InterTech

UNIVERSIDAD DE MÁLAGA

presents

UNIVERSIDAD POLITECNICA DE VALENCIA

Standards

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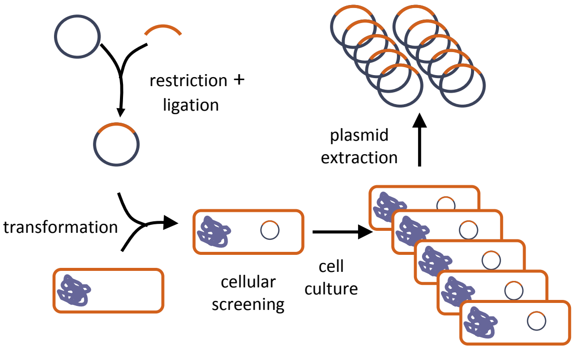
- building a composable DNA library
  - assembly standard
  - enzymes choice
  - assembling DNA
  - assemblies
  - how to make a BioBrick
- Registry
- Parts
  - Promoter
  - RBS
  - Coding Sequence
  - Terminator
- Signal Carrier
  - PoPS
  - RiPS
  - characterization
- Modularity
- Looking for abstraction
  - part characterization
  - plasmids
- Abstraction Hierarchy
  - Parts
  - Devices
    - Biological Inverter
  - Systems
  - Host

typical genetic engineering proceeding



"The **lack of standardization** in assembly techniques for DNA sequences forces each DNA assembly reaction to be both an **experimental tool** for addressing the current research topic, and an **experiment** in and of itself."

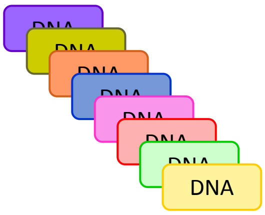
"One of our goals is to replace this ***ad hoc* experimental design** with a set of **standard and reliable engineering mechanisms** to remove much of the tedium and surprise during assembly of genetic components into larger systems "

Tom Knight, MIT

Tom Knight (**MIT**), *Idempotent Vector Design for Standard Assembly of Biobricks*, 2003

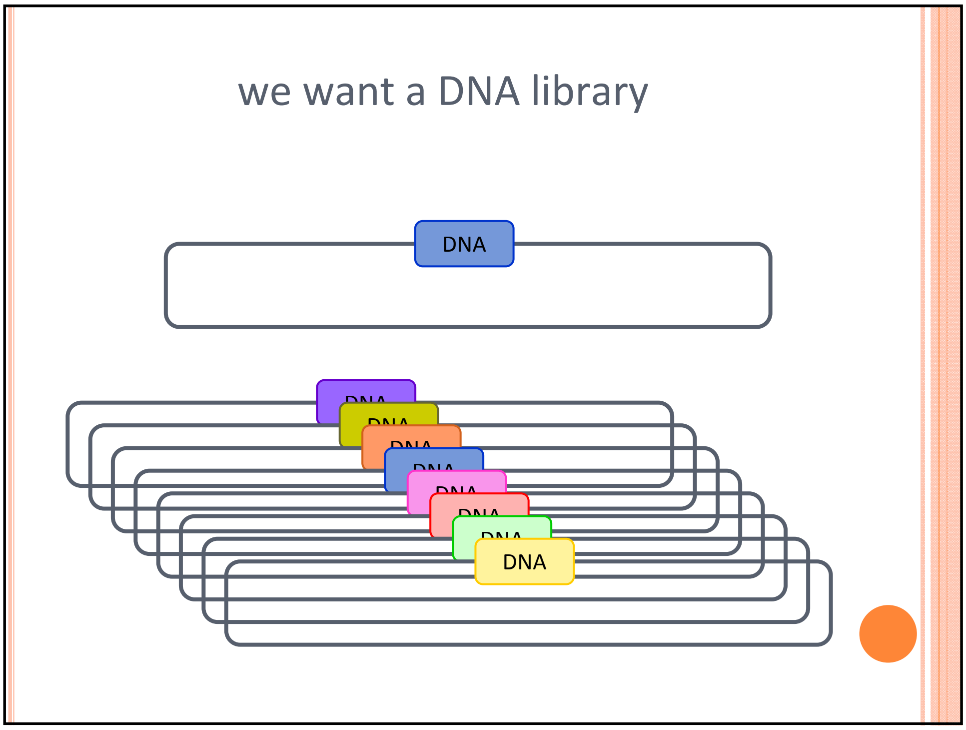
composability

- suppose we have individual parts that work, can we actually put them together such that they work in a well-defined & predictable way ?

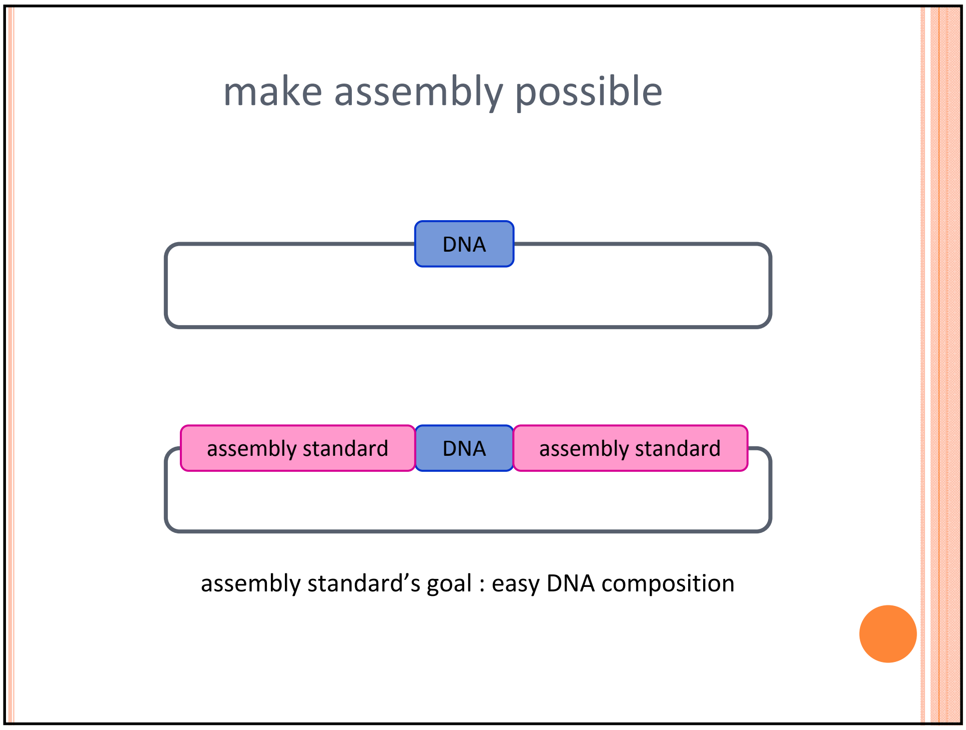


wish list for DNA composition

- Parts that can easily be plugged together
- Efficient and Reliable process
- Support an Abstraction Hierarchy
  - Parts
  - Devices
  - Systems



we want a DNA library

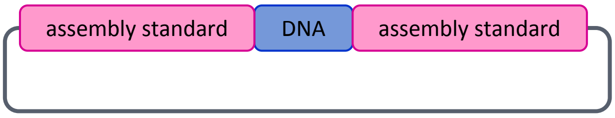


make assembly possible

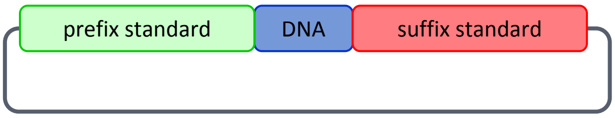
assembly standard’s goal : easy DNA composition

assembly standard

- do they have to be the same ?
  - why ?


assembly standard | DNA | assembly standard

position matters !


prefix standard | DNA | suffix standard

prefix and suffix will have different restriction sites !

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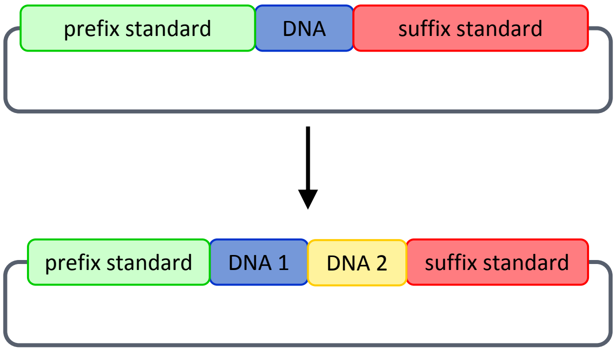
assembling DNA

- we want to get a DNA molecule next to another
  - *cut and paste* DNA

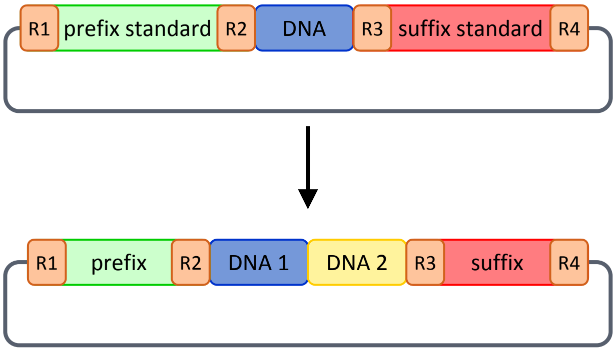

prefix standard | DNA | suffix standard

prefix standard | DNA 1 | DNA 2 | suffix standard

prefix and suffix have to remain the
same and in same order

consecutive assemblies have to be possible

- at the end of the assembly, same restriction sites and in same position !!



idempotence !

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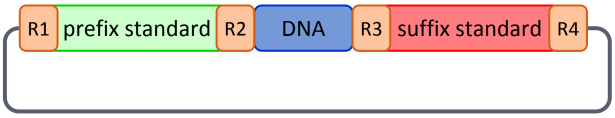
assembling DNA : enzyme choice

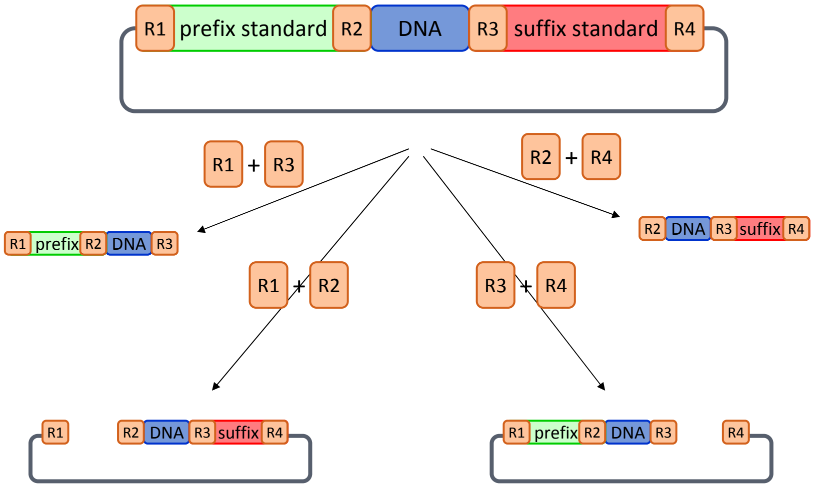


- easy to use
- compatible buffers & temperature
- heat inactivated
- low star activity
- *sticky ends* overhangs to enhance ligation efficiency
- avoiding accidental creation of ATG sequence
- avoid methylation sensitive sites

- must be unique in plasmid

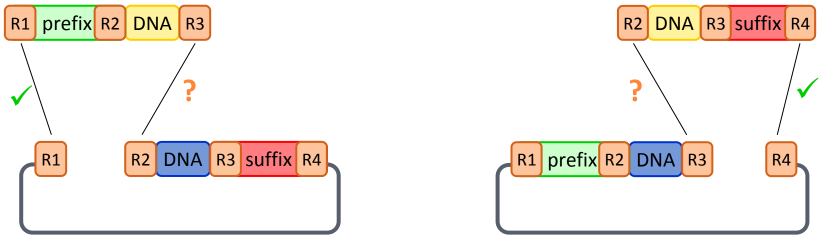
assembling DNA

- *cutting* BioBricks



assembling DNA

- *pasting* BioBricks



R1 : *Eco*RI

R2 : ?

