L3 Hierarchical Design

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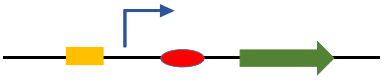
Recall

SynBio applies engineering practices to systematically manipulate biology

Genetic engineering

Synthetic Biology



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This lecture….

- Intro to Engineering design
- SynBio hierarchy
- Biological parts
- Part databases

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Key features of engineering design

1. **Abstraction –** "black boxing" the system to focus on core properties/functions of a system

2. **Modularization** - creation of essential parts with a single function that can be recombined in diverse contexts

3. **Standardization –** parts have fixed interfaces for rapid assembly & prototyping

BE/ChE – Unit ops e.g. Distillation columns, CSTRs
EE – electronic components e.g. resistors, capacitors

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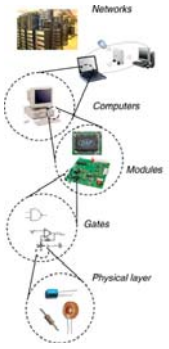

Approach allows for TOP-DOWN or HIERARCHICAL DESIGN where complex problems are broken down to simpler subsystems

Ernesto Andrianantoandro et al. Mol Syst Biol 2006;2:2006.0028

molecular systems biology

Synthetic Biology Hierarchy

Problem — **Microbial production of insulin**

4. Chassis: Host or organism — ***E. coli***

↓

3. System: Combination of devices that execute a specified task — **Insulin producing gene circuit**

↓

2. Device: Combination of parts that achieve a *single* human specified function — **Insulin protein expression, gene regulation cassette (e.g. YES gate)**

↓

1. Part: Minimal DNA required to achieve a biological function — **A promoter, CDS, ribosome binding site, regulatory protein, operator sites, ……**

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0. DNA: Physical specification

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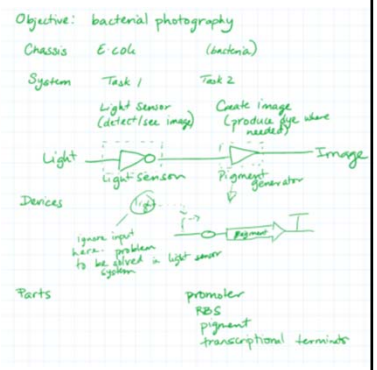
Bacterial Photography

Mask

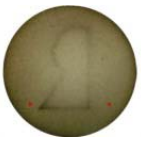
in vivo

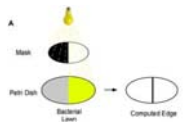


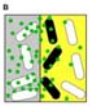
Using the hierarchy, how would you design this system?

Tabor et al, *Cell*, 2010

Edge Detection





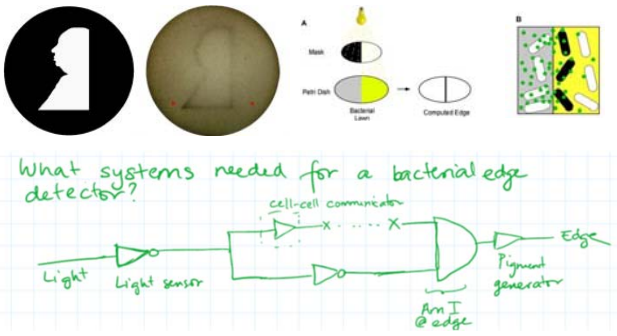


What systems needed for a bacterial edge detector?

Modular nature of systems allow reuse and recombination for new functionality

Tabor et al, *Cell*, 2010

Edge Detection



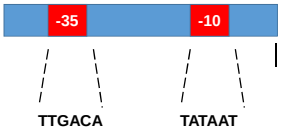
Modular nature of systems allow reuse and recombination for new functionality

Tabor et al, *Cell*, 2010

4 basic biological parts

Promoters – initiates transcription (trxn)

Bacterial Promoters (~40 nts)



-10 and -35 recognized by σ factors + RNAP
→ Binding affinity controls expression strength

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Controlling the regulatory output of a promoter

Regulator proteins (transcription factors) bind to ***operator sites*** within/around a promoter

