Construction of a Bacterial Temperature Sensor

IBE-Tucson Arizona
February 12 2006

Overview

Motivation:

- Bacterial Thermometer
- Temperature Sensor

Picking Promoters:

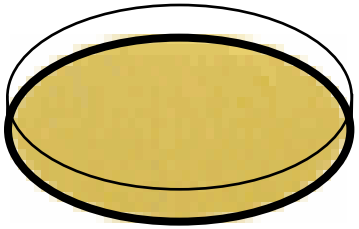
- Background: Heat and Cold Shock response
- Experiment Design
- The Big List

New Circuit “Parts”:

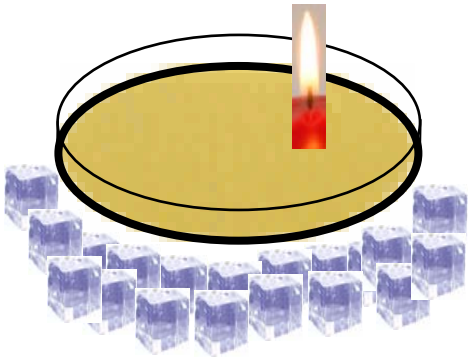
- 3 cold sensing promoters
- Inverter to make heat sensing circuit

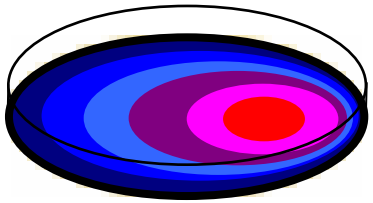
What we learned

Goals:



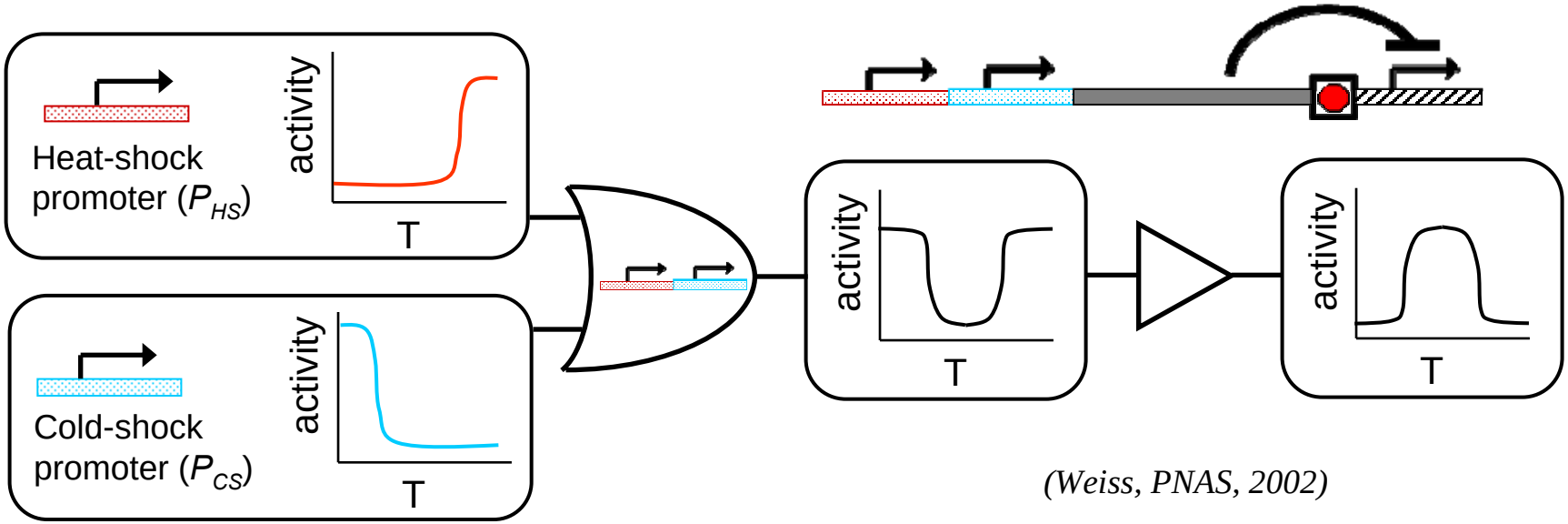
Bacterial Lawn
(*E. coli*)