R3 : ?

R4 : *Pst*I

so, what about R2 & R3 ?

take advantage of mixed restriction sites !!

mixing highly similar specific restriction sites

*Mfe*I (*Mun*I) :

```
5'-C^A A T T G-3'
3'-G T T A A^C-5'
```

*Eco*RI :

```
5'-G^A A T T C-3'
3'-C T T A A^G-5'
```

```
5'-C
3'-G T T A A
```

```
A A T T C-3'
        G-5'
```

```
5'-C A A T T C-3'
3'-G T T A A G-5'
```

neither *Mfe*I nor *Eco*RI can recognize this site !!

a restriction site has been *erased* !

same happens with *Xba*I and *Spe*I …

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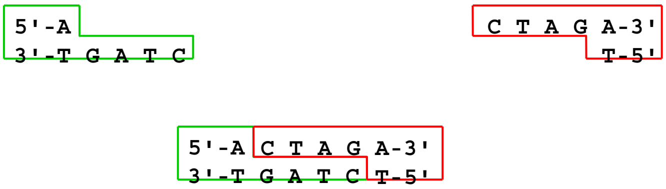
*Xba*I & *Spe*I

*Spe*I (*Bcu*I) :

```
5'-A^C T A G T-3'
3'-T G A T C^A-5'
```

*Xba*I :

```
5'-T^C T A G A-3'
3'-A G A T C^T-5'
```



```
5'-A
3'-T G A T C
```

```
C T A G A-3'
        T-5'
```

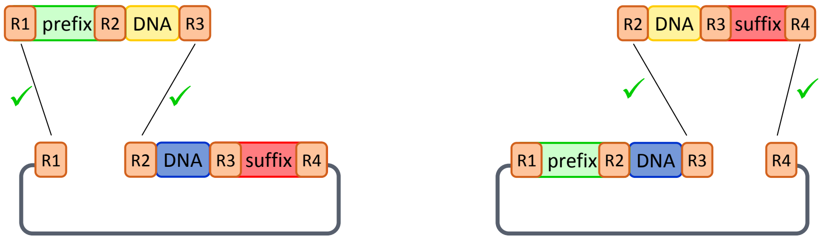
```
5'-A C T A G A-3'
3'-T G A T C T-5'
```

neither *Xba*I nor *Spe*I can recognize this site !!

restriction site has been *erased* !

assembling DNA

- *pasting* BioBricks



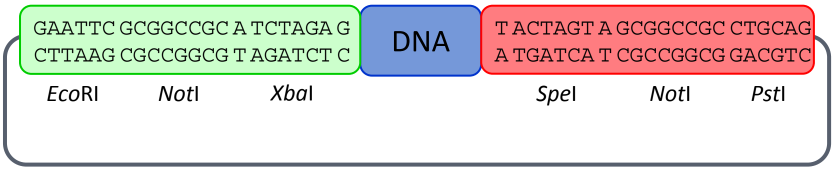
R1 : *Eco*RI

R2 : *Xba*I

R3 : *Spe*I

R4 : *Pst*I

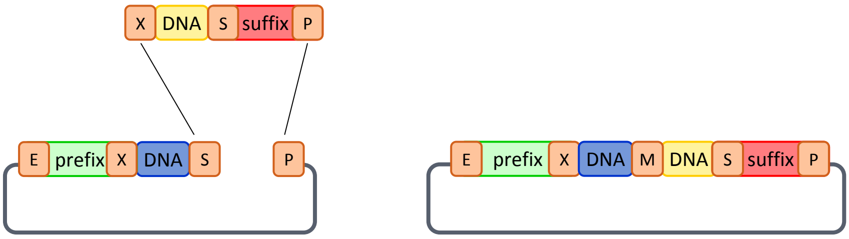
MIT BioBrick Standard



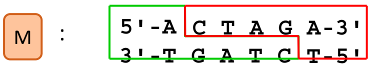
Tom Knight (**MIT**), *Idempotent Vector Design for Standard Assembly of Biobricks*, 2003

assembling DNA

- *pasting* BioBricks : insert DNA *after* vector DNA



*Xba*I 5’ overhang



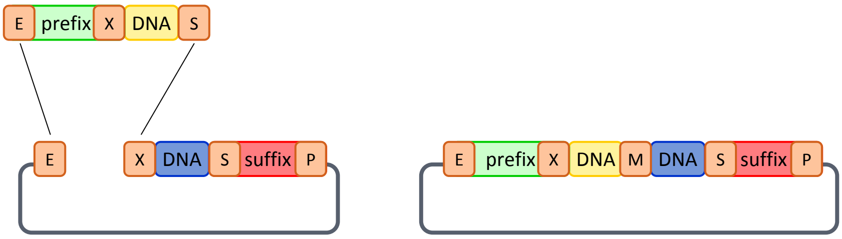
```
5'-A C T A G A-3'
3'-T G A T C T-5'
```

*Spe*I 3’ overhang

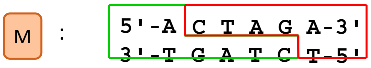
---

assembling DNA

- *pasting* BioBricks : insert DNA *before* vector DNA



*Xba*I 5’ overhang

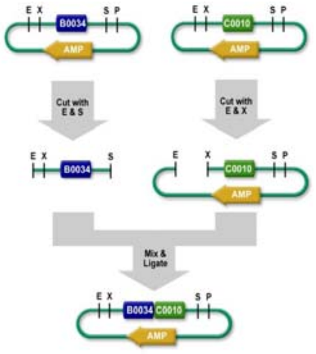


```
5'-A C T A G A-3'
3'-T G A T C T-5'
```

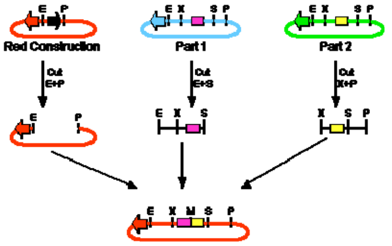
*Spe*I 3’ overhang

assemblies

standard assembly



rolling or parallel assembly



all of them idempotent

3A assembly

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how to make a BioBrick

- PCR

prefix

forward primer

wild DNA

reverse primer

suffix

- synthesis

prefix | desired DNA | suffix

- mutagenesis on DNA constructs
  - be careful : *Eco*RI, *Xba*I, *Spe*I and *Pst*I sites have to be unique !

Registry

- Registry of Standard Biological Parts (MIT)
  - http://parts.mit.edu
- alternative developing registries :
  - BioBrick++, Austin Che, MIT
  - Pam Silver's Lab BioBrick Version, Harvard
  - JC Anderson's Lab BioBrick Version, UC Berkeley

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un-registered knowledge

- Global data bases
  - NCBI – PubMed
    - http://www.ncbi.nlm.nih.gov/
  - KEGG
    - http://www.genome.jp/kegg/
- Protein data bases
  - ExPASy
    - http://expasy.org/
  - Protein Data Bank
    - http://www.rcsb.org

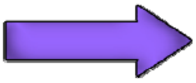
let’s talks about parts…

- what is a part ?
  - just the DNA sequence ?
  - its function ?

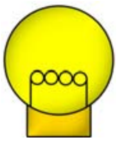
basic functions encoded in DNA sequences

Registry is made of parts

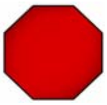
- Promoter
  - regulates transcription



- Coding sequence
  - codes for protein



- Ribosome Binding Site
  - regulates translation



- Terminator
  - stops transcription

Promoters

- regulatory part
  - binding regions for RNA polymerase, which performs transcription (RNA production from a DNA template)
  - binding regions for transcription factors, which regulates the initiation of transcription

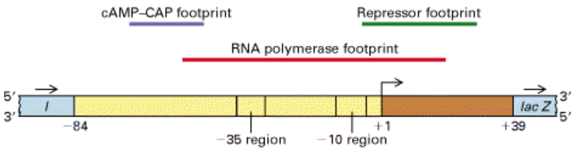


Figure 10-9. (Lodish *et al*, 2000)

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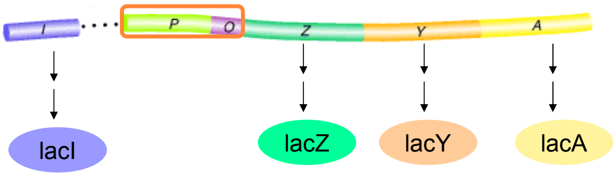
lac operon promoter



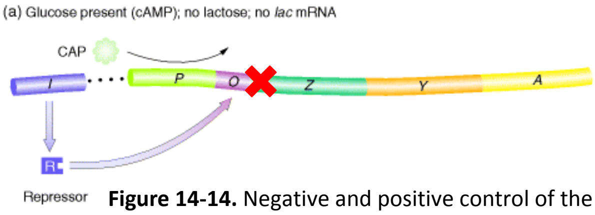


Figure 14-14. Negative and positive control of the *lac* operon by the Lac repressor and catabolite activator protein (CAP), respectively. (Griffiths *et al*, 2000)

lac operon promoter

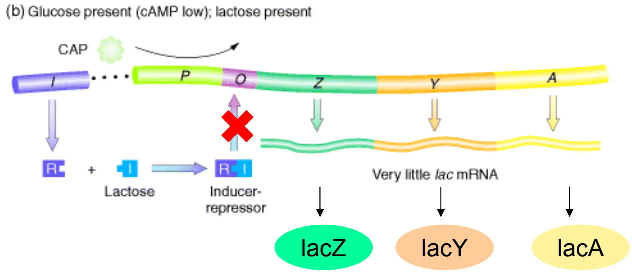


Figure 14-14. Negative and positive control of the *lac* operon by the Lac repressor and catabolite activator protein (CAP), respectively. (Griffiths *et al*, 2000)

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lac operon promoter

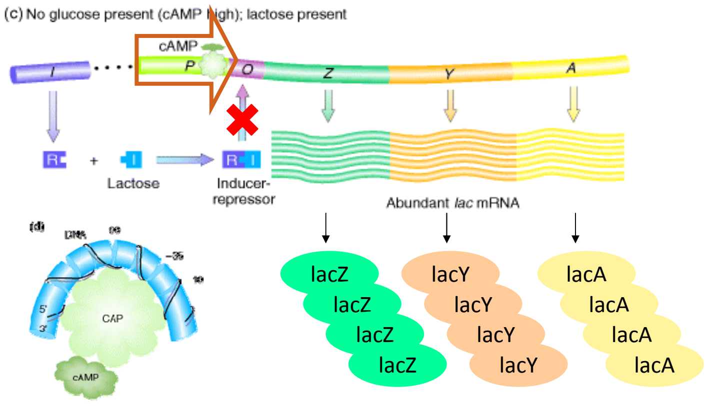


Figure 14-14. (Griffiths *et al*, 2000)

Zinc fingers

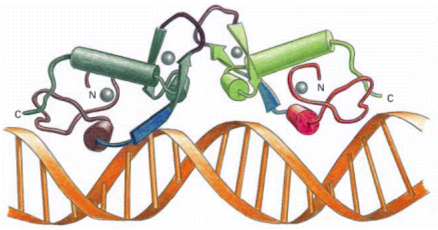


Figure 7-19. (Alberts *et al*, 2002)

