Biomolecular Breadboards for Prototyping and Debugging Synthetic Biocircuits

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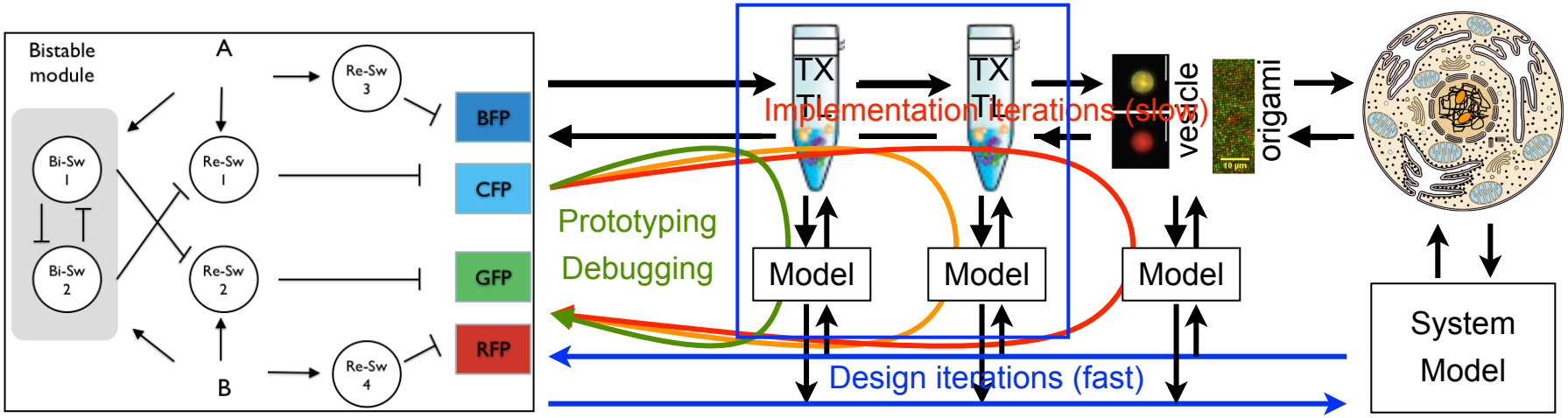
Cell-Free Biomolecular Breadboards



Key characteristics of the cell-free (TX-TL) breadboard (Shin & Noireaux, *ACS Syn Bio*, 2011)

- Inexpensive and fast: ~$0.03/ul for reactions; typical reactions run for 4-6 hours
- Easy to use: works with many plasmids or linear DNA (PCR products!)
  - Can adjust concentration to explore copy number/expression strength quickly
- Flexible environment: adjust energy level, pH, temperature, degradation

TX-TL breadboard components http://www.openwetware.org/wiki/breadboards

- Bulk reactions: 10 ul, 10-25 variations ([DNA], [inducers], etc) in a plate reader
- Droplet-based microfluidics: 0.3 ul, 50-100 variations + merge/split/etc
- Vesicle-based reactions ("artificial cells"): 1-100 fl, 100-1000 phospholipid vesicles
- Spatial localization using DNA origami: 1000 copies w/ 10-100 nm spatial ctrl
- Reaction-based modeling: MATLAB/Simbiology toolbox, with resource limits

Related approaches: Litcofsky et al (*Nature Methods*, 2013); Chappel et al (*NAR*, 2013)

Sample TX-TL Based Design Process

S0: modeling (minutes/cycle, systematic design & analysis)

- Desired function + specs → set of possible designs (circuits) + sensitivity analysis
- Goal at this stage is to determine what circuits to test in TX-TL and predict outputs

S1: linear DNA (4-8 cycles @ 2 cycles/day, 24-96 variants)