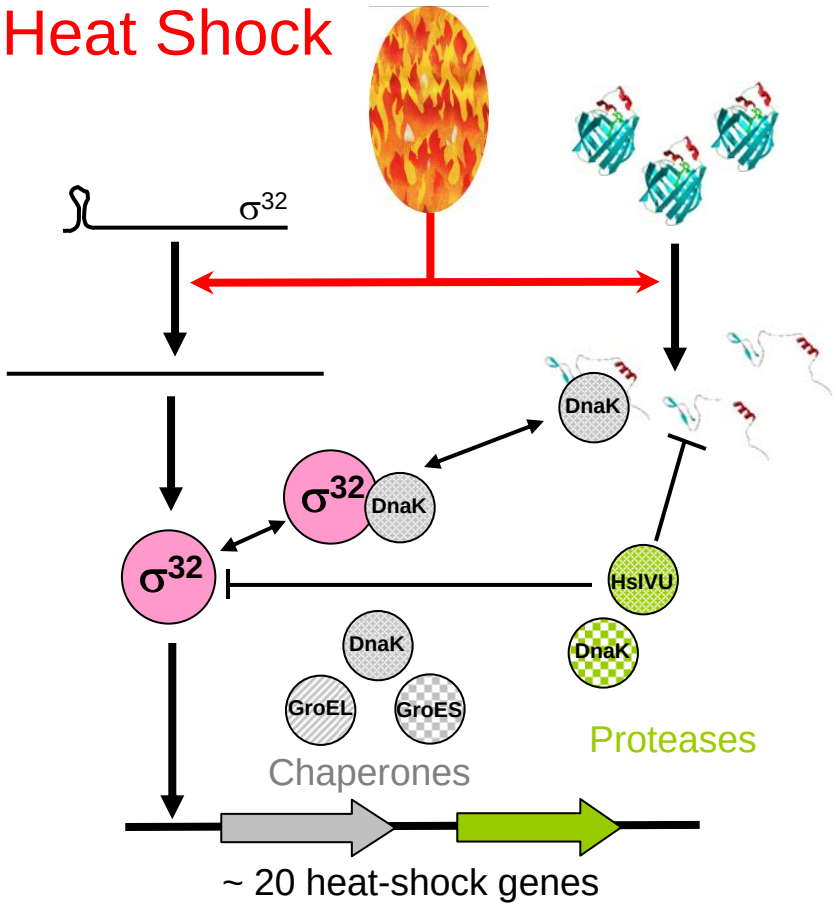
Change in gene
expression

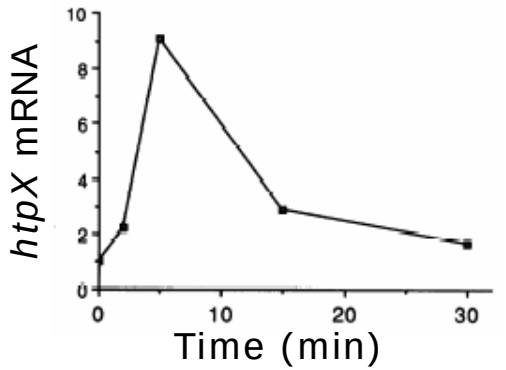
Construct a temperature sensor



(Weiss, PNAS, 2002)

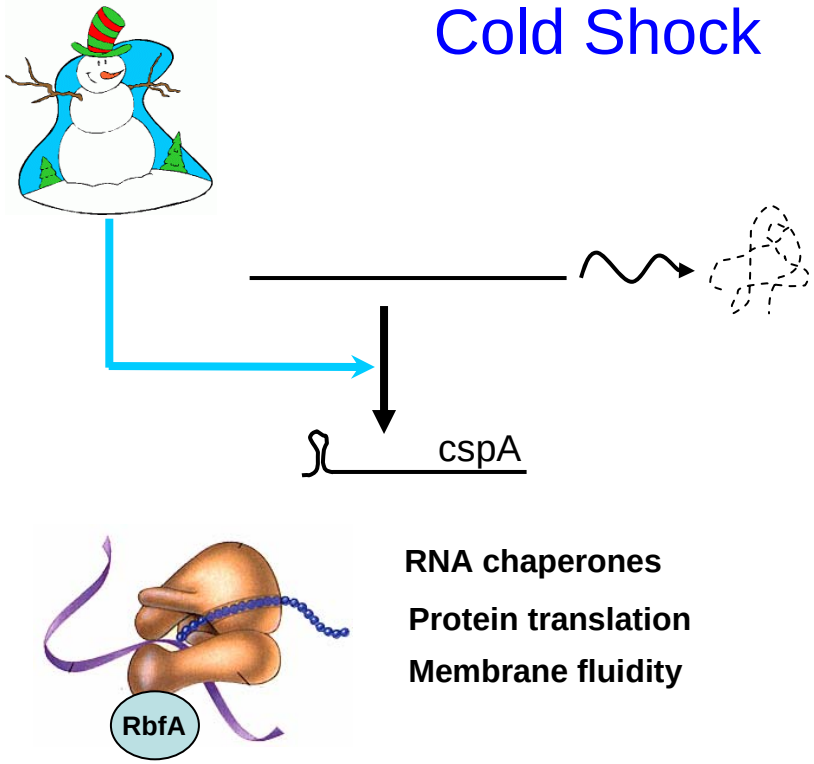
Heat Shock

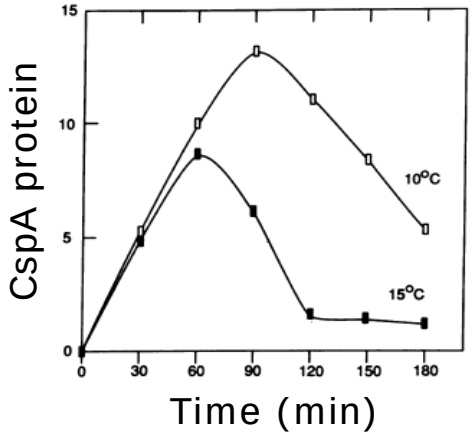




(El-Samad et al., 2005; Kornitzer et al., 1991)

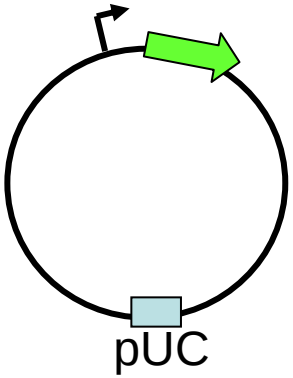
Cold Shock





(Xia et al., 2002; Thieringer et al., 1998; Goldstein, et al., 1990)

