

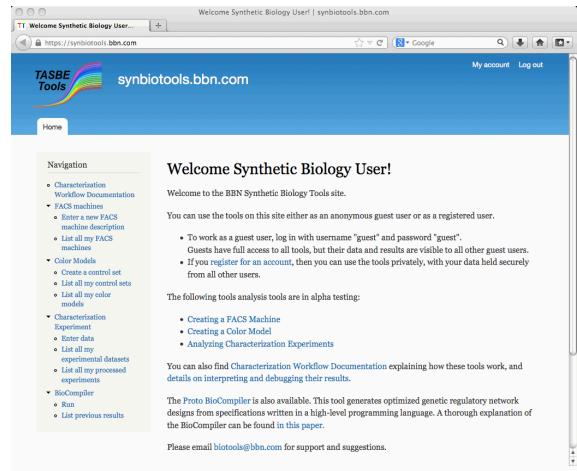
Metrology & Predictive Design For Synthetic Biology

Jacob Beal

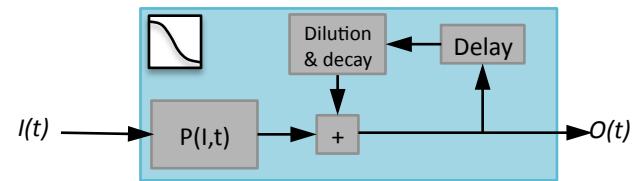
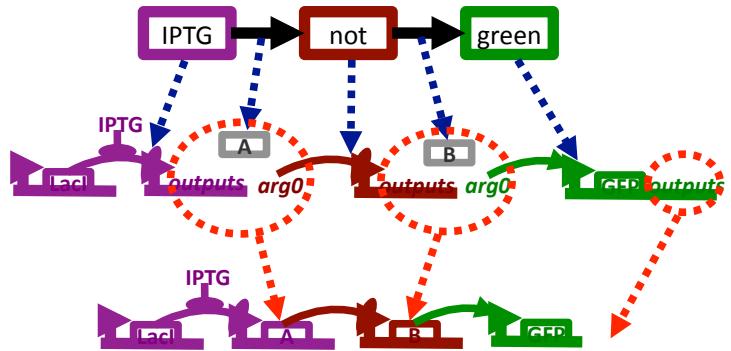
November, 2013

Raytheon
BBN Technologies

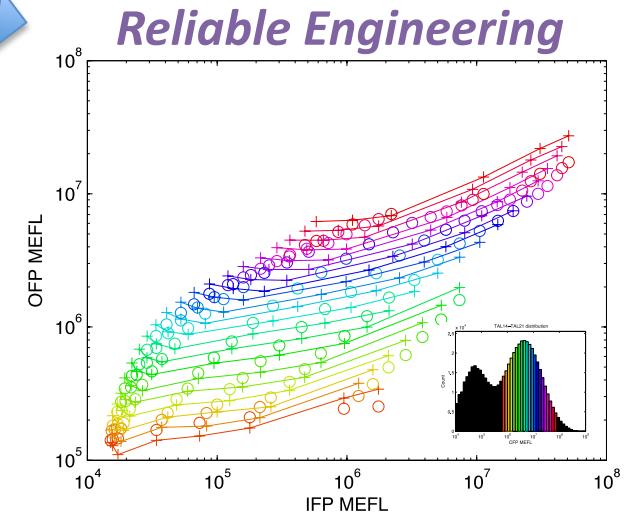
Bringing Wet & Dry Together...



*Computer science
tools and models*



Deeper Understanding

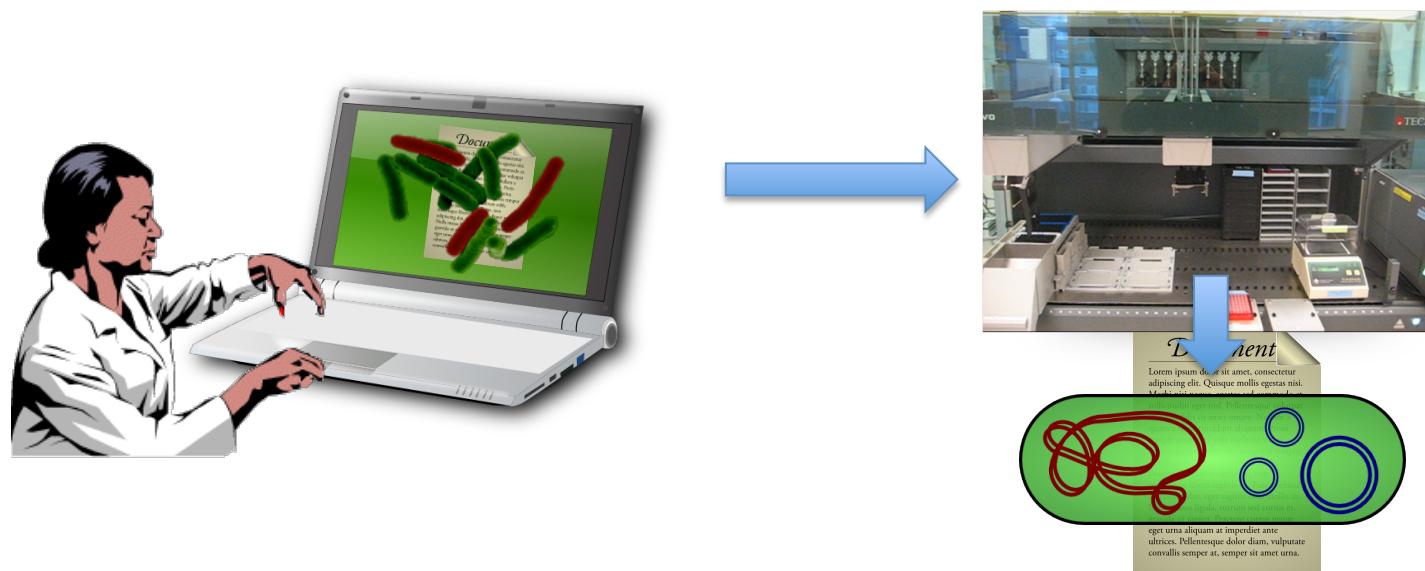


Outline

- **Vision and Motivation**
- Proto BioCompiler
- Calibrating Flow Cytometry
- Building EQuIP Models
- Prediction & Validation

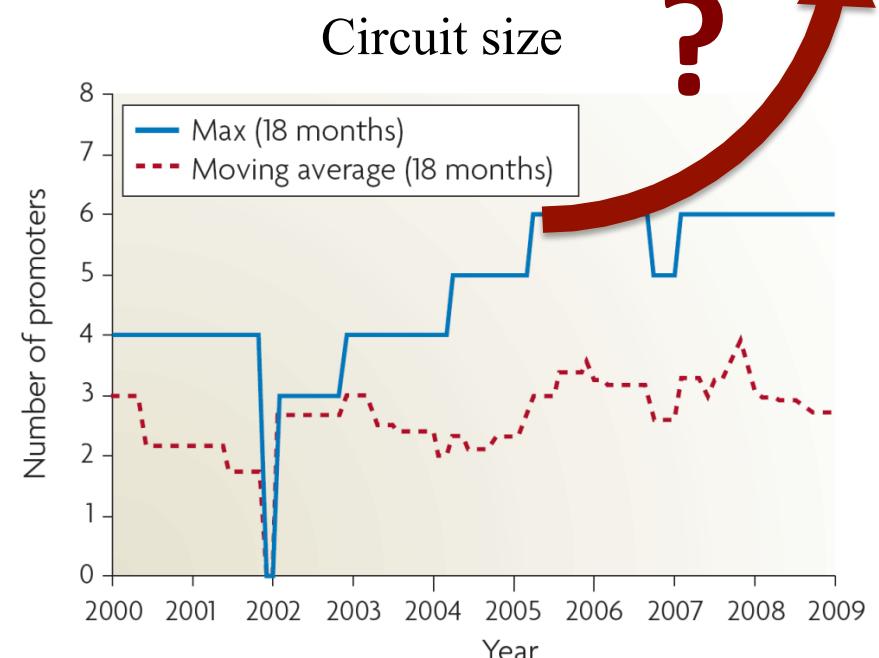
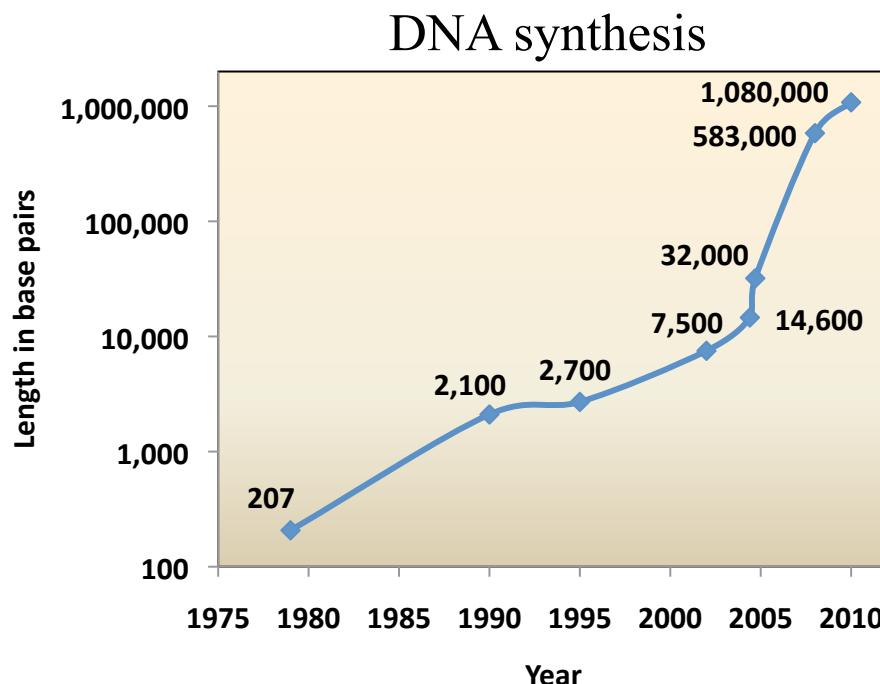
Vision: WYSIWYG Synthetic Biology

Bioengineering should be like document preparation:



Why is this important?

- Breaking the complexity barrier:



[Purnick & Weiss, '09]

- Multiplication of research impact
- Reduction of barriers to entry

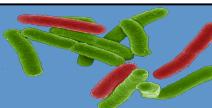
Why a tool-chain?

Organism Level Description

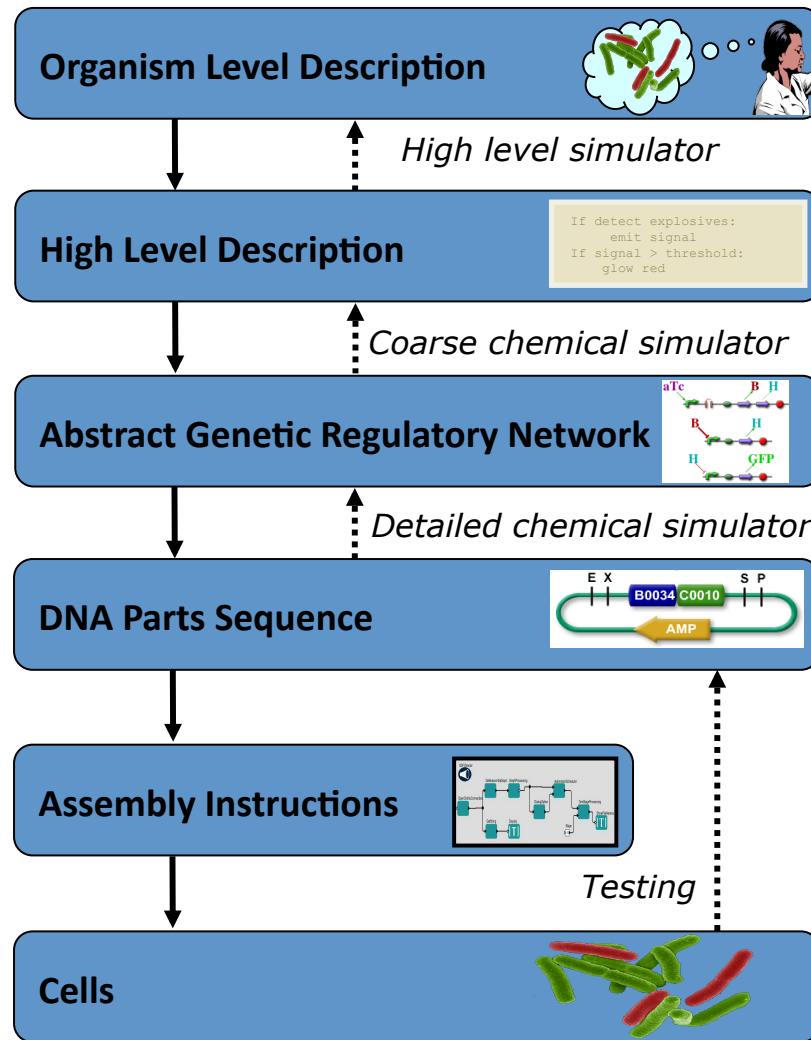


*This gap is too big
to cross with a
single method!*

Cells



TASBE tool-chain



Collaborators:



