terminator to stop RNA pol.

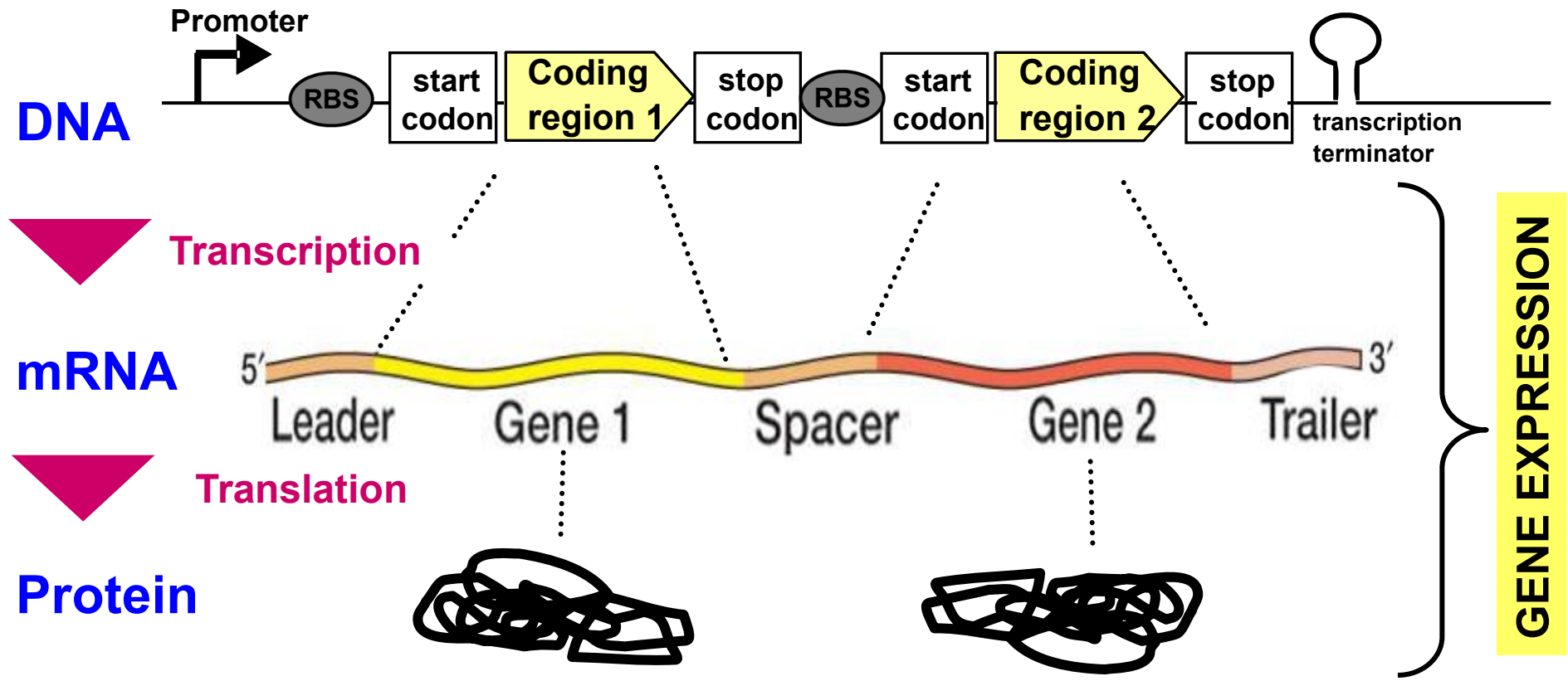
- Translation of mRNA to protein requires a ribosome binding site (RBS), a start codon, and a stop codon



Prokaryotic genes are organised in operons

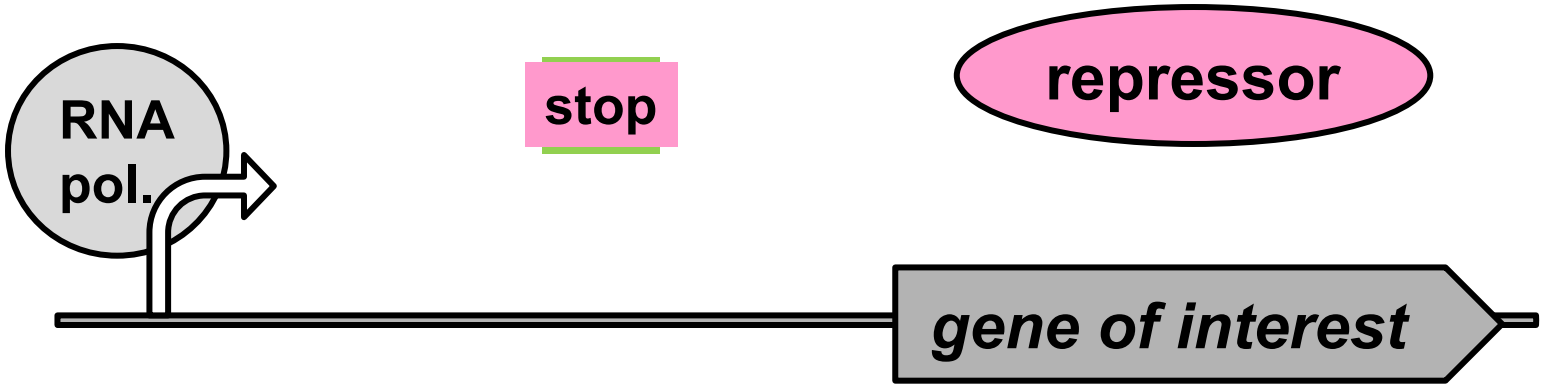
- An operon is a cluster of genes that share a promoter, and are co-transcribed to yield a single strand of mRNA

- Operons usually encode functionally-related proteins eg. sequential enzymes in a single metabolic pathway



Control of gene expression: Regulator proteins

- **Positive regulation: activator protein turns promoter on**
- **Negative regulation: repressor protein turns promoter off**