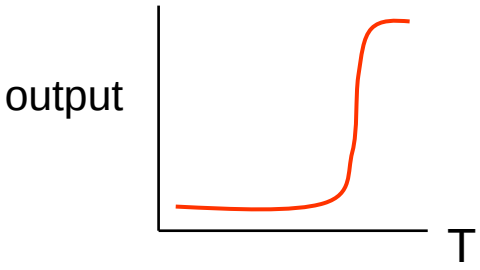
Promoter Strategy



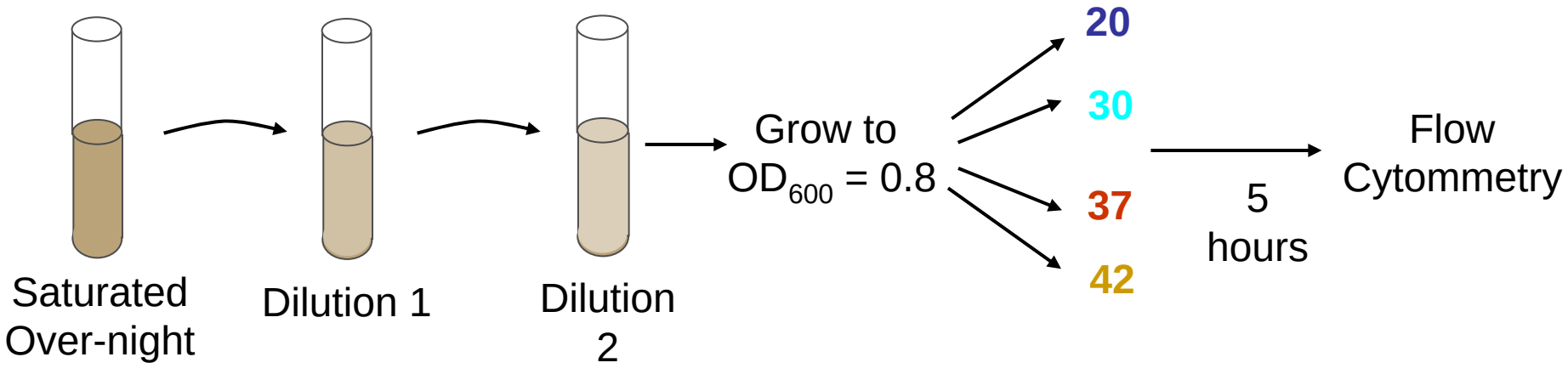
Grow at different temperatures

TG1s

Transfer Function



Transfer Function is defined by the Experiment :



→ *Reduces growth rate dependence*

→ *Response is at "steady-state"*

Candidate heat and cold shock promoters

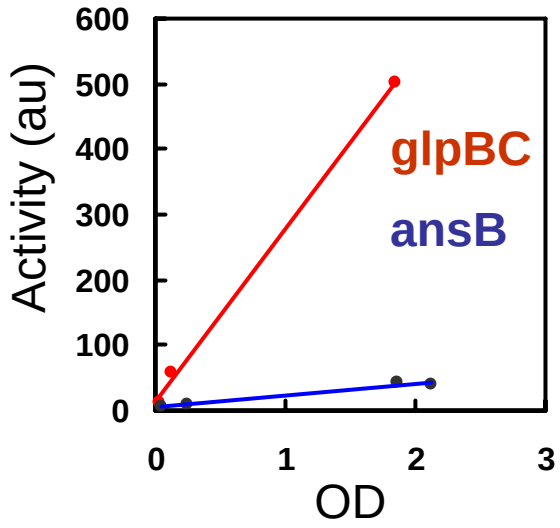
Mostly microarray based

Heat Shock	Cold Shock
~~*clpB*~~	~~*glpBC*~~
~~*groEL*~~	~~*ompT*~~
~~*htpG*~~	~~*nupG*~~
~~*dnaK-p1*~~	~~*katG*~~
~~*dnaK-p2*~~	~~*hypB*~~
~~*dnaJ*~~	*hybB*
~~*grpE*~~	*ansB*
~~*phoBR*~~	~~cspA~~
~~*lon*~~	cspA*
~~*htpX*~~	
~~*ibpA*~~	
~~*ibpA**~~	
~~*ibpB*~~	
~~*yedU*~~	
~~*hslUV*~~	
~~*yccV*~~	

* mRNA truncation

Promoter problems:

- No response
- Toxicity
- Growth phase dependence



F.T. Haddadin *et al.* (2005) Biotech and Bioengineering, **90**(2), 127-153
D.E. Chang *et al.* (2002) Molecular Microbiology **45**(2), 289-306
C.S. Ricjmond et al. (1999) Nucleic Acid Research **27**(19), 3821-3835
S Phadtare *et al*. (2004) J. Bacteriol. **186**(20), 7007-7014
M. A. Schembri *et al*. (2003) Molecular Microbiology **48**(1), 253-267

Cold-shock 1 (hybB promoter)

Parameters:

Cold-induced promoter
(T ≤ 30°C)

$$\frac{x_{on}}{x_{off}} = 12$$

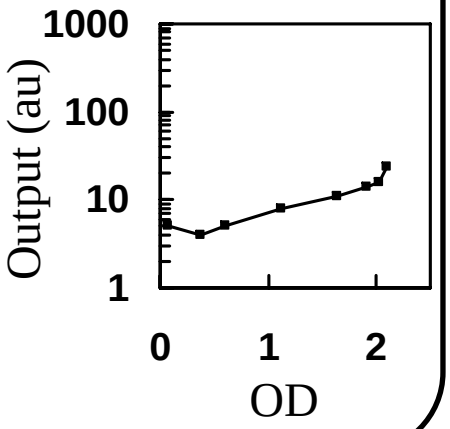
$$x_{off} = 10$$

$$gain = 17 \frac{au}{^{o}C}$$

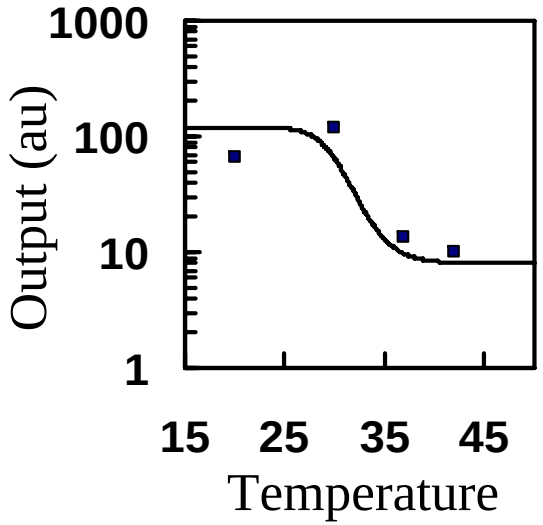
- in pUC plasmid pBAC874t
- in E. coli TG1 cells