Ron
Weiss



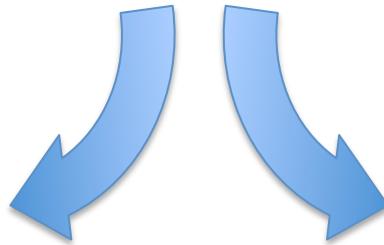
Douglas
Densmore

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

A Tool-Chain Example

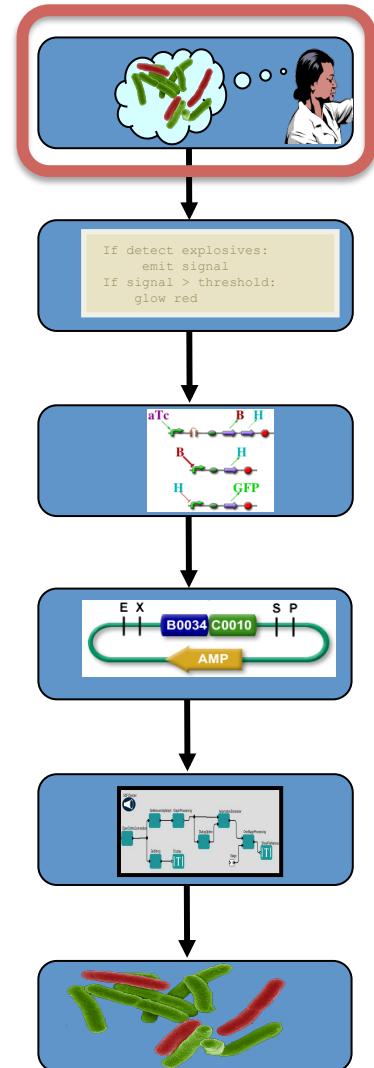
A high-level program of a system that reacts depending on sensor output

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



Mammalian Target

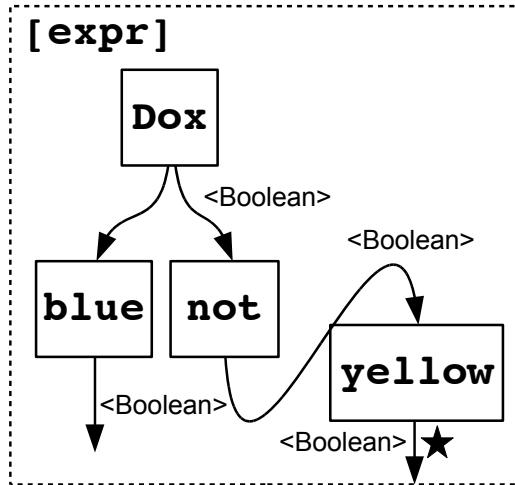
E. coli Target



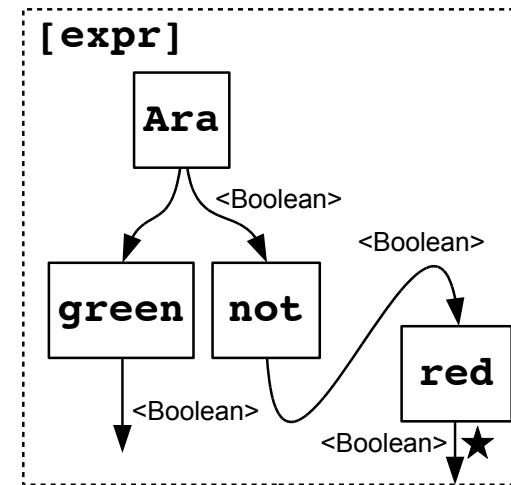
[Beal et al, ACS Syn. Bio. 2012]

A Tool-Chain Example

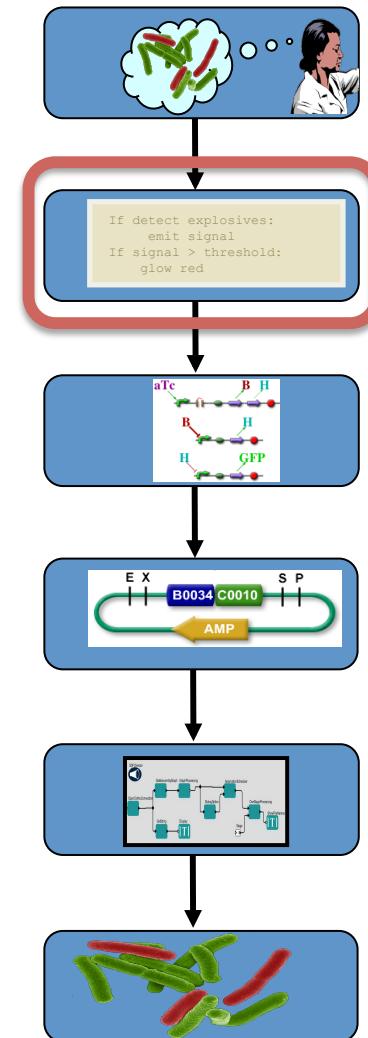
Program instantiated for two target platforms



Mammalian Target



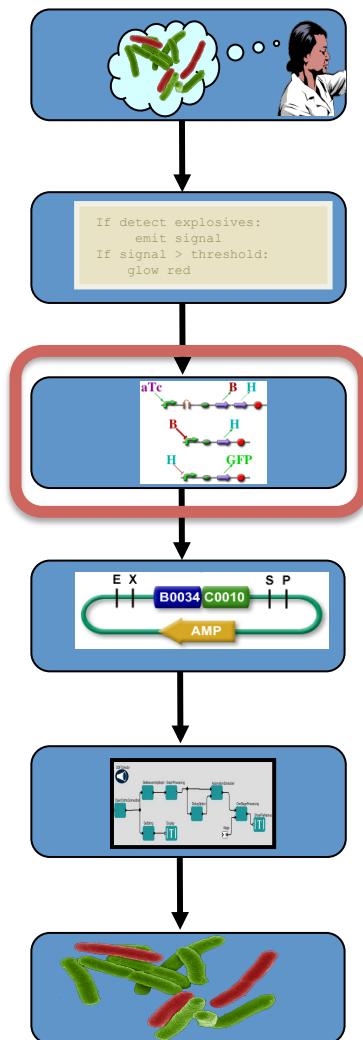
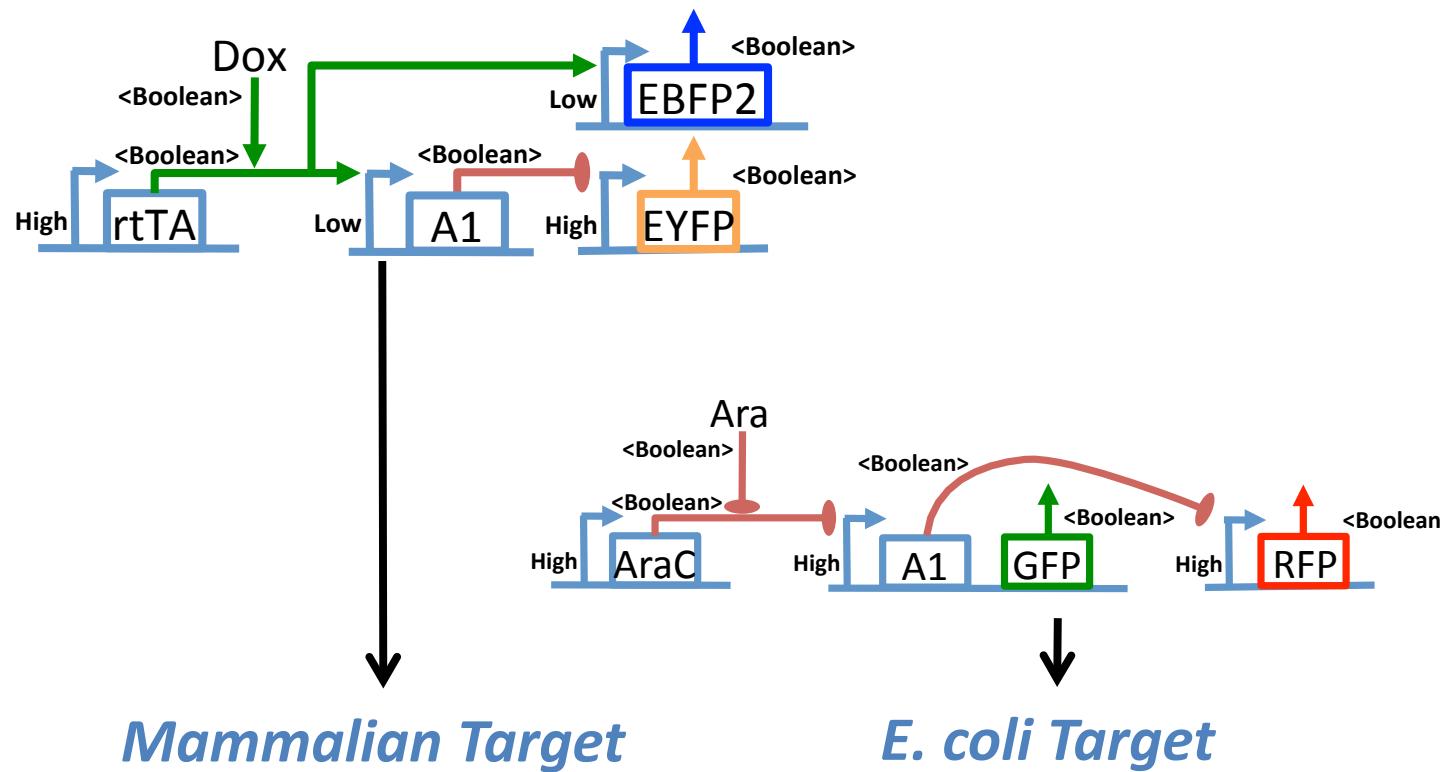
E. coli Target