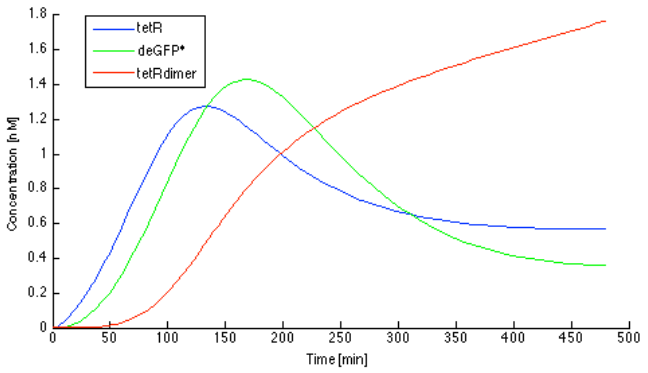
- Components from std library or PCR extension (no cloning)
- Test in TX-TL with GamS, ClpX. Try multiple circuits + vary ratios of copy numbers (based on achievable copy #'s)
- Compare w/ models; insure we can model what we see
- Goal: downselect 4-8 designs to test in plasmids

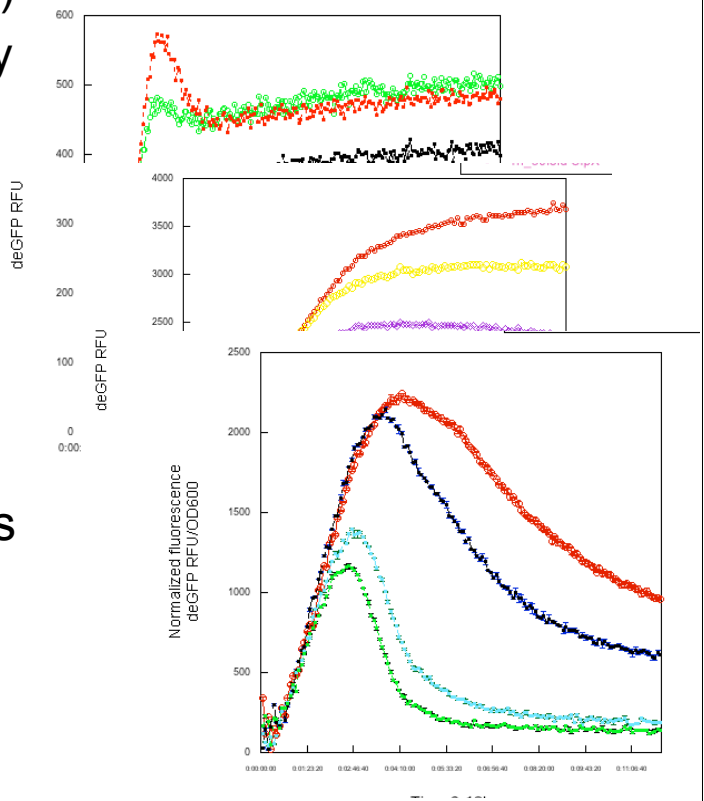
S2: plasmids (2-4 cycles @ 2 days/cycle, 8-24 variants)

- Clone into plasmid(s), using std sequences/protocols
- Verify operation in TX-TL, incl copy number variability
- Test robustness in multiple extracts w/ varying conditions
- Match results to S0 models and S1 linear DNA

S3: validate in cells (1 cycle, 4 days, 1-4 variants)

- Test top constructs from plasmid-based TX-TL assay





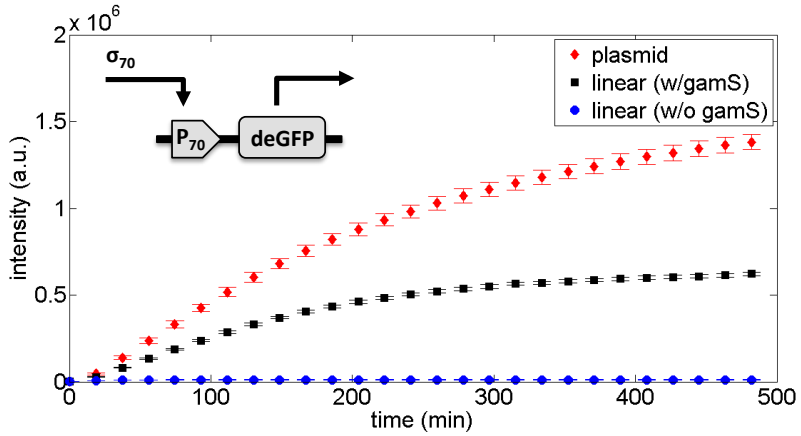
Sun, Hayes et al, 2013 (in preparation)

