Engineering Complex Behaviors in Biological Organisms

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University of Iowa
December, 2015

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BBN Technologies

Vision: WYSIWYG Organism Engineering

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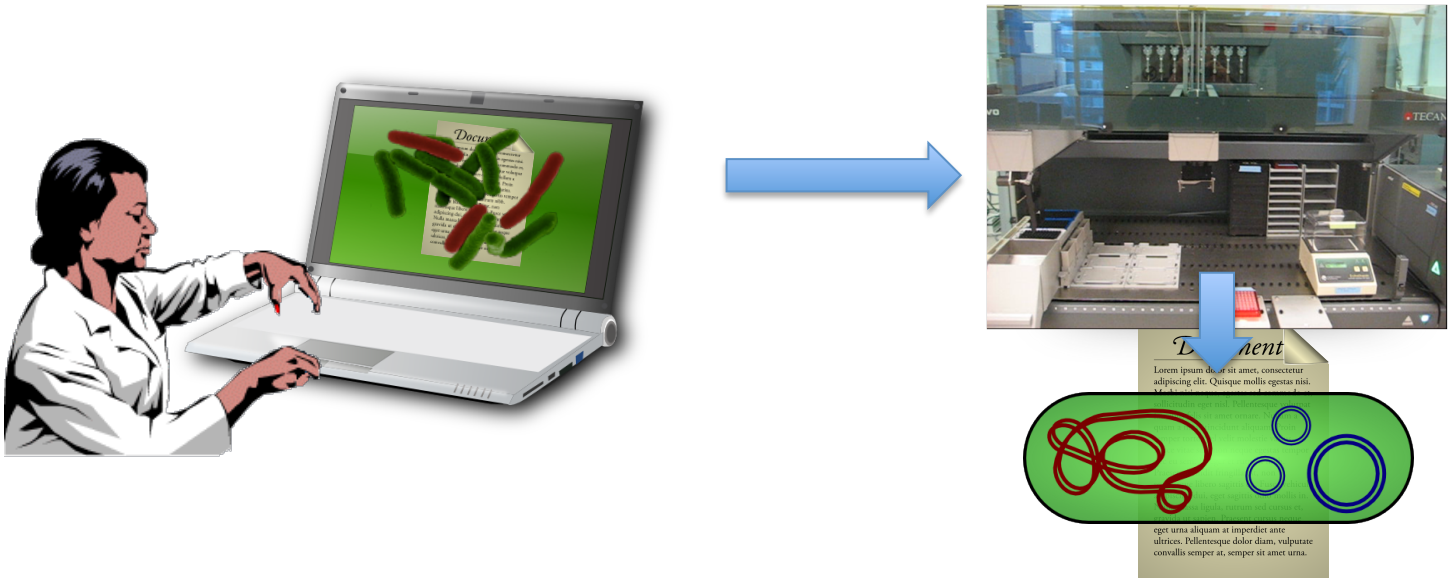
Bioengineering should be like document preparation:



Focus: Genetic Circuits

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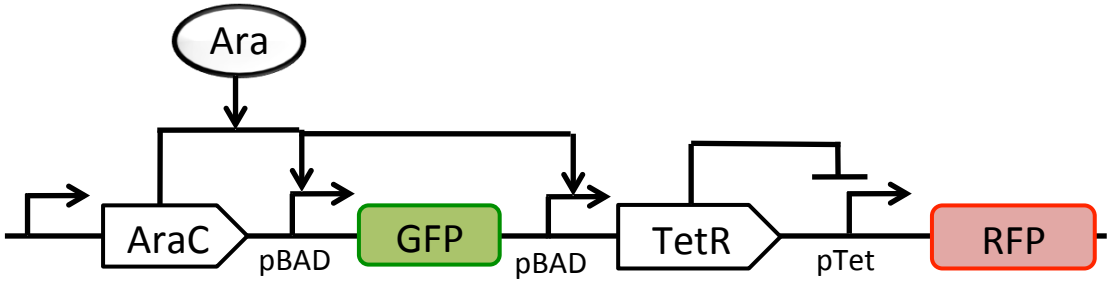
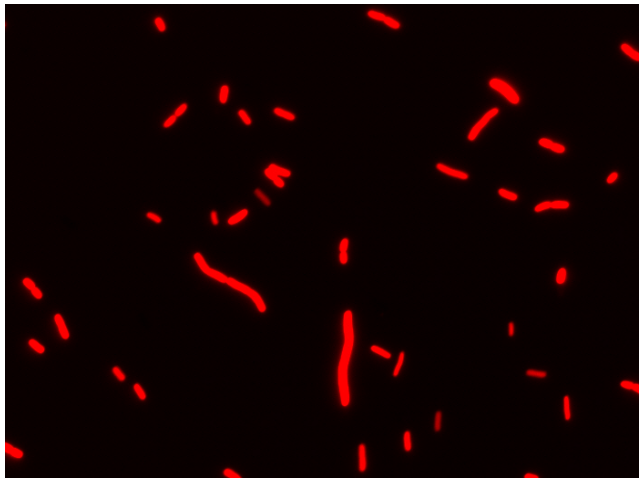
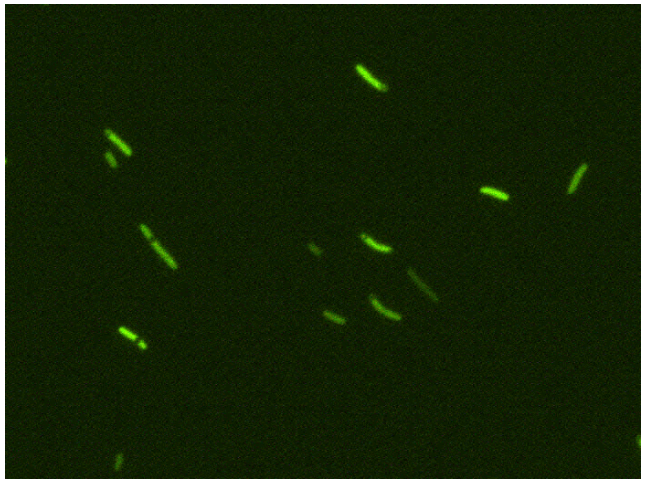


No Arabinose



High Dose Arabinose



Example genetic circuit applications

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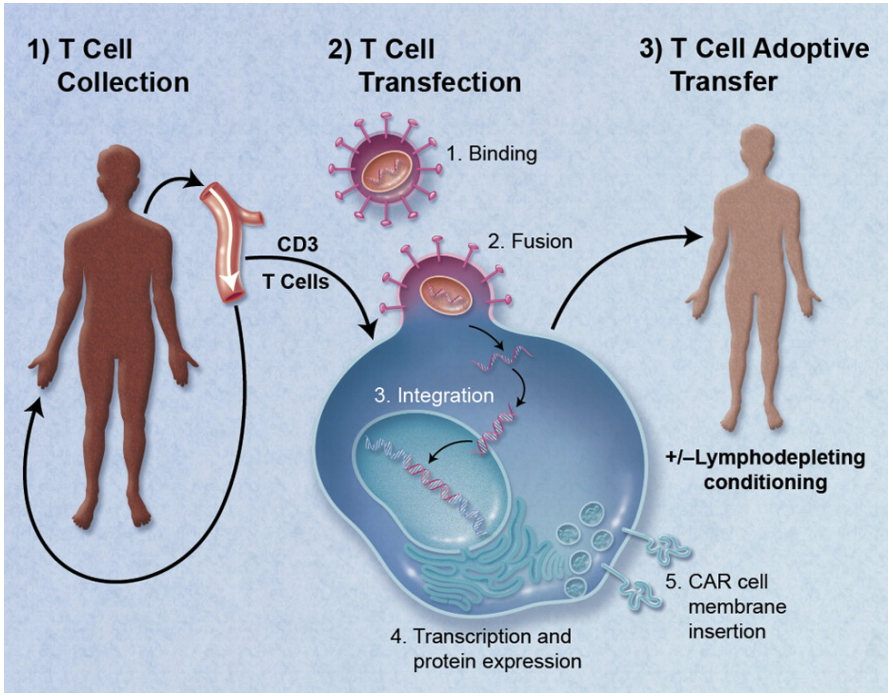
Fermentation control

CAR T-cell Therapy



High-Level Genetic Circuit Design

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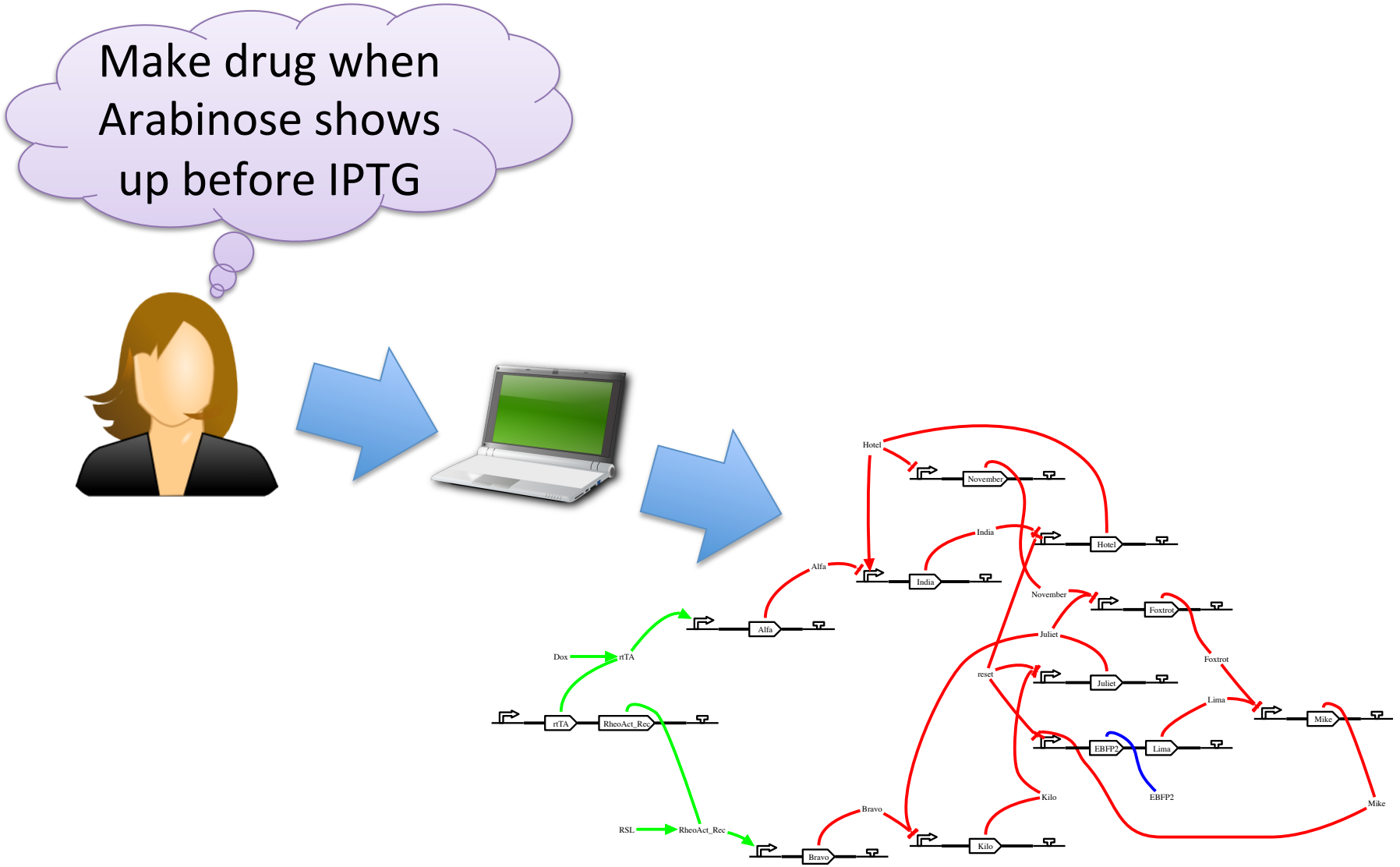

Why is this important?