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

A Tool-Chain Example

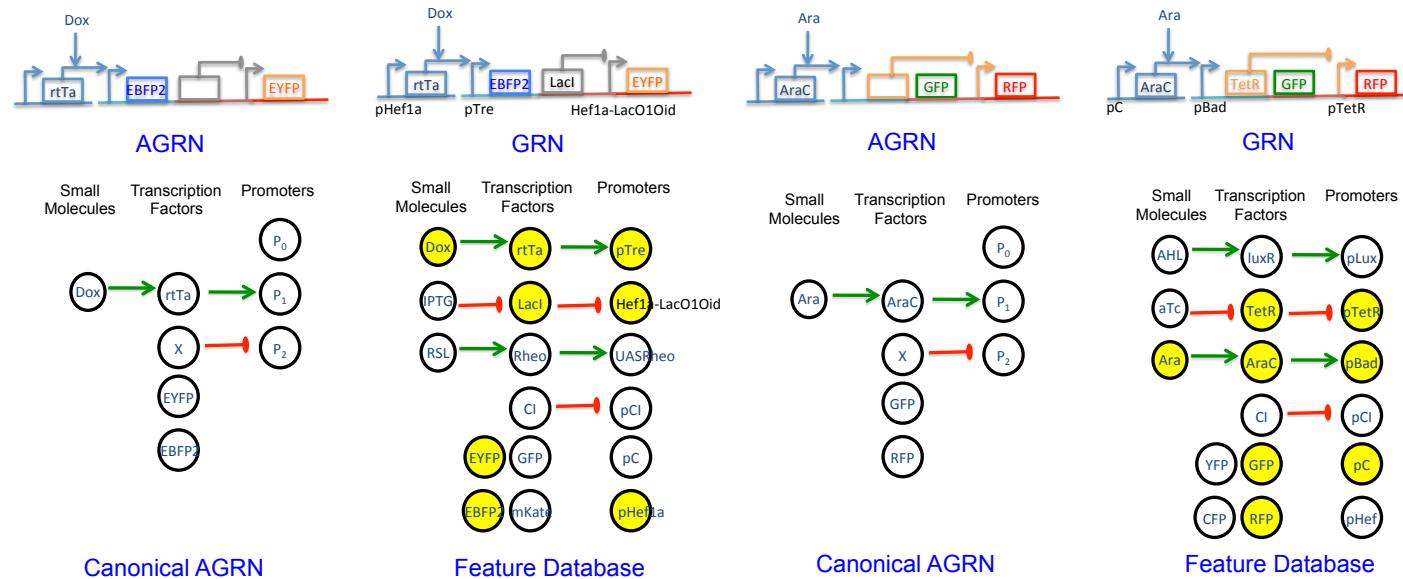
Abstract genetic regulatory networks



[Beal et al, ACS Syn. Bio. 2012] ¹⁰

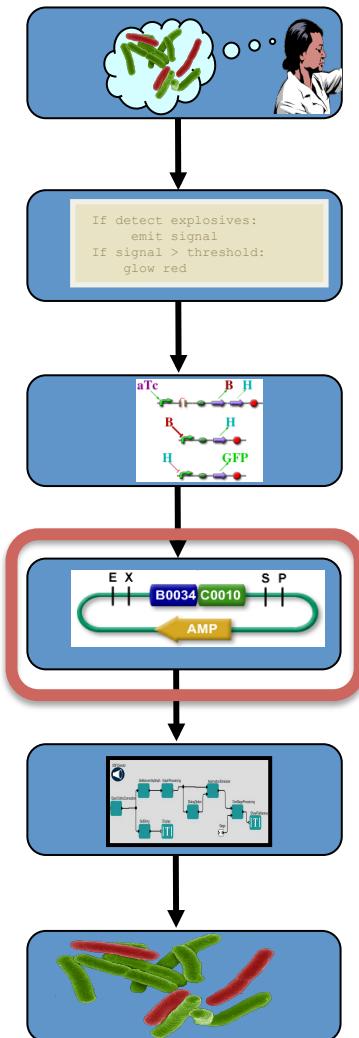
A Tool-Chain Example

Automated part selection using database of known part behaviors



Mammalian Target

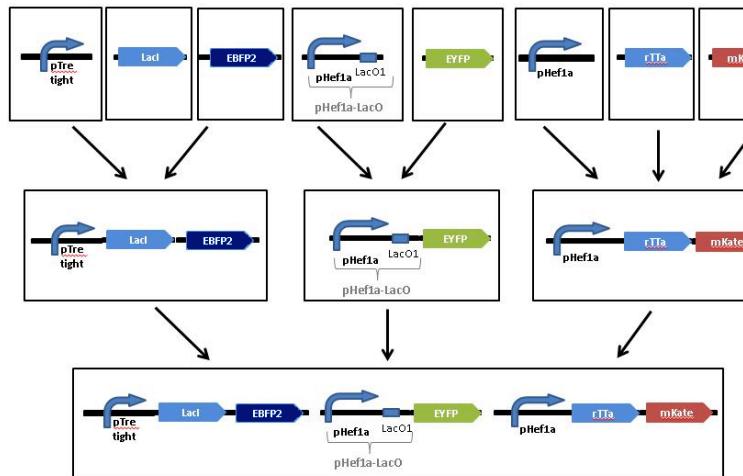
E. coli Target



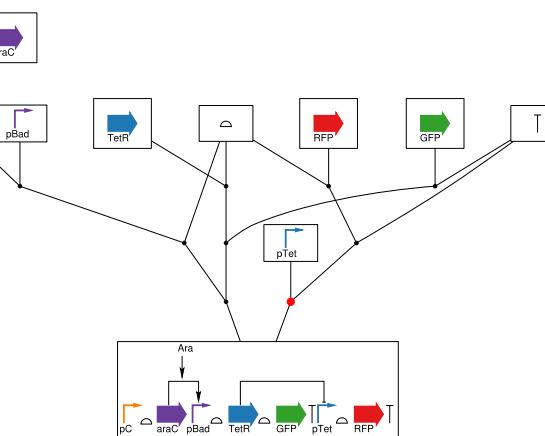
[Beal et al, ACS Syn. Bio. 2012] ¹¹

A Tool-Chain Example

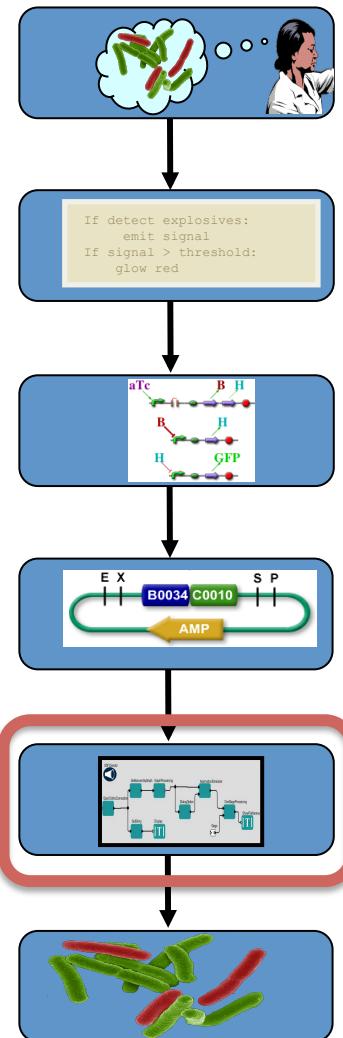
Automated assembly step selection for two different platform-specific assembly protocols



Mammalian Target



E. coli Target

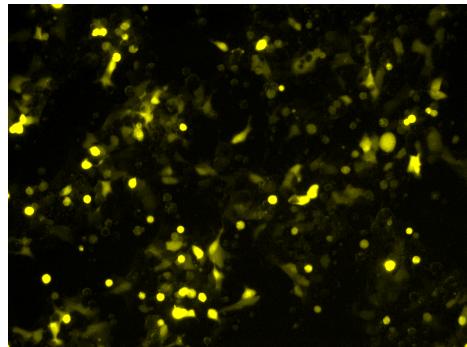


[Beal et al, ACS Syn. Bio. 2012]¹²

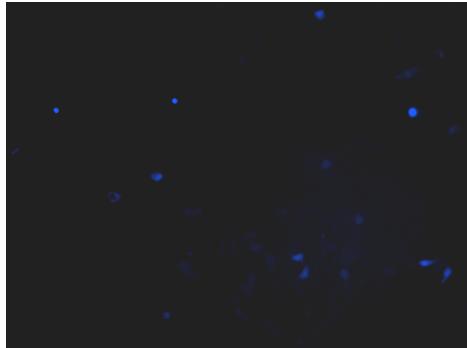
A Tool-Chain Example

Resulting cells demonstrating expected behavior

Uninduced

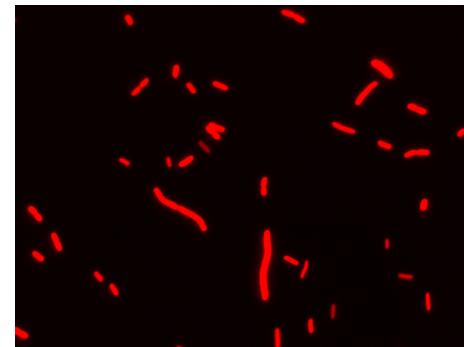


Induced

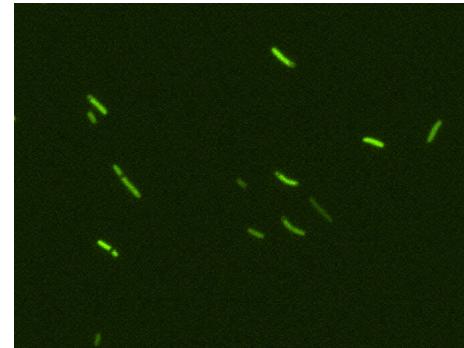


Mammalian Target

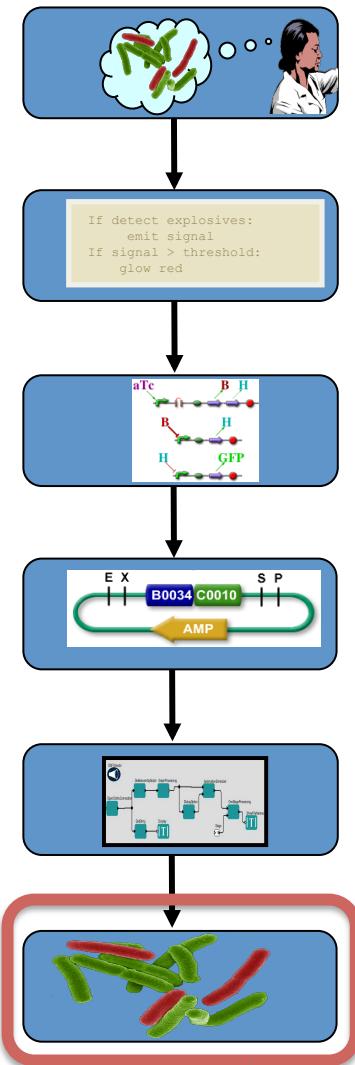
Uninduced



Induced



E. coli Target



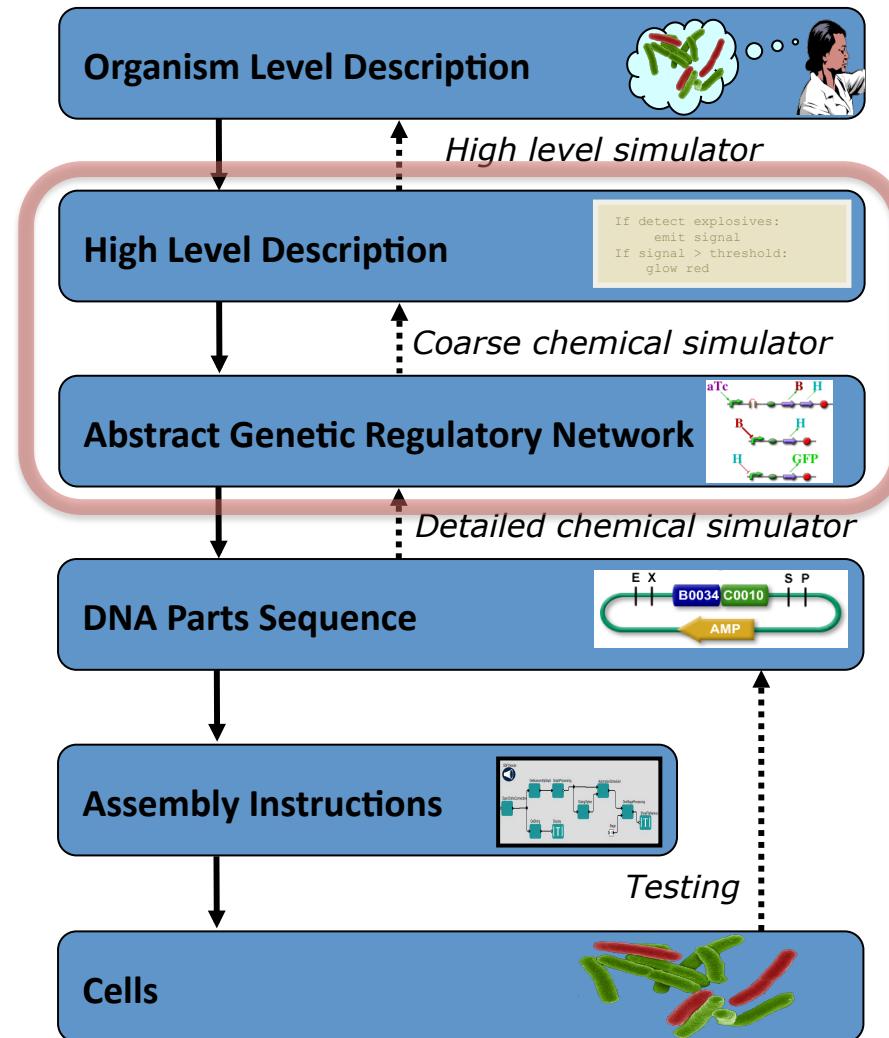
[Beal et al, ACS Syn. Bio. 2012]¹²

Outline

- Vision and Motivation
- **Proto BioCompiler**
- Calibrating Flow Cytometry
- Building EQuIP Models
- Prediction & Validation