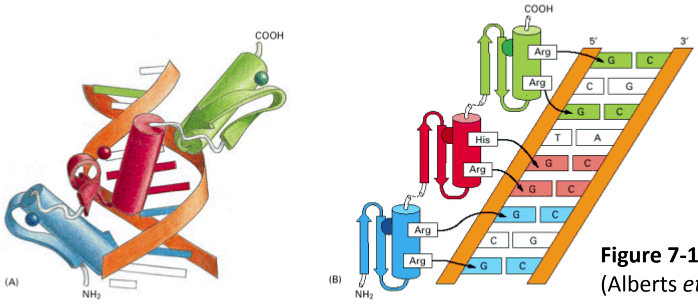


Figure 7-18.
(Alberts *et al*, 2002)

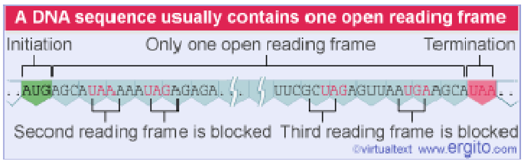
Zinc fingers

- ***Zinc Finger Tools***
  - a web-based tool
    - find possible Zinc finger target sequences
    - design new Zinc finger transcription factors
    - predict a target DNA sequence from a Zinc finger amino acid composition
  - http://www.zincfingertools.org

Mandell & Barbas. **Nucleic Acids Res**. 2006

Coding sequences

- bears DNA sequence that codes for a protein
  - DNA will be transcribed to mRNA
  - mRNA will be translated to protein



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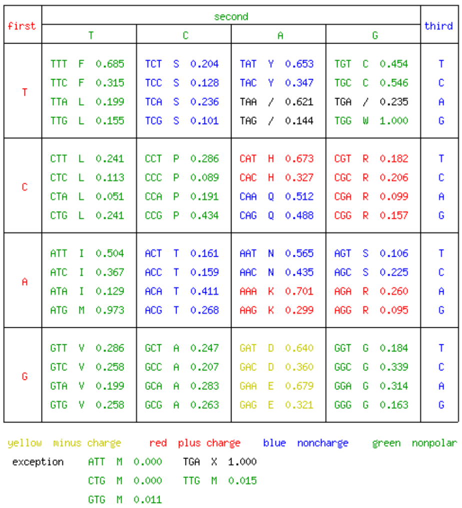
codon usage



first	second				third
	T	C	A	G	
T	TTT F 0.685	TCT S 0.204	TAT Y 0.653	TGT C 0.454	T
	TTC F 0.315	TCC S 0.128	TAC Y 0.347	TGC C 0.546	C
	TTA L 0.199	TCA S 0.236	TAA / 0.621	TGA / 0.235	A
	TTG L 0.155	TCG S 0.101	TAG / 0.144	TGG W 1.000	G
C	CTT L 0.241	CCT P 0.286	CAT H 0.673	CGT R 0.182	T
	CTC L 0.113	CCC P 0.089	CAC H 0.327	CGC R 0.206	C
	CTA L 0.051	CCA P 0.191	CAA Q 0.512	CGA R 0.099	A
	CTG L 0.241	CCG P 0.434	CAG Q 0.488	CGG R 0.157	G
A	ATT I 0.504	ACT T 0.161	AAT N 0.565	AGT S 0.106	T
	ATC I 0.367	ACC T 0.159	AAC N 0.435	AGC S 0.225	C
	ATA I 0.129	ACA T 0.411	AAA K 0.701	AGA R 0.260	A
	ATG M 0.973	ACG T 0.268	AAG K 0.299	AGG R 0.095	G
G	GTT V 0.286	GCT A 0.247	GAT D 0.640	GGT G 0.184	T
	GTC V 0.258	GCC A 0.207	GAC D 0.360	GGC G 0.339	C
	GTA V 0.199	GCA A 0.283	GAA E 0.679	GGA G 0.314	A
	GTG V 0.258	GCG A 0.263	GAG E 0.321	GGG G 0.163	G

yellow minus charge   red plus charge   blue noncharge   green nonpolar

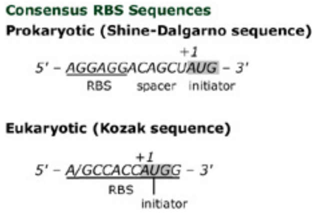
exception ATT M 0.000 TGA X 1.000
CTG M 0.000 TTG M 0.015
GTG M 0.011

http://www.g-language.org/data/haruo/codon_table.gif

- triplets (codons) of RNA code for amino acids
- codon usage is organism specific
- re-coding amino acids can result in improved or reduced translation

Ribosome Binding Sites

- landing site for ribosomes on mRNA
- approximately 10 nt away from AUG



www.ambion.com

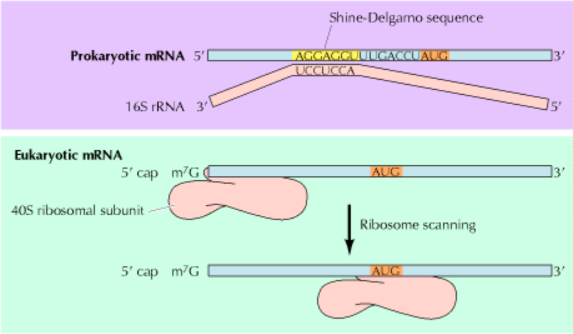


Figure 7.7. Signals for translation initiation. (Cooper, 2000)

RBS manipulation

- mutate RBS sequence
- add secondary structures to alter binding

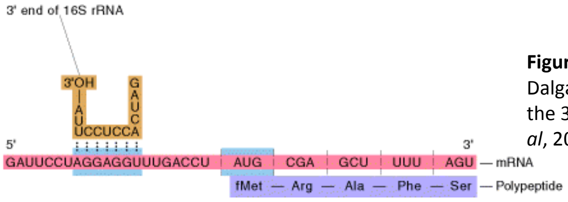


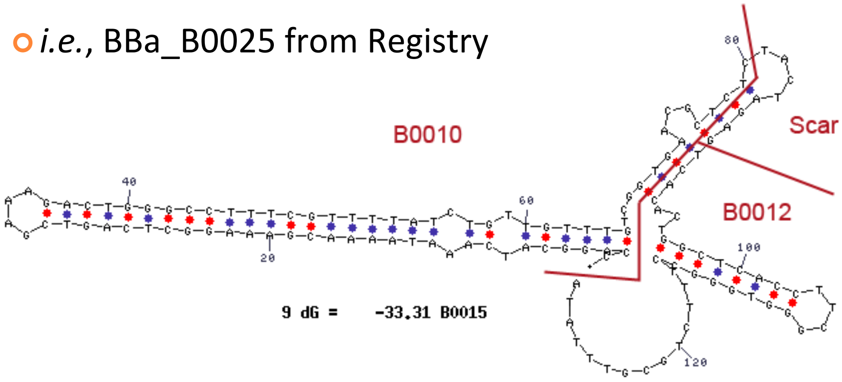
Figure 10-35. Binding of the Shine-Dalgarno sequence on an mRNA to the 3′ end of 16S rRNA. (Griffiths *et al*, 2000)

Orig:	ATTAAAGAGGAGAAATTAAGCATG	strong
RBS-1:	TCACACAGGAAACCGGTTCGATG	↓
RBS-2:	TCACACAGGAAAGGCCTCGATG	
RBS-3:	TCACACAGGACGGCCGGATG	weak

Weiss, *et al*. **Oxford Unviersity Press.** 2004.

Terminators

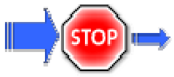
- stops transcription
- must be forward and reverse
- *i.e.*, BBa_B0025 from Registry



Terminator Efficiency

- single terminators
  - forward and reverse efficiency
  - current range -1.09 to .984
    - negative means it acts as a promoter
  - terminators can be combined and inversed
    - B0021 = B0010 + B0012
    - B0015 inversed is B0025

BBa_B0025



forward efficiency	0.295
reverse efficiency	0.984

taming nature

- lots of natural parts !

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taming nature

- lots of natural parts !
- get them and tame them !!

Riding into Arrakeen – Bryce Homick

Signal Carrier

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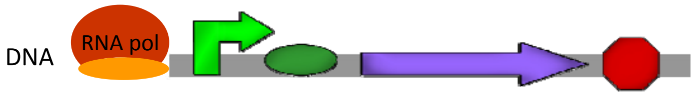
biology

- goal : a protein generator
- transcription & translation



biology

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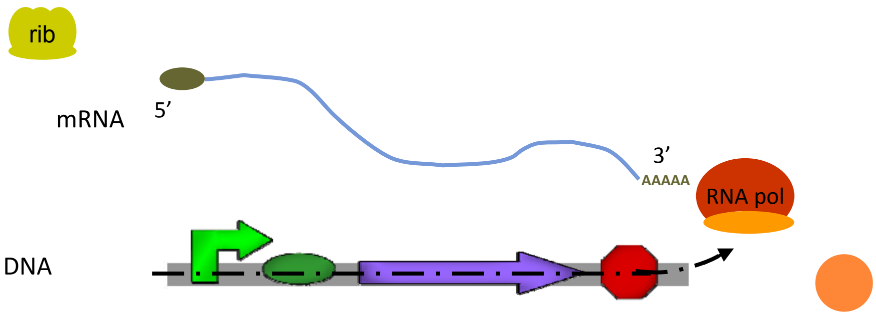

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biology

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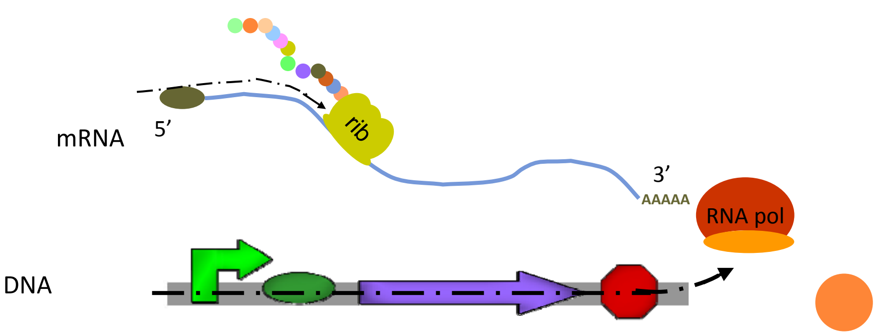
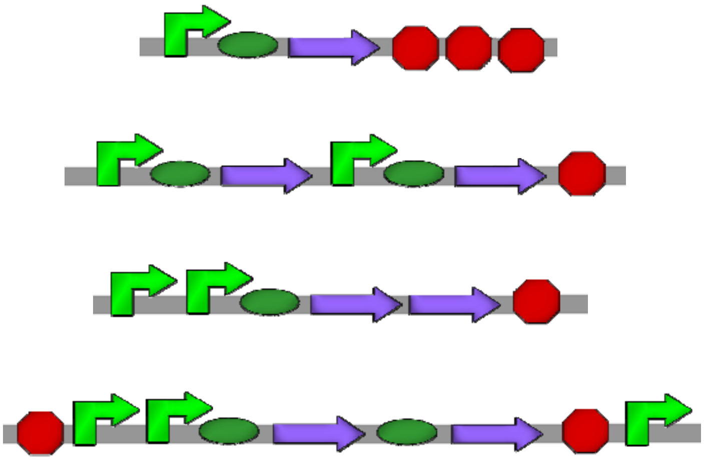