TX-TL Core Processes

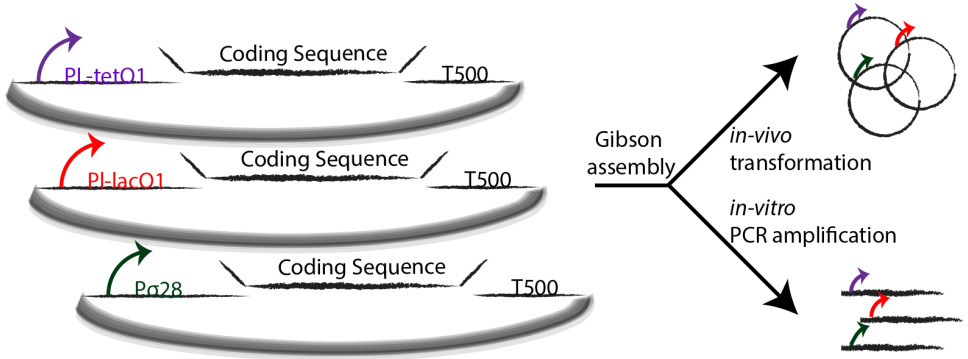
Zachary Sun, Vincent Noireaux

Rapid prototyping using linear DNA

- Use PCR products with GamS to get expression levels of ~60% of plasmid

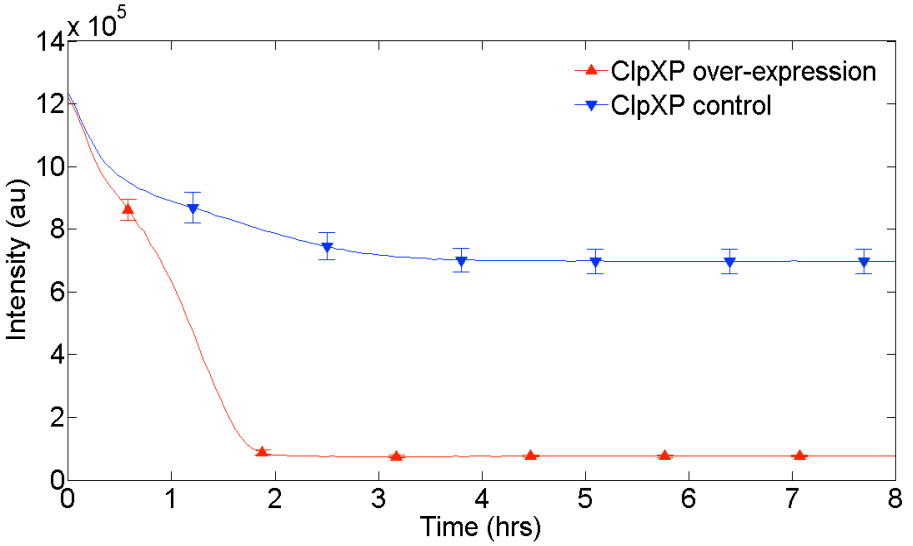


- Allows rapid assembly of constructs
  - PCR extension for simple circuits
  - IDT gBlocks + isothermal ass'y