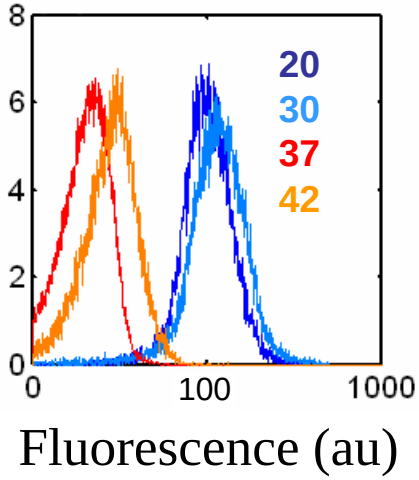
Source / Response:

- from E. coli K12
- controls hydrogenase II transcription
- anaerobic induction
- no OD dependence



Transfer function:





Cold-shock 2 (ansB promoter)

Parameters:

Cold-induced promoter
(20°C ≤ T ≤ 30°C)

$$\frac{x_{on}}{x_{off}} = 5$$

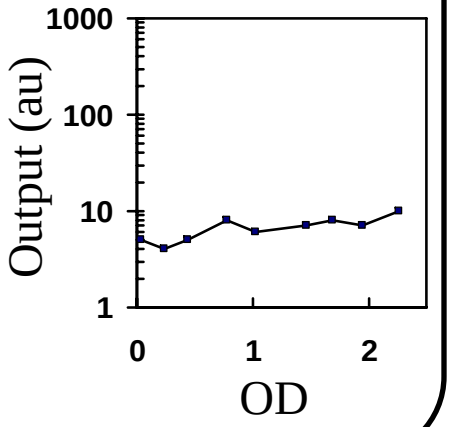
$$x_{off} = 10$$

$$gain = 7\frac{au}{^oC}$$

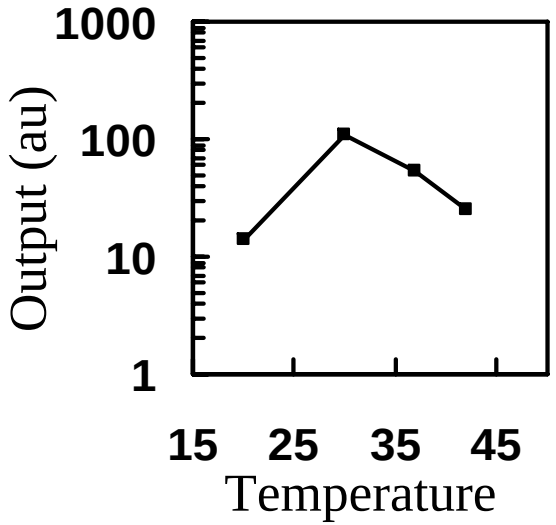
- in pUC plasmid pBAC874t
- in E. coli TG1 cells

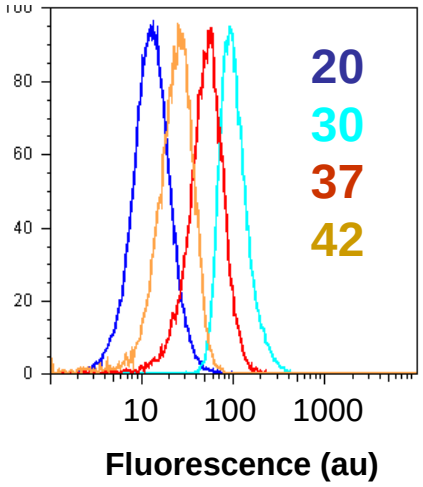
Source / Response:

- from E. coli K12
- controls trancription of an asparaginase
- no OD dependence



Transfer function:





Cold-shock 3 (cspA* promoter)

*mRNA truncation

Parameters:

Cold-induced promoter
(30°C ≤ T)

$$\frac{x_{on}}{x_{off}} = 2$$

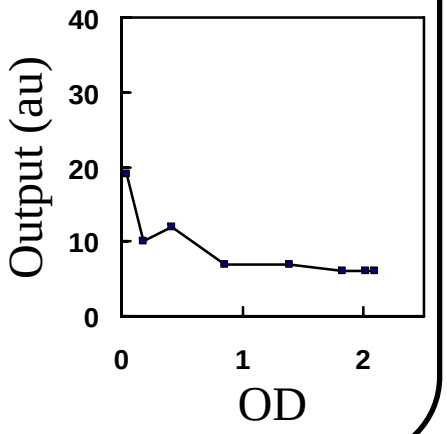
$$x_{off} = 10$$

$$gain = 1.5 \frac{au}{^{o}C}$$

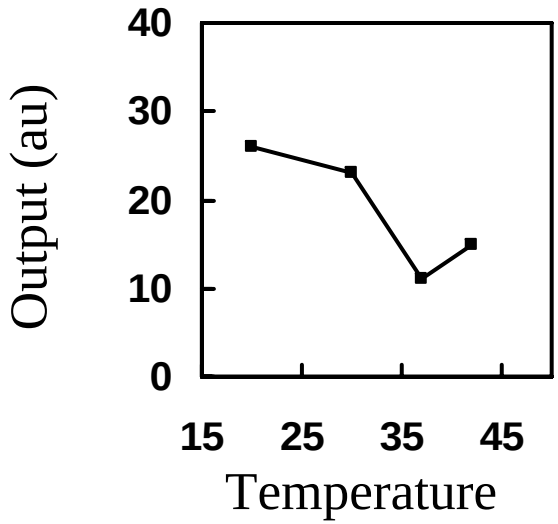
- in pUC plasmid pBAC874t
- in E. coli TG1 cells