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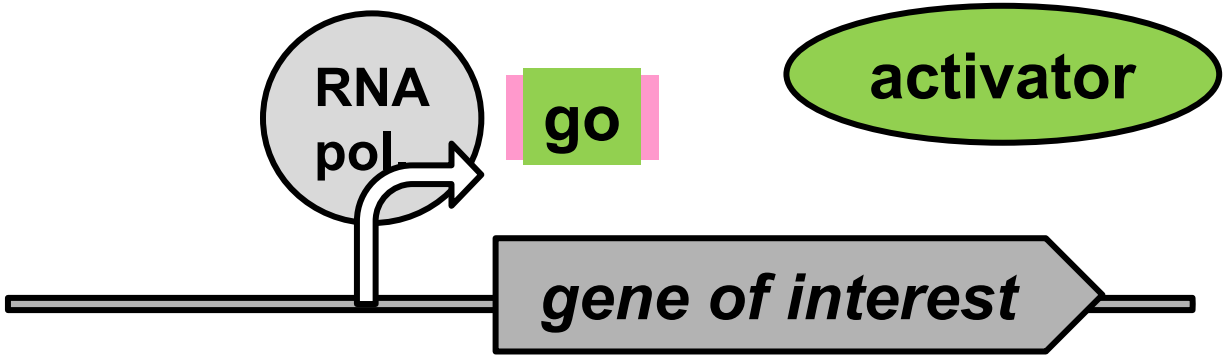
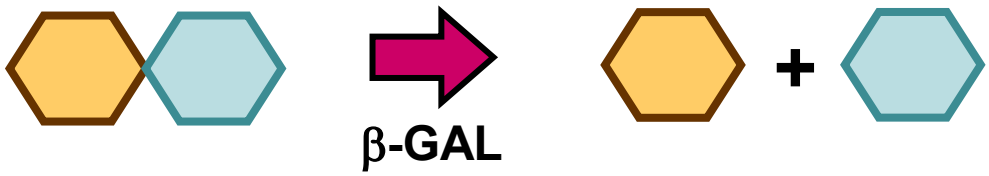
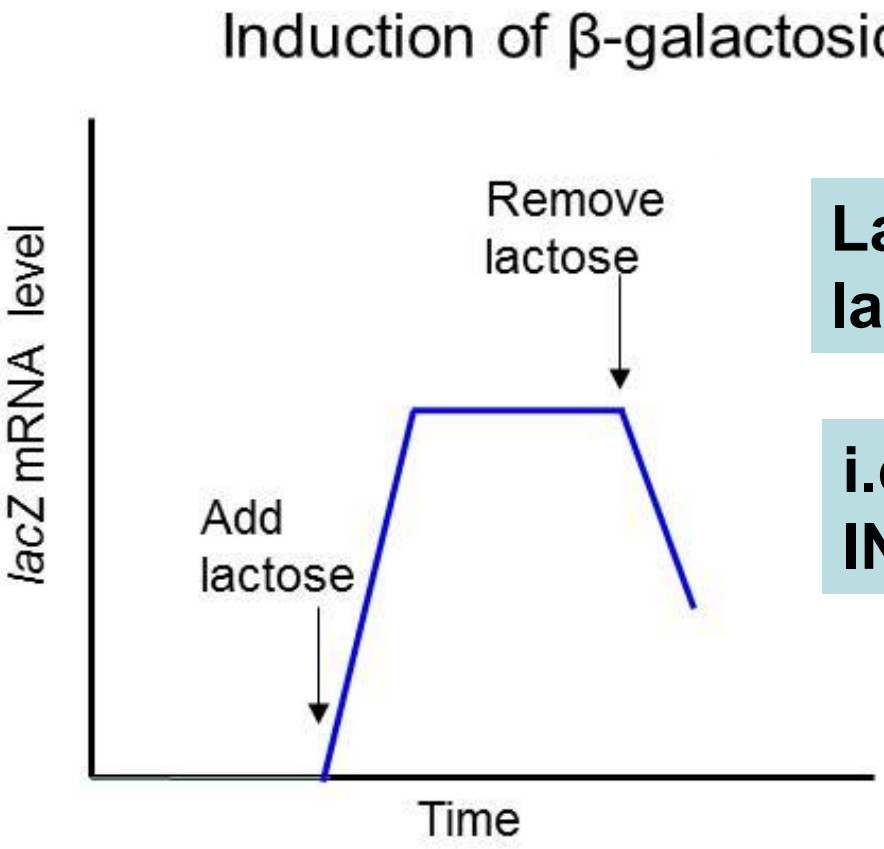


Lac operon: example of negative regulation

- **lacZ gene codes for LacZ protein: beta-galactosidase**



Beta-gal. converts lactose into glucose plus galactose

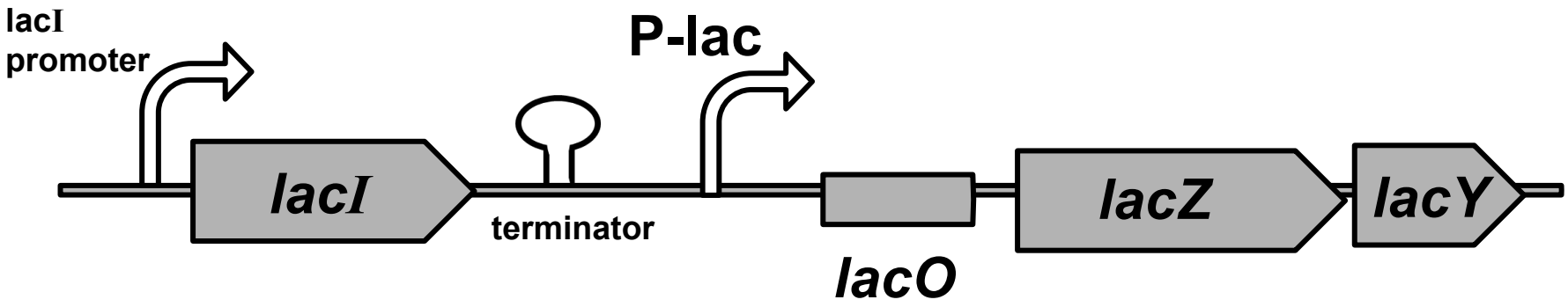


Lactose is an INDUCER of lacZ gene expression in *E.coli*

i.e. the lac operon is INDUCIBLE by lactose

Lac operon: example of negative regulation

- **The LAC OPERON includes structural genes, a regulatory gene & regulatory DNA (promoter, operator)**

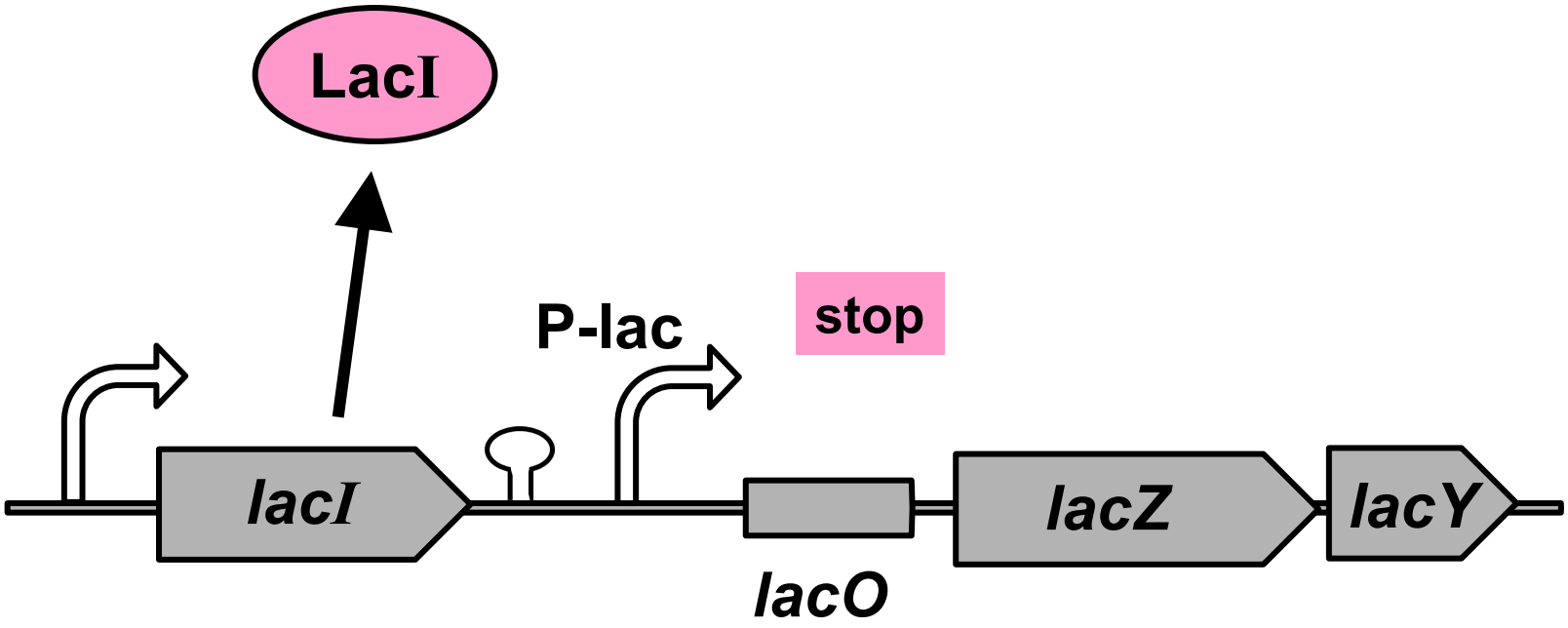


- **Structural genes: *lacZ, lacY* → galactosidase, permease**
- **Regulatory gene: *lacI* → LacI repressor protein**
- **Regulatory DNA: Promoter sequence P-lac (approx 30 bp) and an adjacent operator site lacO (approx 20 bp)**
  - **Operator: where the regulatory protein binds**