Protein degradation

- Use clpXP machinery to degrade tagged proteins

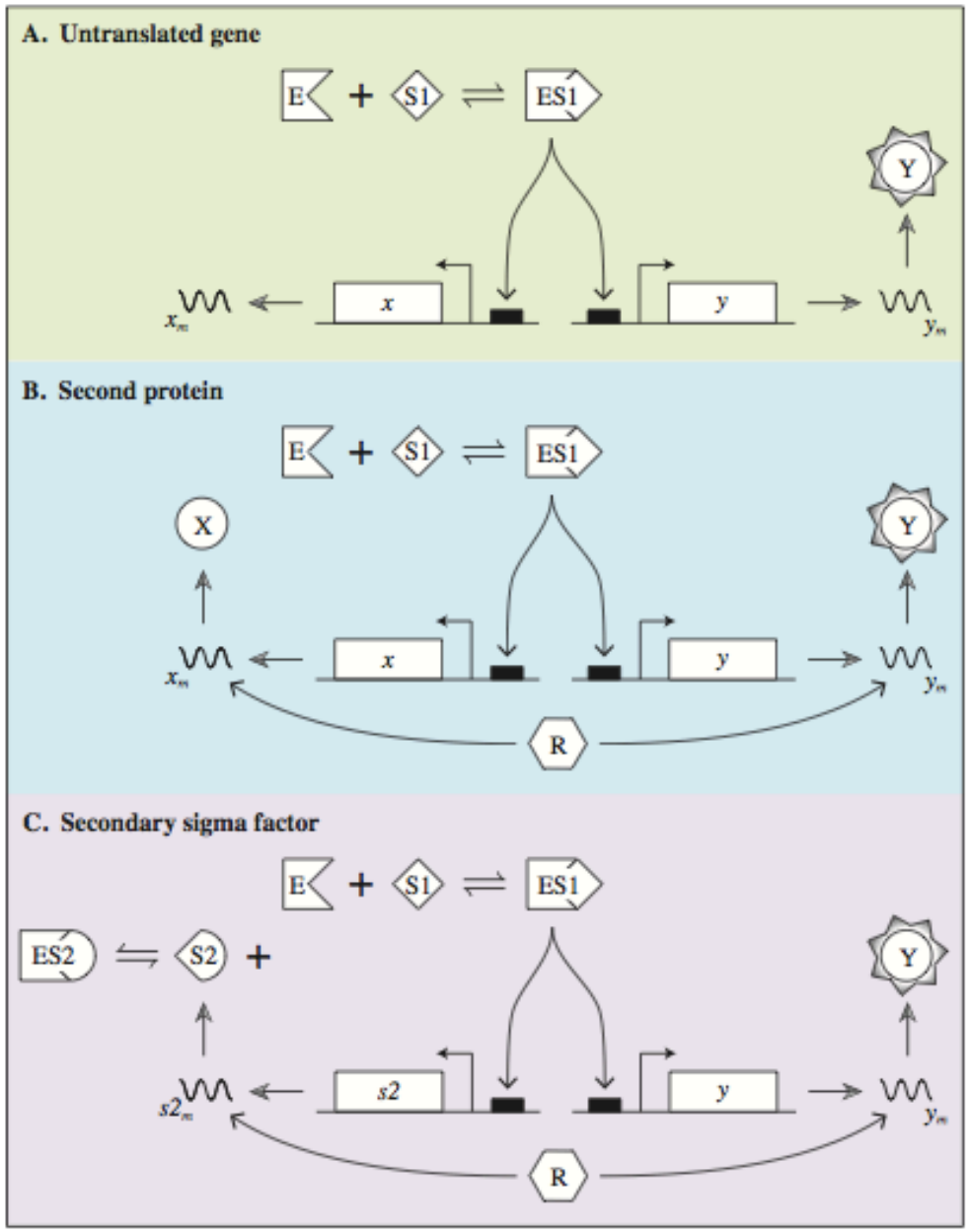


Tested components

- RNA polymerases: E. coli*, T7
- Activators: sigma28*, AraC*
- Repressors: TetR*, LacI*
- Reporters: deGFP*, MG, mSpinach
- Phosphorylation: NRI/pglnA
- DNA/RNA/protein deg: gamS*, clpXP*