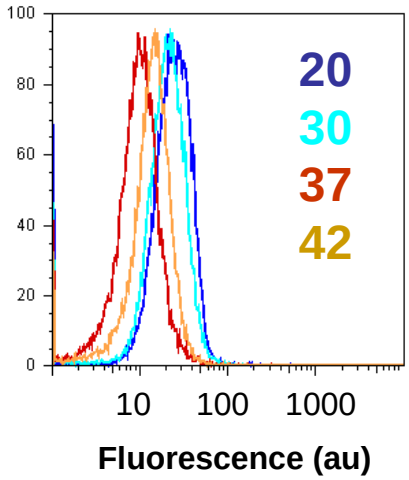
Source / Response:

- from E. coli K12
- controls transcription of cold shock protein A
- no OD dependence

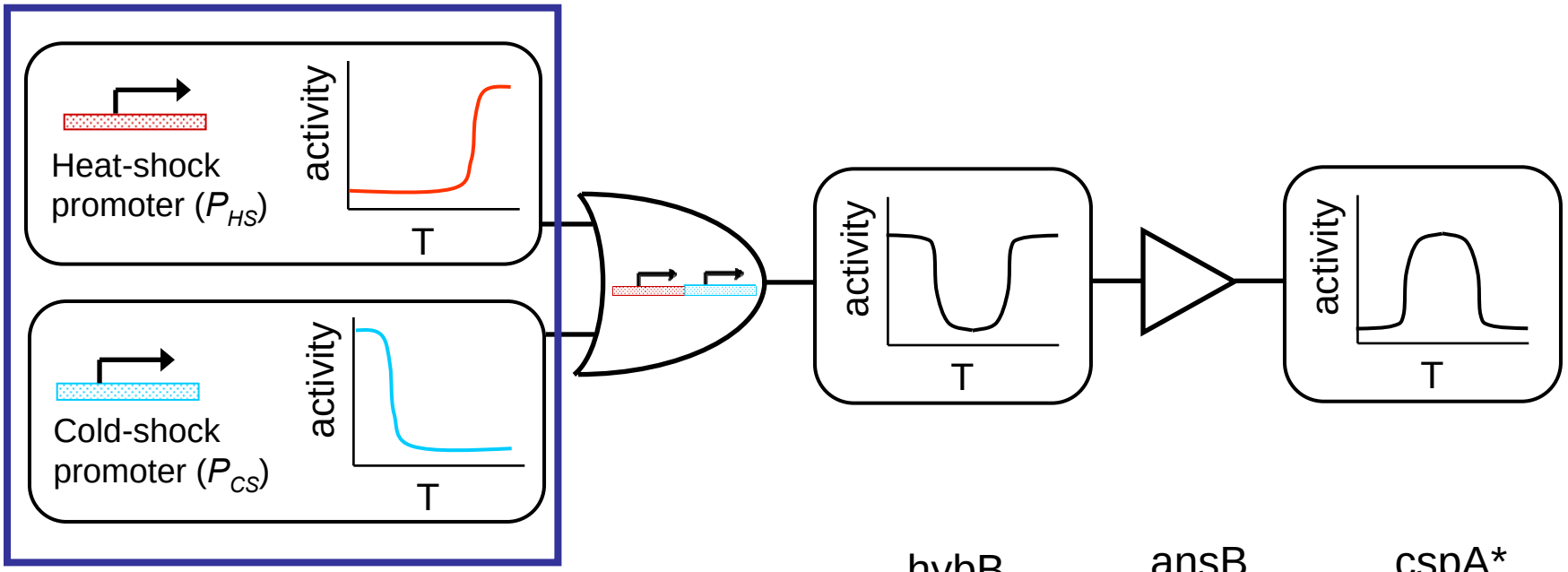


Transfer function:



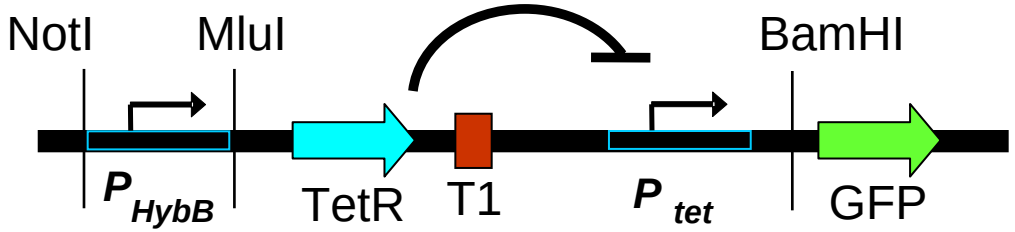


Road-Map



	hybB	ansB	cspA*
$\frac{x_{on}}{x_{off}}$	12	5	2
x_{off}	10	10	10
$gain\left(\frac{au}{^{o}C}\right)$	17	7	1.5