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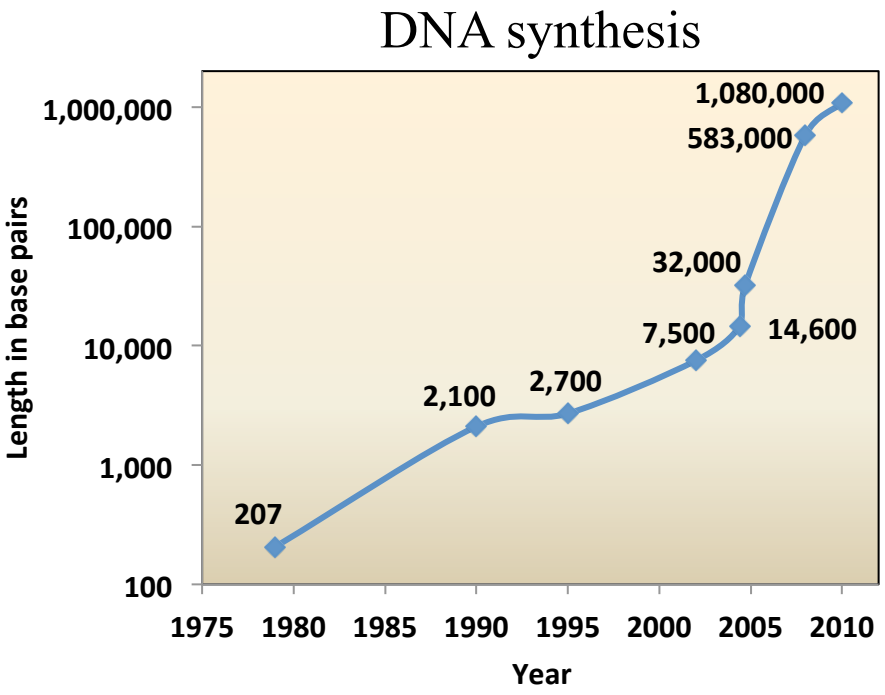
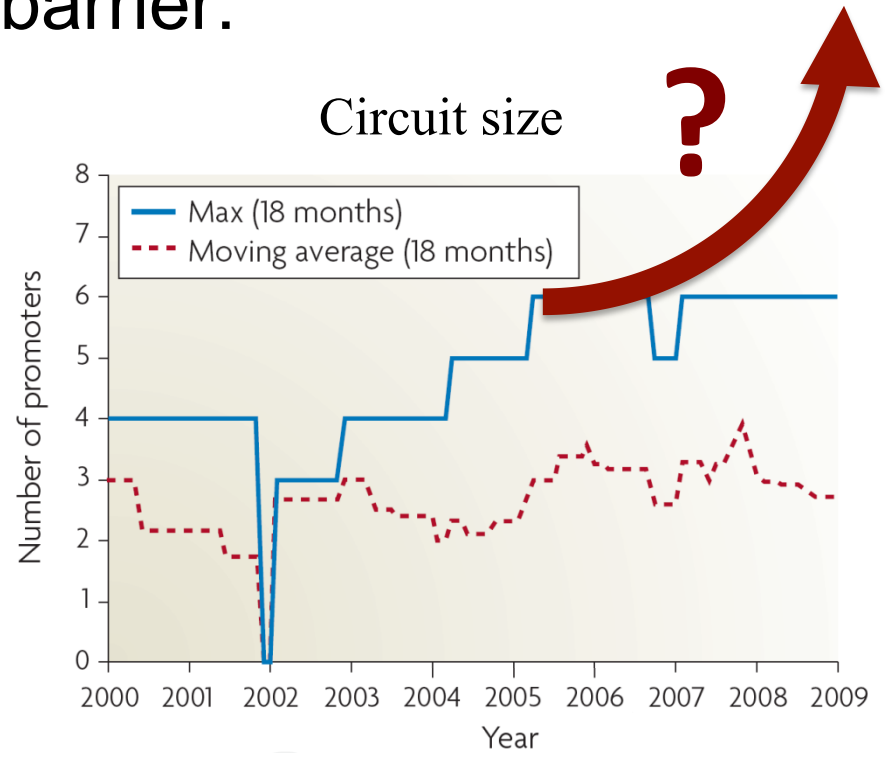
- Breaking the complexity barrier:





[Purnick & Weiss, '09]

- Multiplication of research impact
- Reduction of barriers to entry

*Sampling of systems in publications with experimental circuits

Why a tool-chain?

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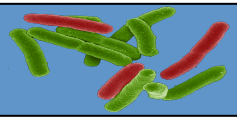
Organism Level Description

This gap is too big to cross with a single method!

Cells



TASBE tool-chain

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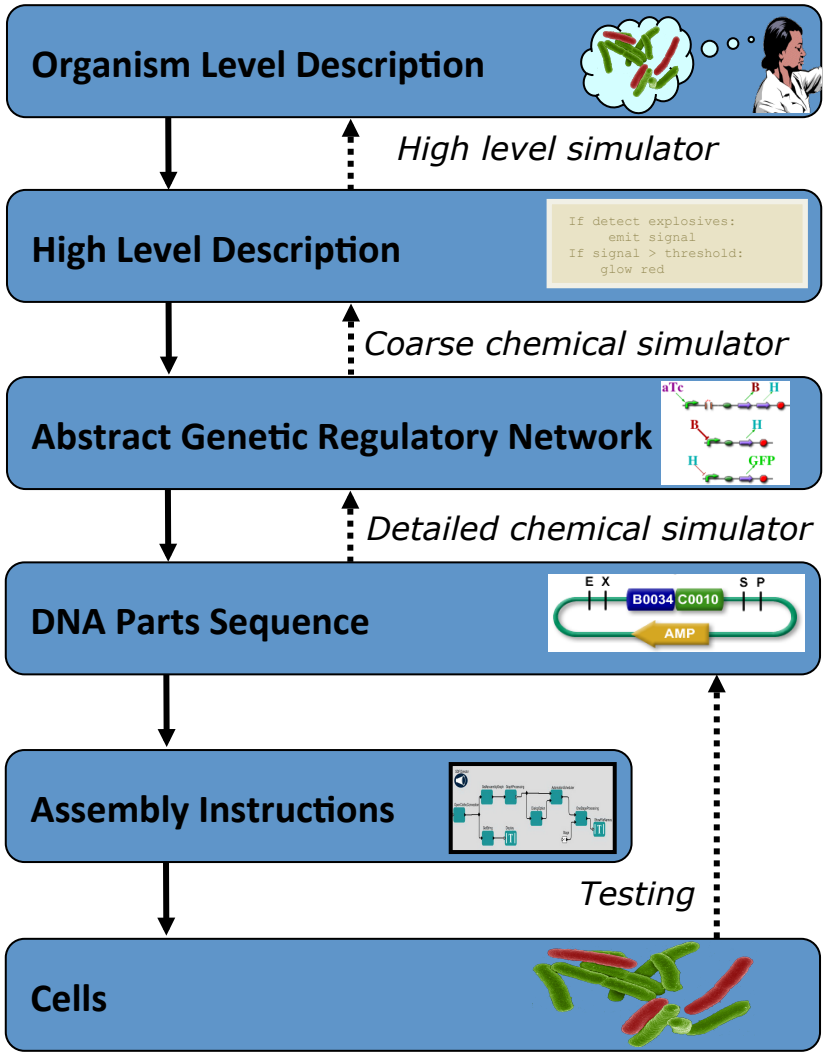

Collaborators:

Ron Weiss

Douglas Densmore

Modular architecture also open for flexible choice of organisms, protocols, methods, …

[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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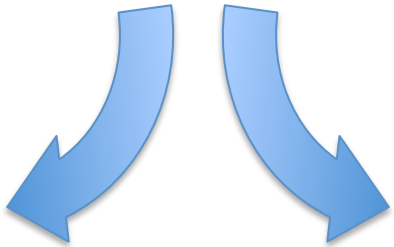
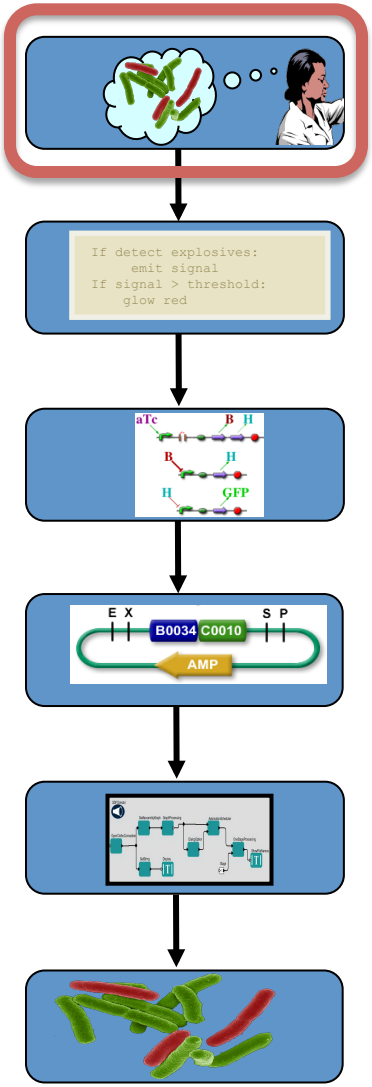
A high-level program of a system that reacts depending on sensor output

```
(def simple-sensor-actuator ()
  (let ((x (test-sensor)))
    (debug x)
    (debug-2 (not x))))
```



Mammalian Target

E. coli Target



[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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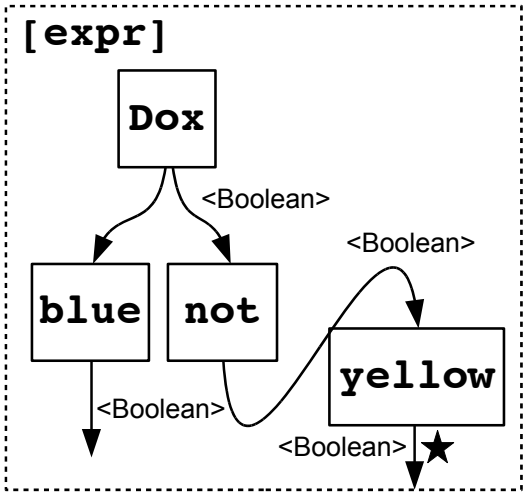
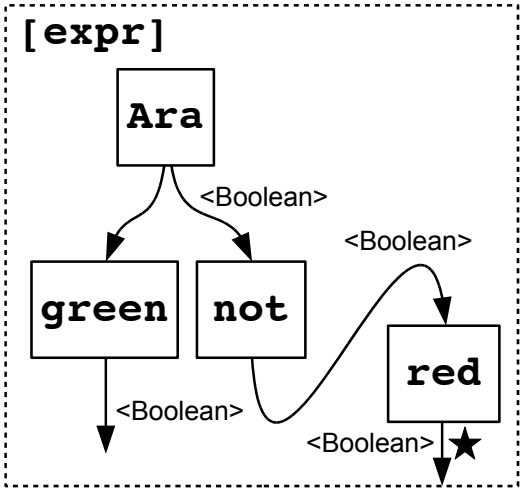
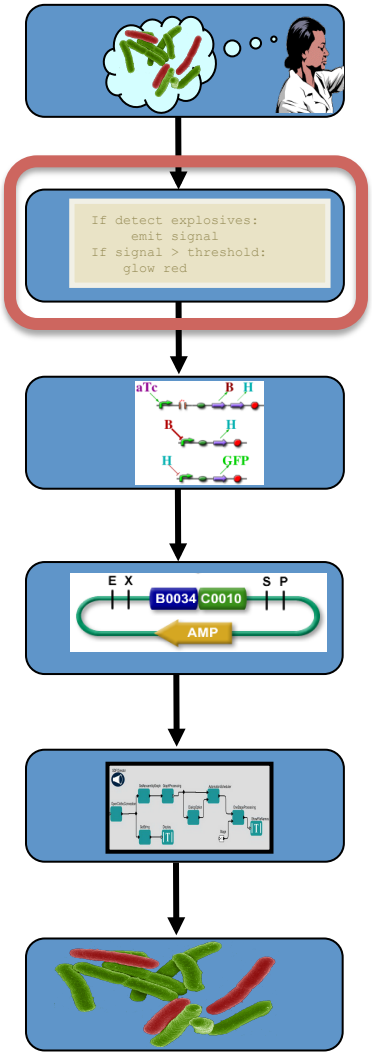
Program instantiated for two target platforms





Mammalian Target

E. coli Target



[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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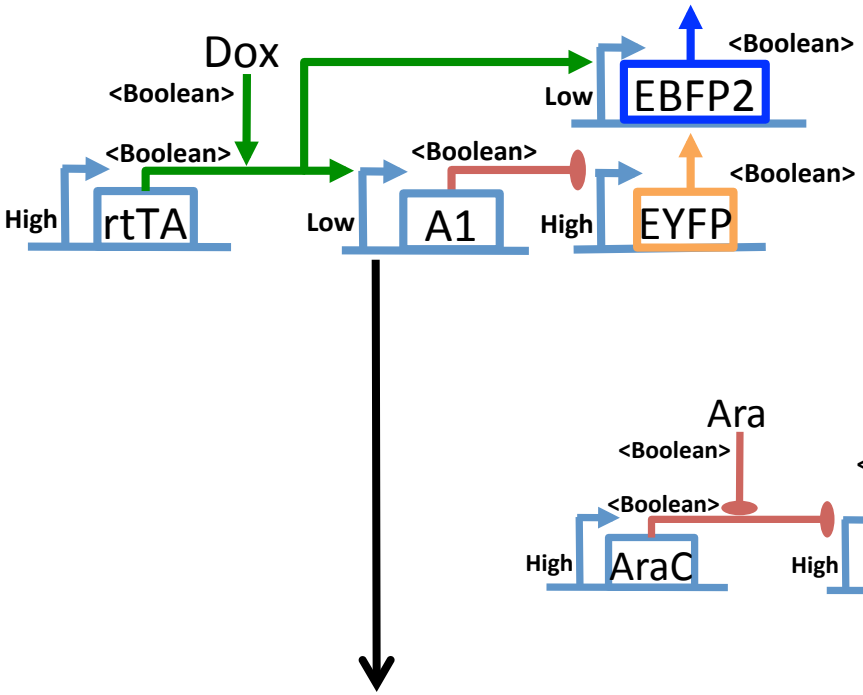
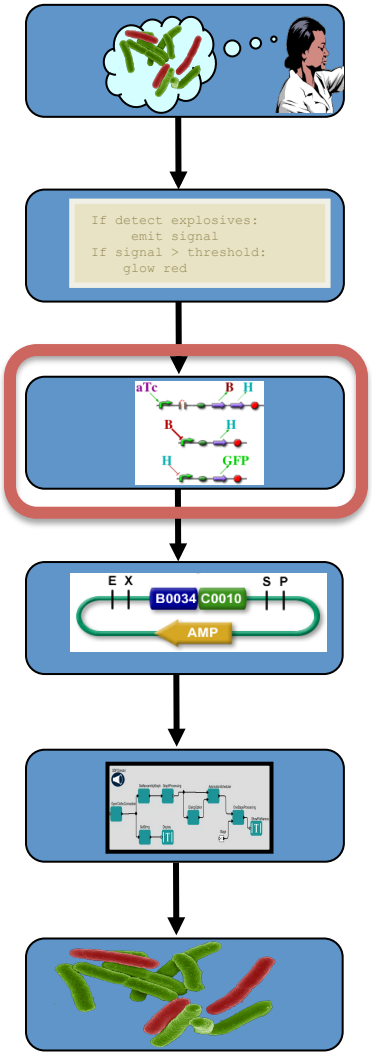
Abstract genetic regulatory networks