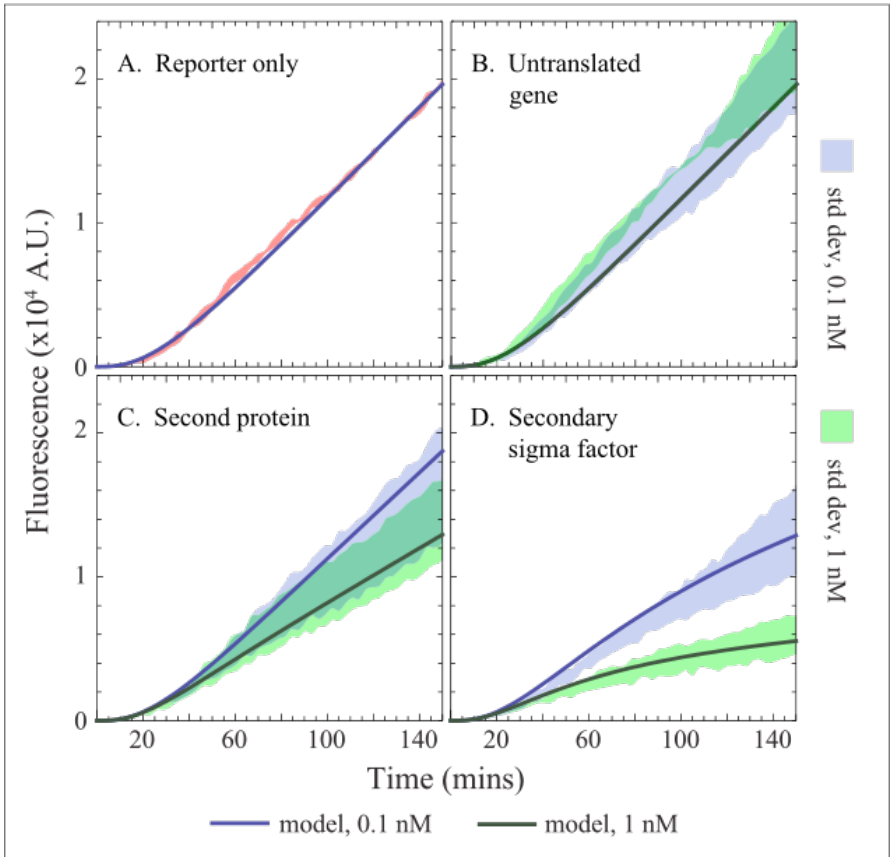
* preliminary models also available

Effects of Resource Limits



Which resources are limited?

- No evident transcriptional limits [B]
- Limited protein resources (AA, ATP) generate significant coupling [C]
- Sigma factors sequester RNAP [D]