functionality



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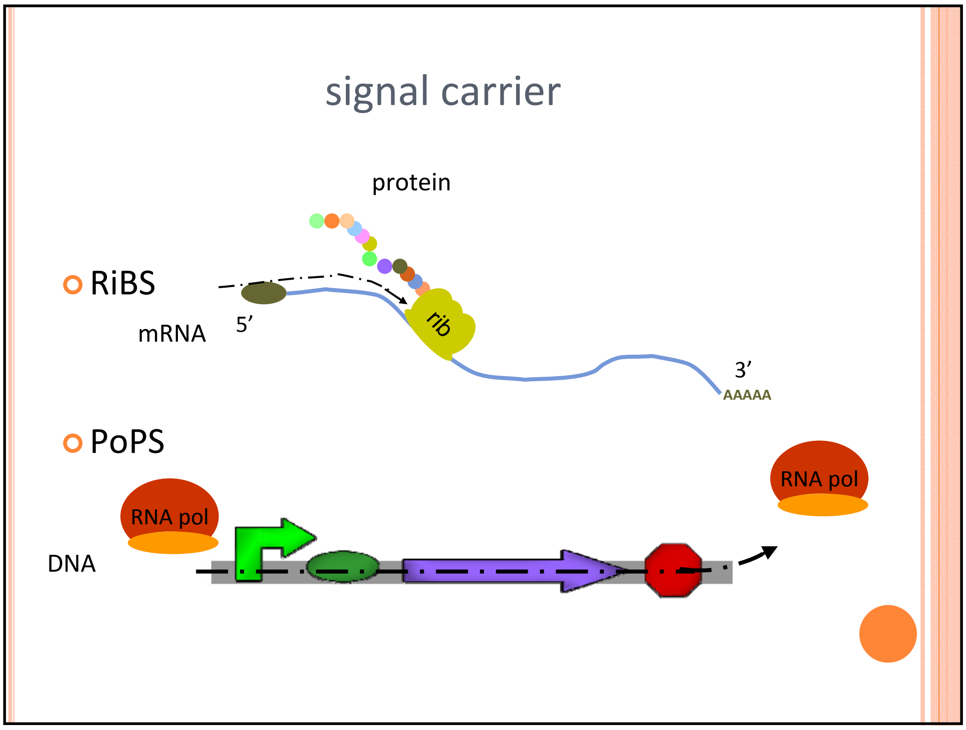
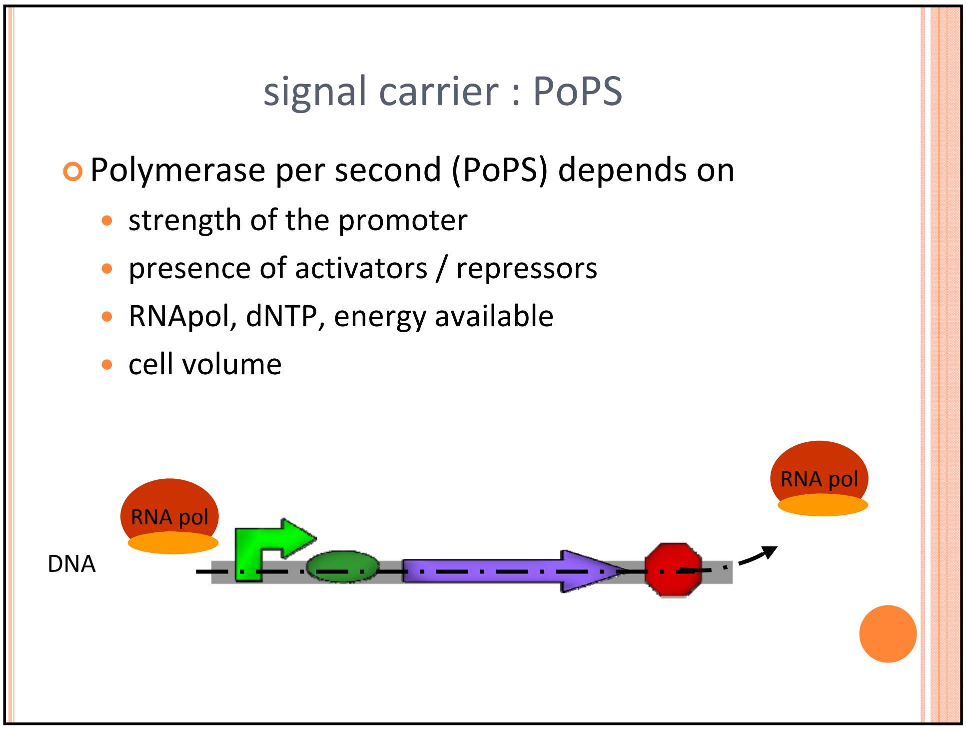
functionality

- find a set of design rules to insure
  - functional property of the assembly
  - modularity of the device created
  - re-usability of the device/system
- make possible
  - abstraction
  - standarization



signal carrier

- RiBS
- PoPS

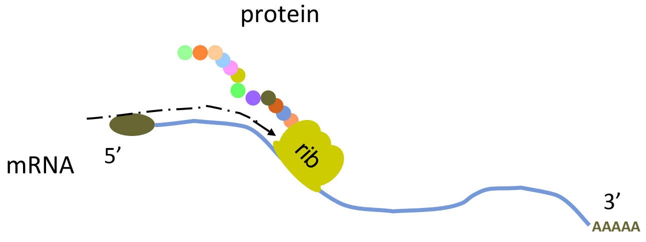


signal carrier : PoPS

- Polymerase per second (PoPS) depends on
  - strength of the promoter
  - presence of activators / repressors
  - RNApol, dNTP, energy available
  - cell volume

signal carrier : RiPS

- Ribosome per second (RiPS) depends on
  - strength of the RBS
  - presence of activators / repressors
  - ribosomes, tRNA, energy available
  - cell volume



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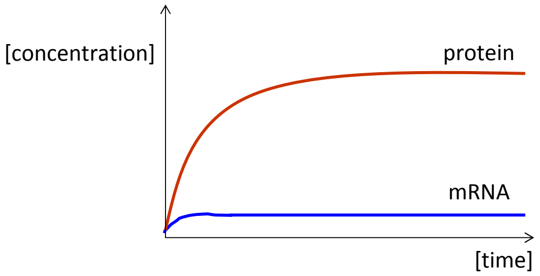
dynamics



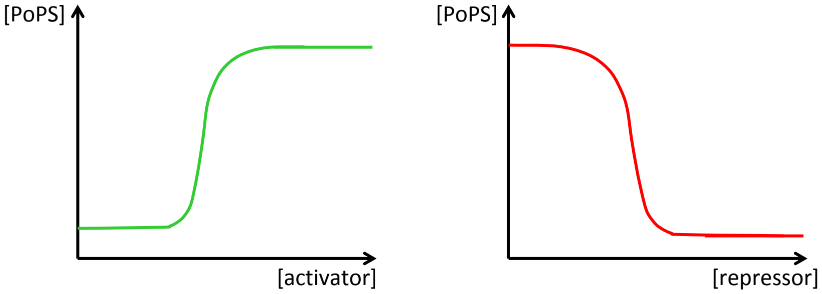
$$\frac{d[mRNA]}{dt} = k_1 - d_1[mRNA]$$

$$\frac{d[protein]}{dt} = k_2[mRNA] - d_2[protein]$$

PoPS characterization

- large variety of promoters

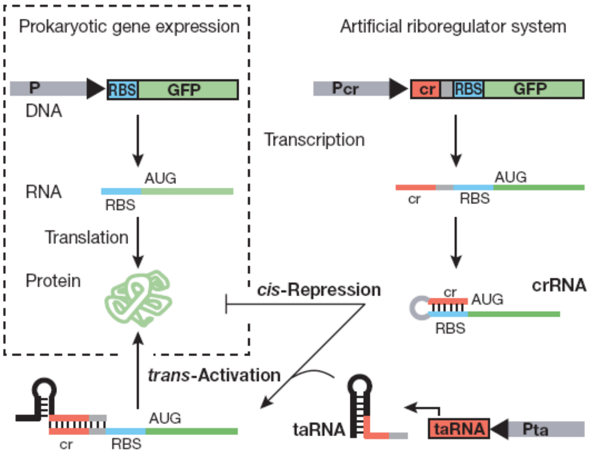
- constitutives : with a given *strength*
- tunable : respond to activators or repressors



RiPS characterization

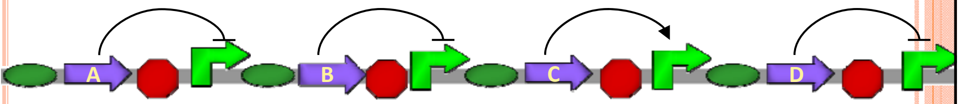
- variations can be made

- tunable too !
  - miRNA
  - riboswitches



Isaacs *et al*. (2004)
Nature Biotech.

modularity



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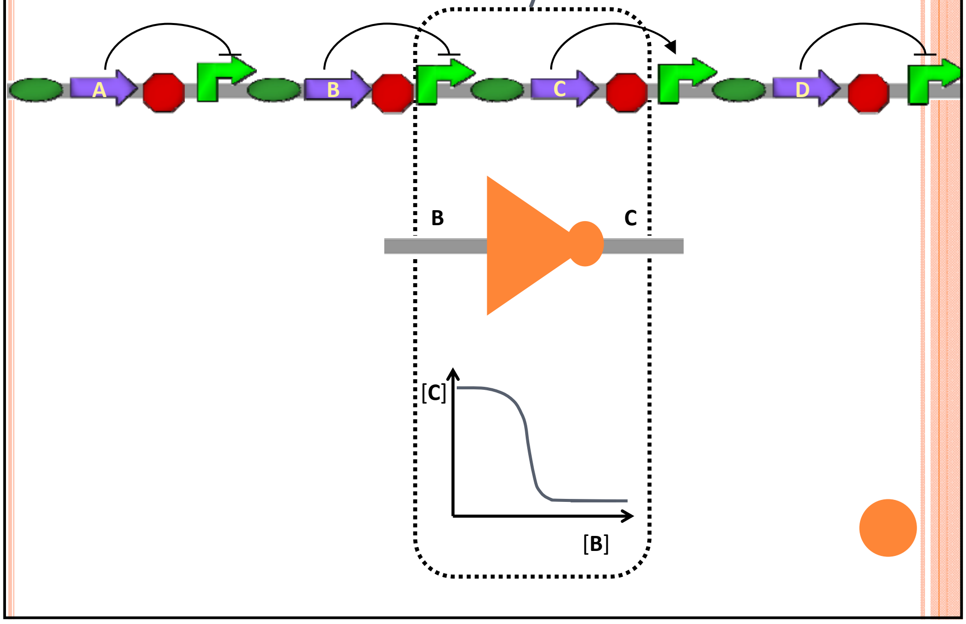
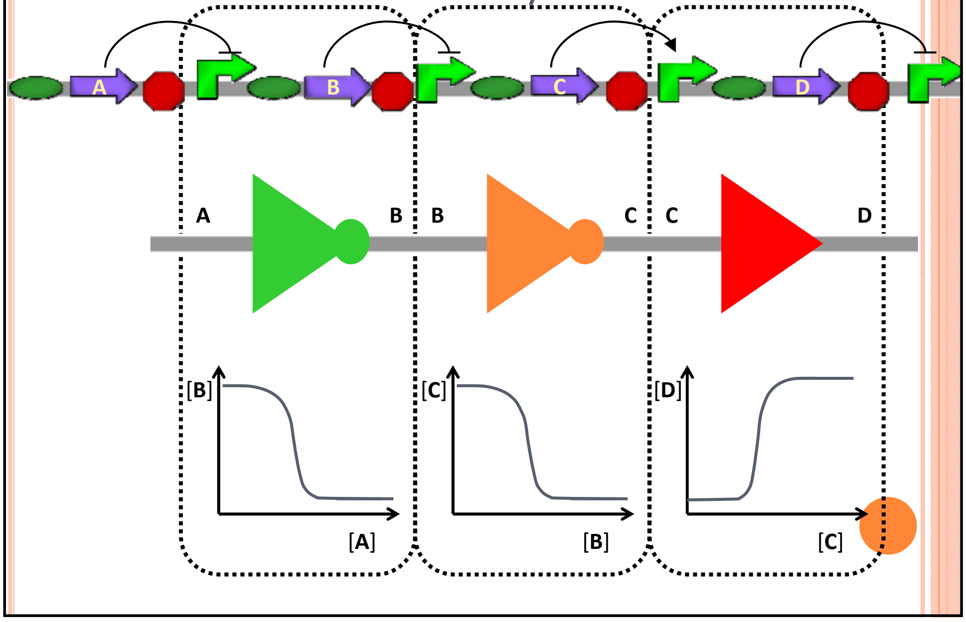
modularity