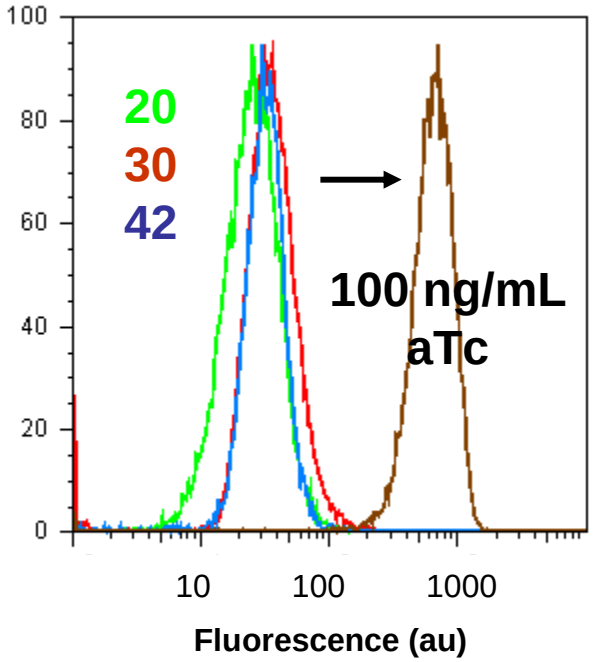
Inverter: A potential heat-shock promoter



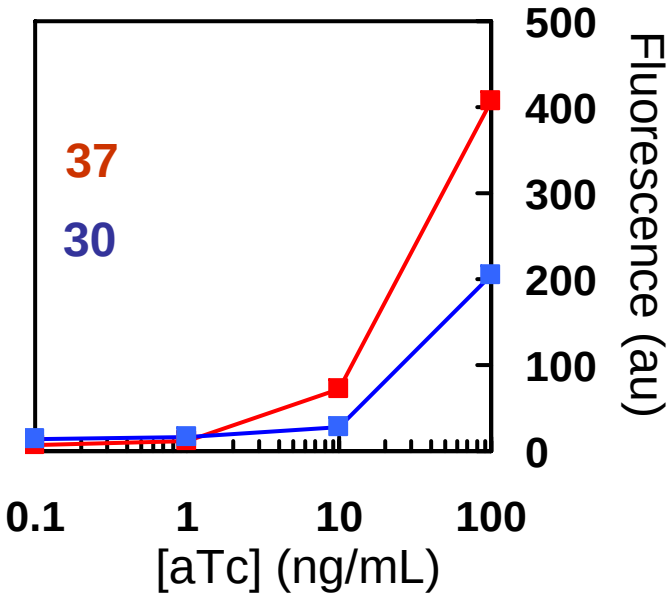
λ	***BBa_Q04510***
tetR	***BBa_Q04400***

MIT Registry of Standard Biological Parts

(Weiss, PNAS, 2002)



De-bugging



Modifying the Ribosome Binding Site

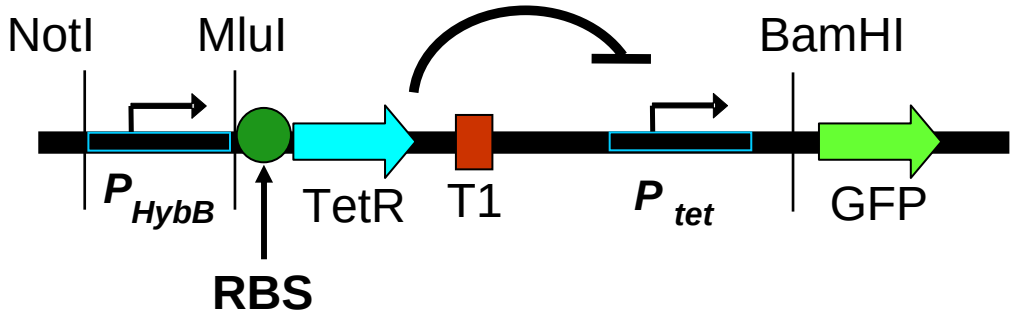
RBS-1: TCACACAGGAAACCGGTTCGATG
RBS-2: TCACACAGGAAAGGCCTCGATG
RBS-3: TCACACAGGACGGCCGGATG

strong
↓
weak

BBa_B0031	0.07	(weak)
BBa_B0032	0.3	(medium)
BBa_B0033	0.01	(weakest)