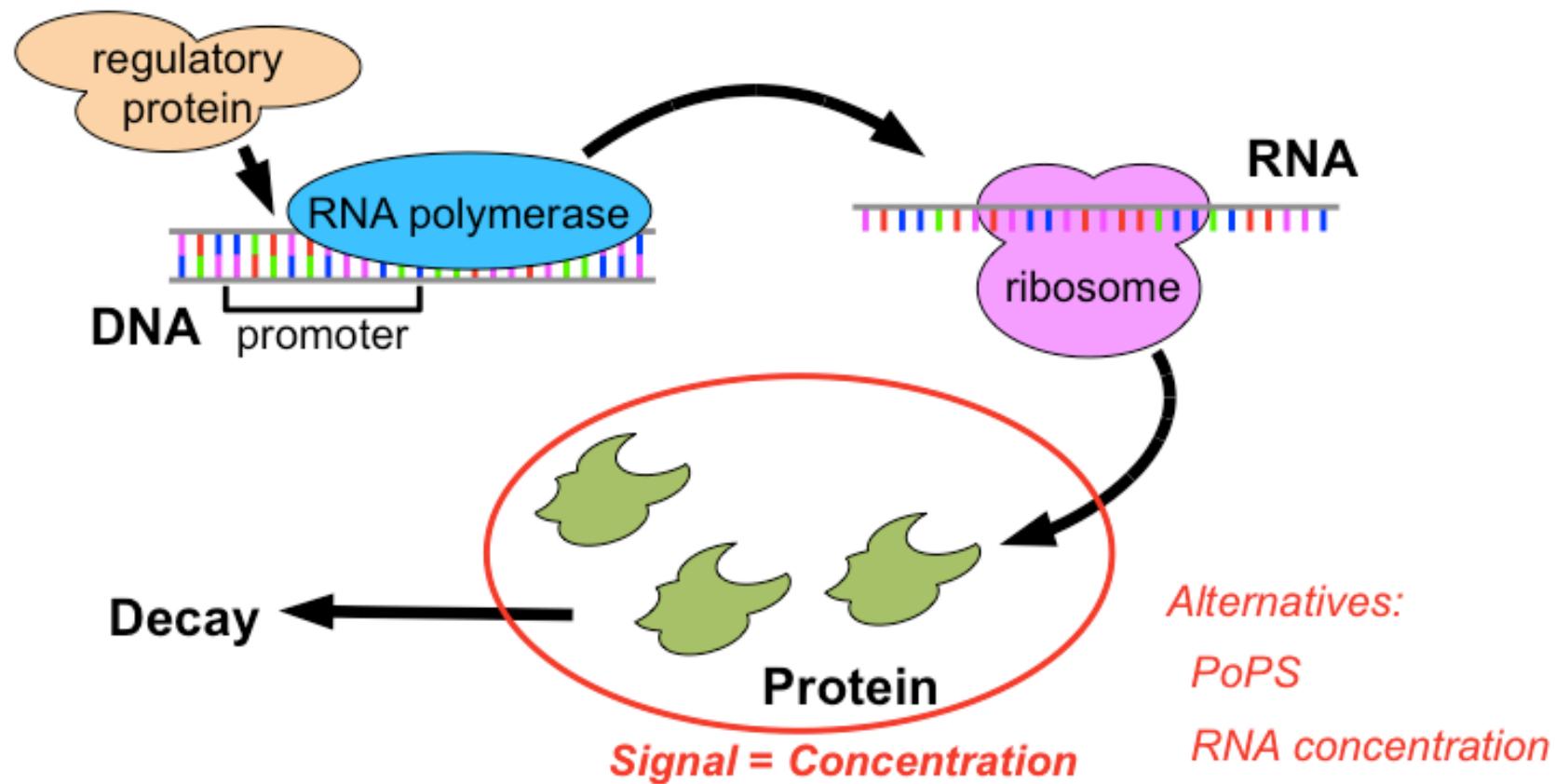
Focus: BioCompiler

Compilation & Optimization



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

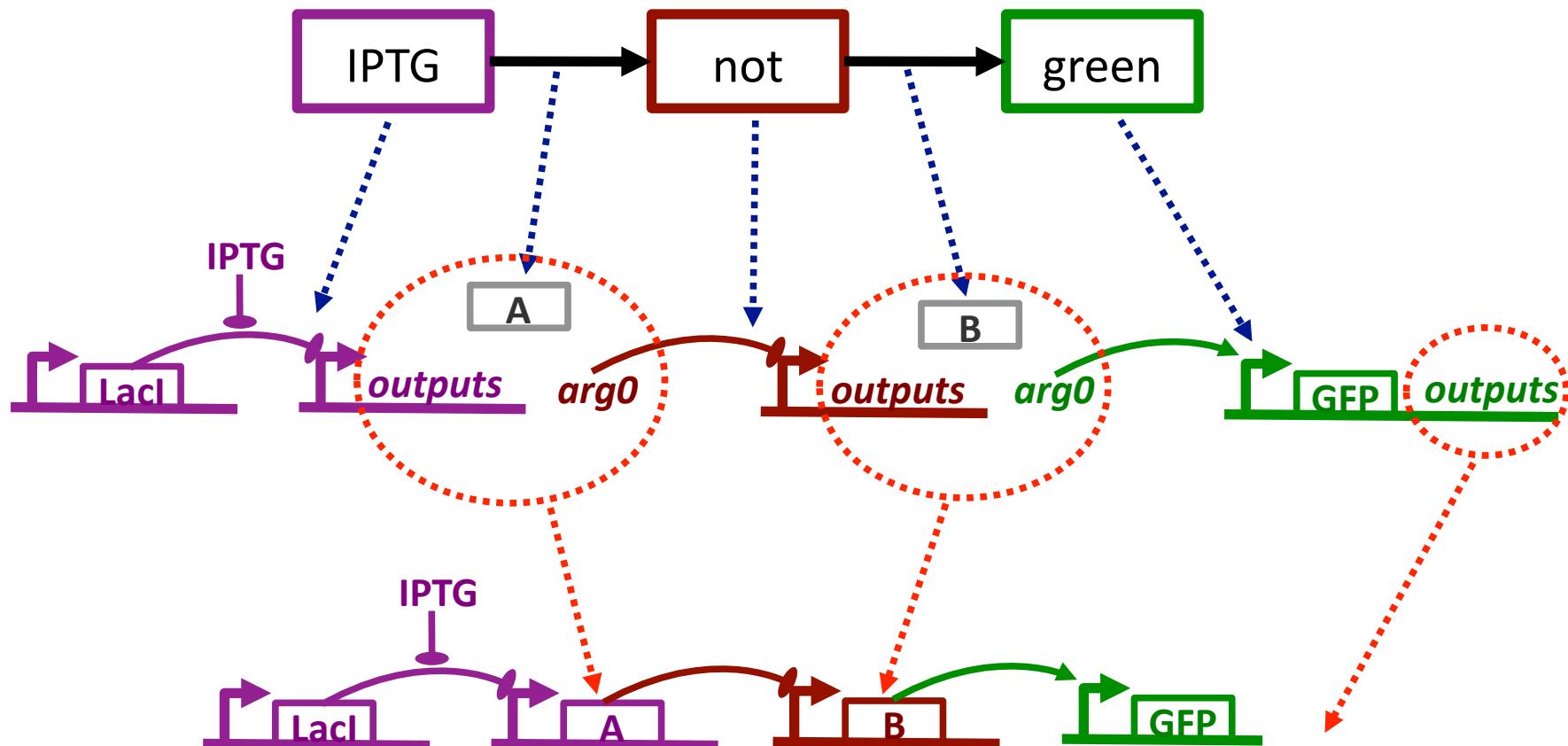
Transcriptional Logic Computations



Stabilizes at $\text{decay} = \text{production}$

Motif-Based Compilation

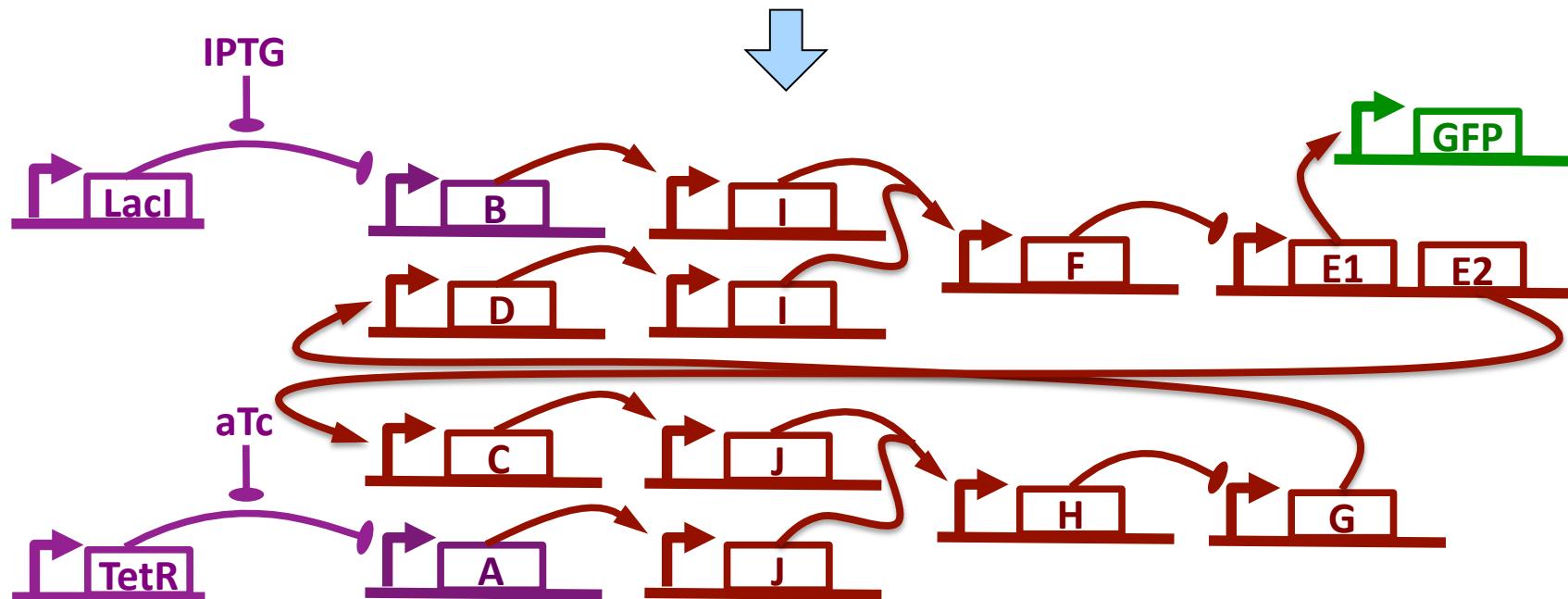
- Operators translated to motifs:



Design Optimization

```
(def sr-latch (s r)
  (letfed+ ((o boolean (not (or r o-bar)))
            (o-bar boolean (not (or s o))))
    o))  

(green (sr-latch (aTc) (IPTG)))
```

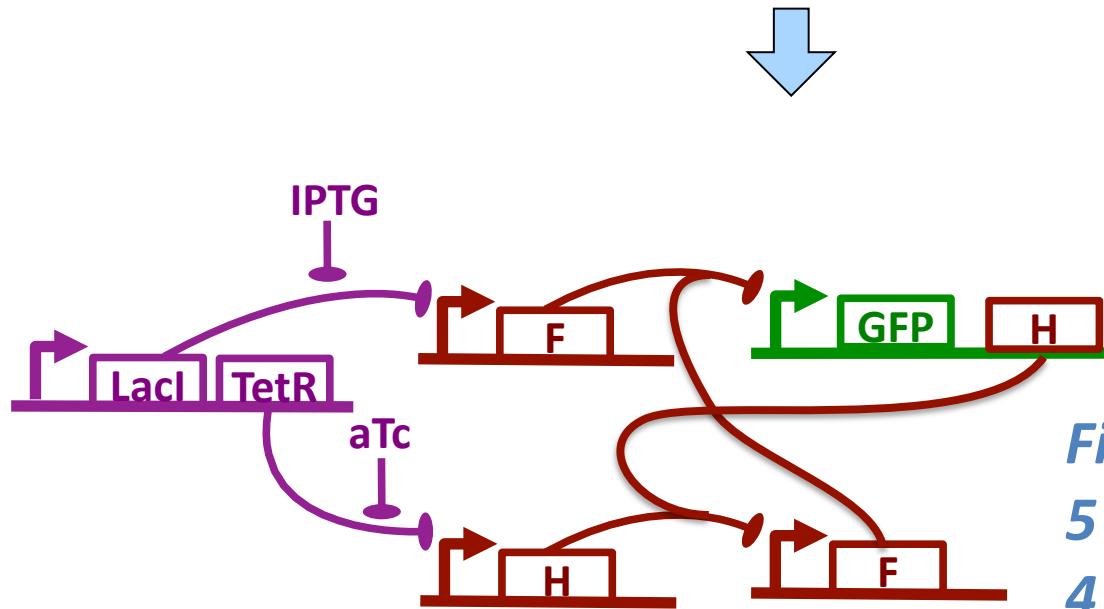


Unoptimized: 15 functional units, 13 transcription factors

Design Optimization

```
(def sr-latch (s r)
  (letfed+ ((o boolean (not (or r o-bar)))
            (o-bar boolean (not (or s o))))
    o))
```

(**green** (**sr-latch** (**aTc**) (**IPTG**)))

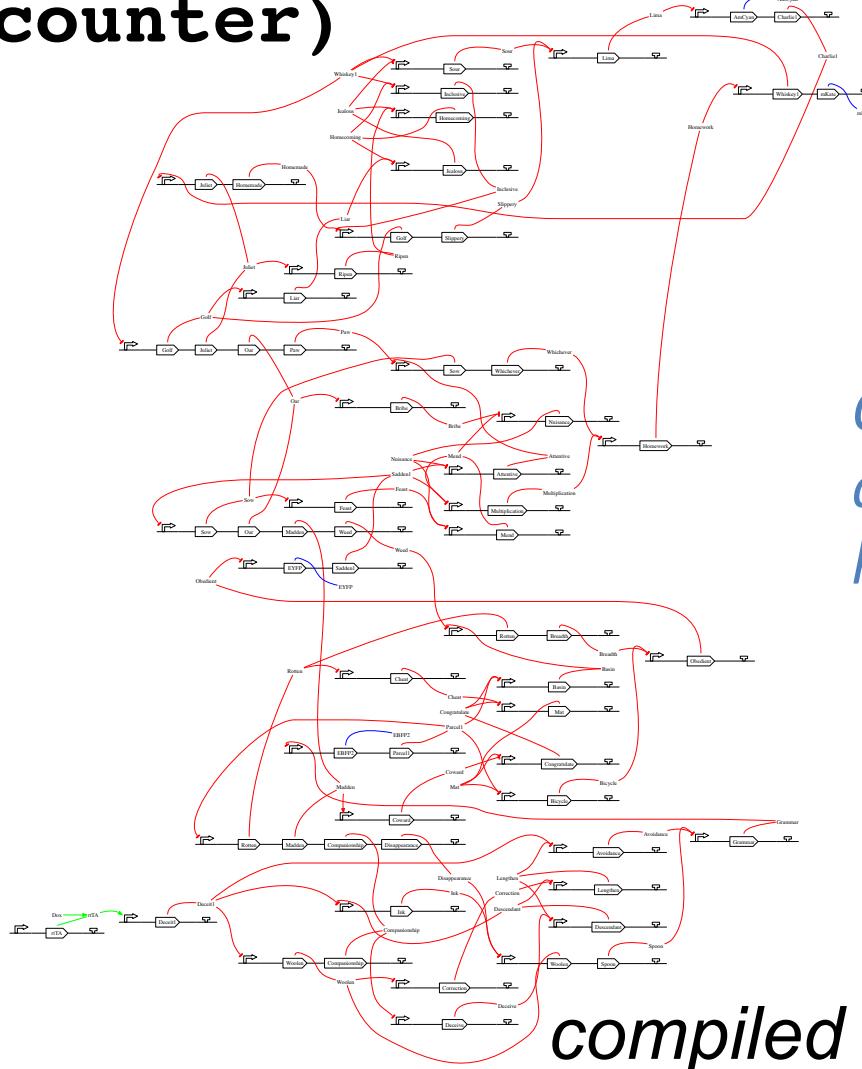


Final Optimized:
5 functional units
4 transcription factors

Unoptimized: 15 functional units, 13 transcription factors

Complex Example: 4-bit Counter

(4-bit-counter)