modularity



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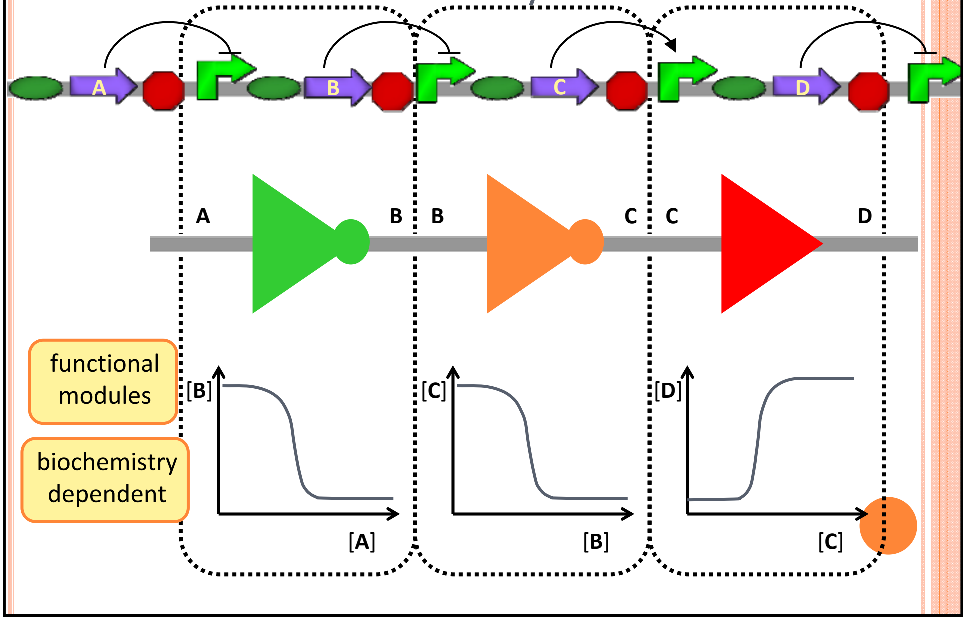
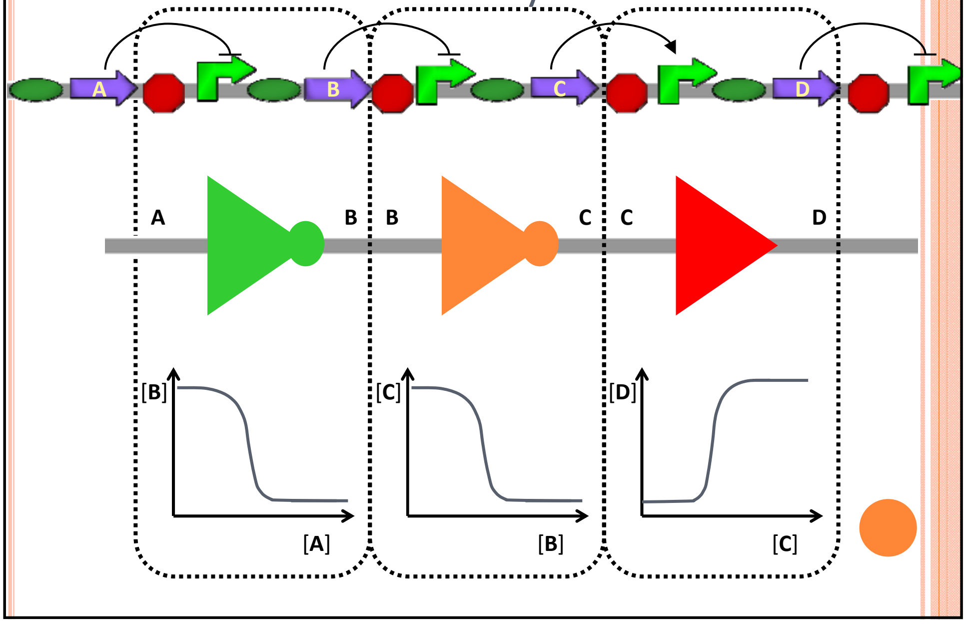
modularity



functional modules

biochemistry dependent

modularity



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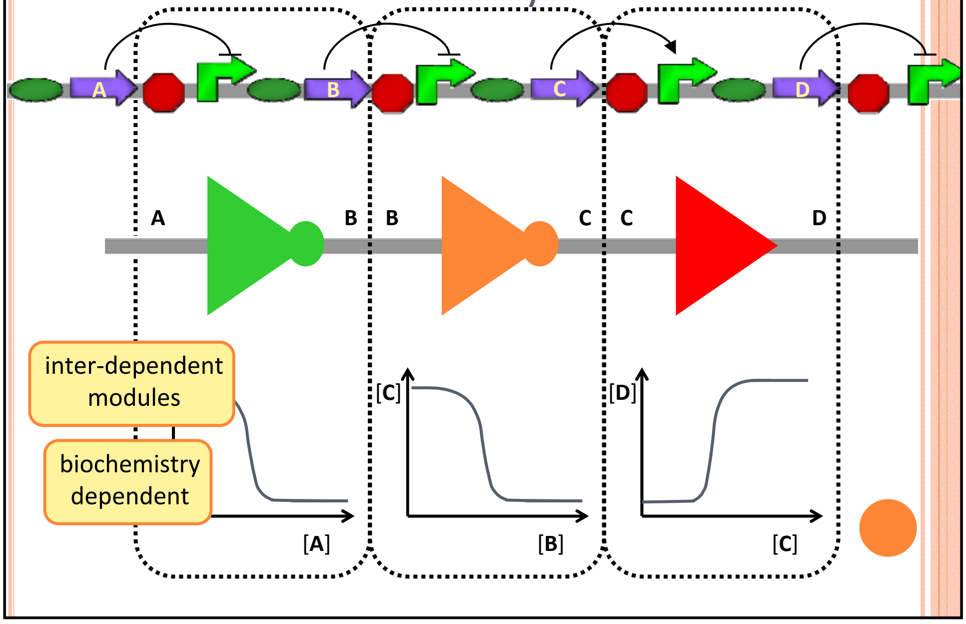
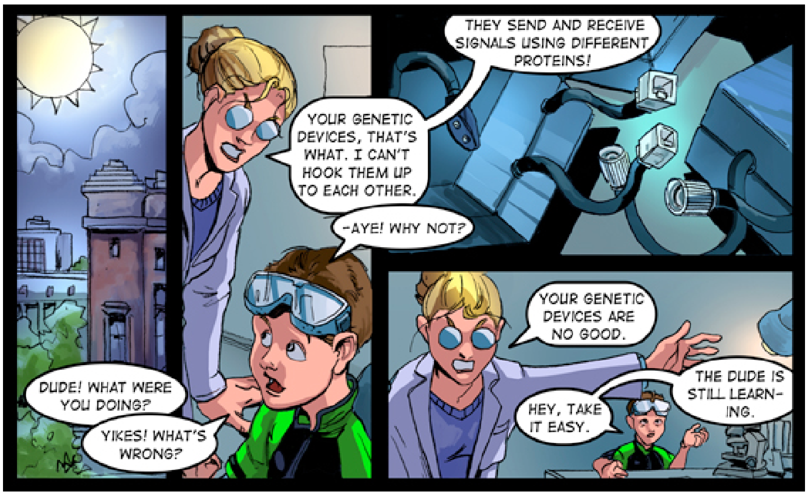
modularity



inter-dependent modules

biochemistry dependent

modularity



Adventures in Synthetic Biology – http://openwetware.org/wiki/Adventures

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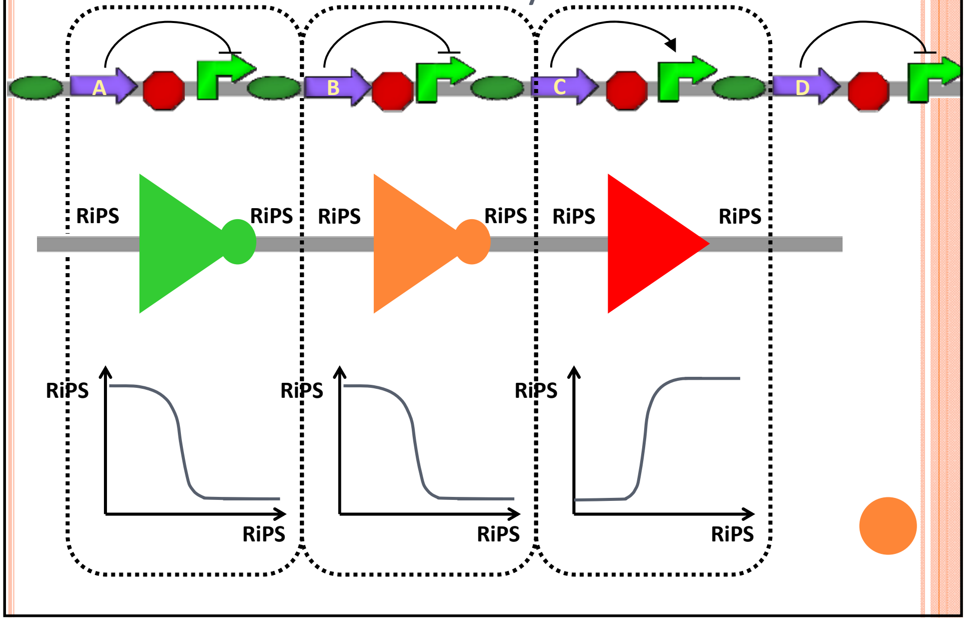
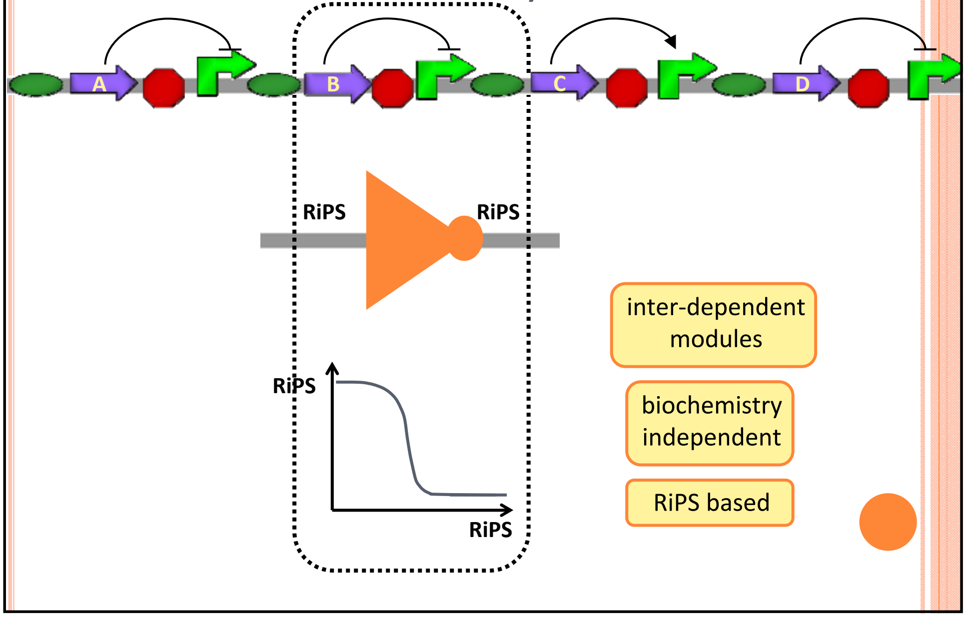
modularity