The lac operon: example of negative regulation

No lactose present

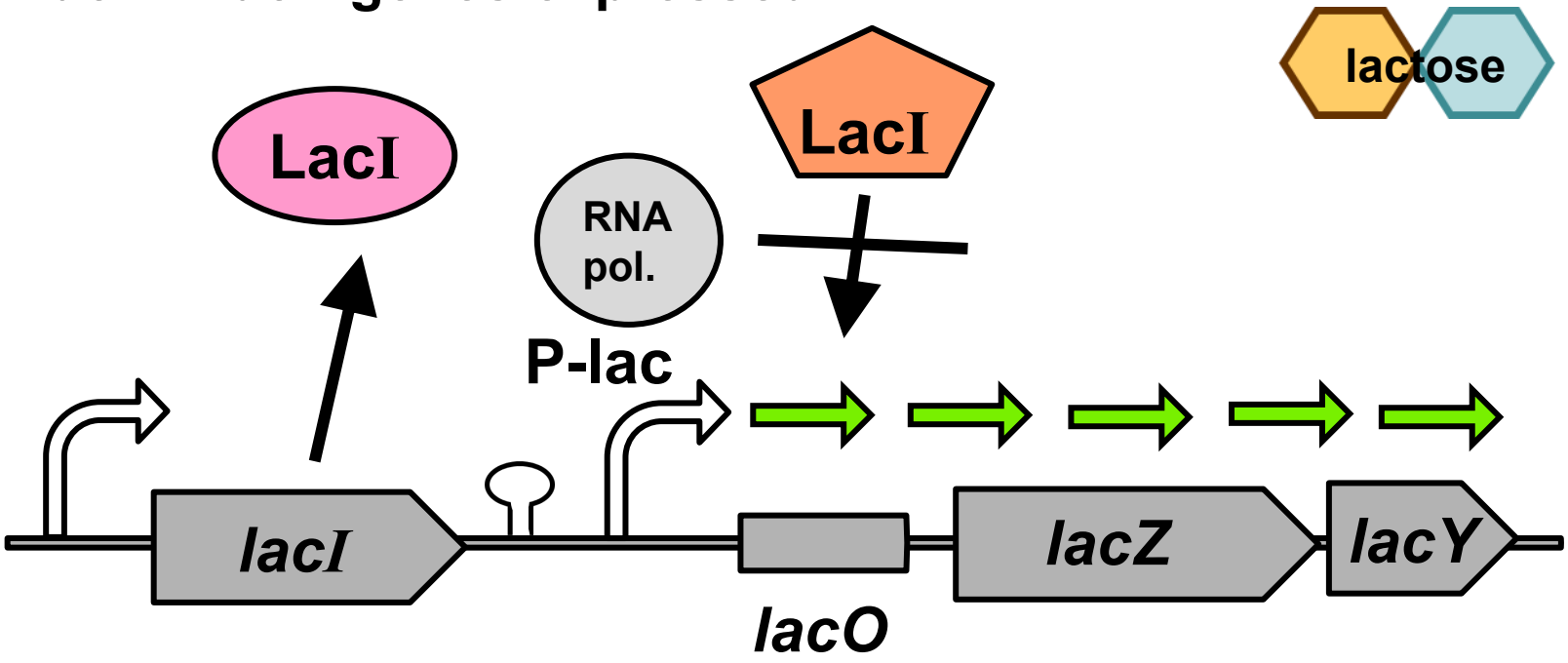
- LacI repressor protein made
- LacI repressor binds to *lacO*
- RNA polymerase cannot transcribe from P-lac
- *lacZ* + *lacY* genes not expressed



The lac operon: example of negative regulation

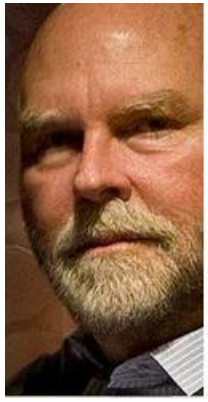
Lactose present

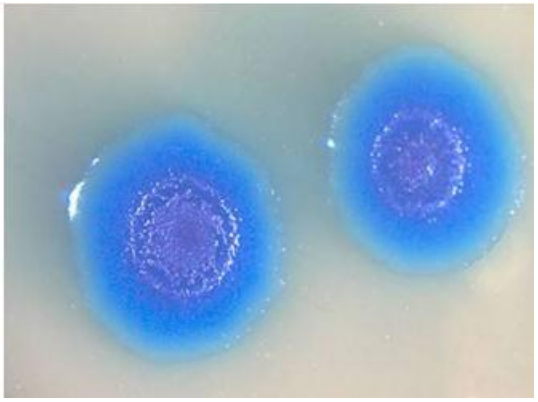
- LacI repressor protein made
- LacI binds to lactose, changes conformation
- LacI cannot bind to *lacO*
- RNA polymerase transcribes from P-lac
- *lacZ* + *lacY* genes expressed



Part 2.

Designing new microbes via synthetic biology





Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

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What is Synthetic Biology? (SynBio)

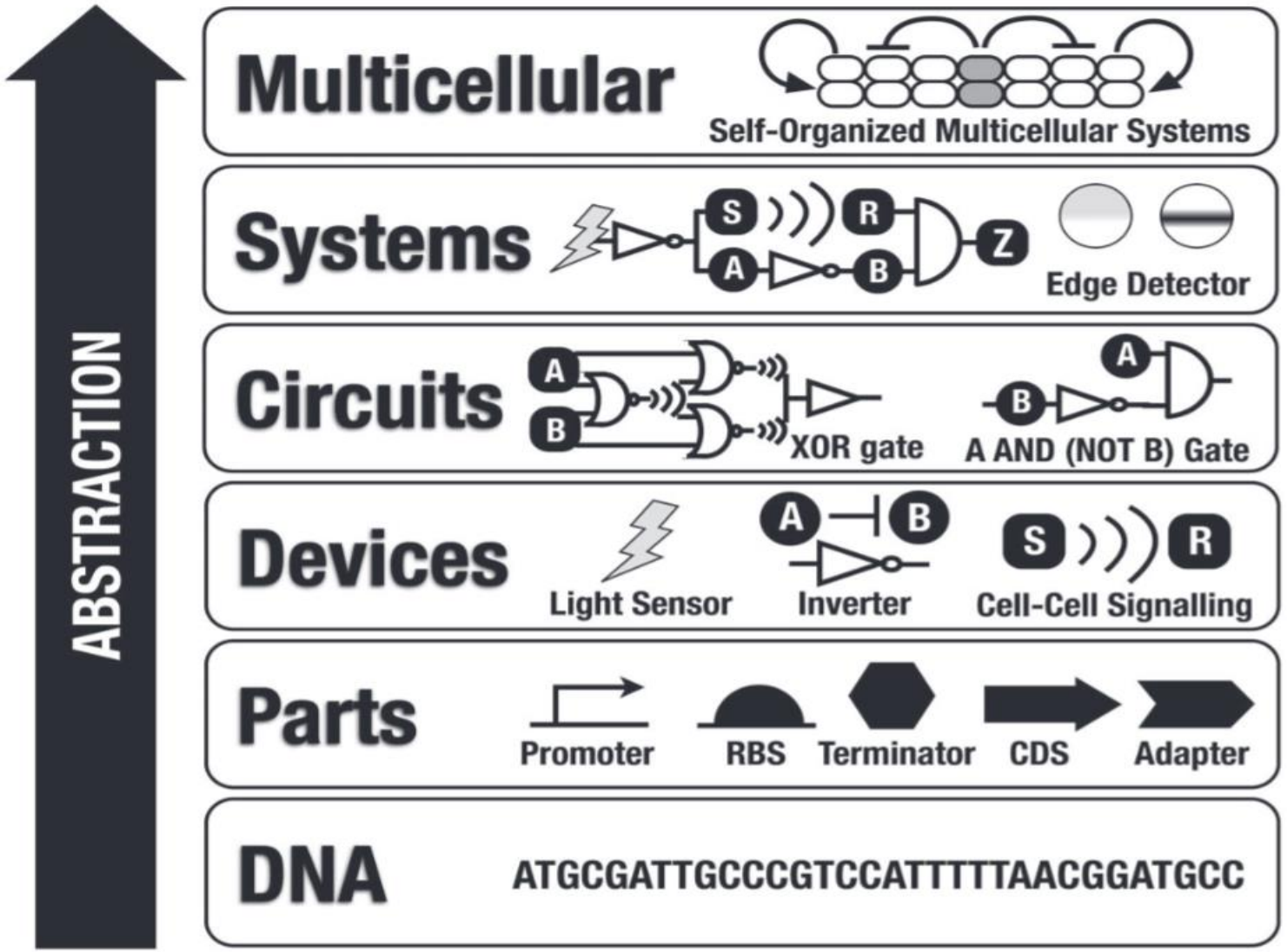
- **SynBio proposes that new biology can be built from the 'bottom up' using standard DNA parts**

