L5 Part Libraries and Qualitative Properties

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Recall…

Parts, devices and systems can be assembled in modular fashion with designed input/output for a function

Chassis → **Systems** → **Devices** → **Parts** → **DNA**

What properties must be true for a design to "work" as intended?

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This lecture….

- Recap IO relationship and physical design
- Properties required for function
- Part libraries

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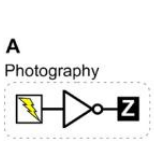
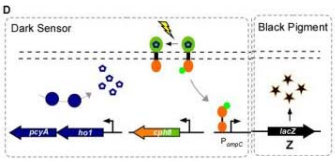
Bacterial Photography

If no light, produce pigment

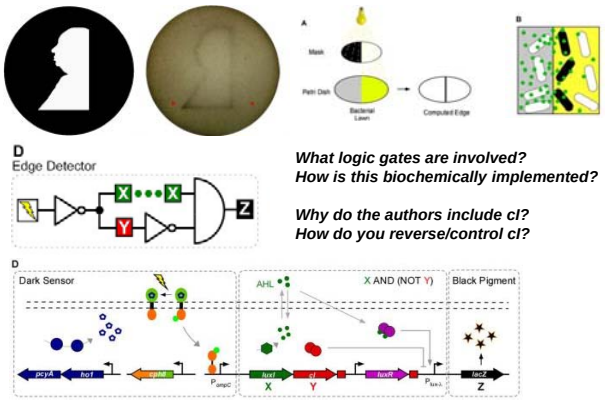
What logic gates are involved?
How is this biochemically implemented?





Tabor et al, *Cell*, 2009

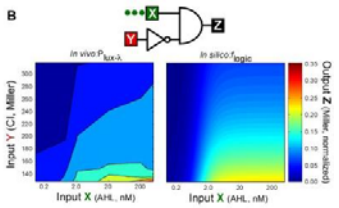
Edge Detection



Tabor et al, *Cell*, 2009

cI added to tune system properties

- cI repressor prevents **leaky** (non-zero background) expression, negates effects of low concentrations of AHL
- System is more sensitive to AHL (log vs linear scales)
- luxI is catalytic, AHL will accumulate and ultimately overpower cI



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To ensure proper functioning, parts must be

1. Impedance matched
   - IO ranges of adjacent parts must be compatible for maximum signal transfer
2. Independent
   - Parts that perform the same distinct function in isolation and in interaction with other systems. These are typically *reliable* and can be combined with other systems (*composable*)
3. Orthogonal
   - Parts that do not cross talk or cross react

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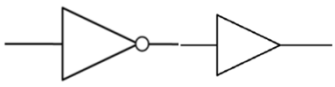
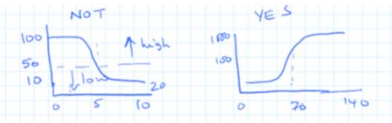
Impedance matching





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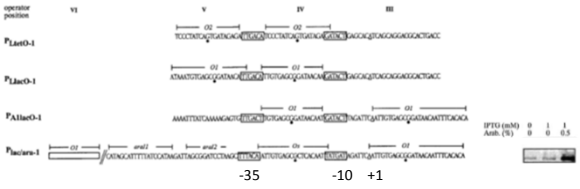
Independence

Parts have distinct functions that allow use in multiple contexts without interaction

- E.g. LacO (O1), TetO (O2), and AraO (ara1/ara2) are distinct
- P_{lac}/lacI, P_{BAD}/AraC, P_{tet}/TetR can be used simultaneously or combined to make chimeric promoters (***composable***)
- Components don't compete for binding across syste/don't titrate components (fixed IO, ***reliable***)



Lutz and Bujard, NAR, 1997

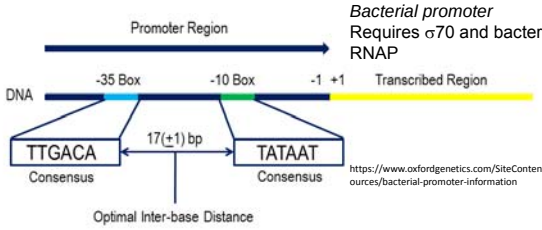
Orthogonality

Parts do not cross-talk or cross react

T7 Promoter (~18 bps) – requires T7 RNAP

5′ TAATACGACTCACTATAG 3′

Bacterial promoter
Requires σ70 and bacterial RNAP



https://www.oxfordgenetics.com/SiteContent/TeamResources/bacterial-promoter-information

Part libraries

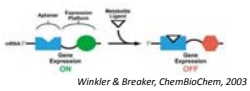
We enforce these properties and create a variety of input/output relationships with engineered part libraries (see http://parts.igem.org)

- Promoter engineering
- Machinery from different organisms (e.g. T7, tetON/tetOFF)
- Directed evolution

Apart from canonical parts (promoters, etc), libraries come in other forms such as RNA, RNAP, ribosomes, etc