[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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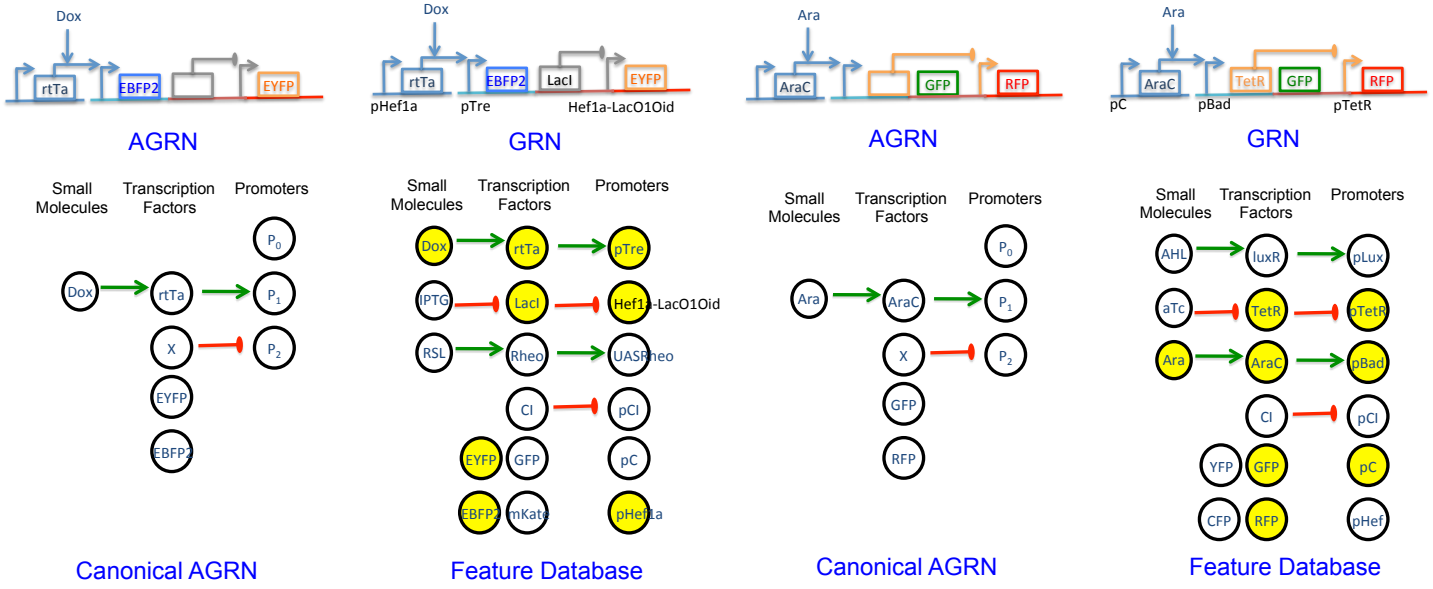
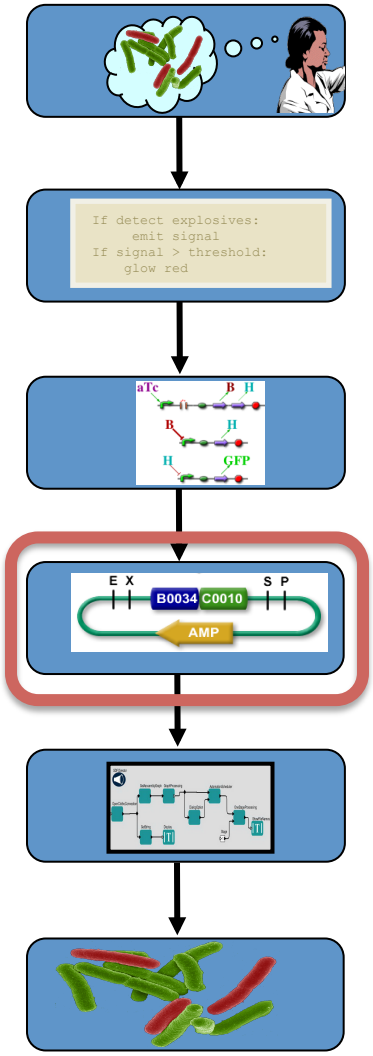
Automated part selection using database of known part behaviors





Mammalian Target

E. coli Target

[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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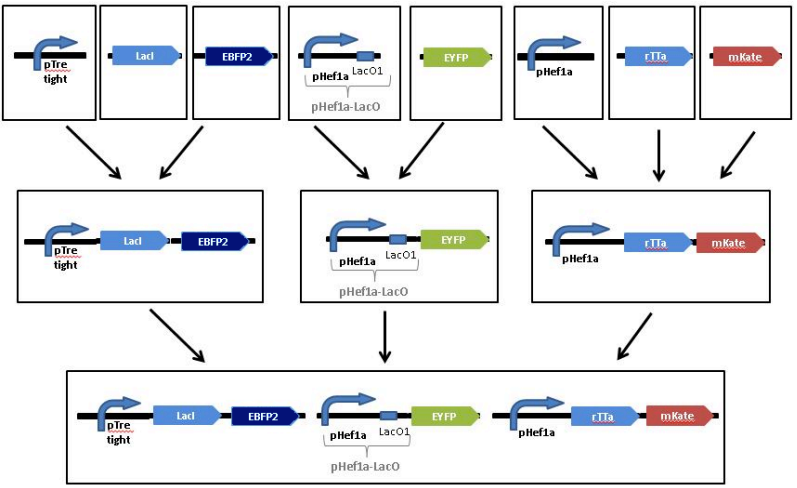
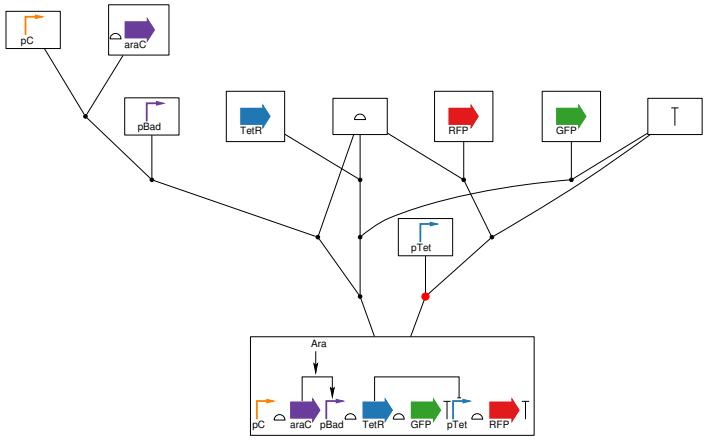
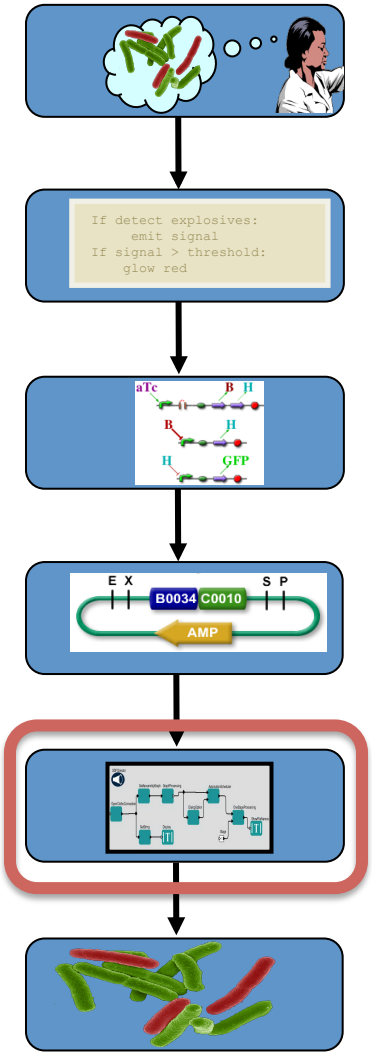
Automated assembly step selection for two different platform-specific assembly protocols







Mammalian Target

E. coli Target

[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

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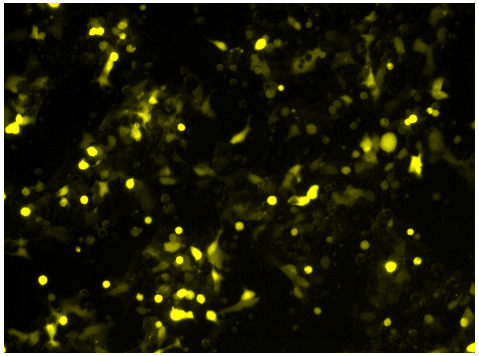
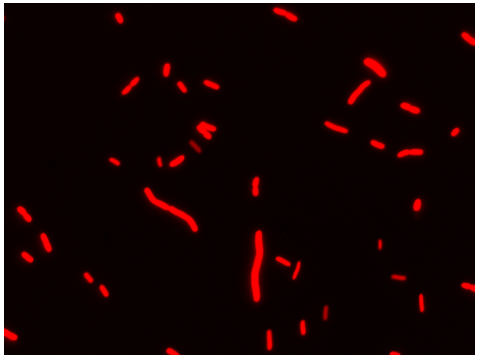
Resulting cells demonstrating expected behavior

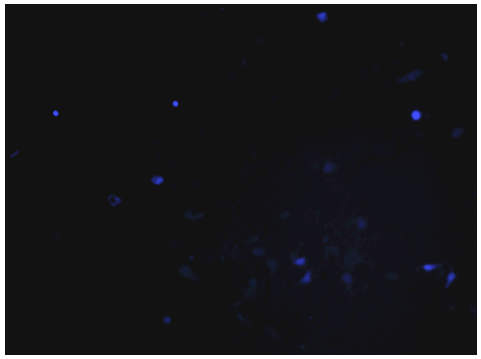
Uninduced



Uninduced

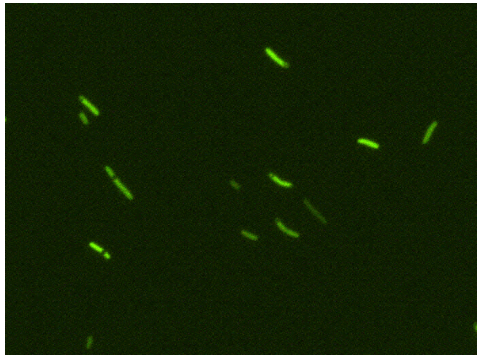


Induced

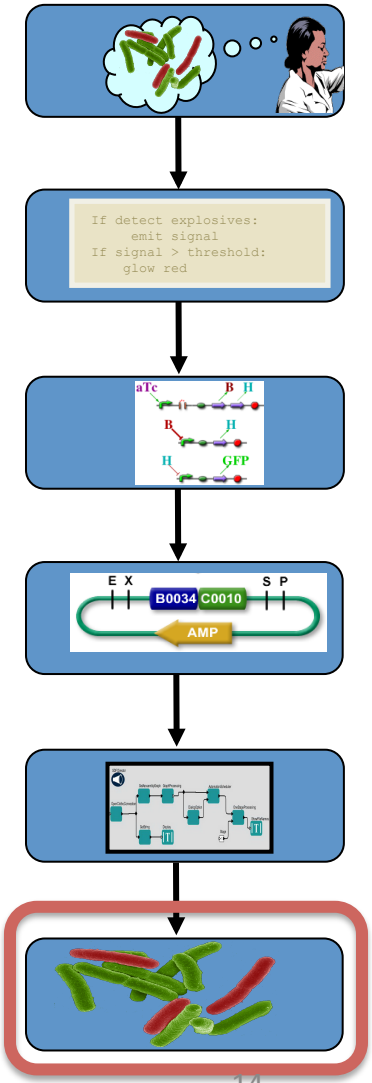


Mammalian Target

Induced



E. coli Target



[Beal et al, ACS Syn. Bio. 2012]

Synthetic Biology Open Language

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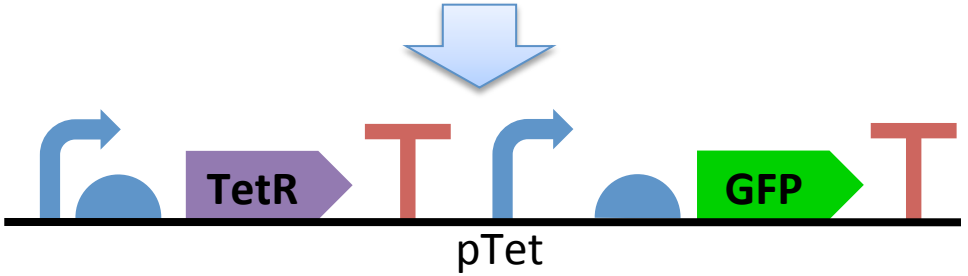
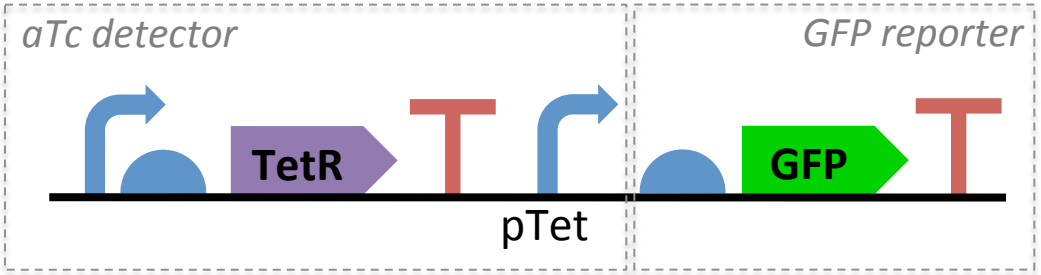
FASTA