- **Hypothesis: biological parts will behave in a predictable way, even when they are part of complex systems**

Genes
= Lego ?

SynBio design concepts

1. Abstraction



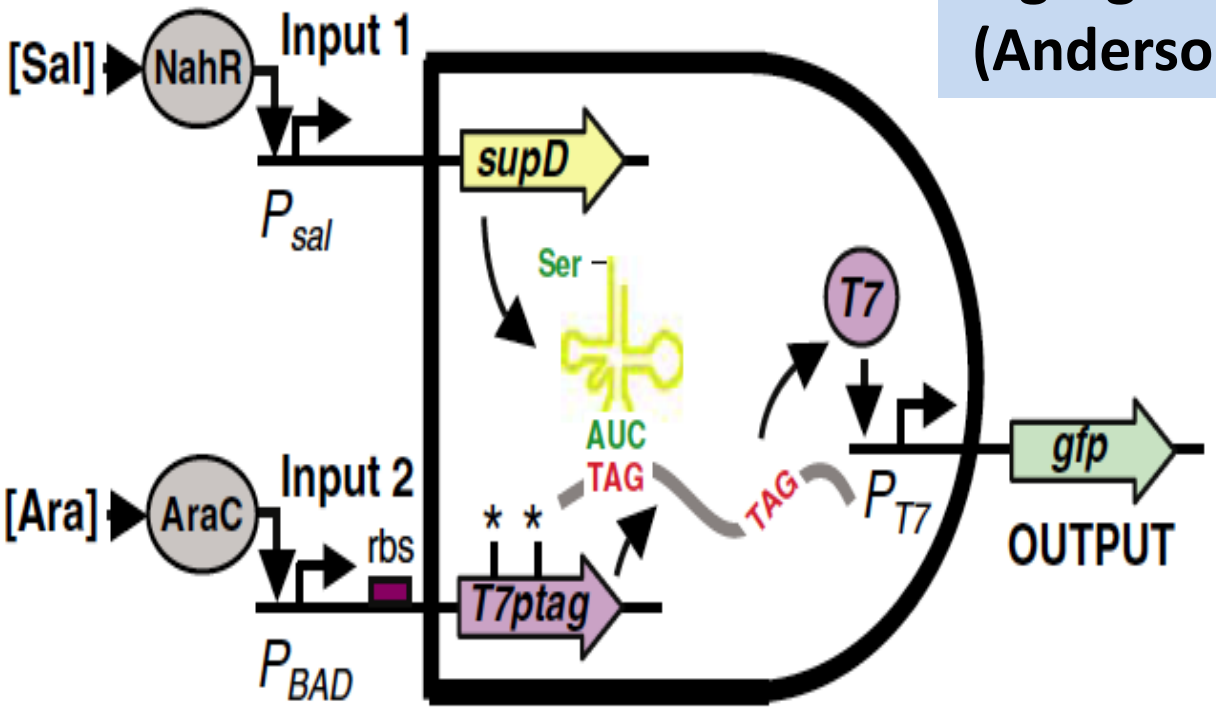
Federici et al (2013) Biol Res 46:383-393

SynBio design concepts

2. Modularity

- **Parts, devices, systems can combine in multiple ways**

Logic gate built from DNA (Anderson lab, UC Berkeley)