RNA Parts

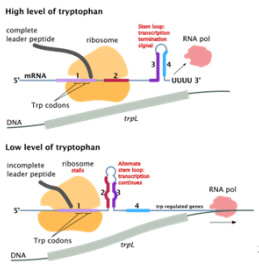
are specific to individual genes (orthogonal) as they rely on RNA:RNA intxns



Winkler & Breaker, ChemBioChem, 2003

Operate in:

i) *Cis* – gene & regulatory RNA are on same transcript
e.g. B12 riboswtiches
Transcriptional attentuation



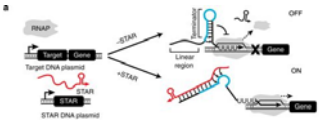
Wikipedia.org

RNA parts

Operate in:

ii) Trans - gene and regulatory RNA on separate transcripts

e.g. siRNA, trans activating RNA, antisense RNA



Chappell et al, Nature Comm, 2017.

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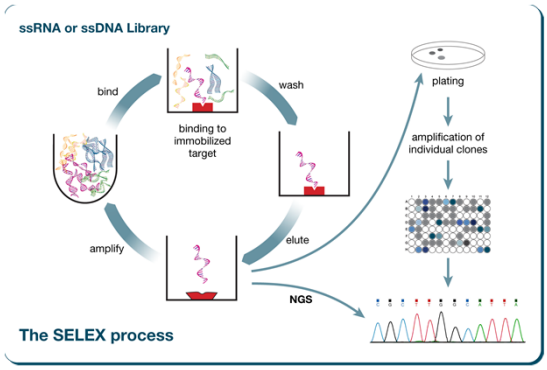
Selection & Directed Evolution can be used to make RNA parts that bind (SELEX – systematic evolution of ligands by exponential enrichment)



https://ngsdataanalysis.com/technology/selexaptamer/

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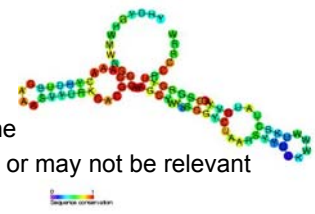
RNA interactions can be modeled

Computational design can be used to predict RNA properties via thermodynamics

- Vienna, NuPACK, Mfold
  Determine minimum free energy structure and/or likeliest structure



These tools only predict the final structure, which may or may not be relevant

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Genome mining

Can look to nature for natural solutions

e.g. B12 swtich – can reuse that aptamer in other contexts

All these approaches (selection and directed evolution, computational design, and mining) can be used for **any** type of part library

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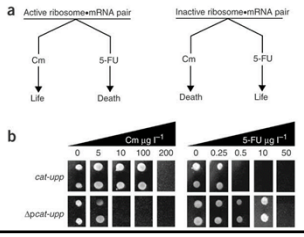
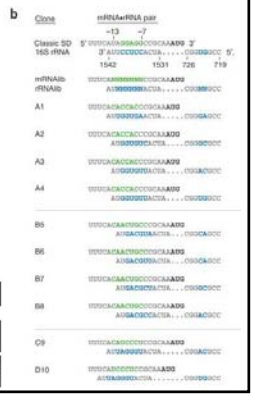
Orthogonal ribosomes

- Ribosomes that recognize non-canonical RBS
- +ve and –ve screens used to identify functional mutants via directed evolution
- O-ribosomes are independent, and can't be titrated away (fixed I/O)

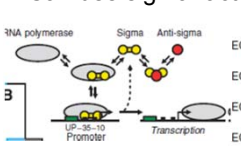


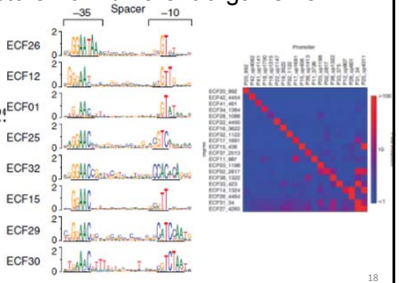


Rackham & Chin, *Nat Chem Biol, 2005*

Orthogonal transcriptional machinery

- Use viral trxn machinery that is host independent (e.g. T7) – no titration
- Can use sigma factors from different organisms





Rhodius et al, *MSB*, 2013

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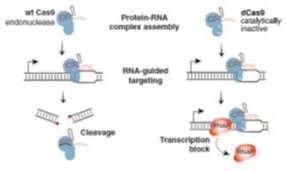
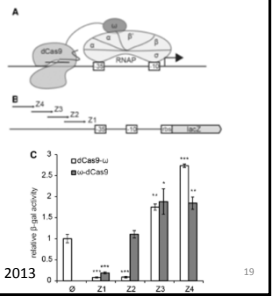
CRISPRi/a

RNA guided technologies can be used to target individual genes for expression through presence/absence of guide (orthogonality)



CRISPRi
Qi et al, Cell, 2013



CRISPRa
Bikard et al, NAR, 2013

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Next time….

- What are the *quantitative* properties that are needed for function?

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