*Optimized compiler
already outperforms
human designers*

compiled for mammalian

Barriers & Emerging Solutions:

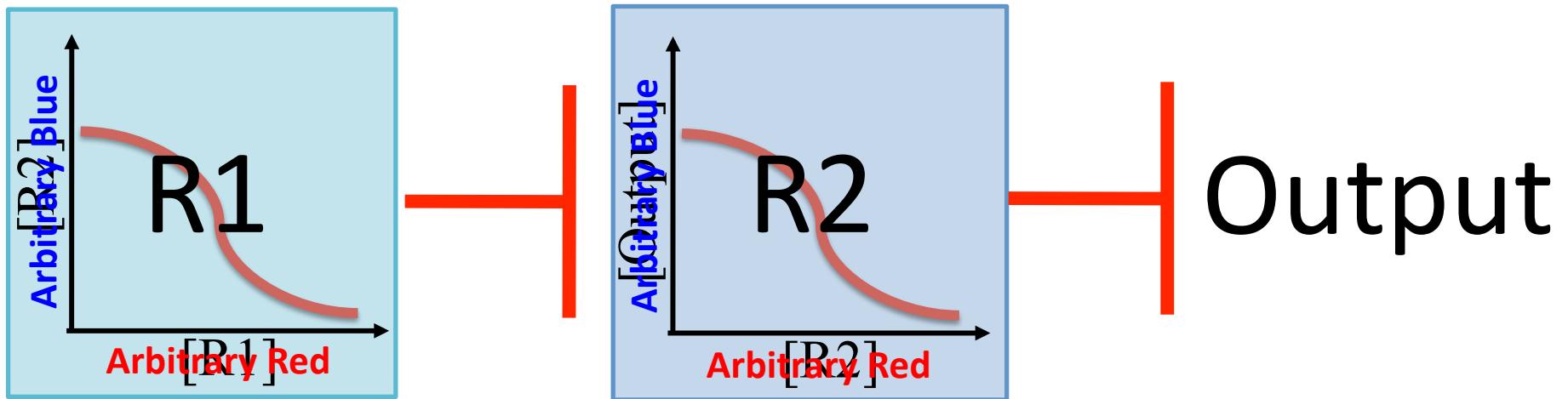
- Barrier: Availability of High-Gain Devices
 - Emerging Solution: combinatorial device libraries based on TALs, ZFs, miRNAs
- Barrier: Characterization of Devices
 - Emerging solution: TASBE characterization method
- Barrier: Predictability of Biological Circuits
 - Emerging solution: EQuIP prediction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

Outline

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- Proto BioCompiler
- **Calibrating Flow Cytometry** *TASBE Method*
- Building EQuIP Models
- Prediction & Validation

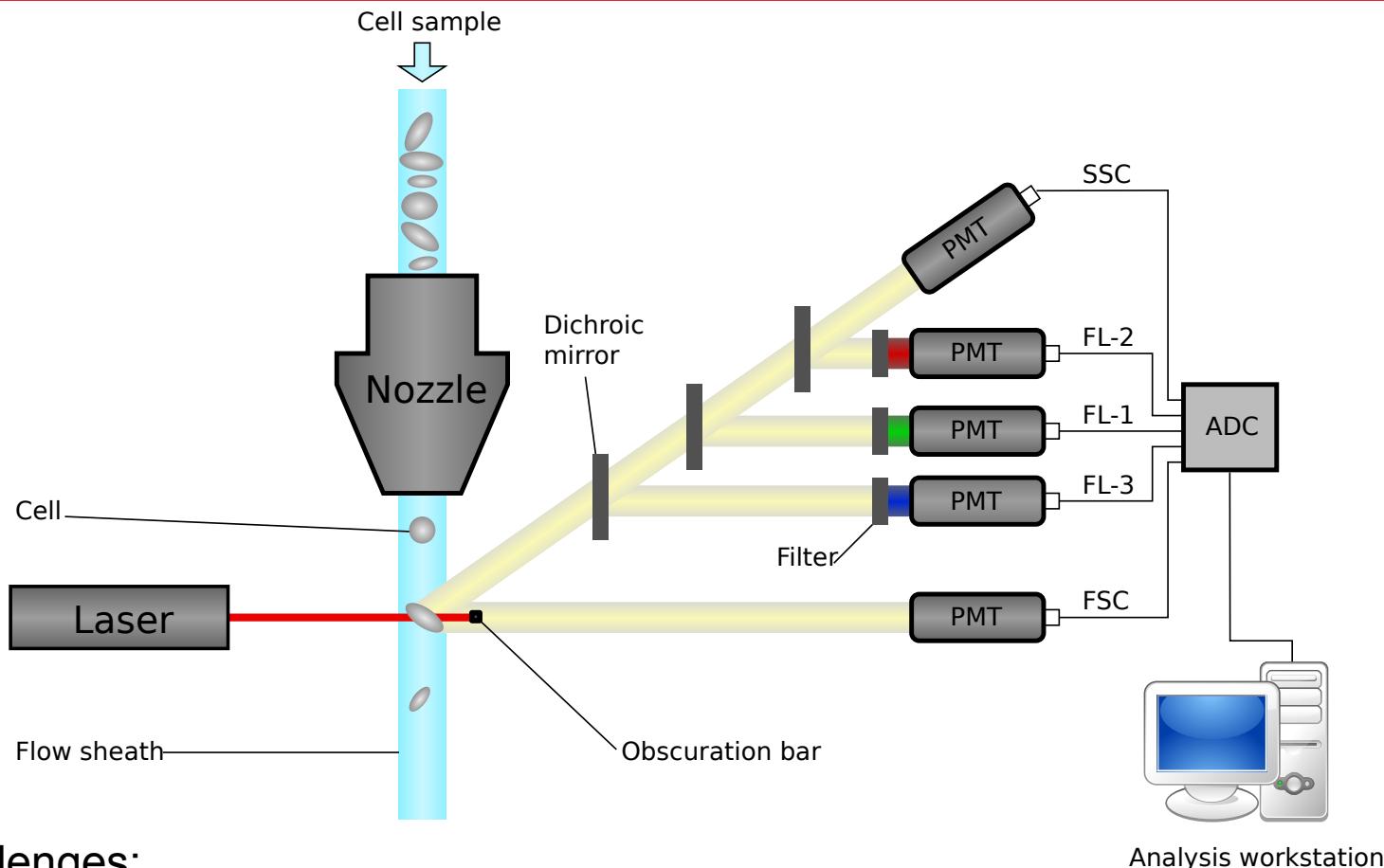
[Beal et al., Technical Report: MIT-CSAIL-TR-2012-008, 2012]

First, some metrology...



Unit mismatch!

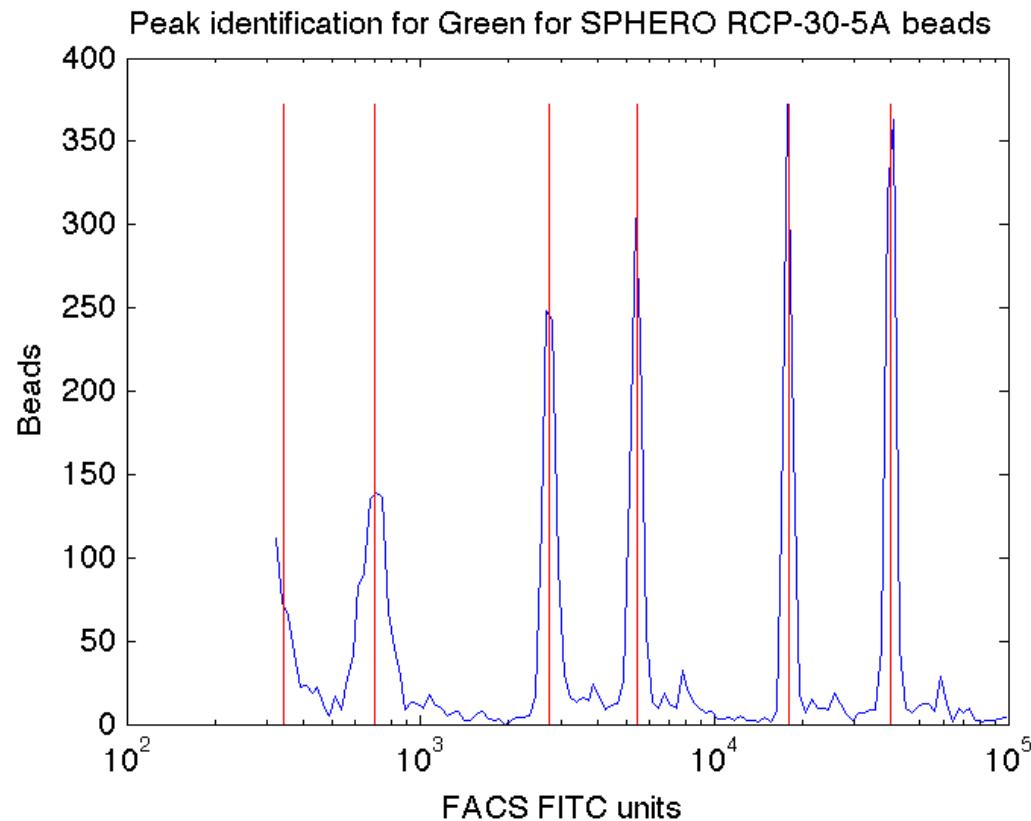
How Flow Cytometry Works



Challenges:

- Autofluorescence
- Variation in measurements
- Spectral overlap
- Time Contamination
- Lots of data points!
- Different protein fluorescence
- Individual cells behave (very) differently

Fluorescent Beads → Absolute Units

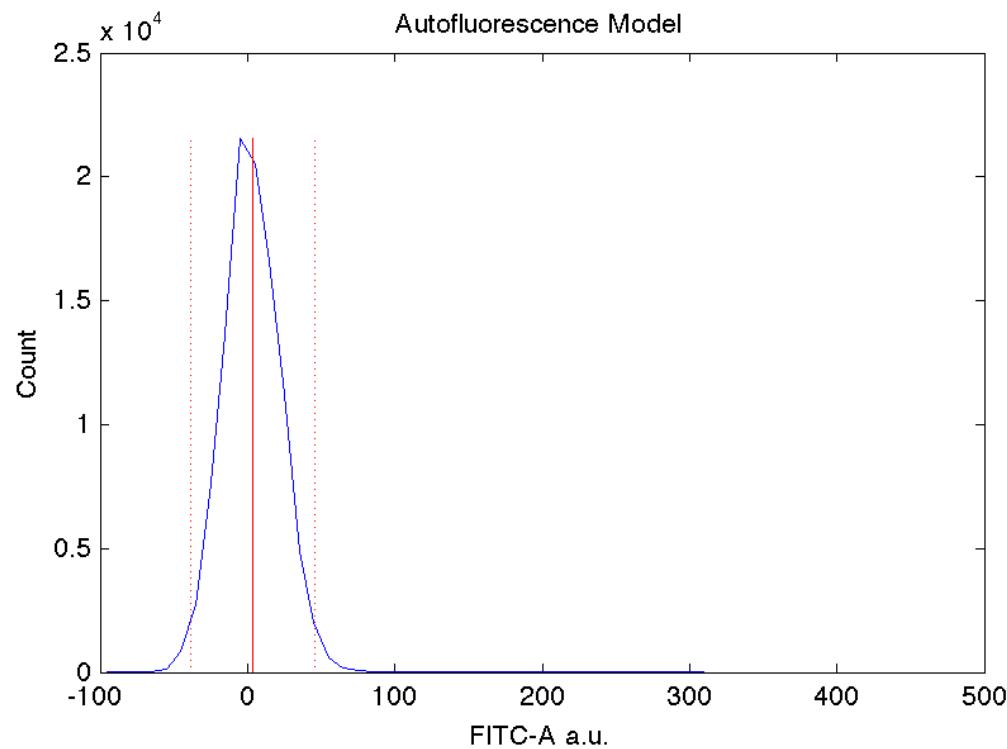


SpheroTech RCP-30-5A

PEAK #	CH #	MEFL
1	22.42	
2	77.13	692
3	108.17	2192
4	135.42	6028
5	164.11	17493
6	183.31	35674
7	217.49	126907
8	239.84	290983