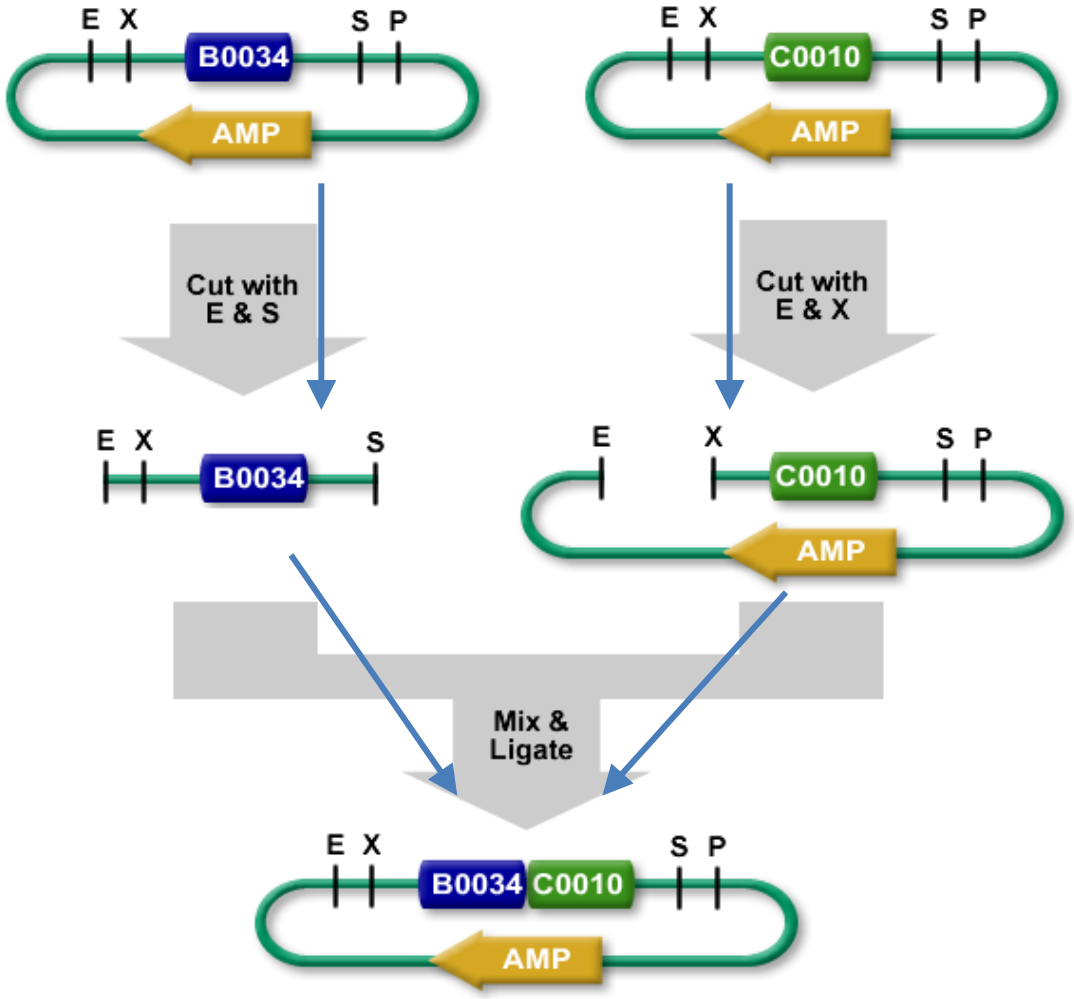


http://www.bio.davidson.edu/projects/gcat/synthetic/what_is_synbio.html

SynBio design concepts

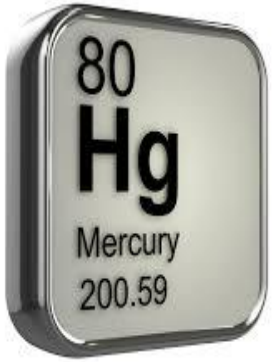
3. Standardisation

- **Standard connections allow new and old parts to join**



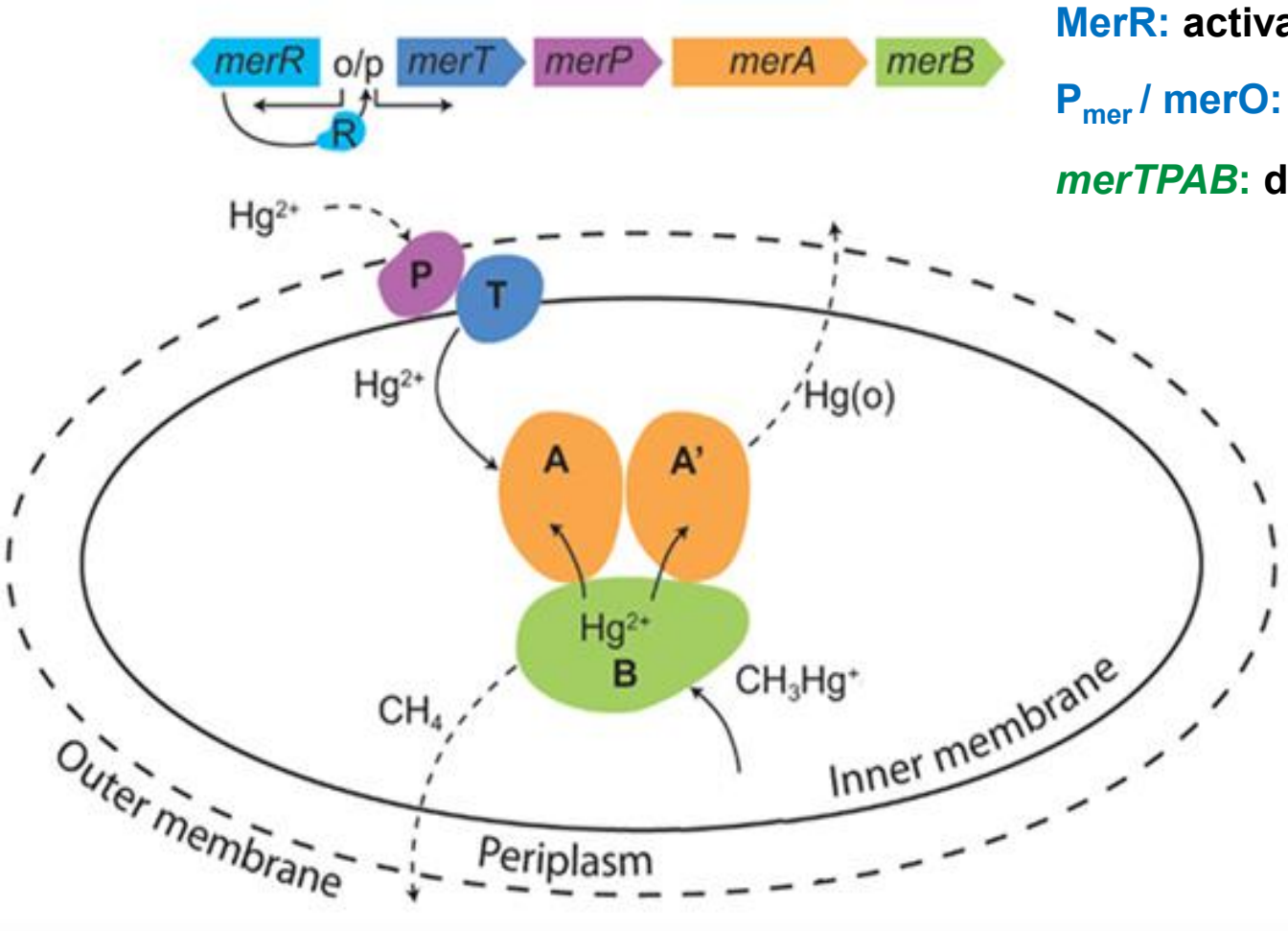
Example: making a mercury biosensor

- **Mercury is a toxic metal, and a common environmental contaminant**
- **A biosensor uses biological molecules as sensors for specific chemicals**
- **A good biosensor will be:**
  - **Sensitive**
  - **Specific**
  - **Rapid**



Sensing module: MerR protein / P_{mer} promoter

- **Many bacteria contain the *mer* operon, which provides mercury resistance in response to mercury**