ACTGTGCCGTTAAACGTGATTAAATCCGTACTGATAT…

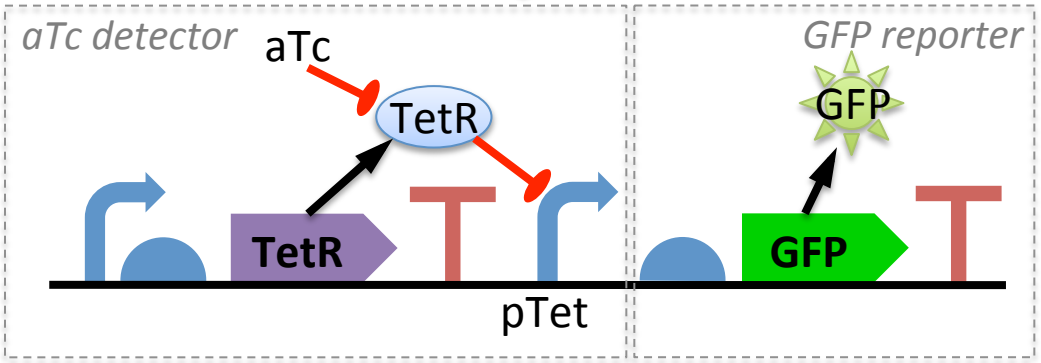
GenBank



SBOL 1.1



SBOL 2.0



SBOL supports bio-design interchange

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Lots of different synthetic biology resources…

Repositories & Databases

NCBI GenBank

iGEM

DICOM Digital Imaging and Communications in Medicine

Automation & Integration

Lab Automation

LIMS

HT Assays

SBOL

Modeling

SBML

SO

BioPAX

ChEBI

BioModels.Net

Sequencing & Synthesis

DNA 2.0 IDT Integrated DNA Technologies Gen9 SGIDNA A Synthetic Genomics, Inc. Company

Emerging Approaches

Measurement Data

Strain Data

… SBOL is a "hub" for linking them together

High-Level Design: BioCompiler

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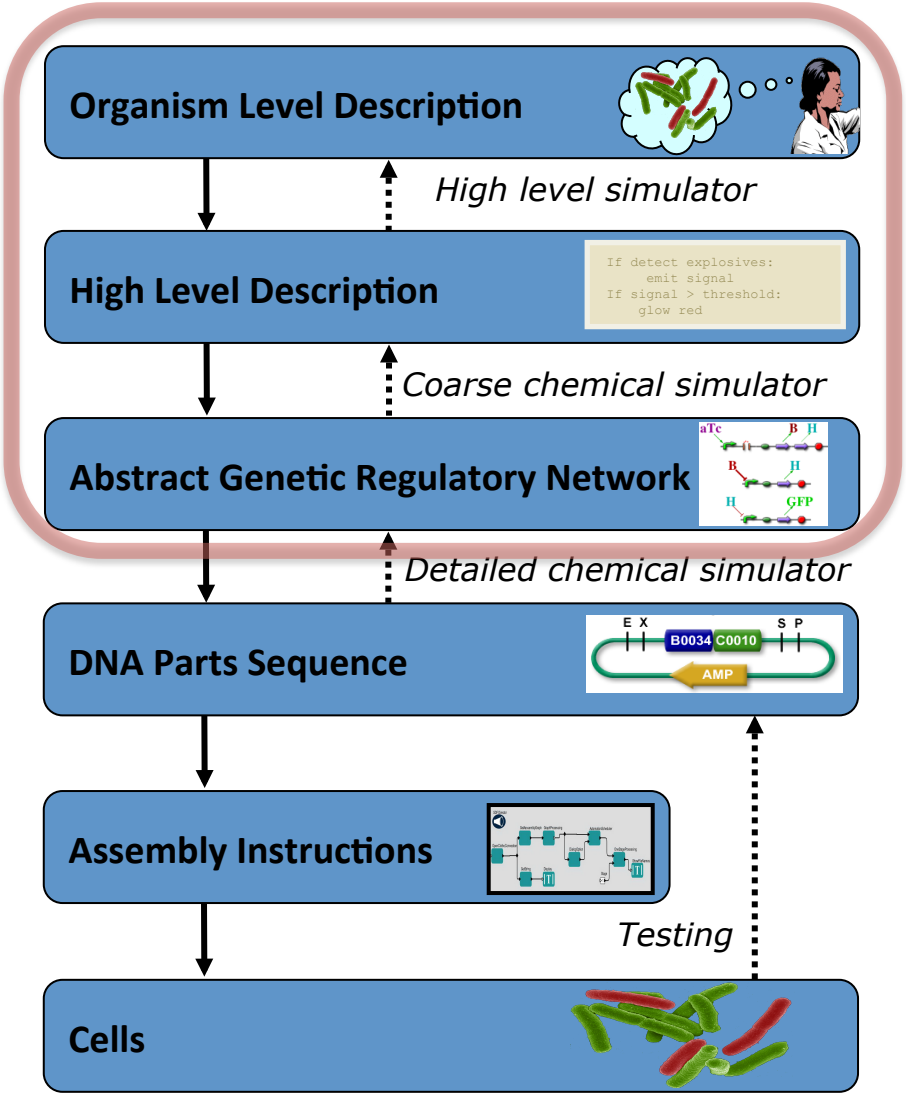
Compilation & Optimization



Other tools aiming at high-level design: Cello, Eugene, GEC, GenoCAD, etc.

[Beal, Lu, Weiss, 2011]

Motif-Based Compilation

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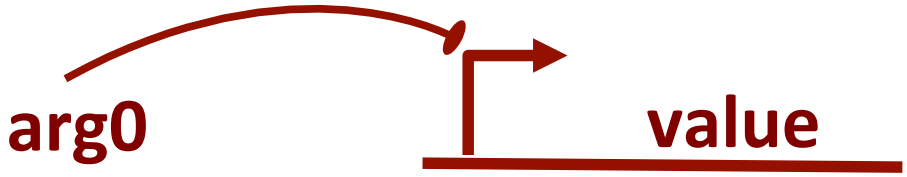
- High-level primitives map to GRN design motifs
  - e.g. logical operators:

```
(primitive not (boolean) boolean
        :grn-motif ((P high R- arg0 value T)))
```

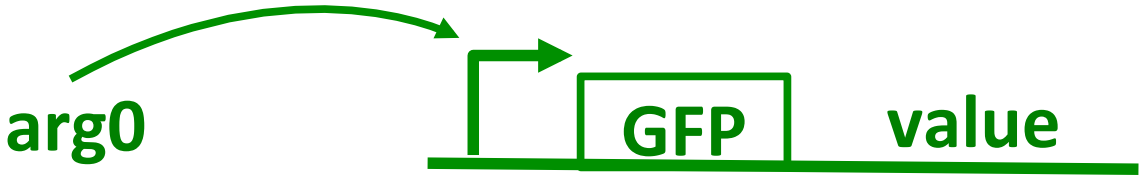


Motif-Based Compilation

Raytheon BBN Technologies

- High-level primitives map to GRN design motifs
  - e.g. logical operators, **actuators**:

```
(primitive green (boolean) boolean :side-effect
  :type-constraints ((= value arg0))
  :grn-motif ((P R+ arg0 GFP|arg0 value T)))
```

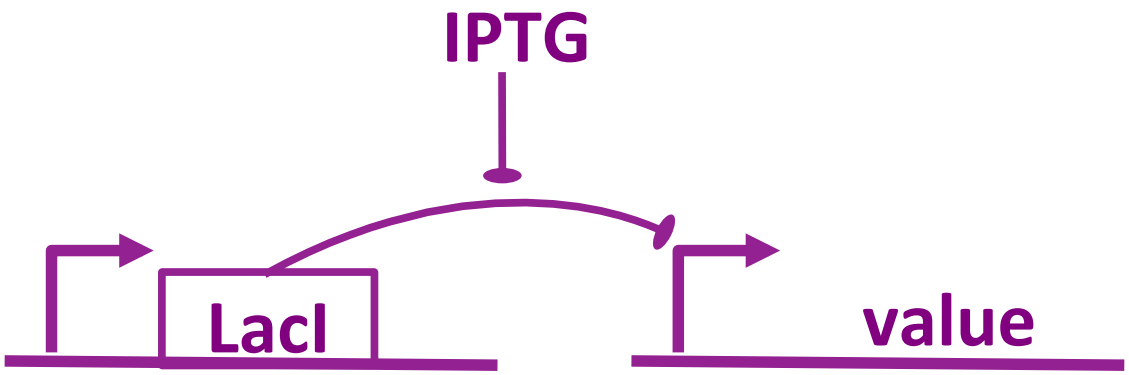


Motif-Based Compilation

Raytheon
BBN Technologies

- High-level primitives map to GRN design motifs
  - e.g. logical operators, actuators, **sensors**:

```
(primitive IPTG () boolean
  :grn-motif ((P high LacI|boolean T)
              (RXN (IPTG|boolean) represses LacI)
              (P high R- LacI value T)))
```



Motif-Based Compilation

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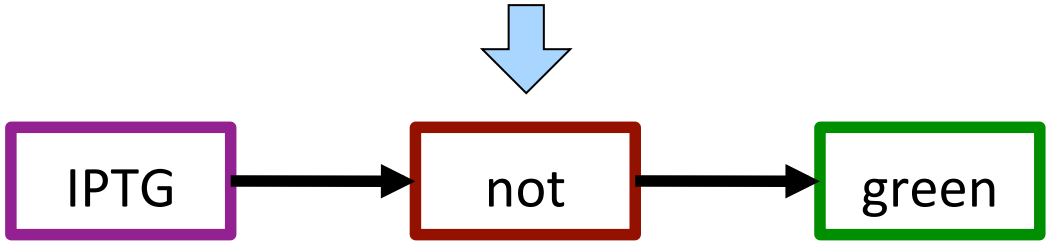
- Functional program gives dataflow computation:

```
(green (not (IPTG)))
```



Motif-Based Compilation

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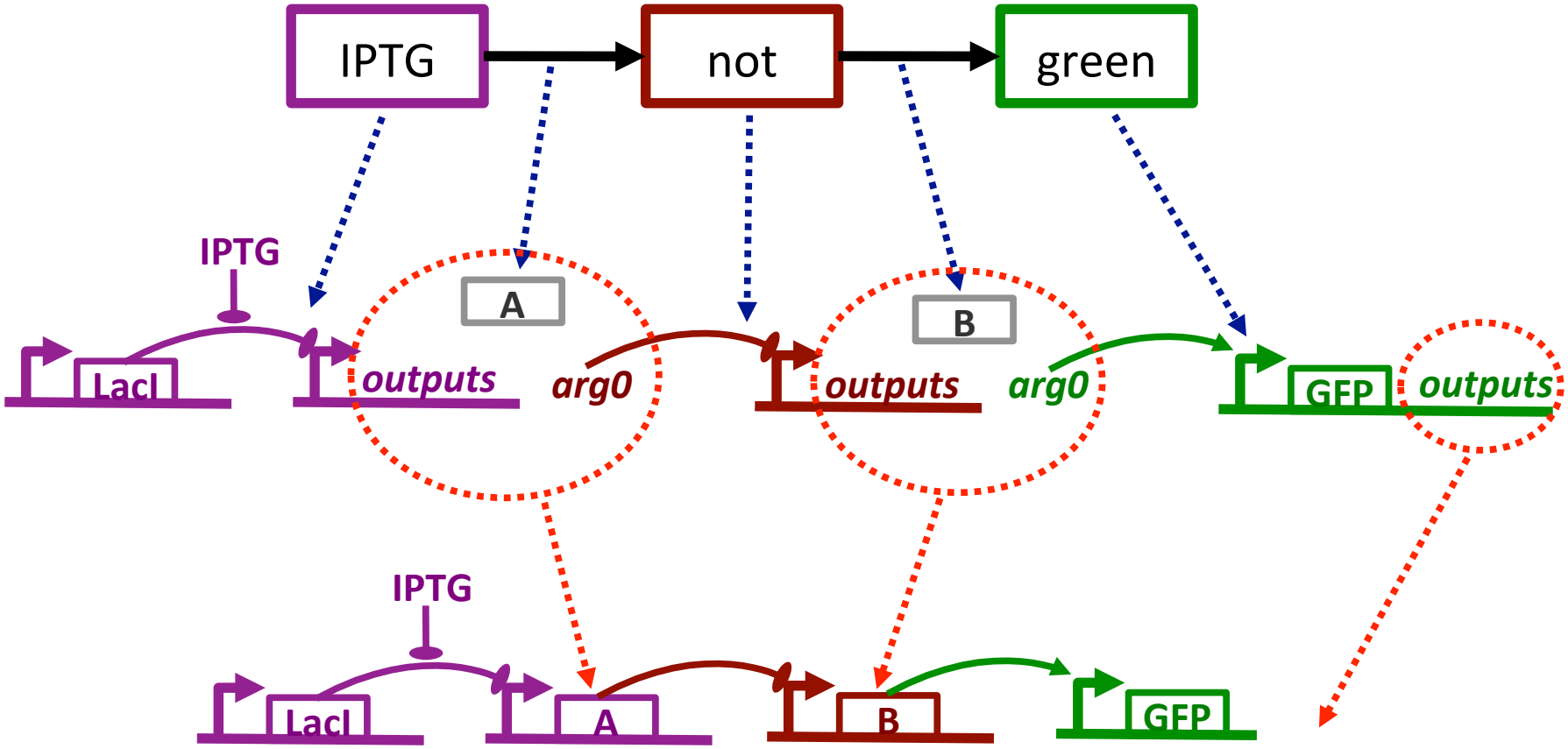
- Operators translated to motifs:



Optimization

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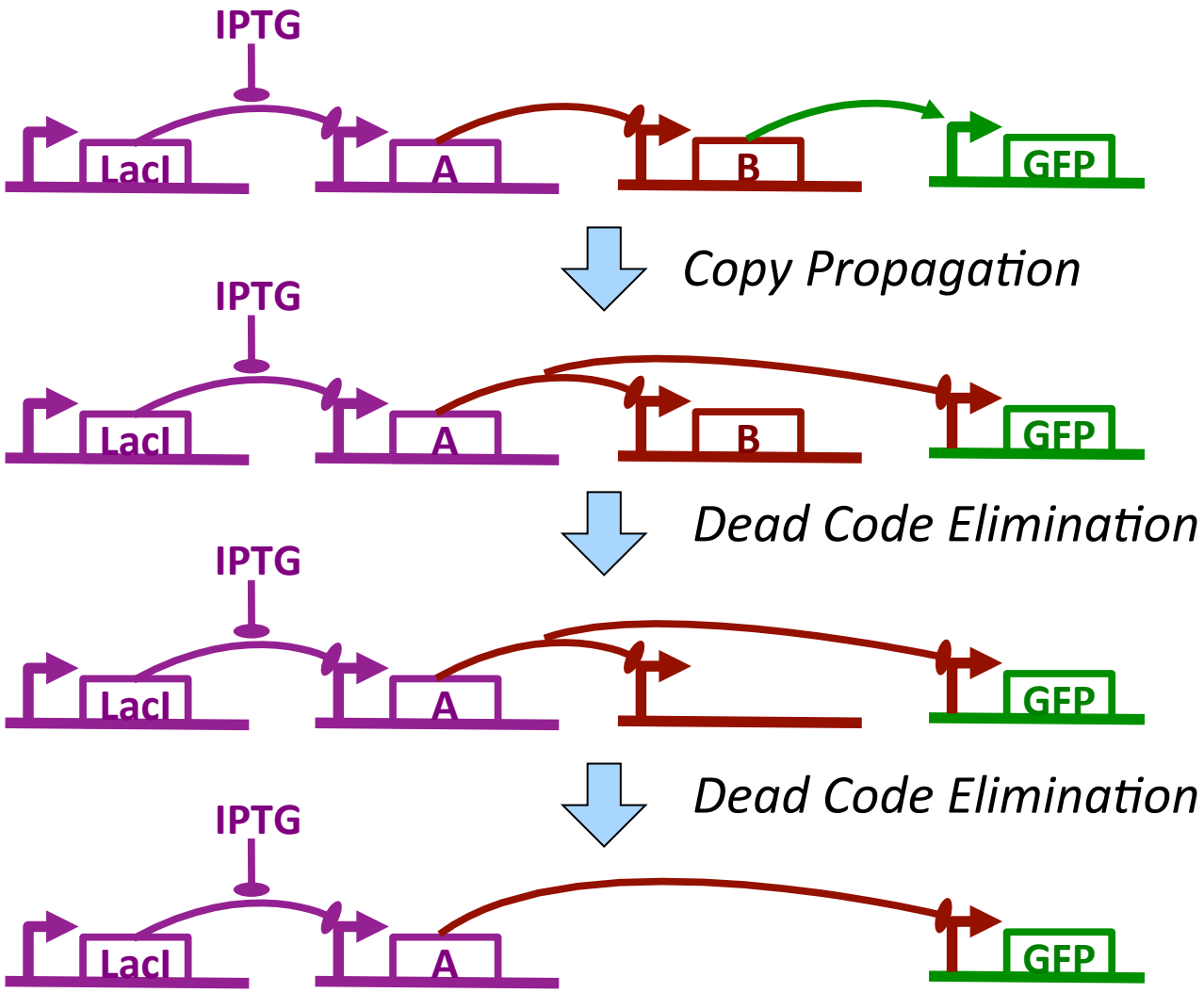

Design Optimization

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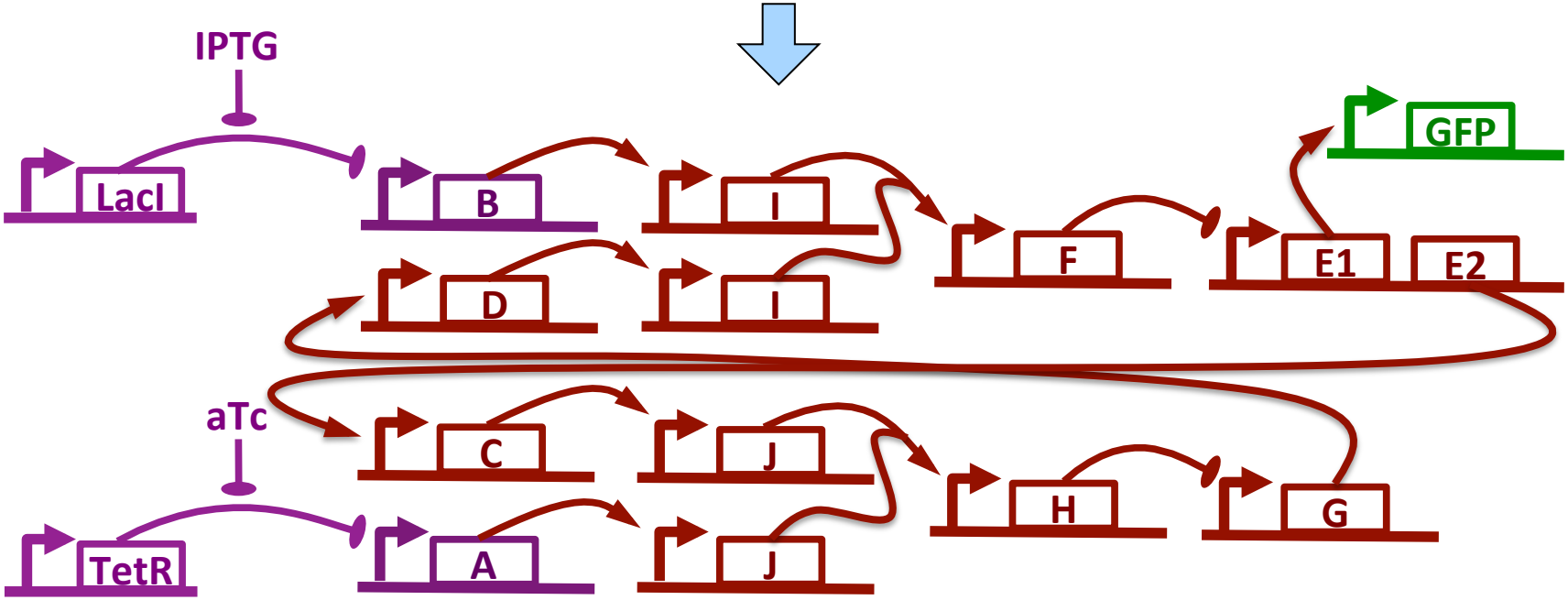
```
(def one-bit-memory(set reset)
  (letfed+ ((o boolean (not (or reset o-bar)))
            (o-bar boolean (not (or set o))))
    o))

(green (one-bit-memory (aTc) (IPTG)))
```



Unoptimized: 15 functional units, 13 transcription factors

Design Optimization

Raytheon BBN Technologies

```
(def one-bit-memory(set reset)
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(green (one-bit-memory (aTc) (IPTG)))
```

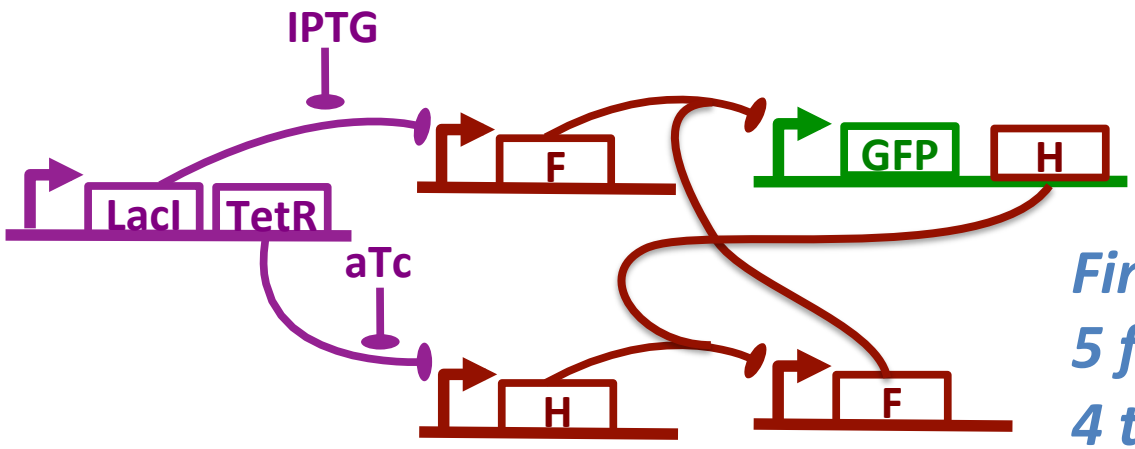

Final Optimized:
5 functional units
4 transcription factors

Unoptimized: 15 functional units, 13 transcription factors

Complex Example: 4-bit Counter

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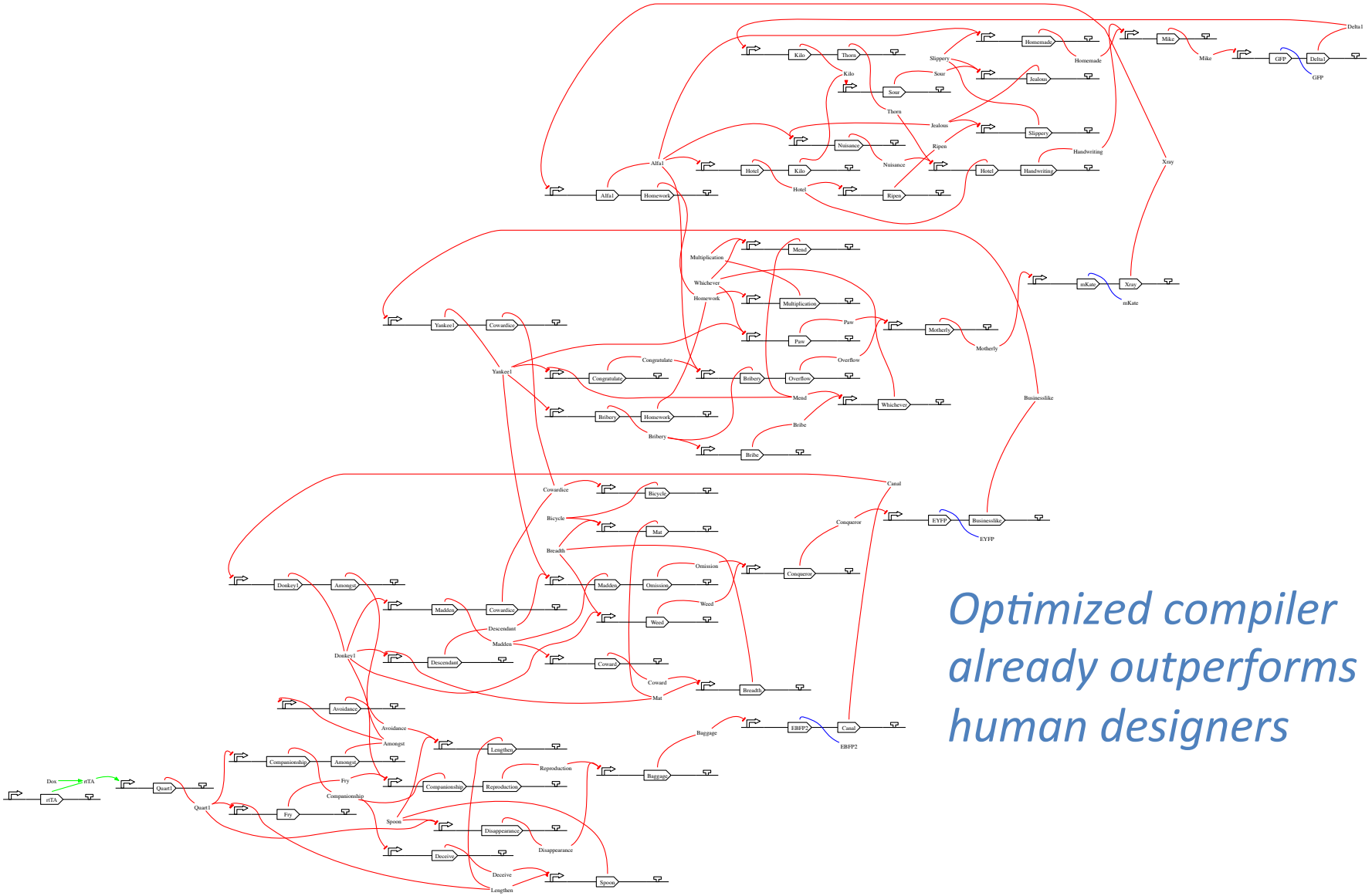

Optimized compiler already outperforms human designers

The Tool-Chain Approach:

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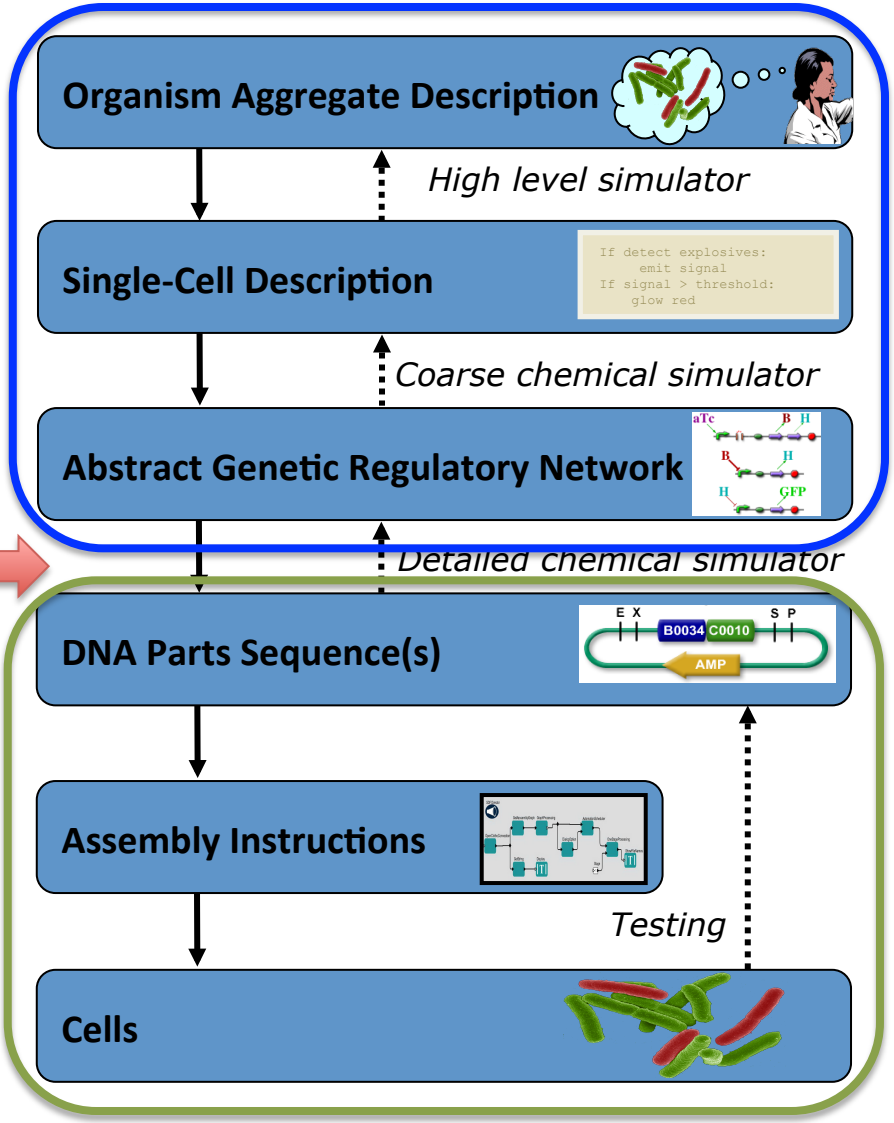

Gap!

Proto BioCompiler

[Beal et al, PLoS ONE 2011]

Alternate 2nd stages:
SBROME, CELLO, GEC, …

Next-Gen Synthesis, Organick, Antha, MAGE, microfluidics, …

Complex Designs: Barriers & Emerging Solutions

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- Barrier: Characterization of Devices
  - Emerging solution: TASBE characterization method
- Barrier: Predictability of Biological Circuits
  - Emerging solution: EQuIP prediction method
- Barrier: Availability of High-Gain Devices
  - Emerging Solution: combinatorial device libraries based on CRISPR, TALs, miRNAs, recombinases, …

Characterization & reproducibility

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iGEM Interlab Study:

- Build three constitutive GFP constructs
- Culture & measure fluorescence
- 3 biological replicates (Extra: x 3 technical rep.)

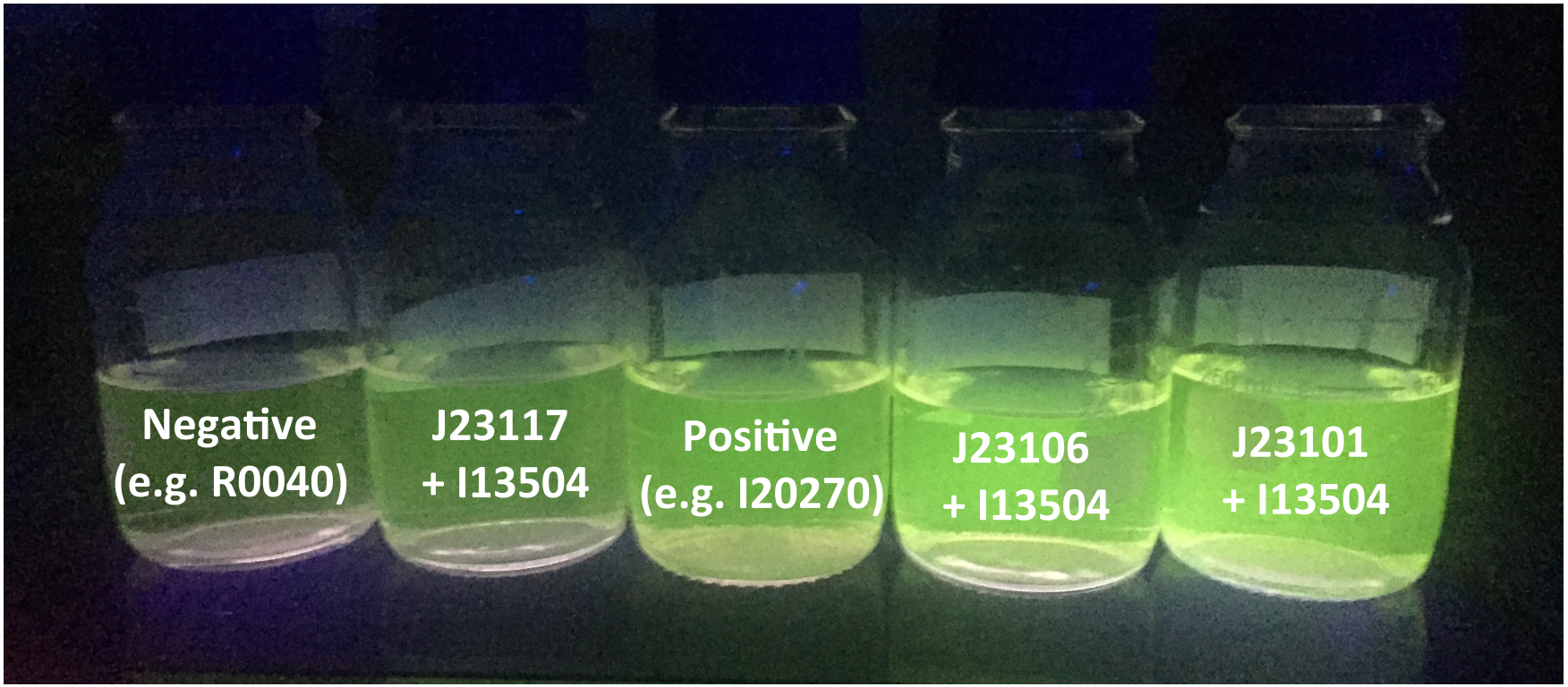

Image from iGEM Oxford 2015

2015 iGEM Interlab Study Participation

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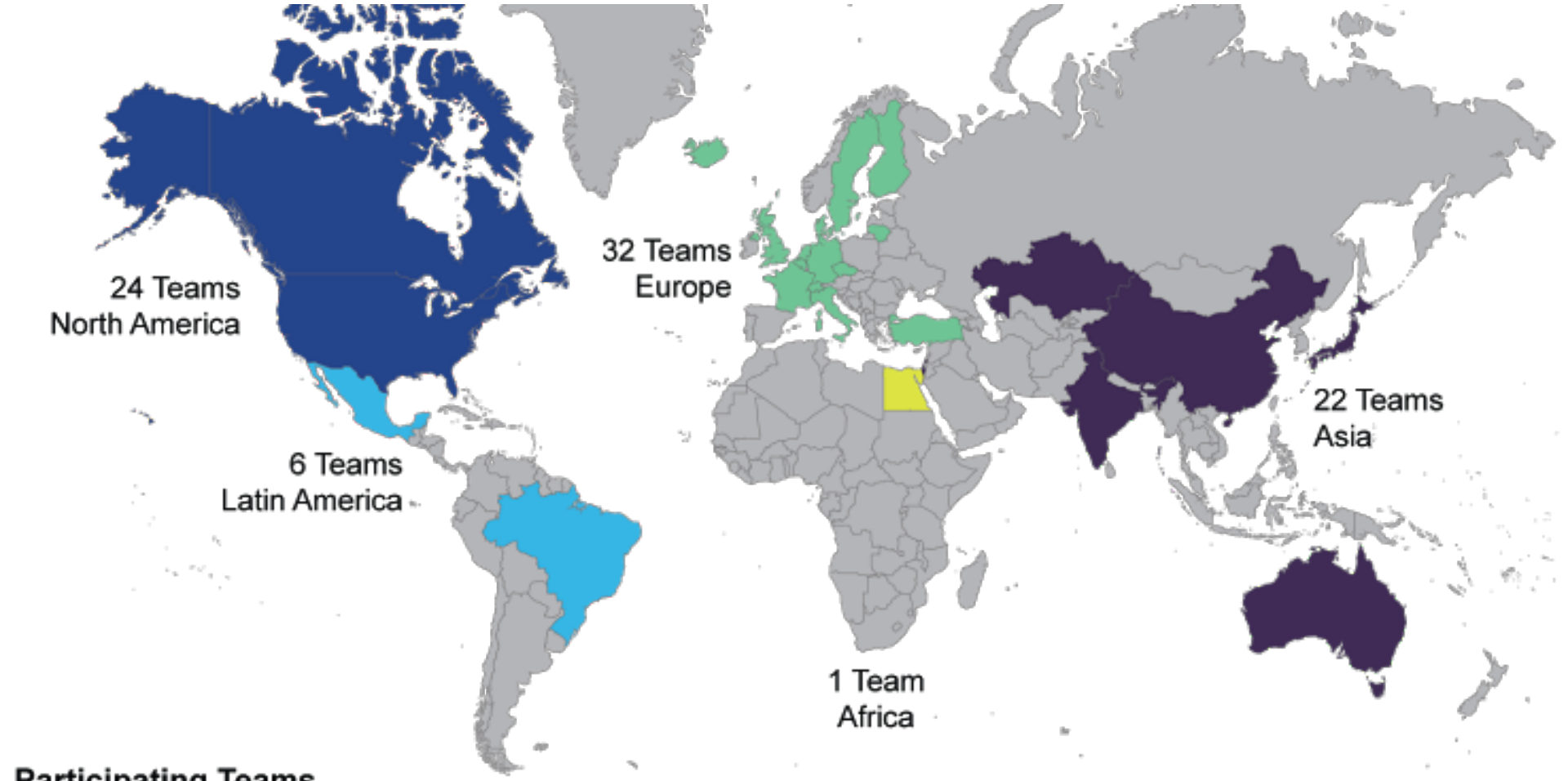

Participating Teams

Aalto-Helsinki	Birkbeck	CU Boulder	Glasgow	London Biohackspace	NJAU_China	Rock Ridge Virginia	TecCEM HS	Tufts	Waterloo
Aix-Marseille	BIT	Czech_Republic	Harvard_BioDesign	LZU	Northeastern	SCUT	Tec_Monterrey	UCL	William and Mary
Amoy	Boston University	Duke	Hong_Kong-CUHK	Marburg	NRP-UEA	SDU-Denmark	Tokyo Tech	UCLA	WLC-Milwaukee
ANU-Canberra	Brasil-USP	Edinburgh	HUST-China	METU_Turkey	NTNU-Trondheim	SPSingapore	Toronto	UC San Diego	WPI-Worcester
ATOMS-Turkiye	Cairo_Egypt	EPF_Lausanne	HZAU-China	Minnesota	NU_Kazakhstan	Stanford-Brown	Trento	UFMG_Brazil	
Austin_UTexas	Carnegie Mellon	ETH Zurich	IISER_Pune	MIT	OUC-China	Stockholm	TrinityCollegeDublin	UMaryland	
BHSF_Beijing	CityU_HK	Exeter University	KU Leuven	Nanjing_NFLS	Oxford	SYSU-Software	TU Delft	Utah_State	
Bielefeld	Cork	Freiburg	Leicester	Nankai	Paris-Saclay	SZMS_15_Shenzhen	TU_Eindhoven	Vanderbilt	
BIOSINT Mexico	CSU_Fort_Collins	Gifu	Lethbridge	NEAU-China	Pasteur	TecCEM	Tuebingen	Vilnius-Lithuania	