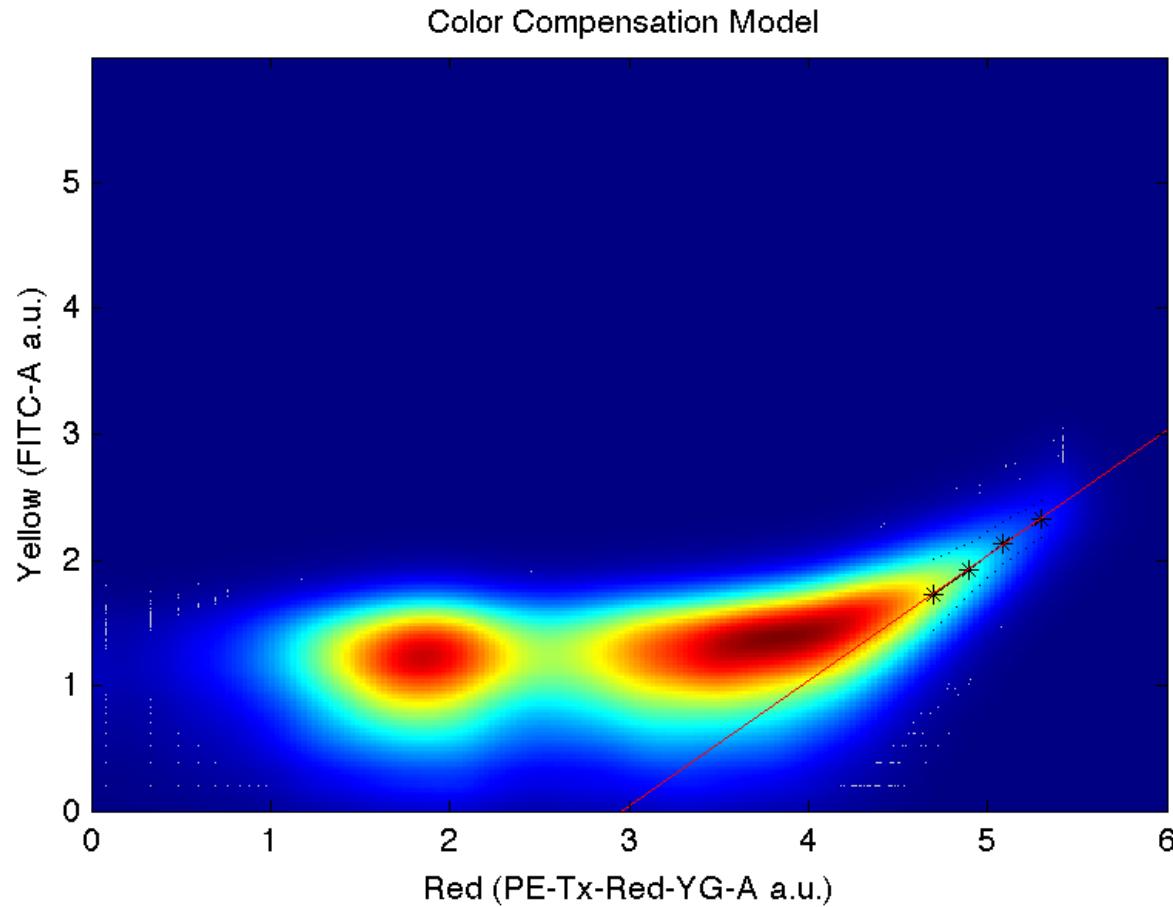
*Run beads every time: flow cytometers drift up to 20 percent!
Also can detect instrument problems, mistakes in settings*

Compensating for Autofluorescence



Negative control used for this

Compensating for Spectral Overlap

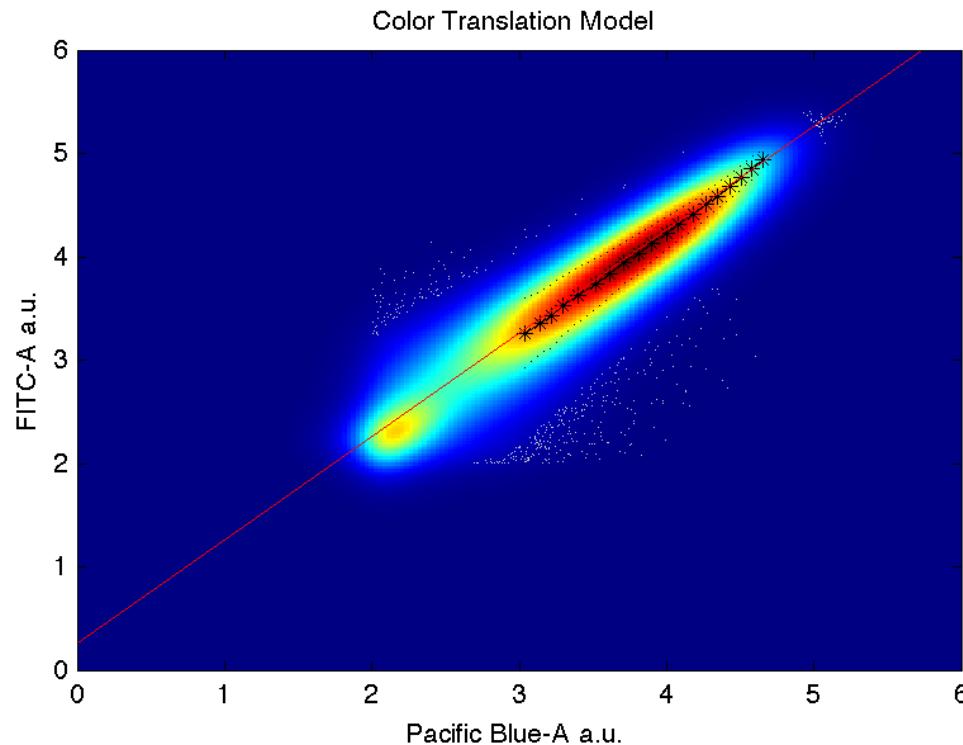


Strong positive control used for each color

Note: only linear when autofluorescence subtracted

Translating Fluorescence to MEFL

- Only FITC channel (e.g. GFP) goes directly



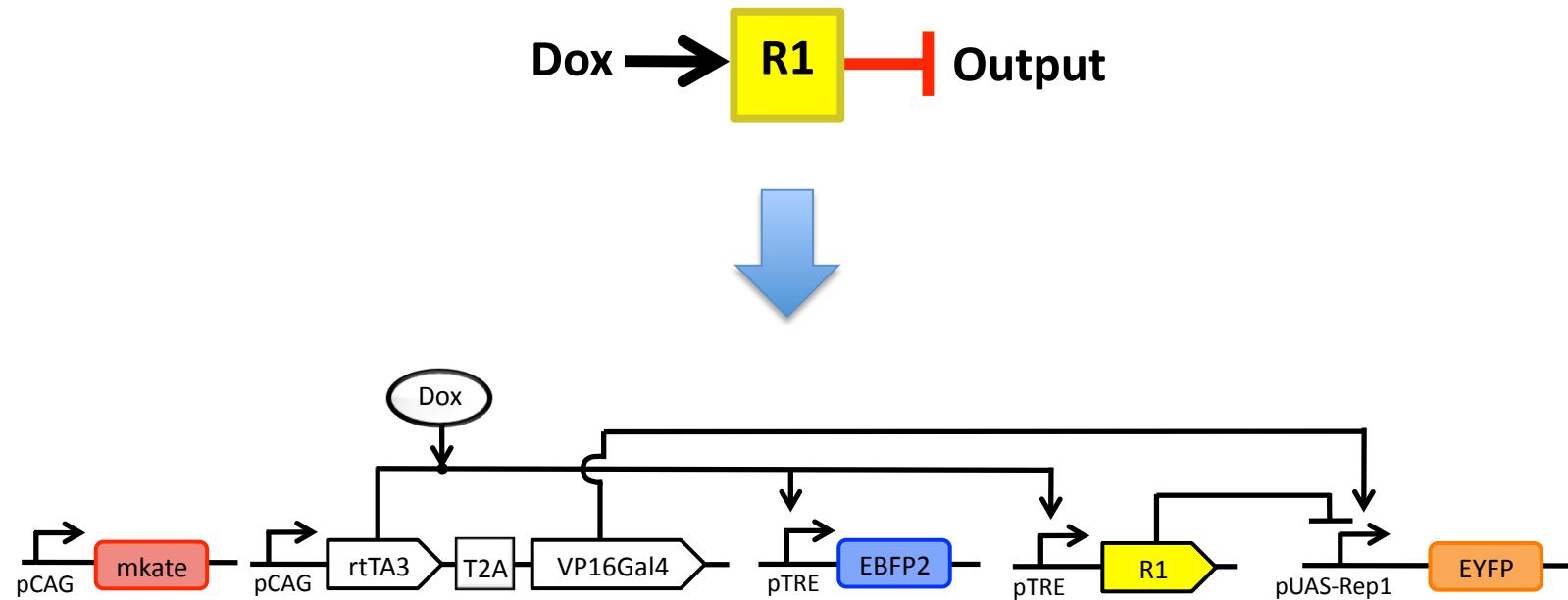
- Others obtained from triple/dual constitutive controls
- **Must have exact same constitutive promoter!**
- **Must have a FITC control protein!**

Outline

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- Prediction & Validation

[Davidsohn *et al.*, IWBDA, 2013]

TASBE Characterization Method



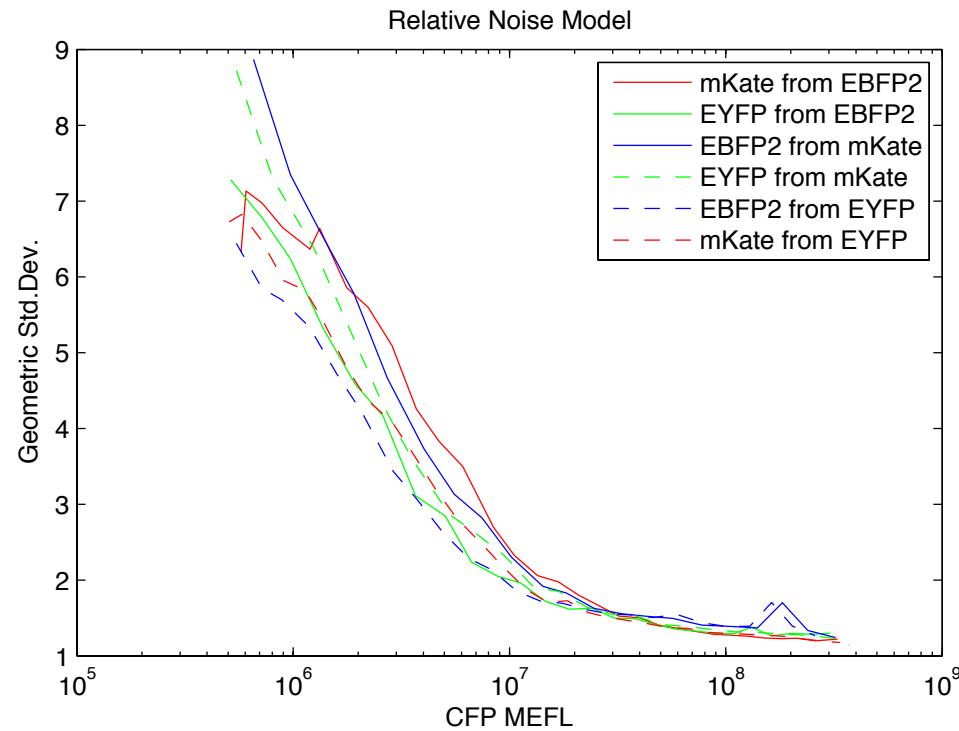
Transient cotransfection of 5 plasmids

Calibrated flow cytometry

Analysis by copy-count subpopulations

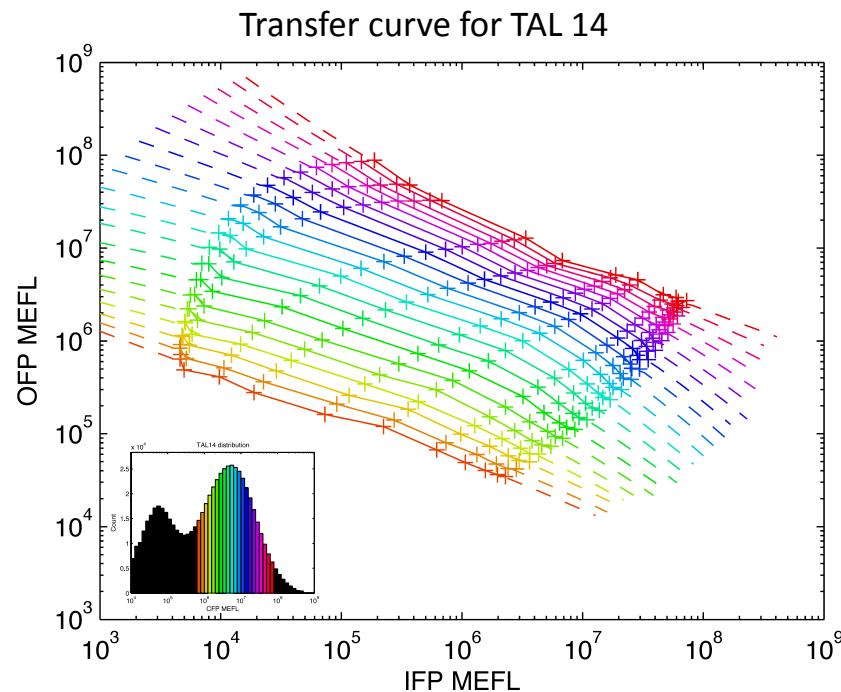
Multi-plasmid cotransfection!?