modularity



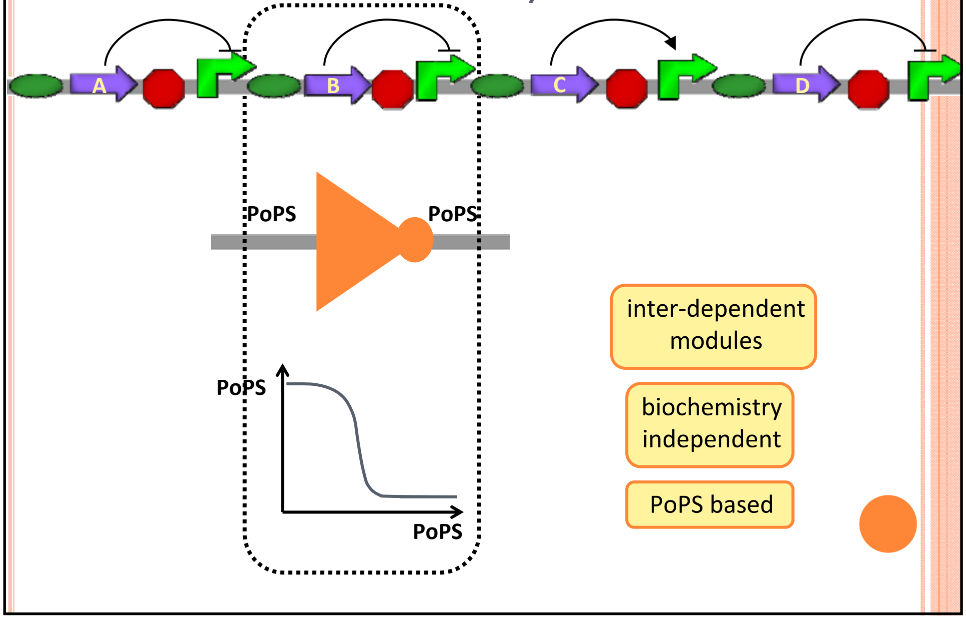
inter-dependent modules

biochemistry independent

RiPS based

---

modularity



inter-dependent modules

biochemistry independent

PoPS based

modularity

Adventures in Synthetic Biology

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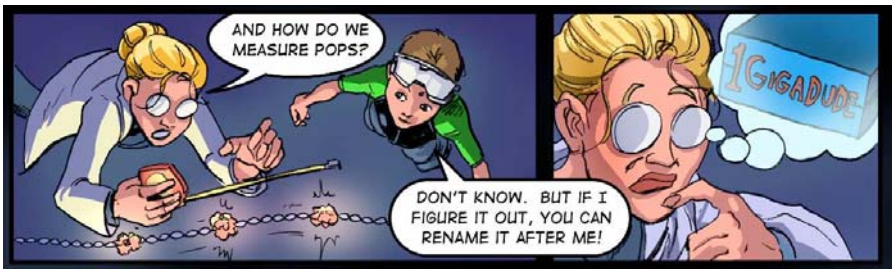
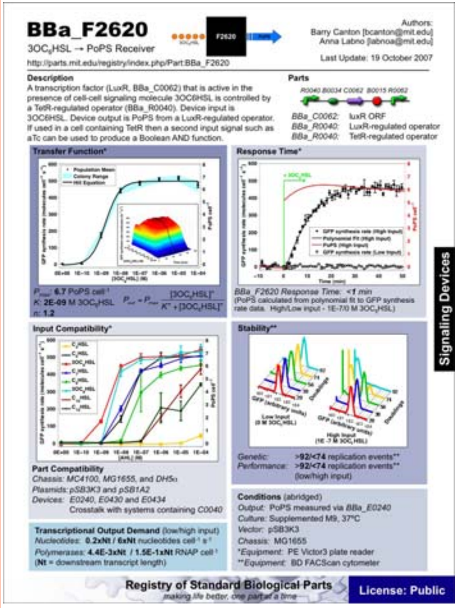
modularity



Adventures in Synthetic Biology

- a question yet to be solved …

looking for abstraction : parts characterization



BBa_F2620

$3OC_6HSL$ → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 19 October 2007

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC6HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC6HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Parts

R0040 B0034 C0062 B0015 R0062

BBa_C0062: luxR ORF
BBa_R0040: LuxR-regulated operator
BBa_R0040: TetR-regulated operator

Transfer Function*

P_{max}: **6.7** PoPS cell^{-1}
K: **2E-09** M $3OC_6HSL$
n: **1.2**

$$P_{out} = P_{max}\frac{[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

Response Time*

BBa_F2620 Response Time: **<1** *min*
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M $3OC_6HSL$)

Input Compatibility*

Stability**

Genetic: **>92/<74** replication events**
Performance: **>92/<74** replication events** (low/high input)

Part Compatibility
Chassis: *MC4100, MG1655, and DH5α*
Plasmids: *pSB3K3 and pSB1A2*
Devices: *E0240, E0430 and E0434*
Crosstalk with systems containing *C0040*

Transcriptional Output Demand (low/high input)
Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
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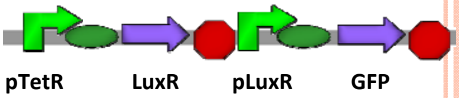
(**Nt** = downstream transcript length)

Conditions (abridged)
Output: PoPS measured via *BBa_E0240*
Culture: Supplemented M9, 37°C
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer



- a good device characterization is
  - the minimum amount of information someone **needs to reuse** the part without any prior knowledge

Barry Canton (Endy's lab)

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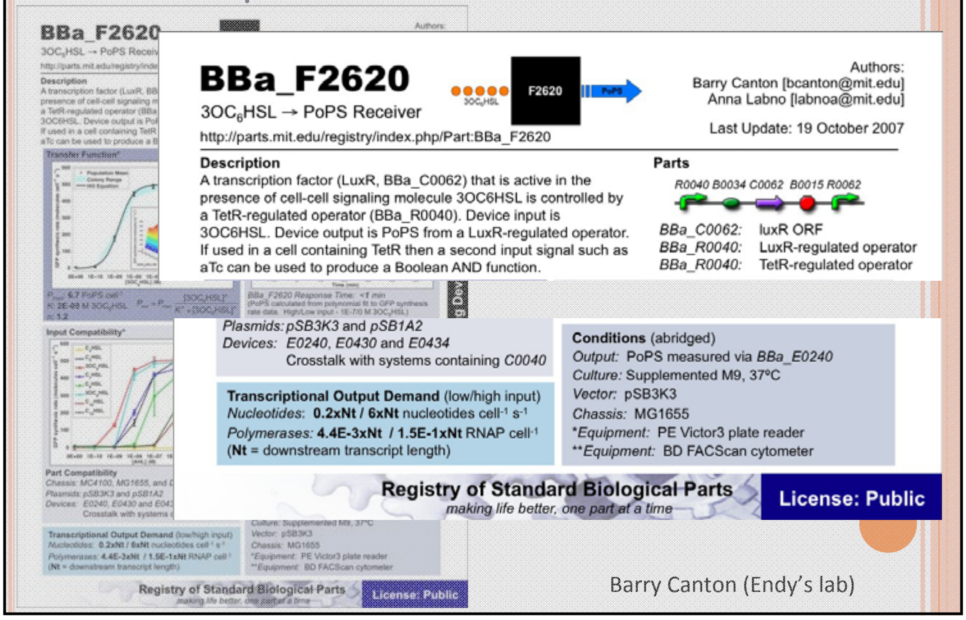
looking for abstraction : parts characterization



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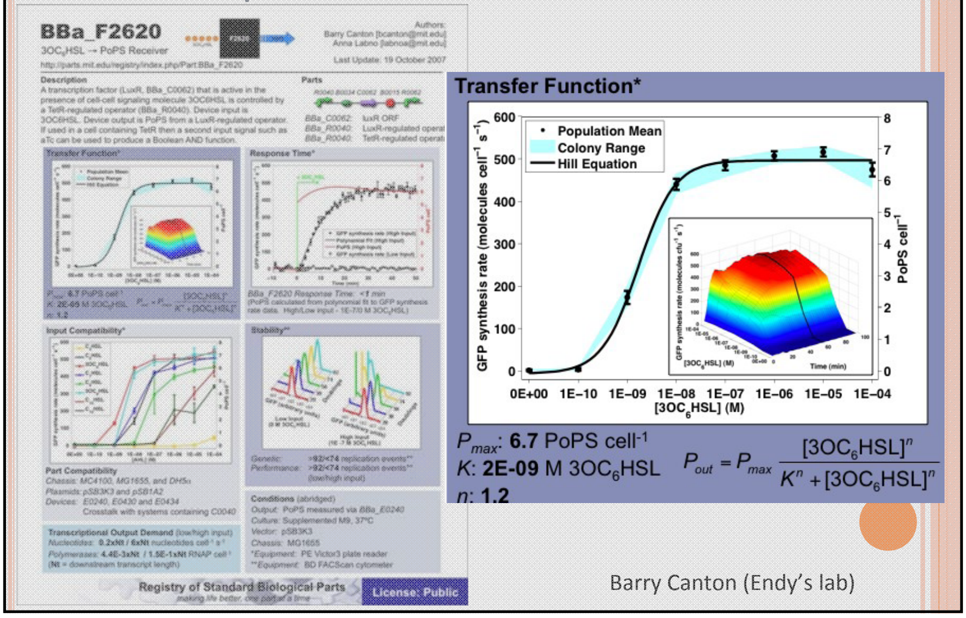
Barry Canton (Endy's lab)

looking for abstraction : parts characterization



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Registry of Standard Biological Parts
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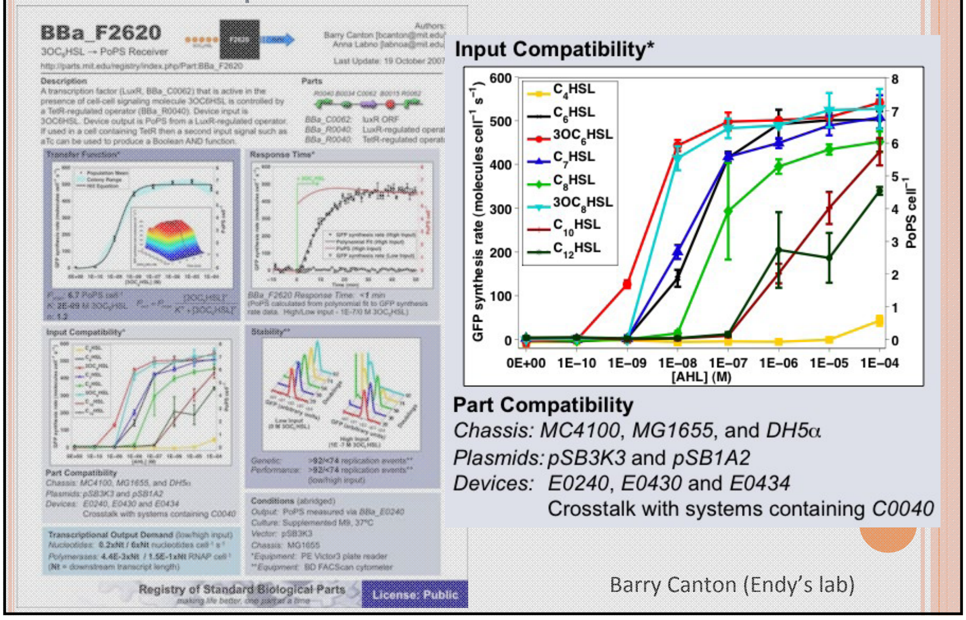
Barry Canton (Endy's lab)