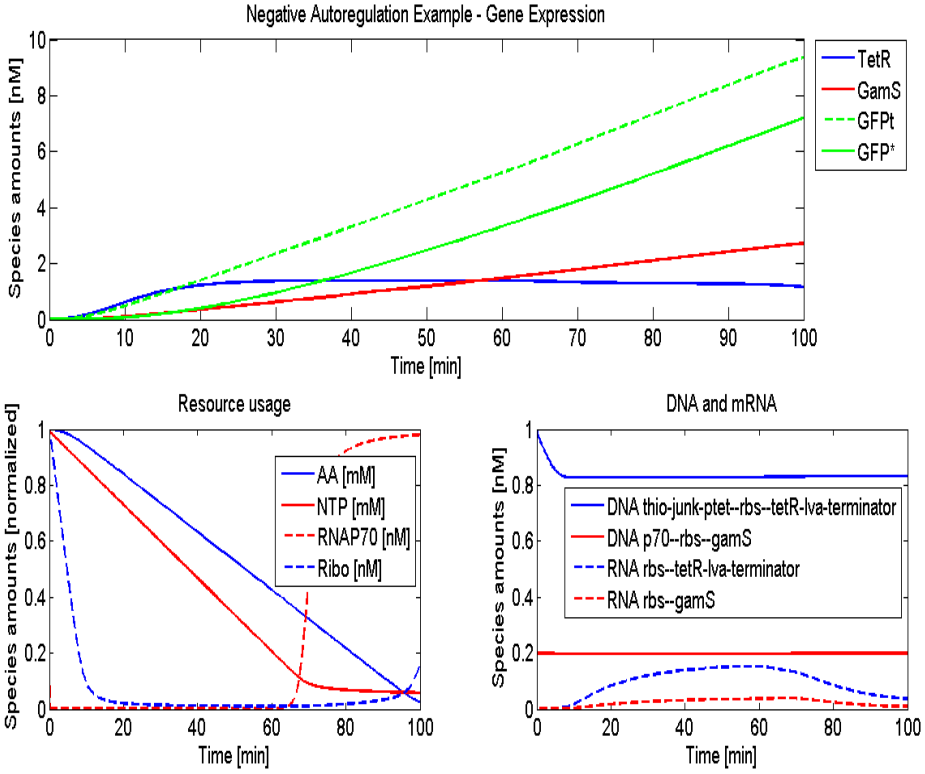


TX-TL Modeling

Zoltan Tuza, Vipul Singhal, Dan Siegal-Gaskins

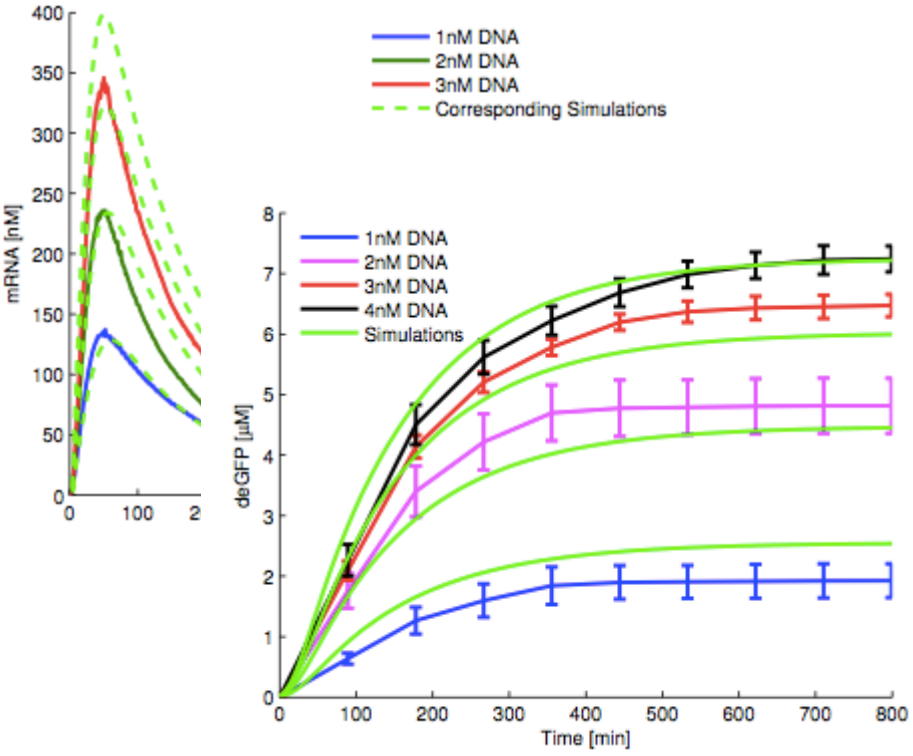
MATLAB toolbox (sf.net/projects/TXTL)

```
% Set up the standard TXTL tubes
tube1 = txtl_extract('e1');
tube2 = txtl_buffer('b1');

% Set up a tube that will contain our DNA
tube3 = txtl_newtube('circuit');
dna_tetR = txtl_dna(tube3, 'ptet', 'rbs', 'tetR', 100, 'linear');
dna_gamS = txtl_dna(tube3, 'p70', 'rbs', 'gamS', 10, 'plasmid');

% Mix the contents of the individual tubes and add some inducer
well_a1 = txtl_combine([tube1, tube2, tube3], [6, 2, 2]);
txtl_addspecies(well_a1, 'aTc', 0.1);

% Run a simulation
[t_ode, x_ode, names] = sbiosimulate(well_a1);
```



Resource utilization effects

- Model+TXTL shows effects of fixed number of RNAPs and ribosomes
- Additional sigma factor gene introduces significant 'crosstalk', reduces output
- Calibrated models that match experimental results



External TX-TL Circuit Testing

Circuit testing (DARPA LF, ONR MURI)

- Stage 1: you send us cells/plasmids; we verify *in vivo* operation (in our hands)
- Stage 2: we perform TX-TL runs, compare to *in vivo*, send you back data
- Stage 3: you perform TX-TL experiments; compare to our TX-TL runs + *in vivo*