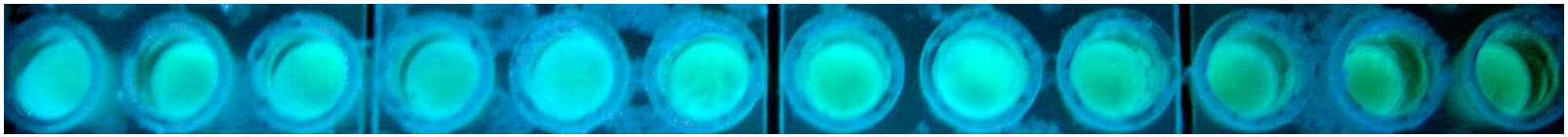
Ron Weiss, 2001

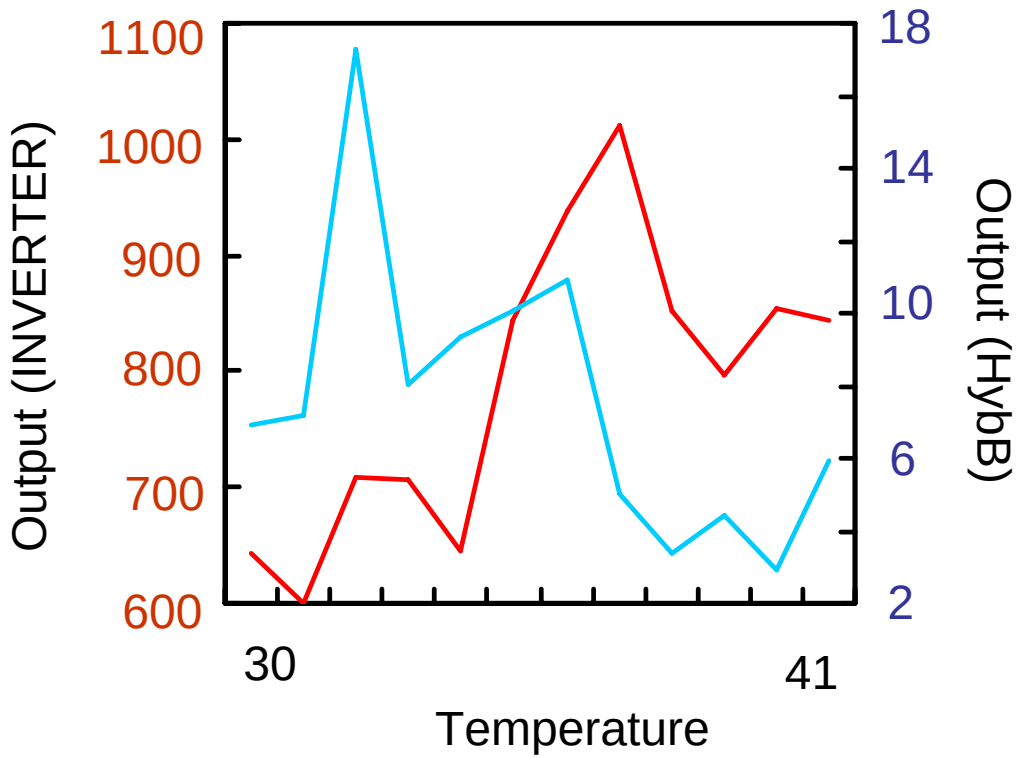
Registry of Standard Parts



Inverter (Heat Shock) Results

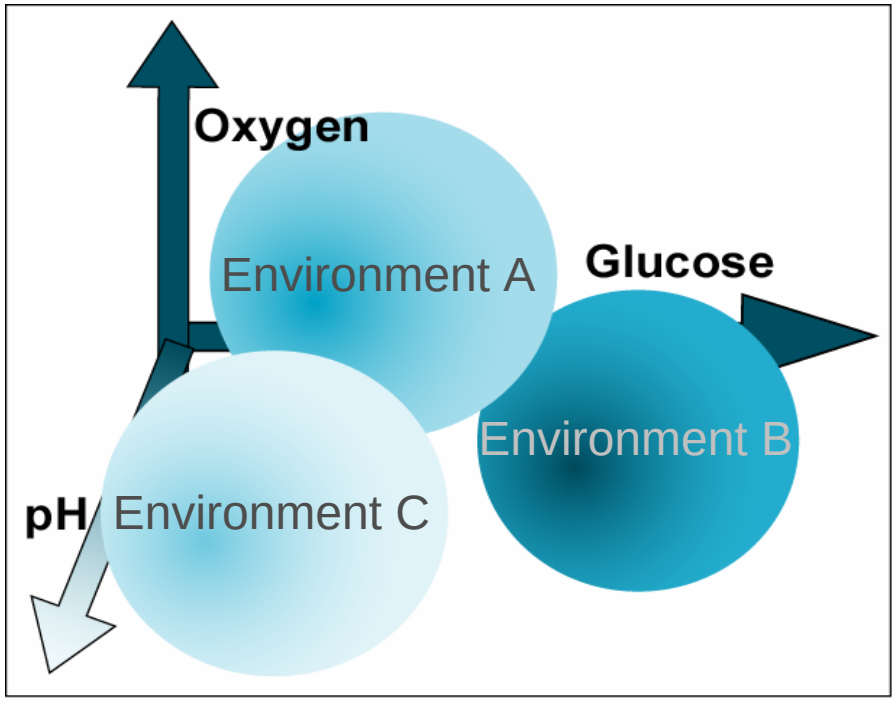
30°C → 41°C

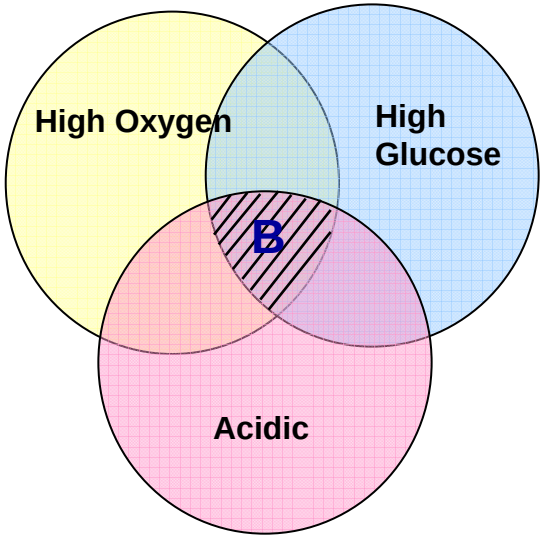




Problems we encountered

Promoters have multiple activators



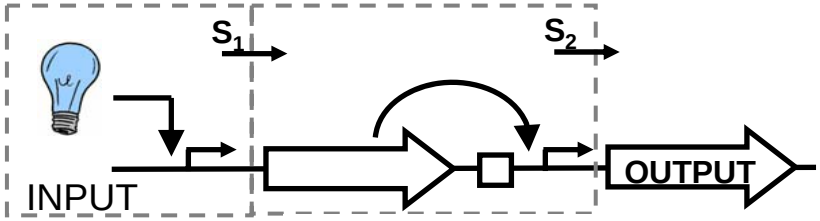


- Specificity can be achieved by integrating multiple sources of non-specific information