- Avoids all problems with adjacency, plasmid size, sequence validations

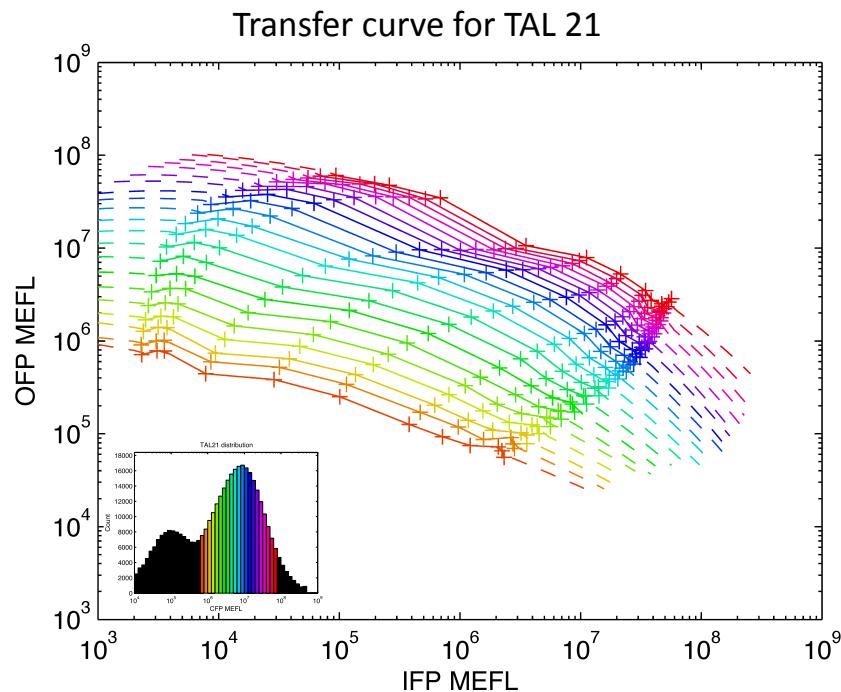


- Variation appears to be independent

Result: Input/Output Relations



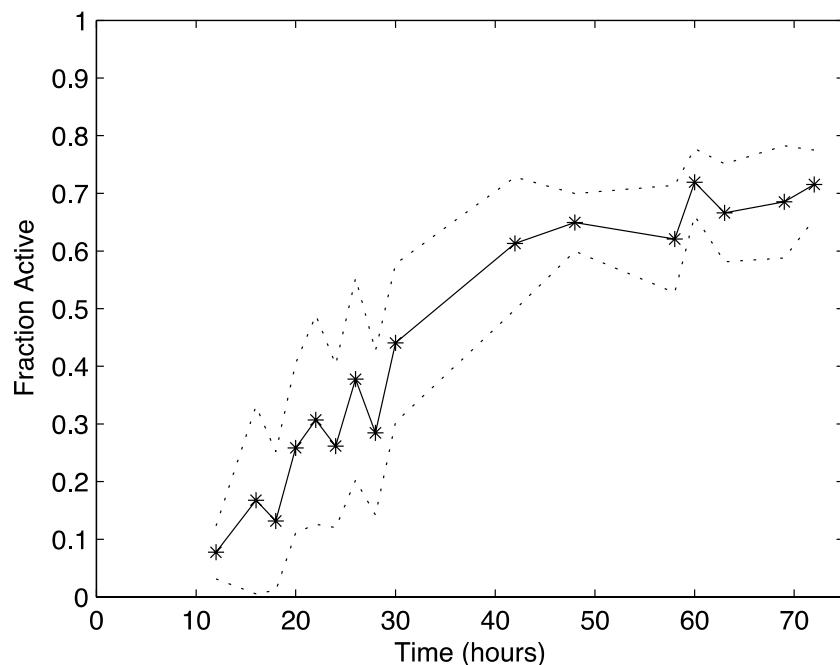
R1 = TAL14



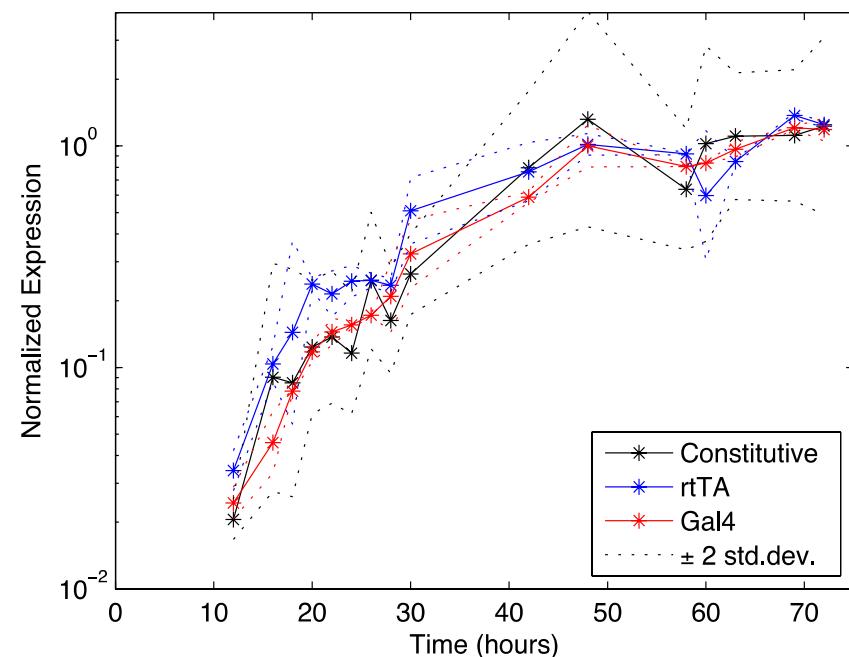
R1 = TAL21

Expression Dynamics

Fraction Active

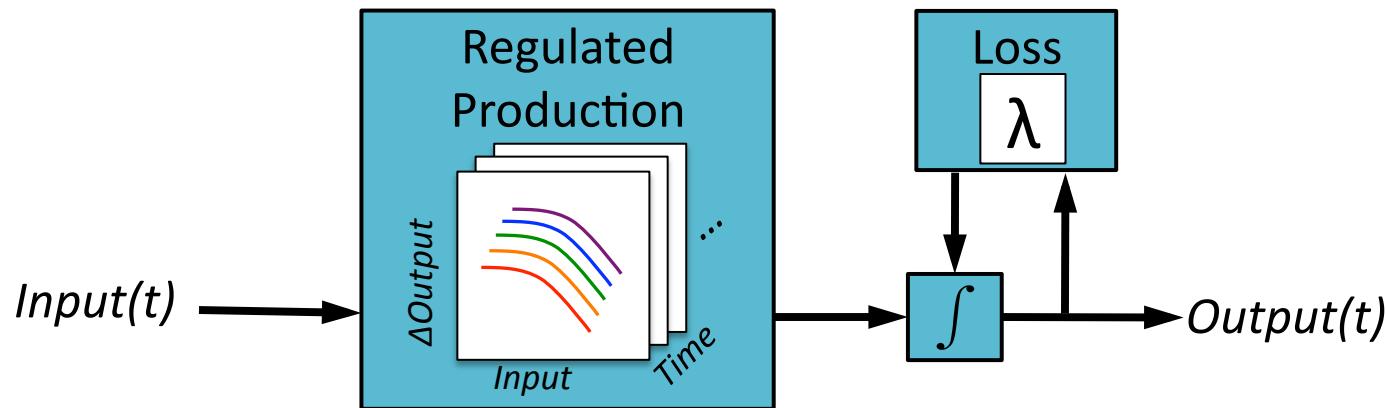


Mean Expression



Results → division rate, mean expression time, production scaling factor

EQuIP model



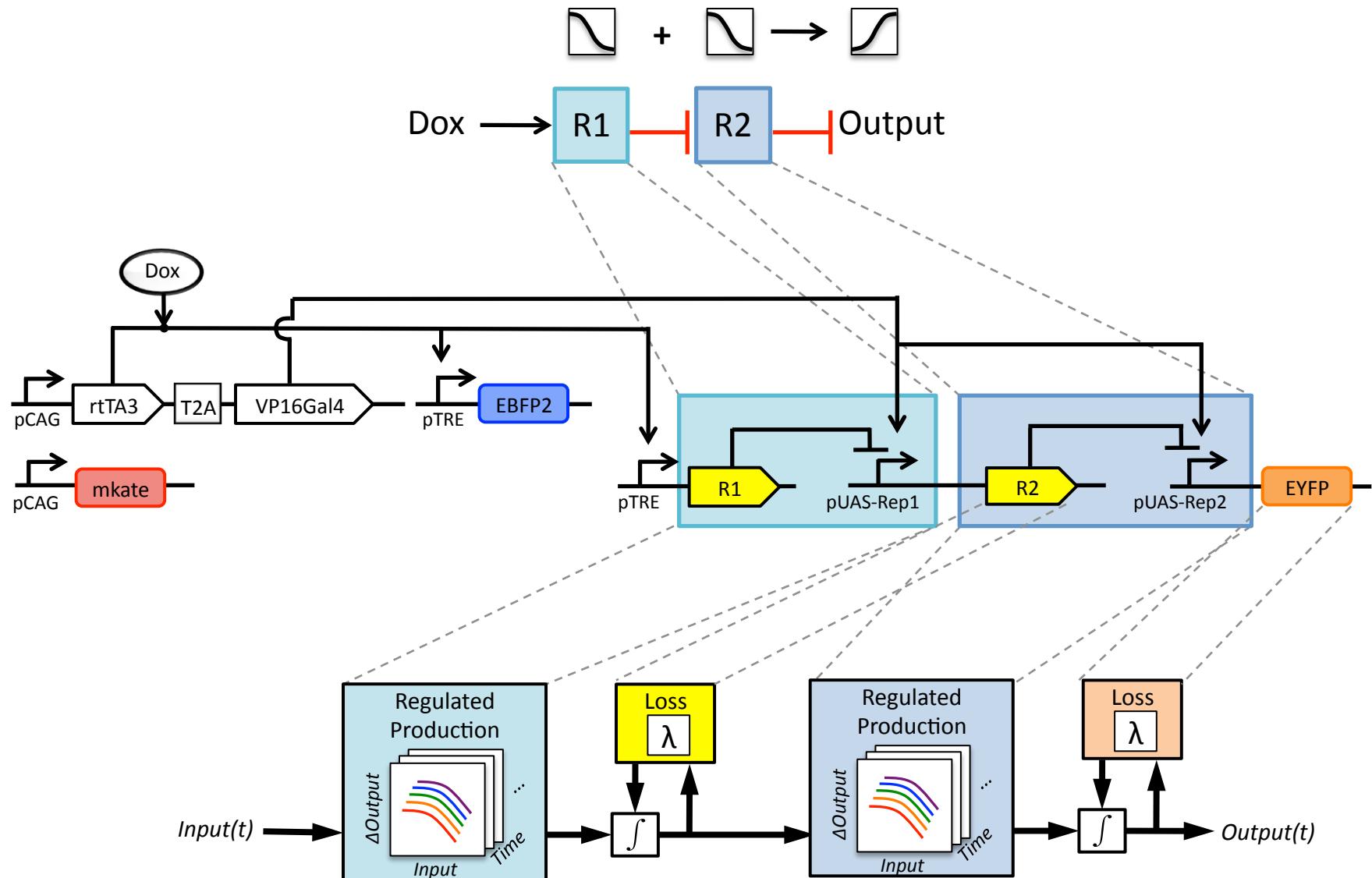
Model = first-order discrete-time approximation