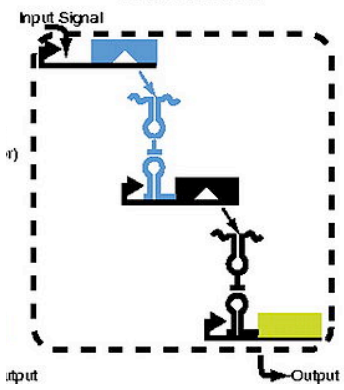
PI (+ contact)	Circuit/Technology	1 2 3
Lucks (CH)	RNA-sensing TFs	✓✓✓
Del Vecchio (EY)	Loading effects	✓✓ -
Temme (VH)	Orthogonal RNAPs	✓? -
Voigt (DSG)	4 input, 11 gene	✓x -
Tabor (JK)	Green light sensor	✓✓○
Endy (VH)	DNA memory	✓ x -
Del Vecchio (SG)	Phospho-insulator	✓✓ -
Kortemme (EdIS)	Molecular sensors	✓○ -
Jewett (YW)	Butanediol pathway	✓✓ -

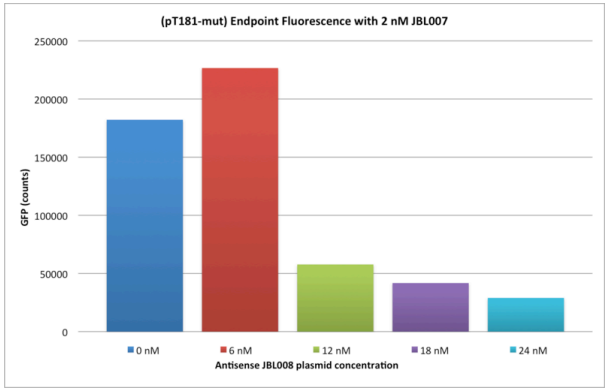
Things that work

- Transcriptional circuits
- RNA-based circuits
- Phosphorylation circuits
- Light-induced transcription factors

Things that haven't worked (yet)

- Multi-layer cascades (resource lims)
- DNA integrase/excisionase (copy #?)
- Modified T7 RNAP (leaky expression)





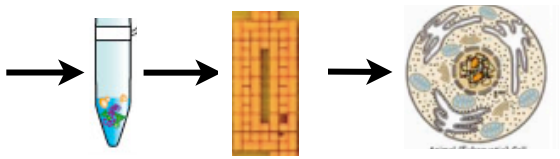
CSHL synthetic biology course: Jul '13

- Will use TX-TL for RNA-based circuits (Lucks) + light-sensitive TFs (Tabor)

Biomolecular Breadboard Suite

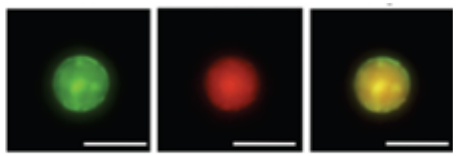
Cell-free breadboard

- Linear DNA assembly (build on work of others)
- Implement/test 6 circuits
- Document design cycle times (vs std cloning)
- Extract preparation video (Sun et al, JoVE, 2013)
- Predictive models for switch, IFFL, neg fbk



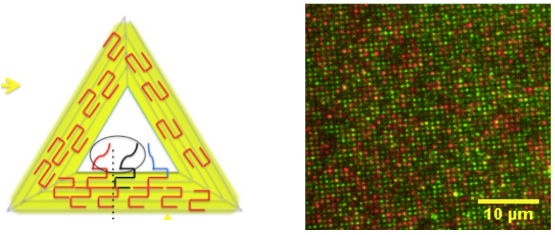
Artificial Cells

- Kinetics of expression inside vesicles
- Statistics of expression and induction (% of vesicles induced)
- Expression (and induction) as a function of vesicle size (1-100 fL)



Spatial Localization

- Control spatial location of DNA, RNA, proteins using DNA origami
- Explore effects of distance on hybridization, binding, scaffolding
- Demo'd transcription of bound DNA



Prototyping and debugging of *in vivo* and *in vitro* circuits

- Very little knowledge/infrastructure required to build in vitro circuits (try it!)
- Exploring use for synthetic biology courses (1 week labs); prototype at CSHL '13

Open source information

- TX-TL protocols, data, tools: http://www.openwetware.org/wiki/breadboards