High precision possible

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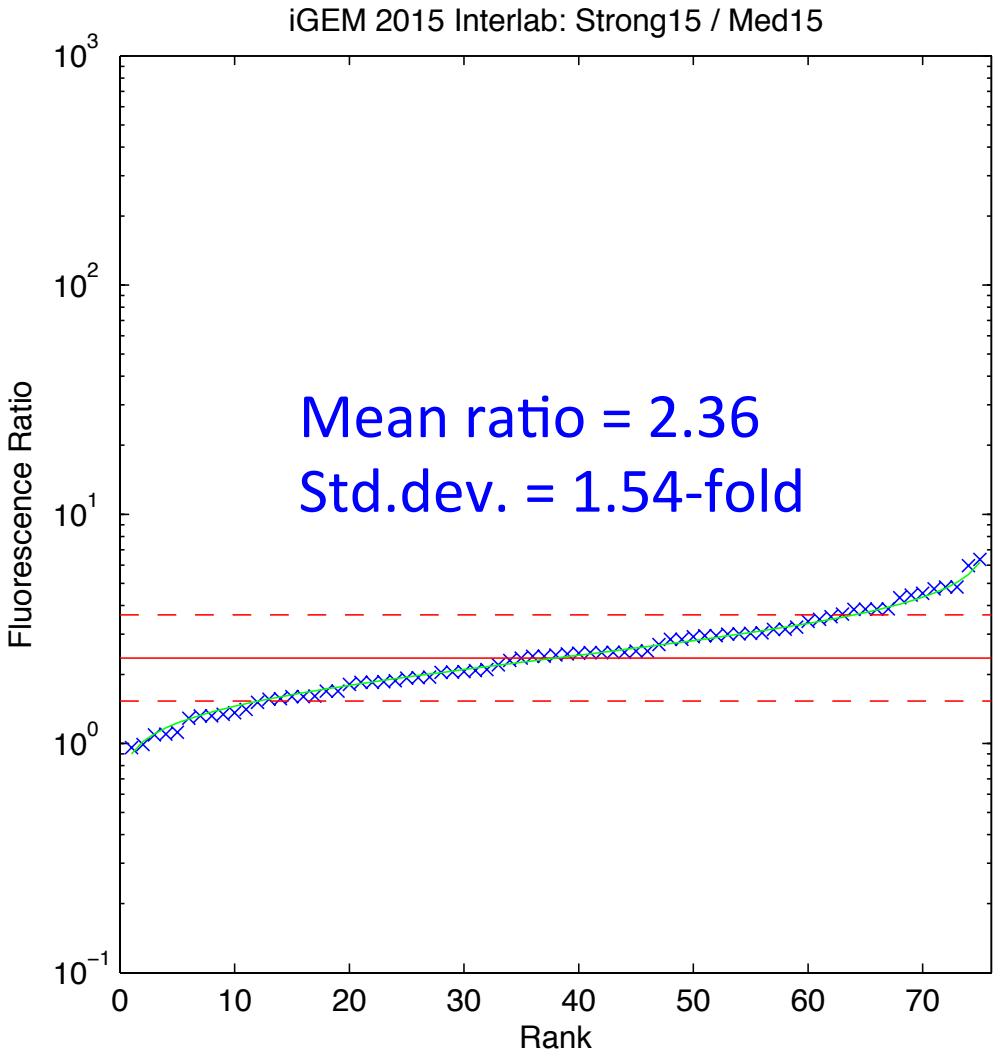

Instrument issues drive variation!

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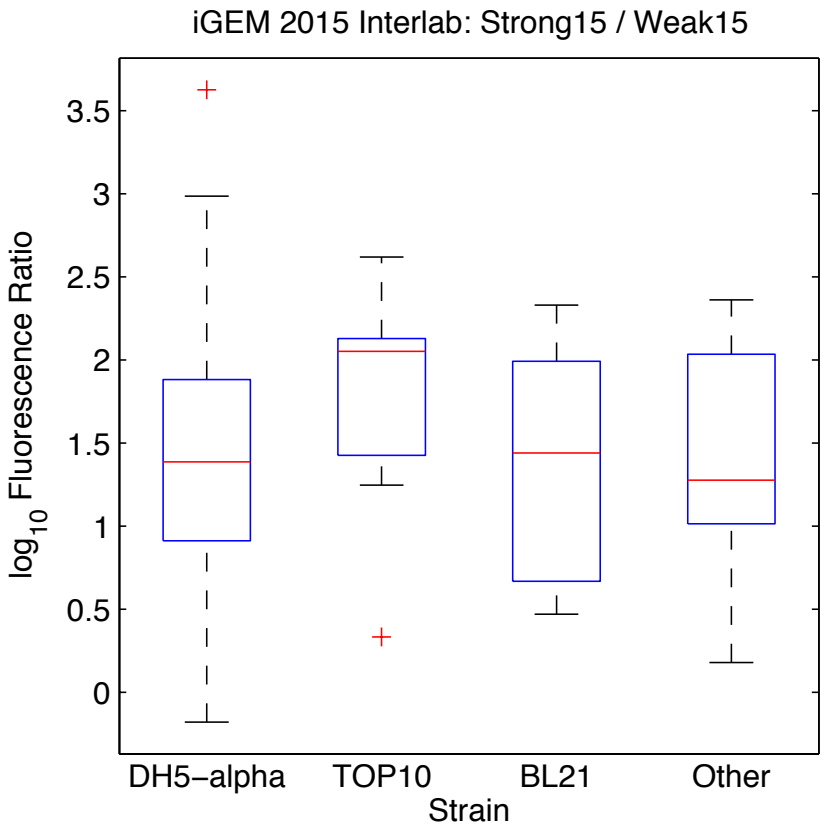
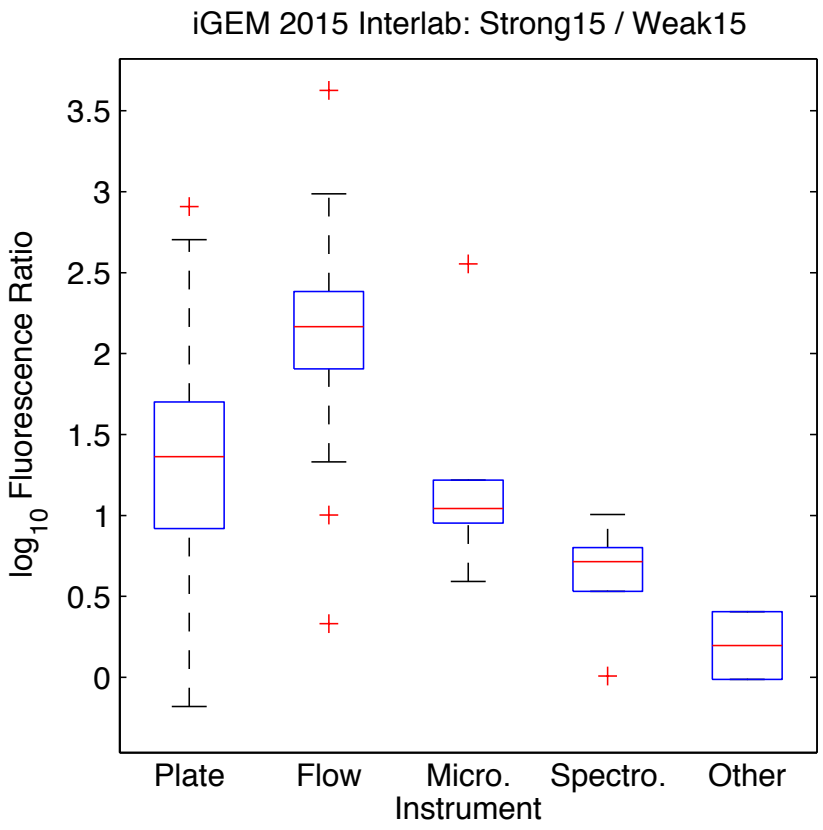
Strain



Instrument



Calibrated Flow Cytometry

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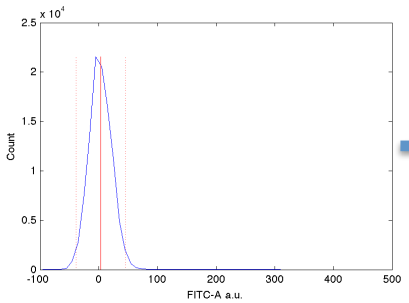
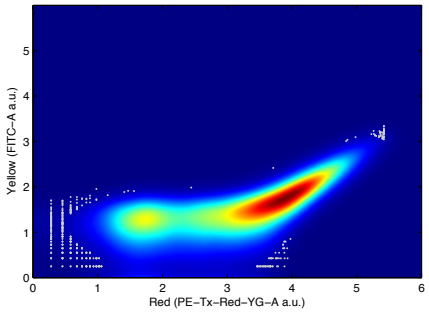


Negative Control: autofluorescence

Arbitrary Units (a.u.)

minus mean autofluorescence

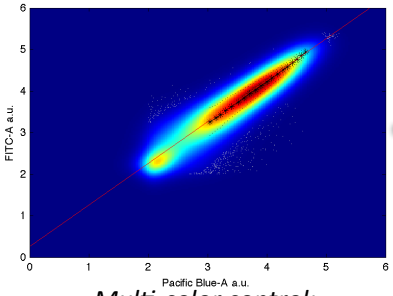
Net a.u.



Single positive controls: compensate for spectral overlap

divide by spectral overlap matrix

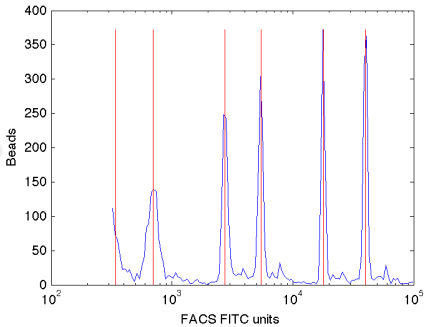
Compensated a.u.



Multi-color control: conversion of non-FITC channels

times mean ratio of FITC / color

FITC a.u.



Calibration beads: Convert FITC a.u. to MEFL

times mean bead peak calibrated / observed

Calibrated MEFL

[Roederer, 2002; Wang et al., 2008; NIST/ISAC, 2012; Beal et al., 2012; Kiani et al., 2014; Beal et al., 2014; Davidsohn et al, 2014]

Example: Predicting Repressor Cascades

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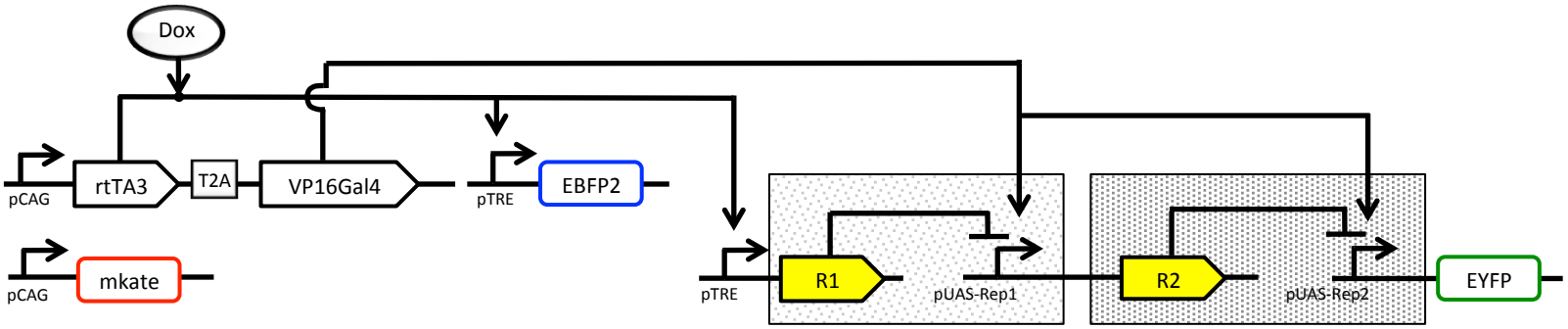
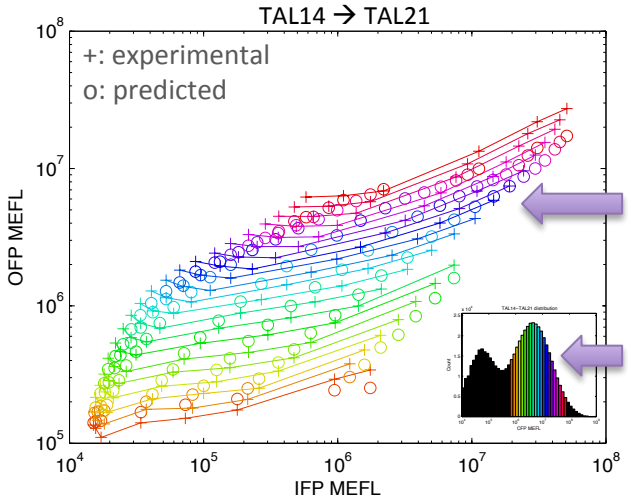
Precision dose-response measurement allows high-precision prediction with quantitative models



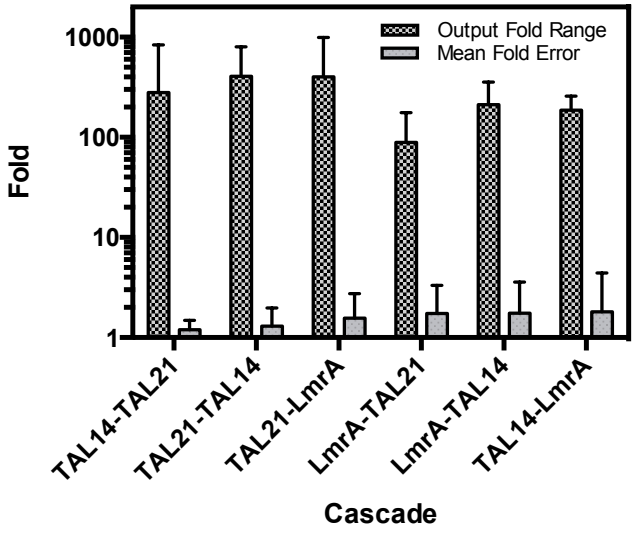
Prediction of Repressor Cascade



Each line is a dose/response curve for a different relative number of circuit copies.

Subpopulation identified by color on inset mKate histogram

Range vs. Error for 6 Cascades



[Davidsohn et al., 2014]

How much does calibration matter?