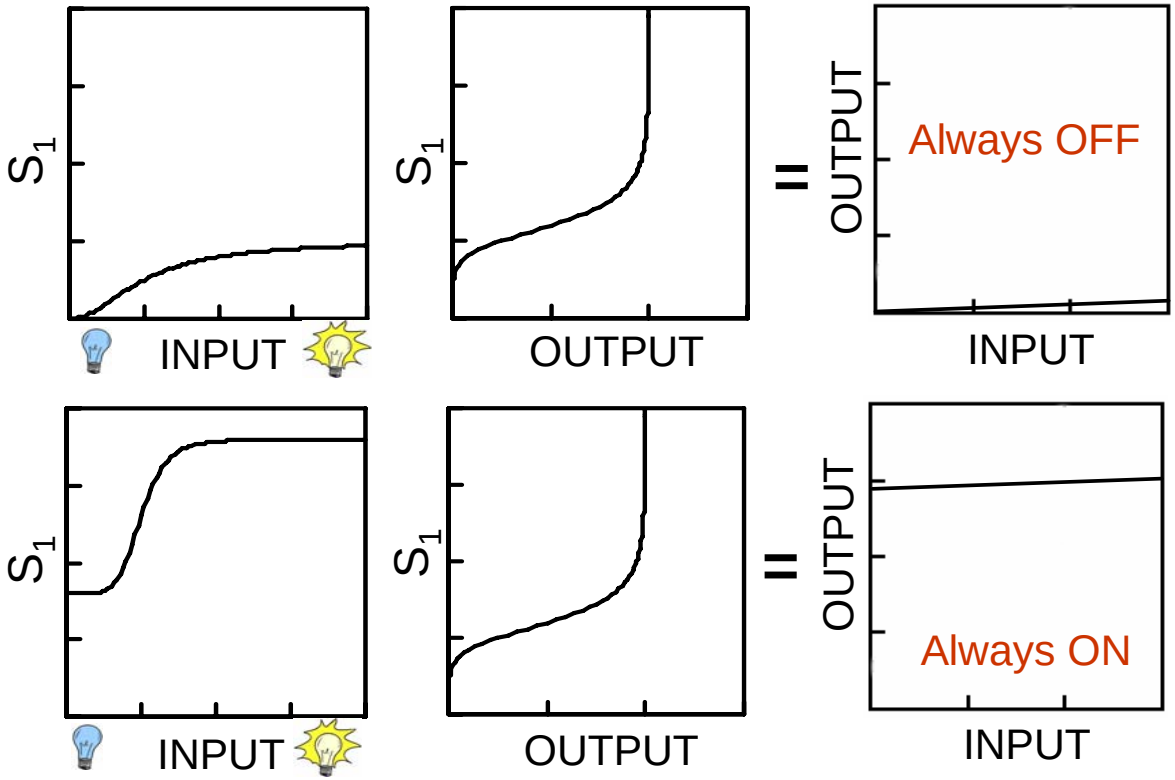
- Need to combine cell-based sensors with genetic circuits

Problems we encountered

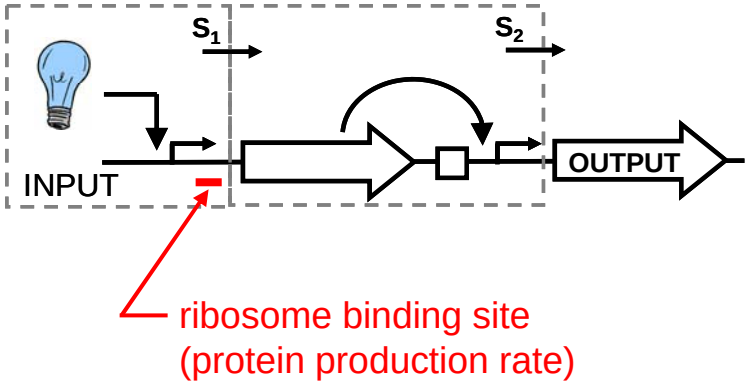
Wiring together parts is difficult!

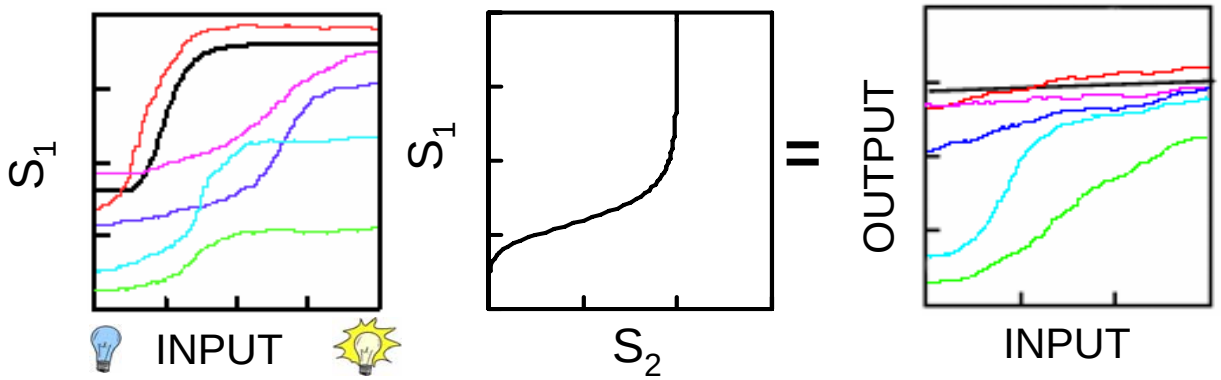


- Use transfer function to predict how multiple genetic parts will perform in series



Problems we encountered





Thanks

Tanja Kortemme

Chris Voigt

Christopher Anderson

Matt Eames

Apple Liu

Nessa Ramos

San Francisco Unified School District