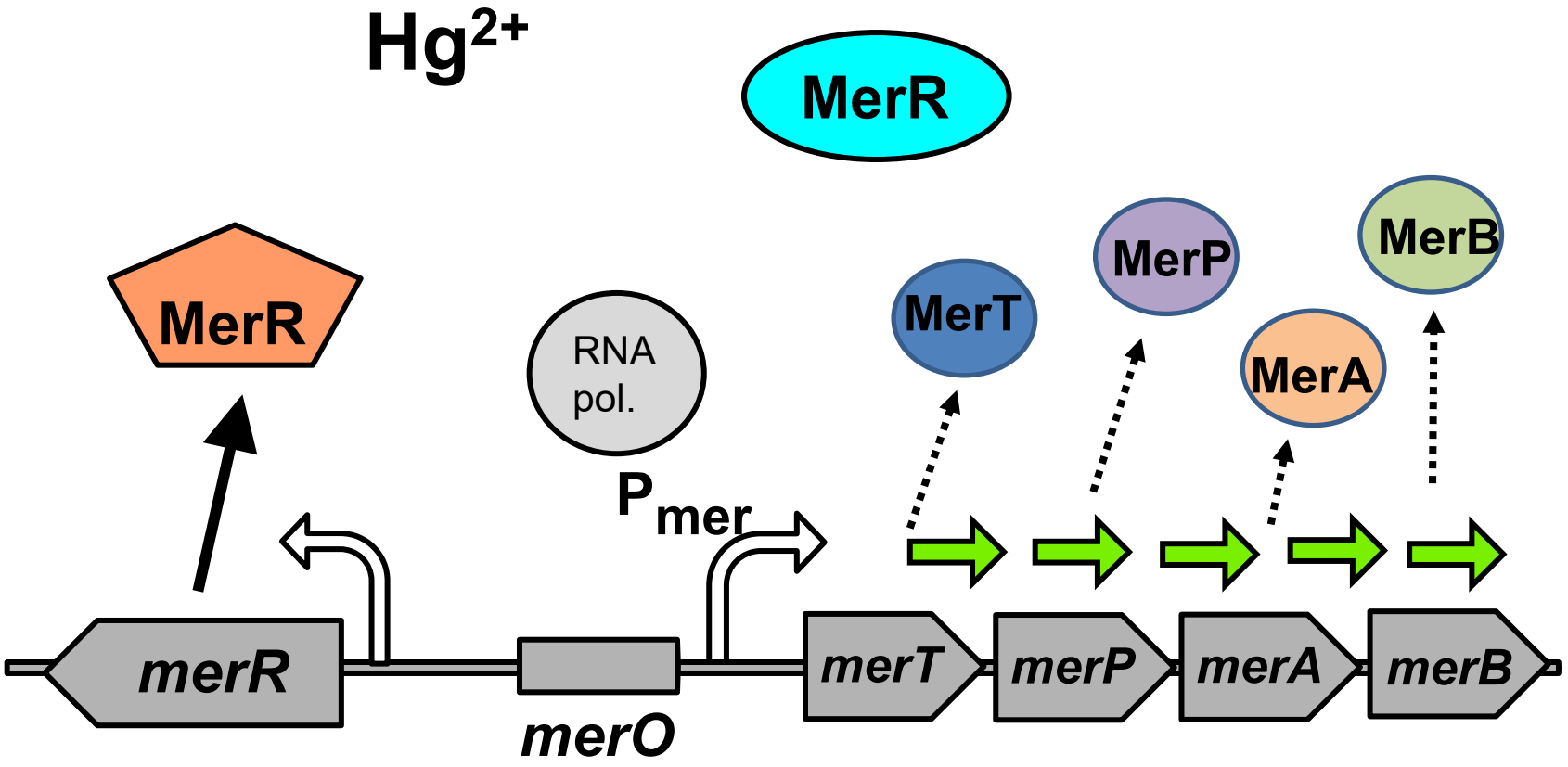
MerR: activator protein

P_{mer} / merO: promoter/operator

***merTPAB*:** detoxify CH_3Hg and Hg^{2+}

Sensing module: MerR protein / *merO* / P_{mer}

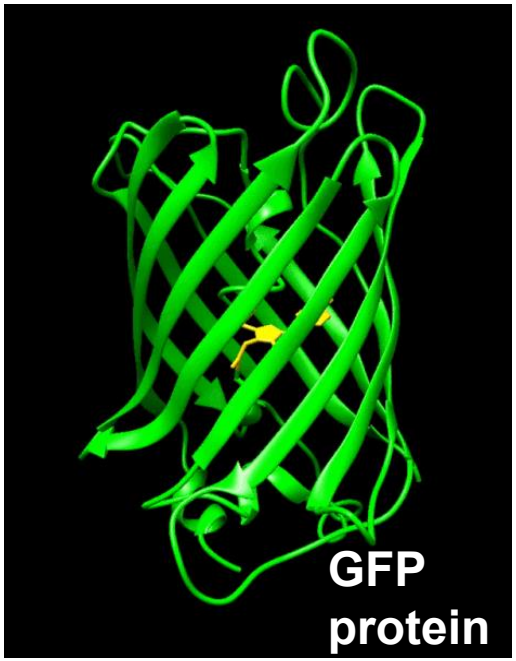
- Need all three regulatory elements MerR, *merO*, P_{mer} to get inducible expression of genes in response to Hg^{2+}



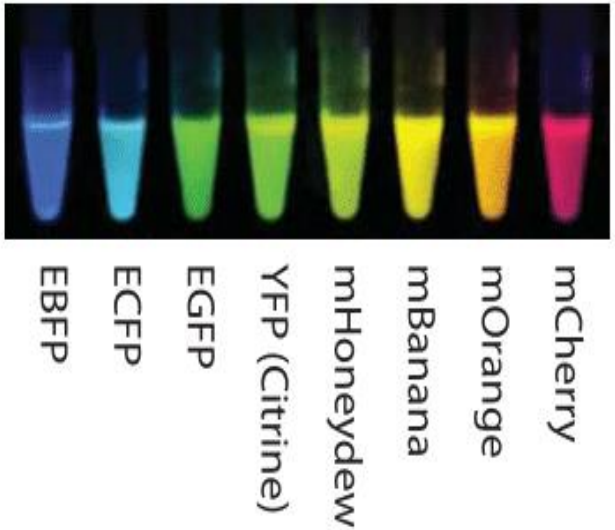
Output module: green fluorescent protein

- Organisms from the phylum CNIDARIA (corals, jellyfish) contain fluorescent proteins

- Green fluorescent protein (GFP) from *Aequoria victoria* is the most widely used marker in biology and medicine

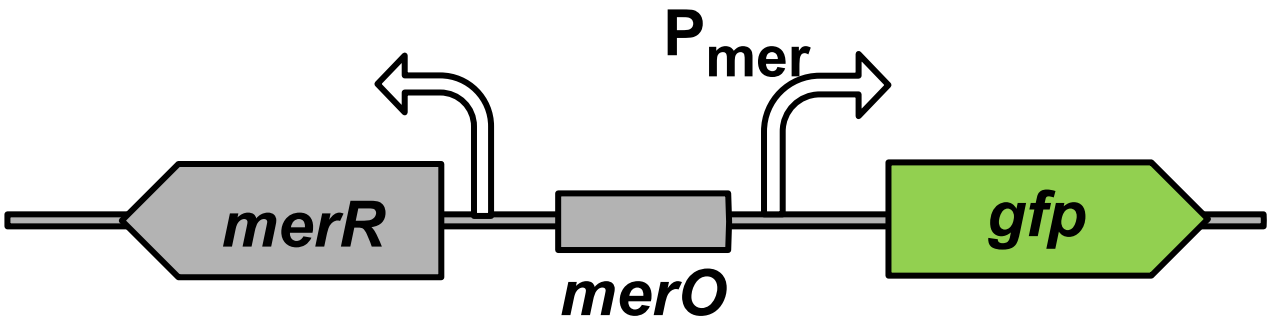


Homologs of GFP



Combining input and output

- **Creation of mercury biosensor is as simple as replacing *merTPAB* with the *gfp* gene (or other detectable output)**



- **But what about <u>host organism</u> and <u>genome location</u>?**

★ *Escherichia coli*

Bacillus subtilis

Pseudomonas sp.

Vibrio sp.