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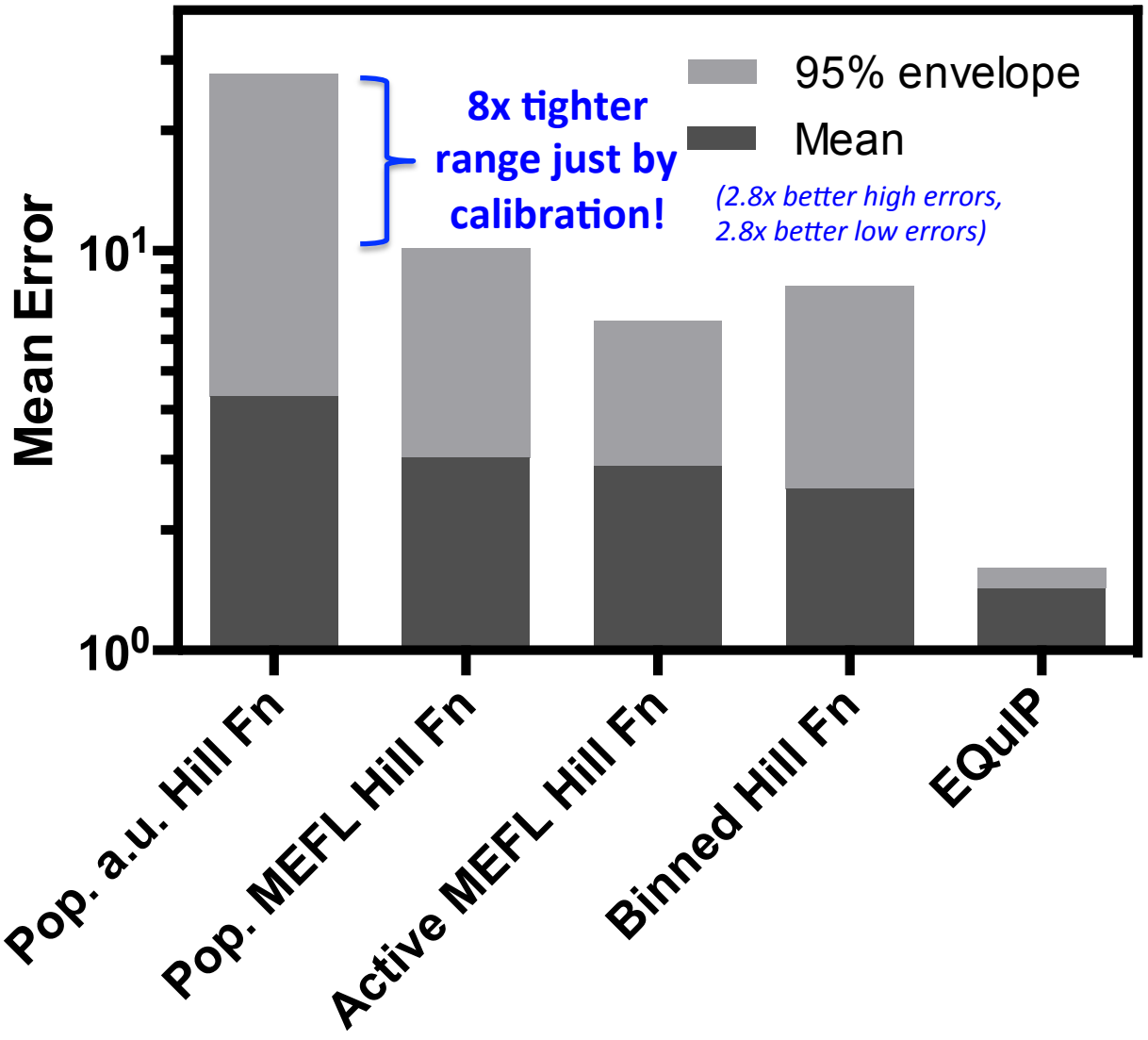

[Davidsohn et al., submitted]

Example: Engineering Replicon Expression

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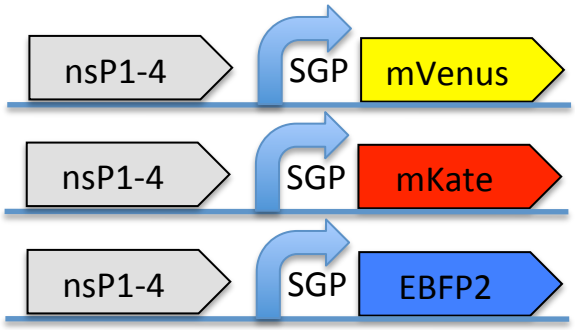
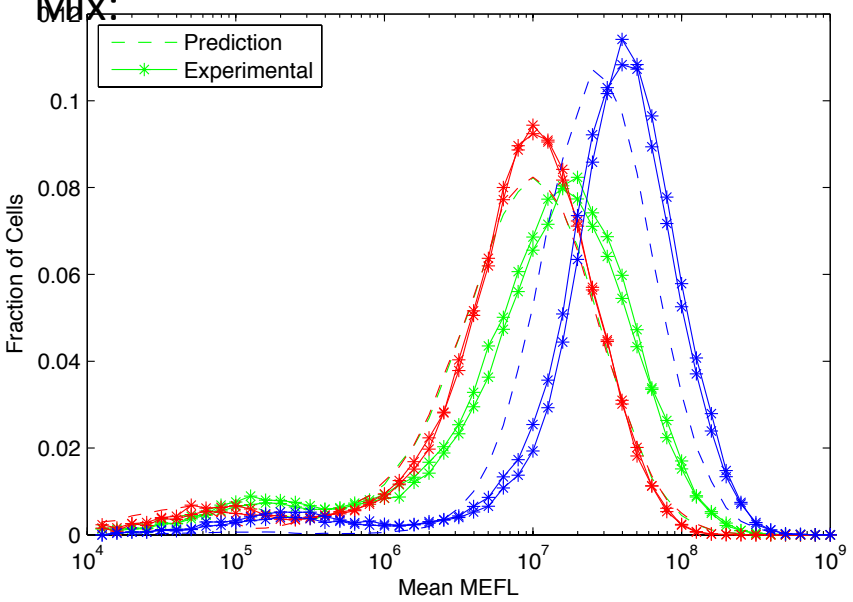
Per-cell measurement of dose-response gives model allowing high-precision control of expression

Example:
Prediction of fluorescence vs. time for novel mixtures of 3 Sindbis RNA replicons

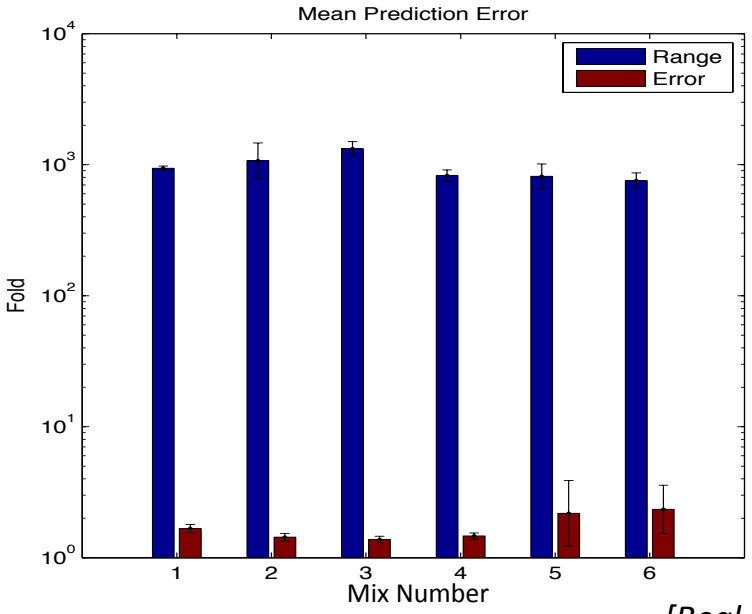


Mix 1: 0.1Y, 0.1R, 0.1B
Mix 2: 0.3Y, 0.3R, 0.3B
Mix 3: 0.1Y, 0.5R, 0.4B
Mix 4: 0.2Y, 0.2R, 0.6B
Mix 5: 0.01Y, 0.1R, 0.5B
Mix 6: 0.4Y, 0.02R, 0.02B

Example Prediction of 3-RNA Replicon Mix:



Range vs. Error for 6 Mixtures



[Beal et al., 2014]

High-performance device libraries

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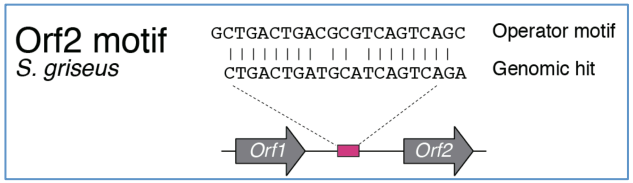
TetR Homologs

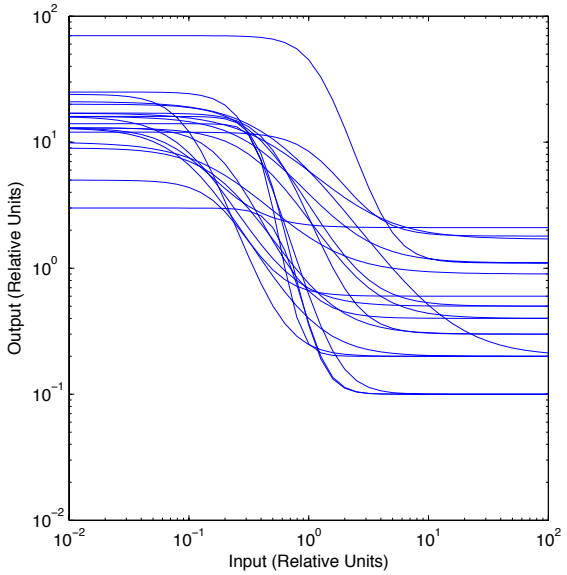
- Variable on/off
- Variable amplification

→*ΔSNR ~0*

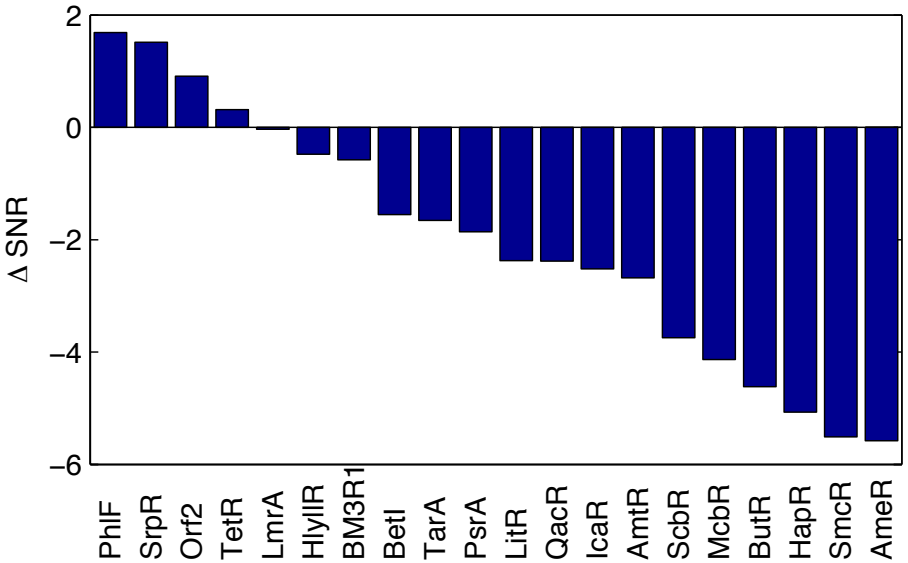


[Stanton et al. 2014]

Transfer Curves:



Best Possible ΔSNR:



- Only 4 devices can have SNR>0
- Few good input/output matches

High-performance device libraries

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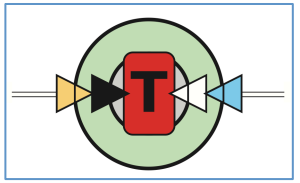
Integrase Logic

- ~1000x on/off, good amplification
- ~1-5% non-responsive

→ *ΔSNR<0*

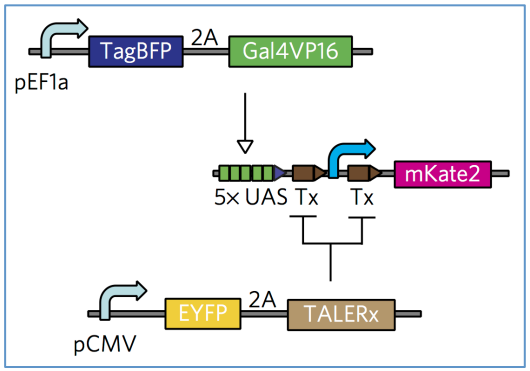


[Bonnet et al. 2013]

TALE Repressors

- ~1000x on/off, poor amplification

→ *ΔSNR<0*

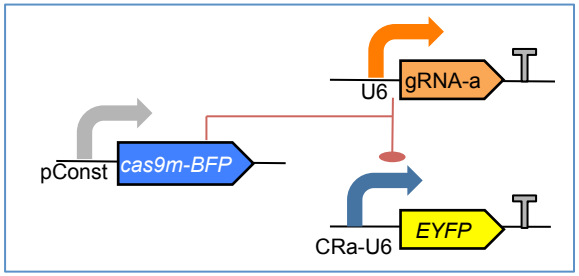


[Garg et al., 2012; Davidsohn et al., 2015; Li et al., 2015]

CRISPR Repressors

- ~100x on/off, amplification ???

→ *ΔSNR unknown*



[Kiani et al. 2014; Kiani et al. 2015]

Summary

Raytheon
BBN Technologies

- Automation-assisted workflows can yield dramatic improvements in organism engineering
- Biological circuits can be “compiled” from high-level specifications of behavior
- New biological devices, measurement, and modeling are starting to enable complex designs

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