→ Repressor proteins bind within or downstream of promoter and compete with RNAP for binding

e.g. tetR/tetO, araC/araO

- can also form a trxn roadblock

e.g. lacI/lacO

→ Activator proteins bind to upstream operators to recruit RNAP or change DNA conformation

e.g. CAP, araC

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Regulator proteins

- Regulator proteins change conformation when exposed to ligands
  - lacI and lactose/IPTG
  - araC and arabinose
  - tetR and tetracycline/doxycycline
- Conformation determines whether bound to DNA or not

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4 basic biological parts

Ribosome binding site – initiates translation (trln) by facilitating ribosome binding

AGGAGG centered ~10 bps upstream of start codon

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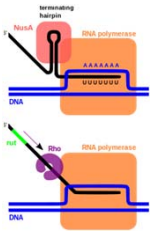
4 basic biological parts

ORF/CDS – genes that encode a function (catalytic, structural or regulatory), 3n bps

Terminators – terminates trxn
- → Rho-independent (T-rich)
- → Rho-dependent (C-rich)



Why no start codons?

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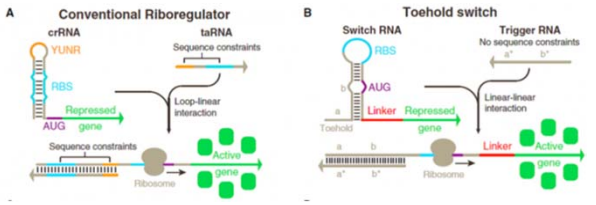
Other parts

- Riboregulators



http://parts.igem.org/Part:BBa_K1639000

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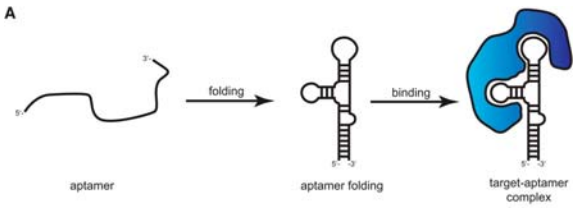
Other parts

- Aptamers – nucleic acids that bind ligands, typically paired with riboswitches



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Parts have properties

- Parts have distinct input/output
- Parts have 'strengths' that control their output
  - E.g. strong promoter == high expression
- Properties controlled by architecture and sequence

What does a promoter that responds to arabinose look like? Tetracycline?

How would you engineer a weaker tetracycline-inducible promoters?

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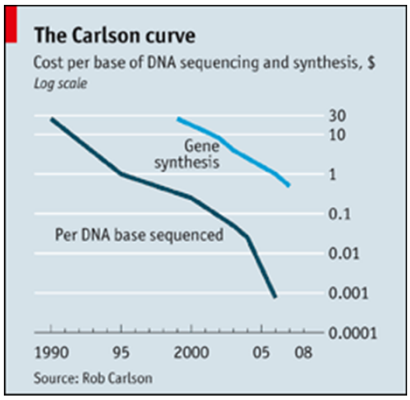

Inexpensive to identify variants from nature and to print them on demand

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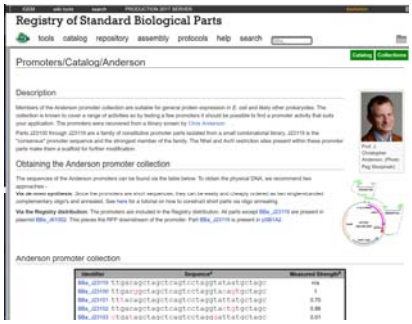
Part libraries exist in public collections

- Registry of Standard Biological Parts (parts.igem.org)



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Part libraries exist in public collections

- BRENDA (www.Brenda-enzymes.org)

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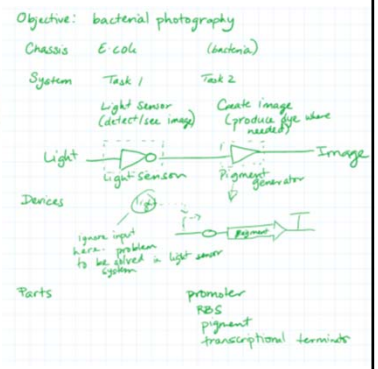
Bacterial Photography



How exactly would you design your light sensor?

Your pigment generator?

Tabor et al, *Cell*, 2010

Next time….

- What are the input-output relationships between parts?

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