looking for abstraction : parts characterization



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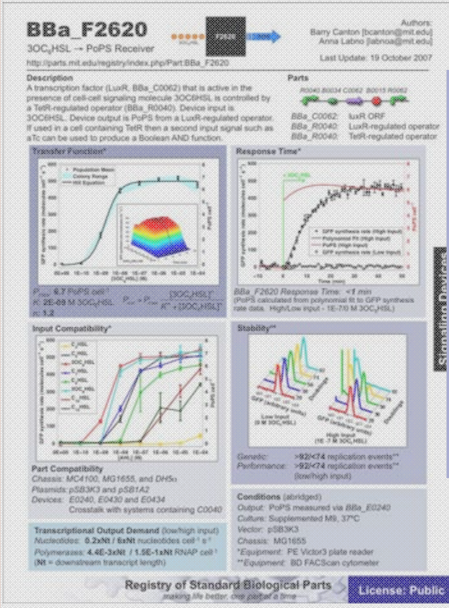
Barry Canton (Endy's lab)

looking for abstraction : parts characterization



BBa_F2620

$3OC_6HSL \rightarrow$ PoPS Receiver

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Signaling Devices

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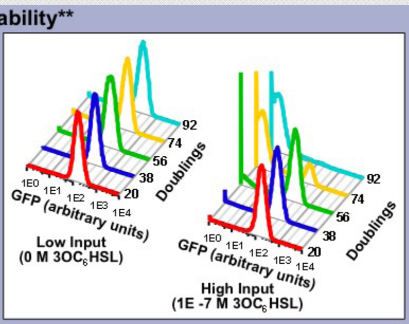
Stability**



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Barry Canton (Endy's lab)

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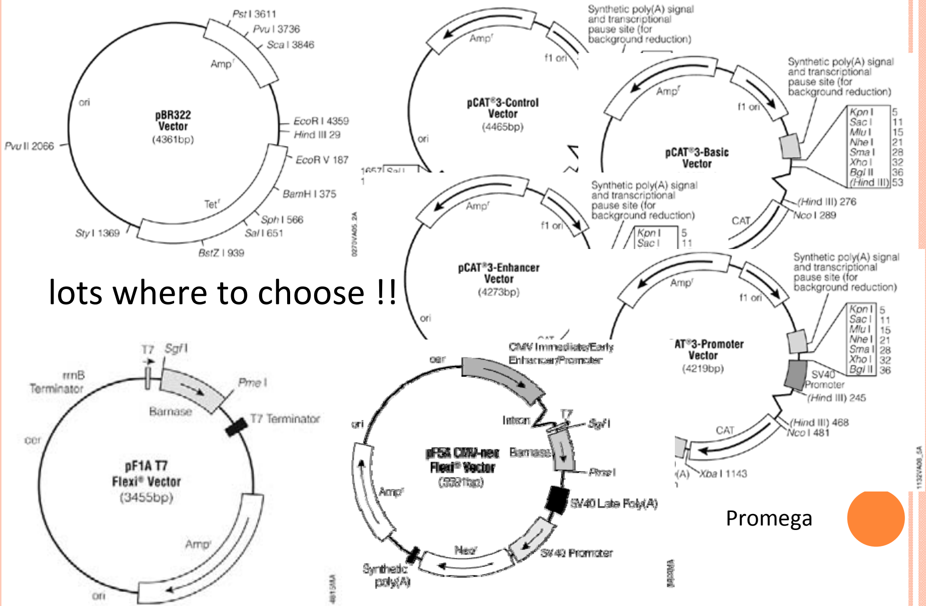
looking for abstraction : plasmids

- expression vector
- bears our parts
- in chassis
  - has to replicate
  - express parts

looking for abstraction : plasmids



lots where to choose !!

Promega

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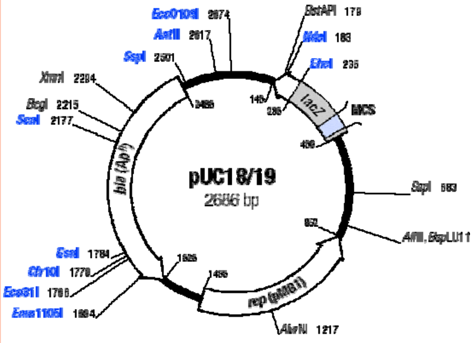
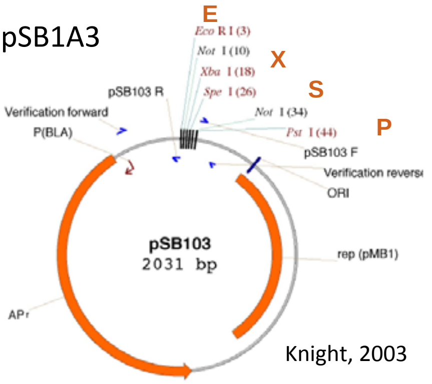
looking for abstraction : plasmids

- pUC19



Fermentas

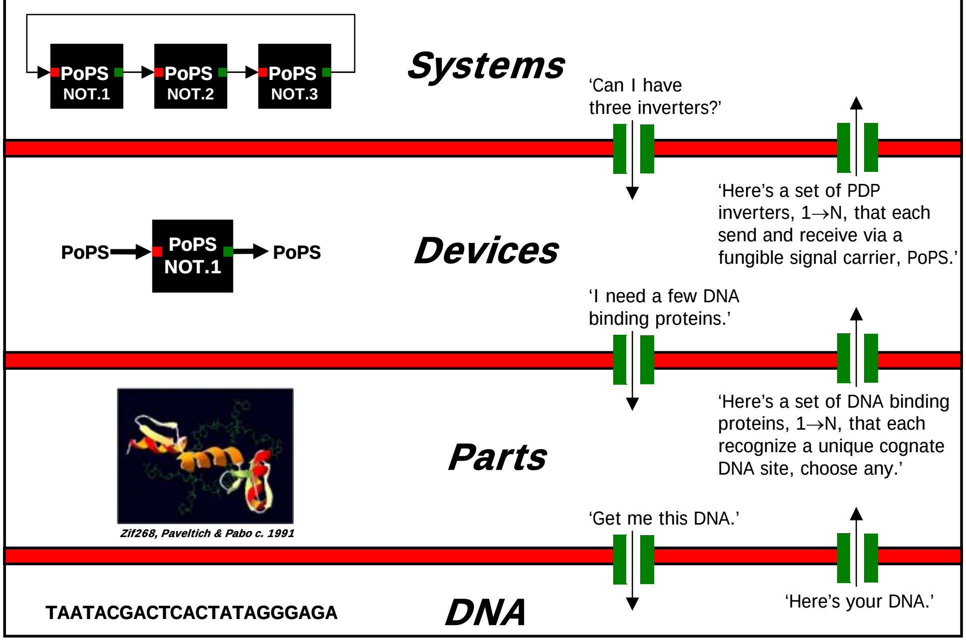
- pSB1A3



Knight, 2003

Figure 4: Structure of the pSB103 vector

Abstraction Hierarchy



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.’

Parts

Zif268, Paveltich & Pabo c. 1991

‘Get me this DNA.’

‘Here’s your DNA.’

DNA

TAATACGACTCACTATAGGGAGA

Parts

- basic biological functions

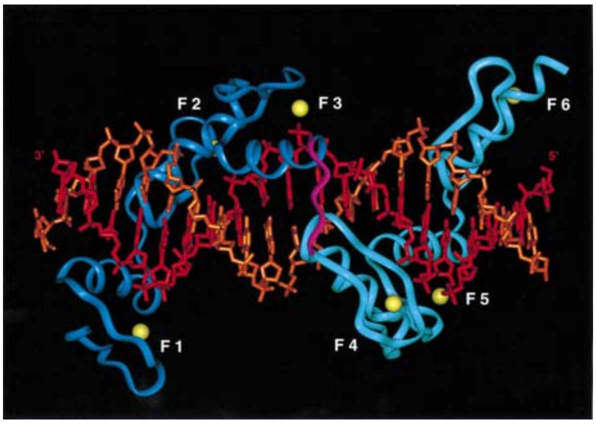
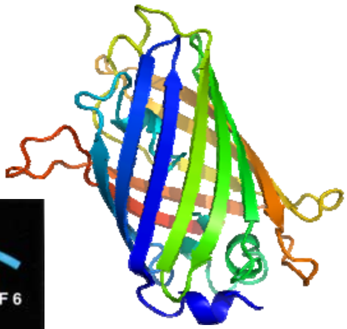


Fig 1. Model of a six zinc-finger–DNA complex.
(Liu *et al*, 1997)



structure of GFP

Devices

- any combination of 'Parts' that perform a human-defined function
- example
  - transcription device
  - sensor device

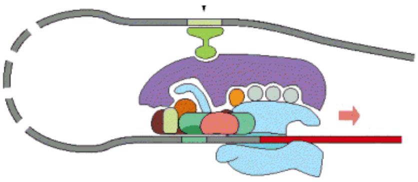
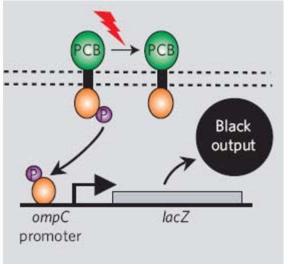
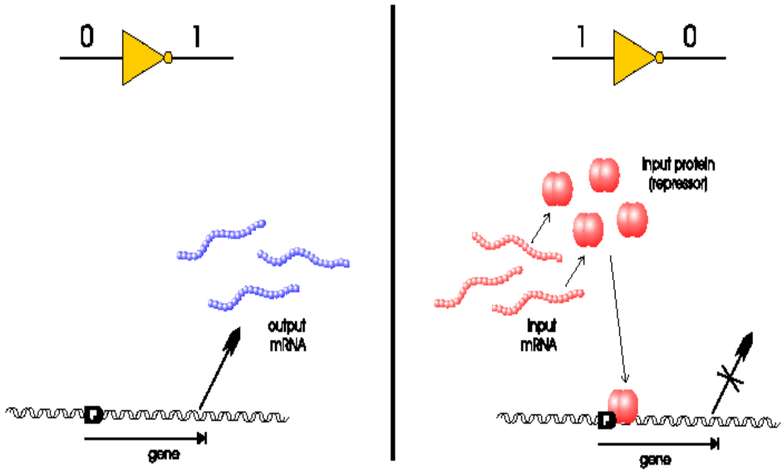


Figure 7-43. (Alberts *et al*, 2002)