Outline

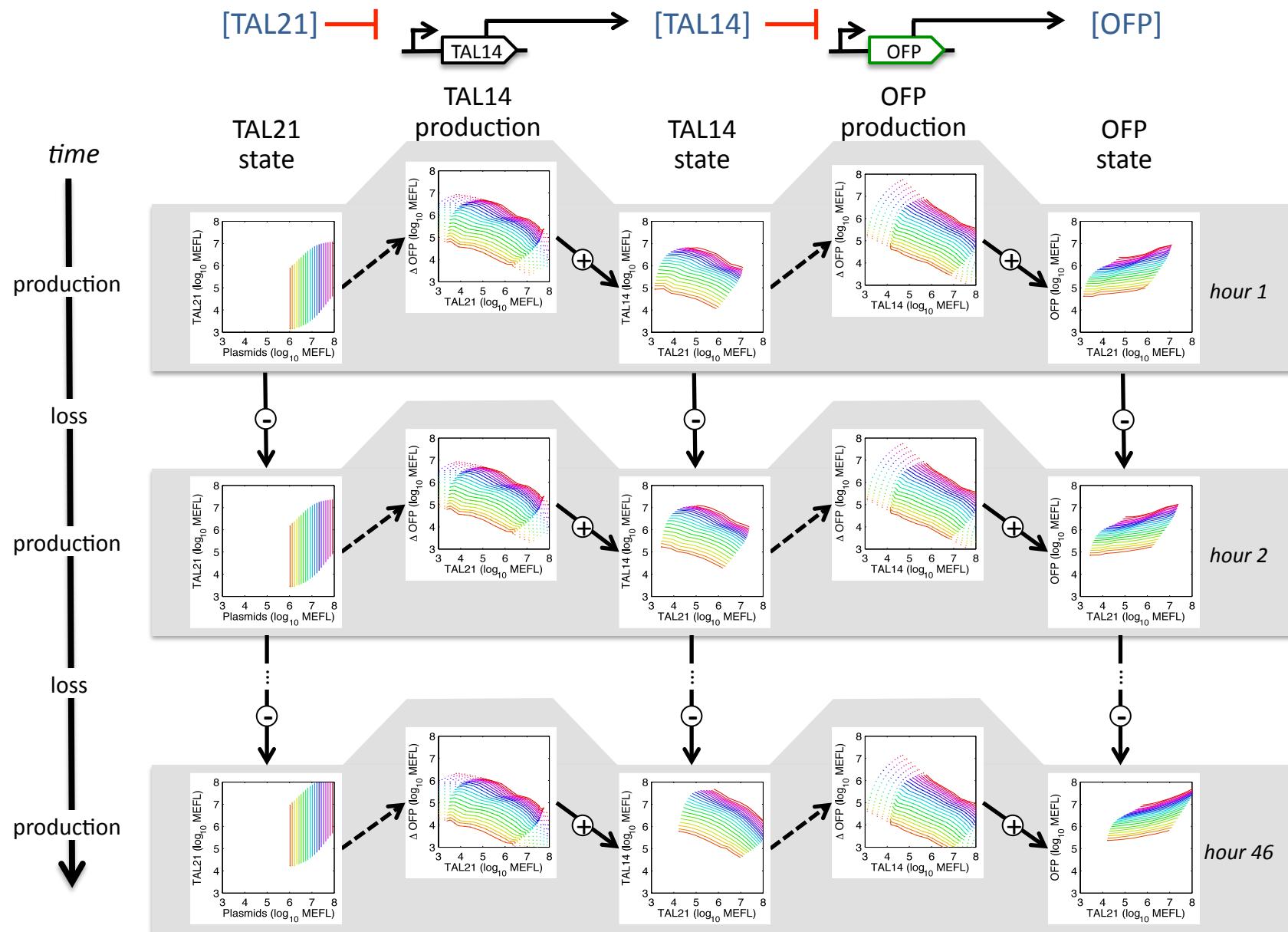
- Vision and Motivation
- Proto BioCompiler
- Calibrating Flow Cytometry
- Building EQuIP Models
- **Prediction & Validation**

[Davidsohn et al., IWBDA, 2013]

EQuIP Prediction



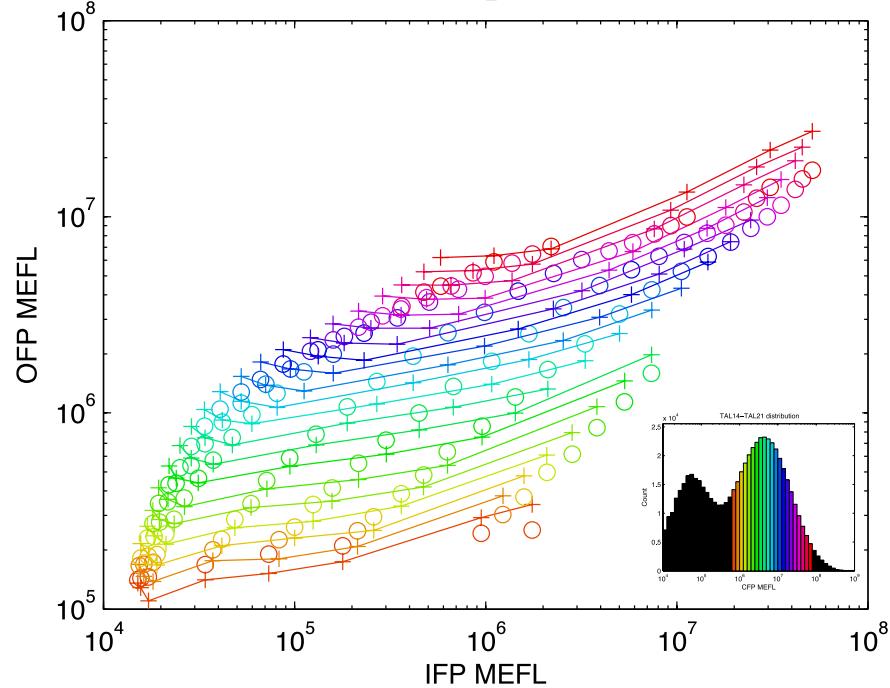
Incremental Discrete Simulation



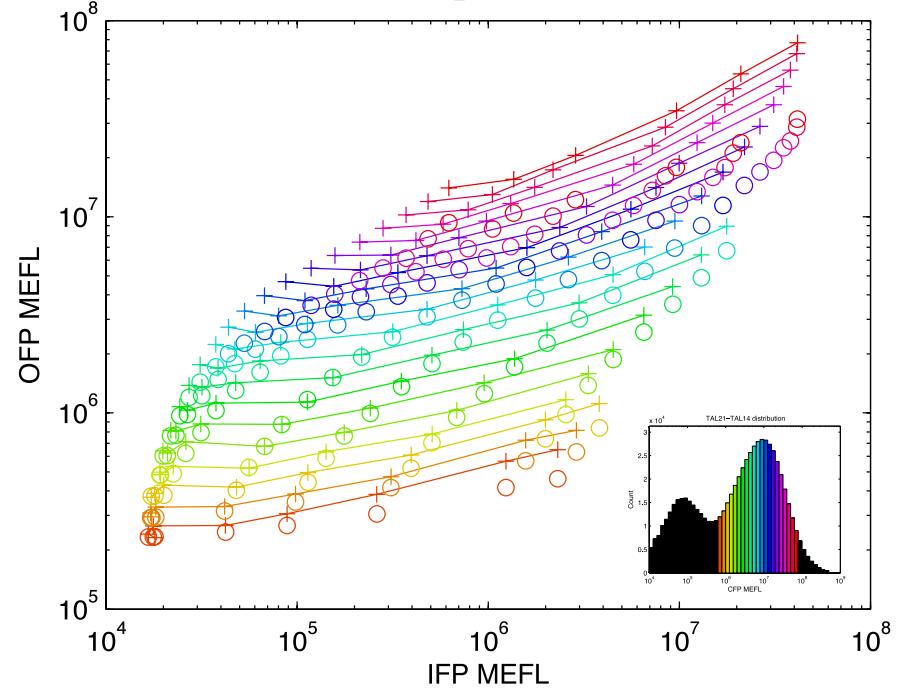
High Quality Cascade Predictions

1.6x mean error on 1000x range!

TAL14 → TAL21



TAL21 → TAL14



*Circles = EQuIP predictions
Crosses = Experimental Data*

Cascade	Mean Prediction Error	
	Output Level	+/- Induction
TAL14-TAL21	1.20x	2.0x
TAL14-LmrA	1.81x	1.8x
TAL21-TAL14	1.30x	1.7x
TAL21-LmrA	1.56x	2.2x
LmrA-TAL14	1.75x	1.5x
LmrA-TAL21	1.74x	1.1x

Summary

Automation supports design and debugging of biological devices, sensors, actuators, circuits

- BioCompiler automates regulatory network design
- TASBE method calibrates flow cytometry data
- Cotransfected test circuits give good models
- EQuIP accurately predicts cascade behavior from models of individual repressors

Looking forward:

- Real measurements → good engineering
- Bigger, better circuits on more platforms

[multiple manuscripts in preparation]

Acknowledgements:

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Ron Weiss
Jonathan Babb
Noah Davidsohn
Ting Lu

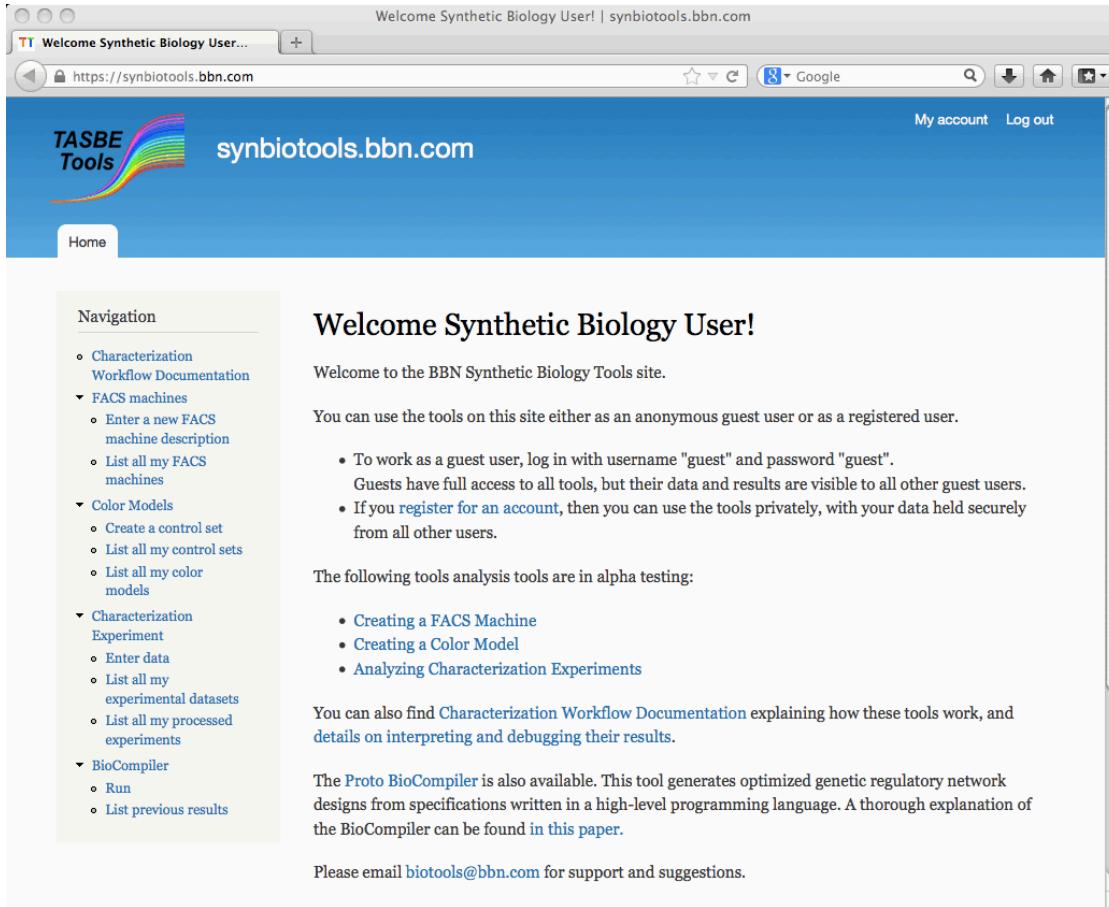


Douglas Densmore
Evan Appleton
Swapnil Bhatia
Traci Haddock
Chenkai Liu
Viktor Vasilev



Characterization Tools Online!

<https://synbiotools.bbn.com/>



The screenshot shows a web browser window for the URL <https://synbiotools.bbn.com/>. The page title is "Welcome Synthetic Biology User! | synbiotools.bbn.com". The header includes the "TASBE Tools" logo and links for "My account" and "Log out". The main content area starts with "Welcome Synthetic Biology User!" and "Welcome to the BBN Synthetic Biology Tools site.". It explains that users can log in as a guest or register for an account. It lists several alpha testing tools: "Creating a FACS Machine", "Creating a Color Model", and "Analyzing Characterization Experiments". It also mentions the "Characterization Workflow Documentation" and the "Proto BioCompiler". A sidebar on the left contains a "Navigation" menu with links to "Characterization Workflow Documentation", "FACS machines", "Color Models", "Characterization Experiment", "BioCompiler", and "Run". At the bottom, there's a note to email biotools@bbn.com for support.

- On first use, you will have to terms of service
- Your data is secure, and can't be shared on site.
- FireFox recommended; Chrome has an image-display bug.

*Register: individual accounts or group account?
Anonymous access also available (but not private)*