★ *High copy number plasmid*

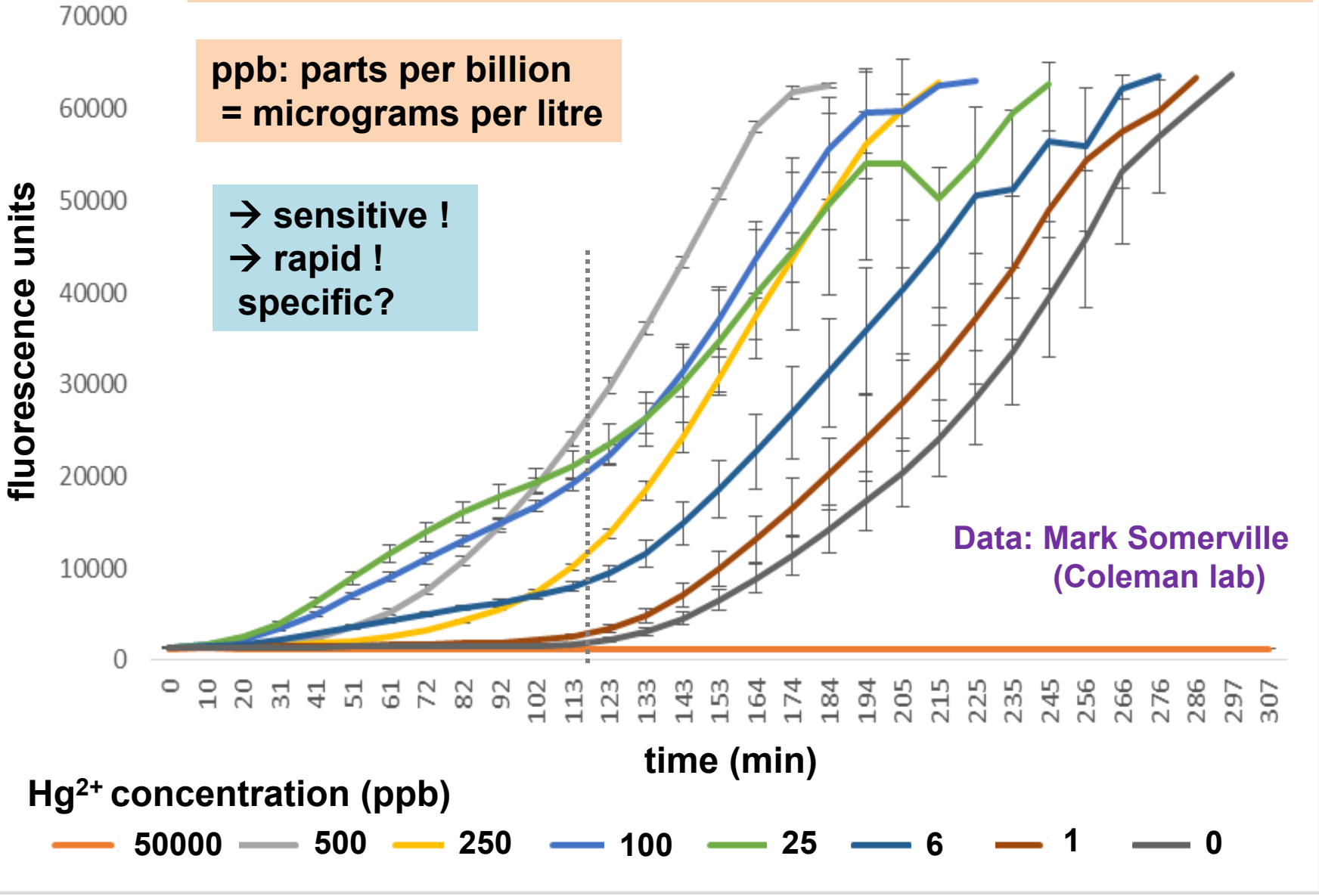
Low copy number plasmid

Bacteriophage

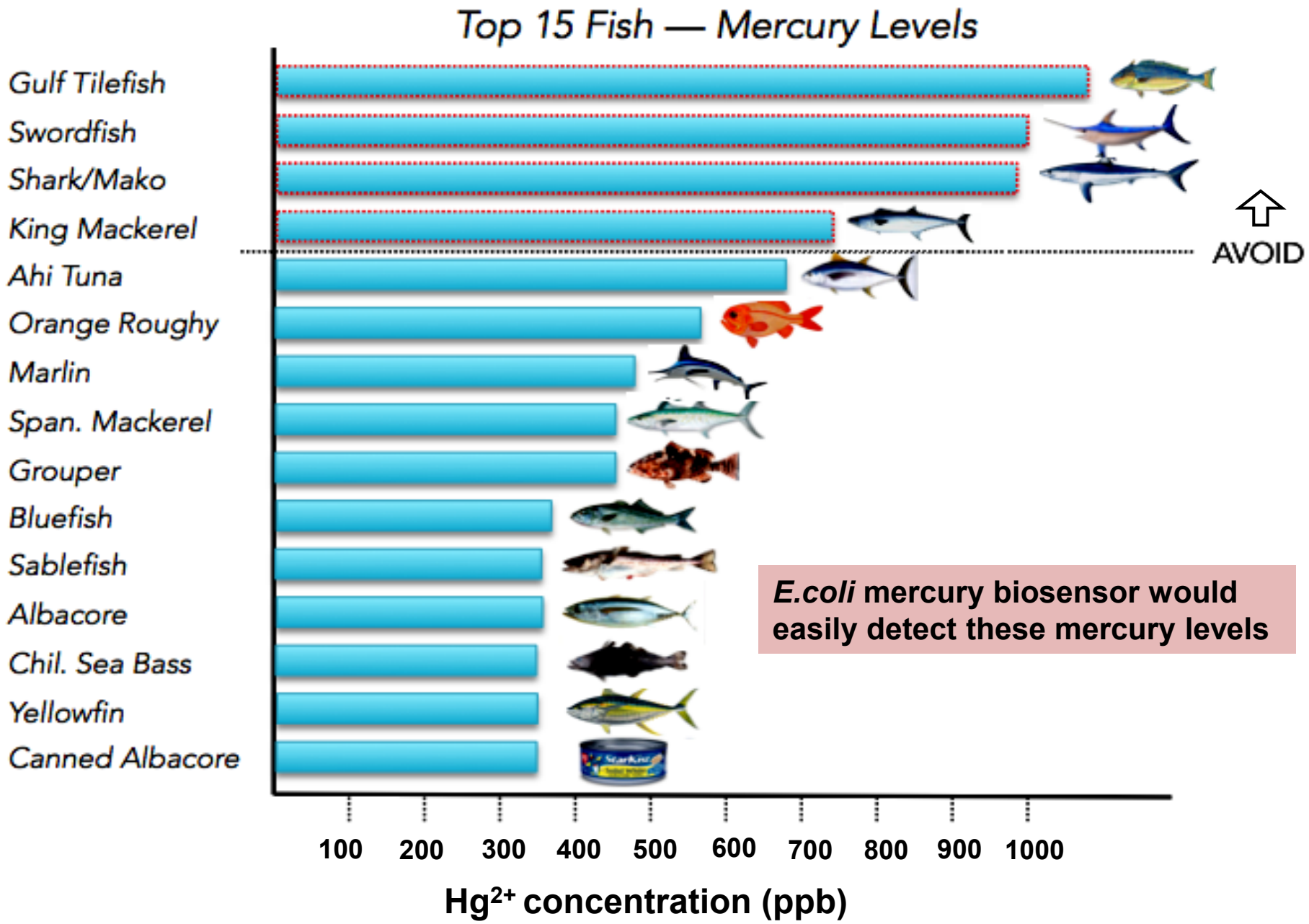
Chromosomal insertion

Does it work?