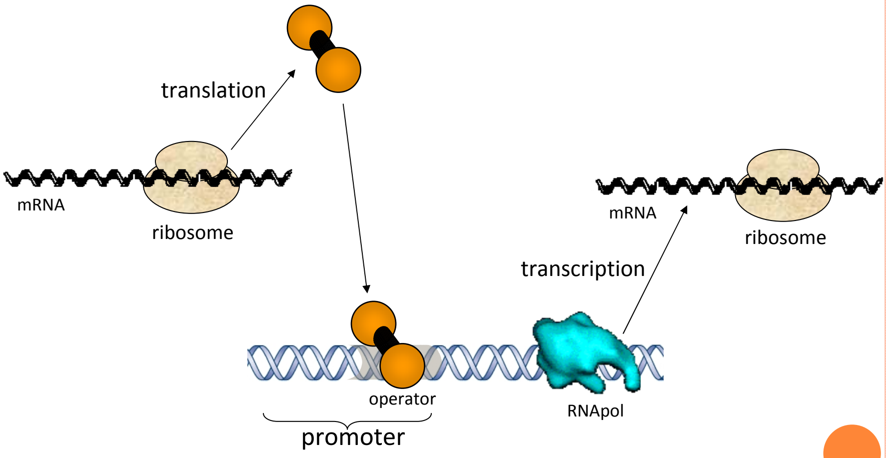
Levskaya *et al.* **Nature**. 2005

Biological Inverter



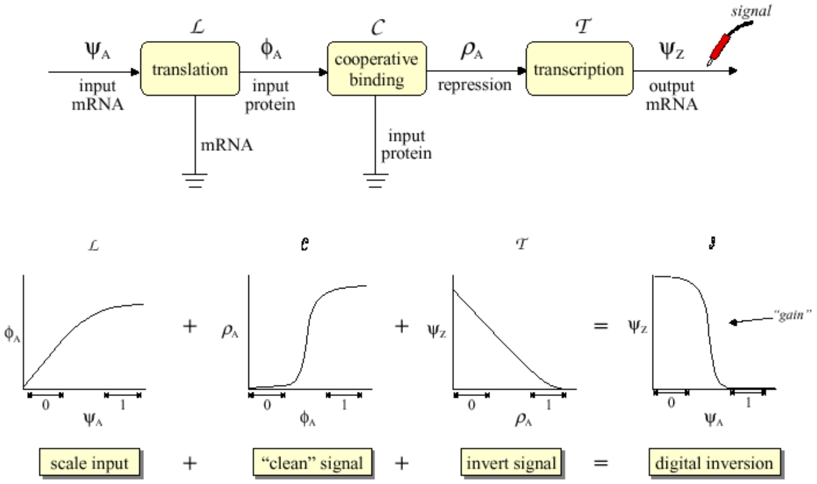
Weiss *et al*. **Oxford University Press.** 2004.

Inverters rely on transcription & translation

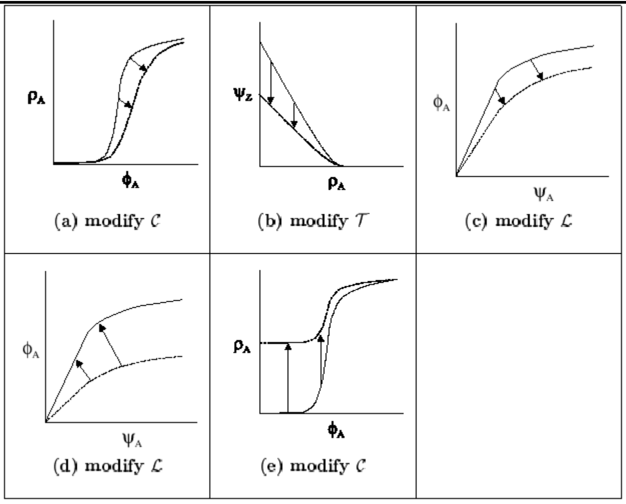


Weiss *et al*. **Oxford University Press.** 2004.

Transfer function



Weiss *et al*. **Oxford University Press.** 2004.



(a) Reducing the repressor-operator binding affinity

(b) Reducing the strength of the promoter

(c) Reducing the strength of the RBS

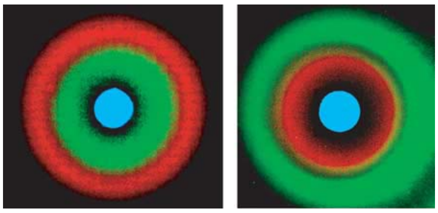
(d) Increasing the translation efficiency

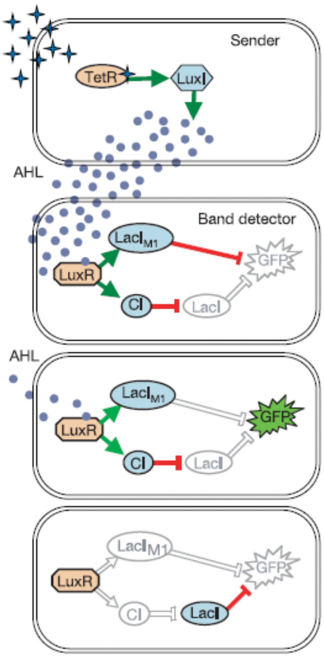
(e) Adding autorepression

Weiss *et al.*
Oxford University Press.
2004.

Systems

- any combination of ‘Devices’
- usually builds up a major level function
- example
  - band detect multicellular system

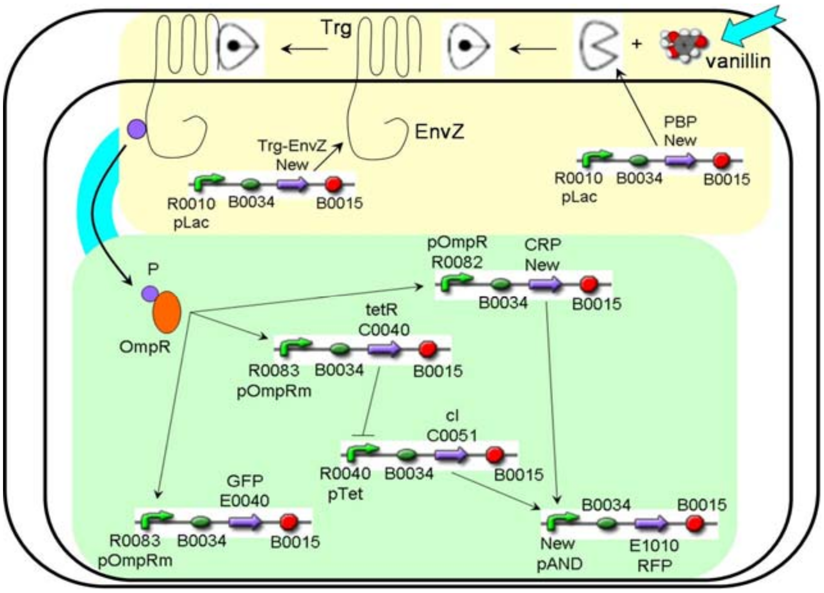




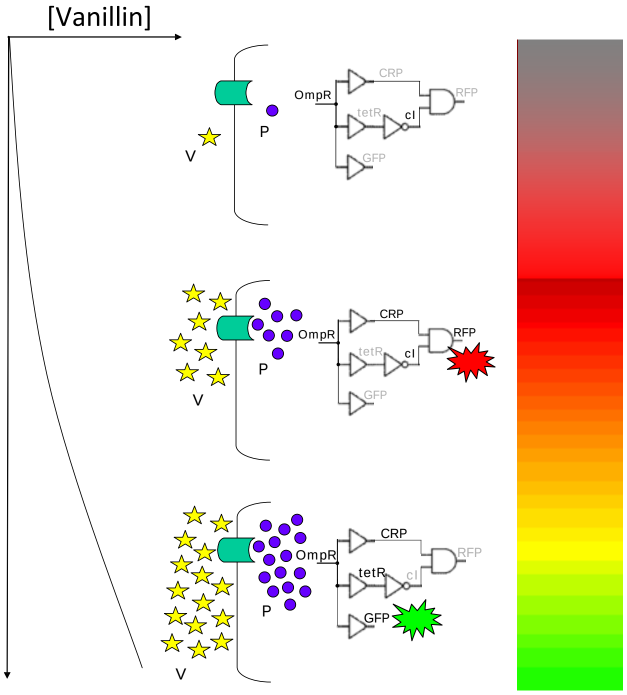
Basu *et al*, **Nature**. 2005

Vanillin Cell Sensor

Rodrigo *et al*, **IET Synthetic Biology**. 2007



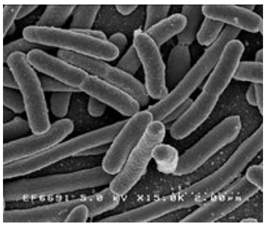
behaviour of the sensor



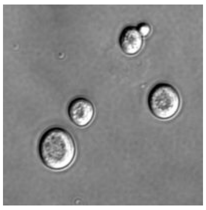
Rodrigo *et al*,
IET Synthetic Biology. 2007

Host

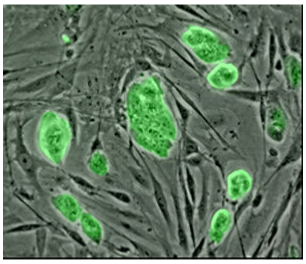
- parts work in a cell, a chassis
  - stability
  - cross-talks
  - cell load



Escherichia coli



Saccharomyces cerevisiae



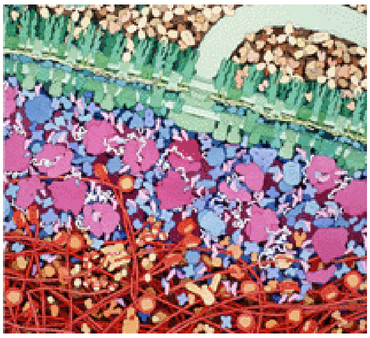
mouse
stem cells

Our Parts aren't necessarily Stable

- parts add load to a cell & reduces its fitness → cells may 'lose' the part
  - mutations : losing a plasmid, alteration of promoters to not work as efficiently (or not at all)
  - antibiotic resistance
- cross-talks
  - parts interact spuriously with some cell part
  - watch out for incompatibilities !

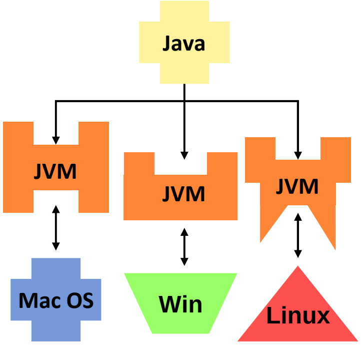
Load

- How many cellular resources does the device use ?
  - dNTPs (DNA replication)
  - rNTPs (RNA Production)
  - Ribosomes (RiPS)
  - Amino Acids (Proteins)
  - ATP for activities



D. S. Goodsell, *The machinery of life,* **Springer**, 1993

Dealing with Load



- Need engineered chassis
  - towards a Java Virtual Machine …
  - reduced genome organisms
    - Mycoplasma genitalium
    - Buchnera sp.
  - eliminate key components
    - recombinases
    - create dependencies
    - unnecessary parts (refactoring genome ?)
  - ortogonal transcription
    - T7 promoters & T7 polymerase

tools

- Zinc Finger Tool
  - http://www.zincfingertools.org
- Gene design
  - http://slam.bs.jhmi.edu/gd/index.html
- Gene Designer –software from DNA2.0
  - http://www.dna20.com/tools.php

Cambridge Dictionaries Online

- **standard**, (USUAL) adjective
  1 **usual rather than special, especially when thought of as being correct or acceptable**:
  These are standard procedures for handling radioactive waste.
  The metre is the standard unit for measuring length in the SI system.

  2 Language described as standard is the form of that language which is considered acceptable and correct by most educated users of it:
  Most announcers on the BBC speak standard English.

  3 [before noun] A standard book or writer is the one that is most commonly read for information on a particular subject:
  Her book is still a standard text in archaeology, even though it was written more than twenty years ago.

- **standard**, noun
  1 [C] a song or other piece of music which has been popular and often played over a long period of time

  2 [C usually singular] **a pattern or model that is generally accepted**:
  This program is an industry standard for computers.

  3 [C] US a car with gears that are changed by hand

- **standardize**, UK USUALLY standardise, verb
  to make things of the same type all have the same basic features:
  We standardize parts such as rear-view mirrors, so that one type will fit any model of car we make.

sources

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- www.ergito.com
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