Rapid increase in fluorescence seen at Hg^{2+} conc. >25 ppb



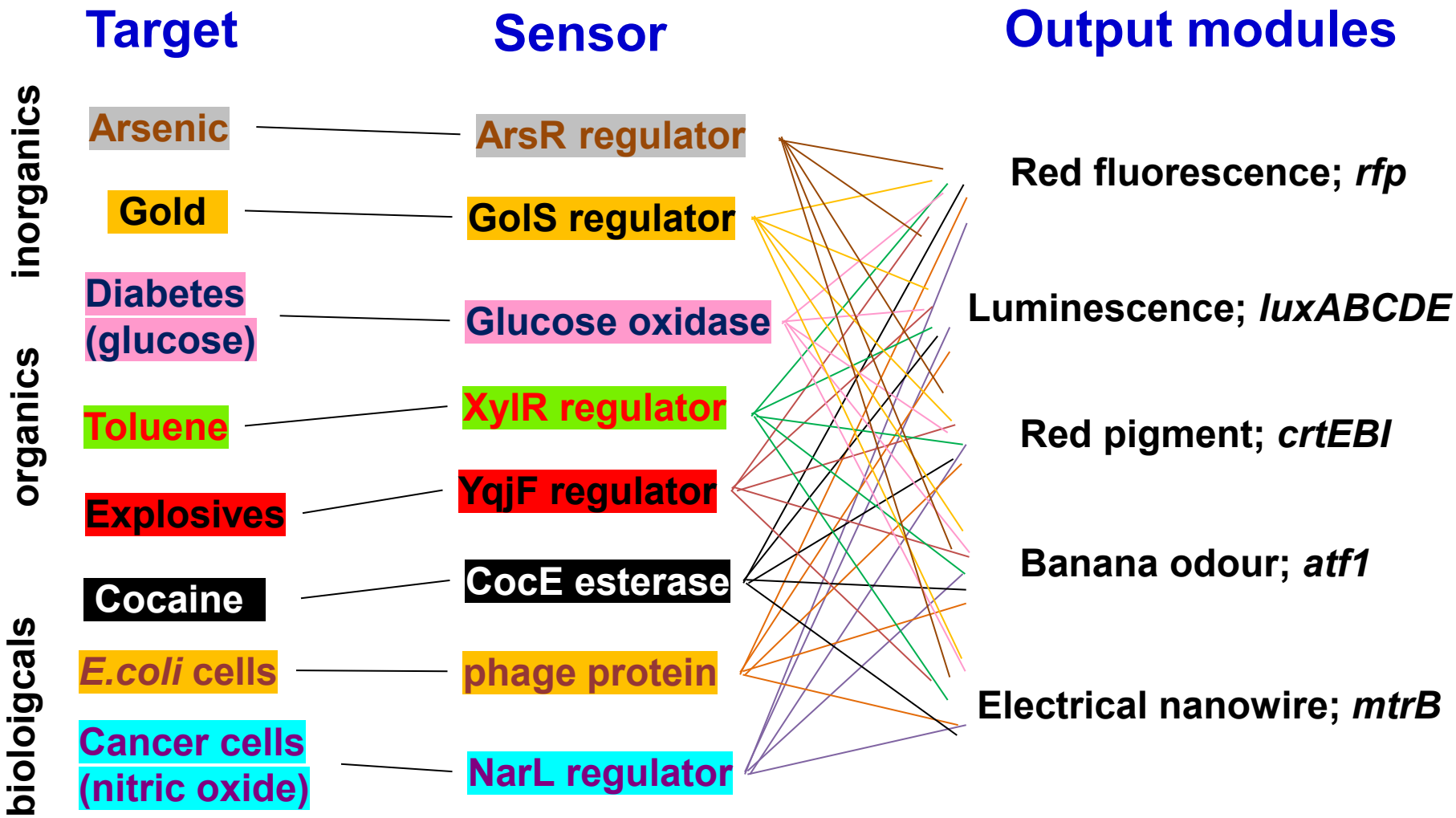
Real world significance?



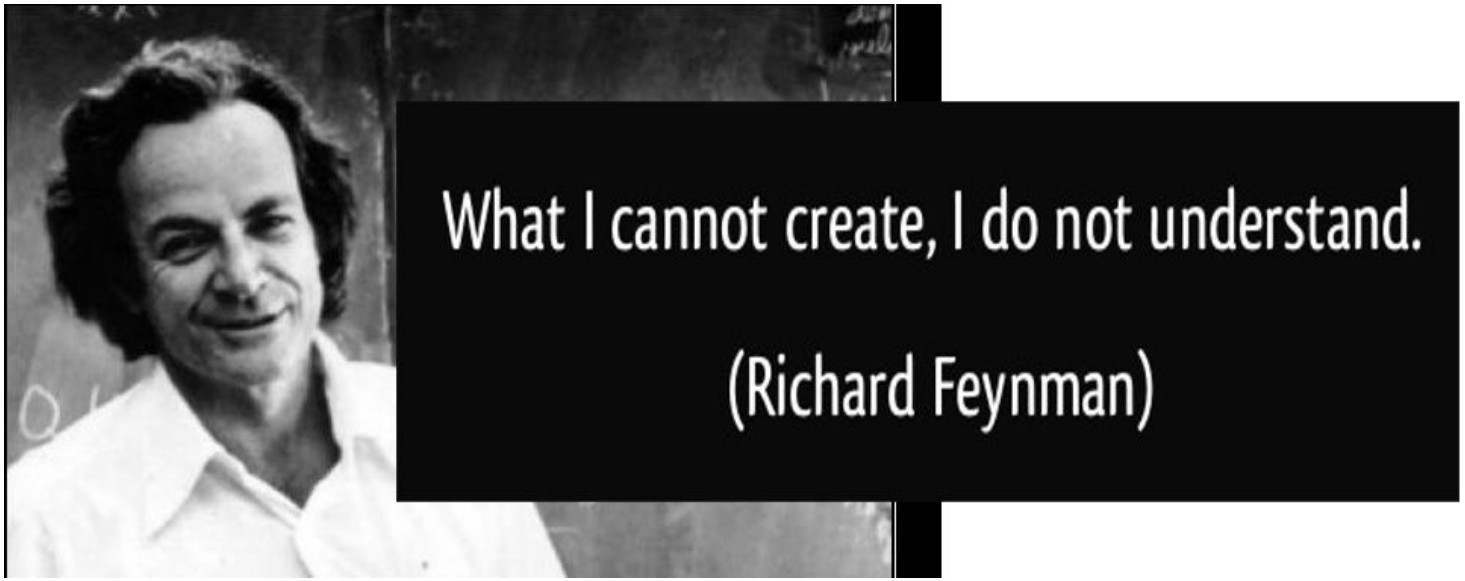
E.coli **mercury biosensor would easily detect these mercury levels**

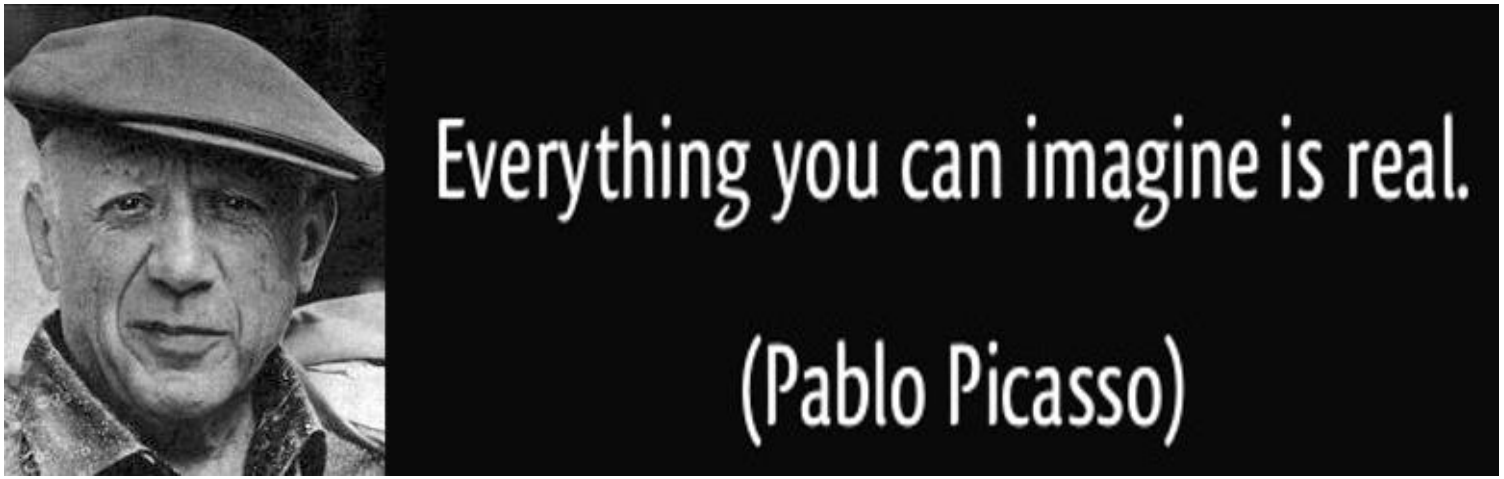
Biosensor modularity

- **Nearly infinite possibilities for biosensors are provided by the massive biodiversity of bacteria and their genomes**



SynBio – the possibilities are endless





… but powerful tools lead to large risks

https://www.youtube.com/watch?v=UaRfbJE1qZ4