Rational Diversification of a Promoter Providing Fine-Tuned Expression and Orthogonal Regulation for Synthetic Biology

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Presented by Jessica and Natalie

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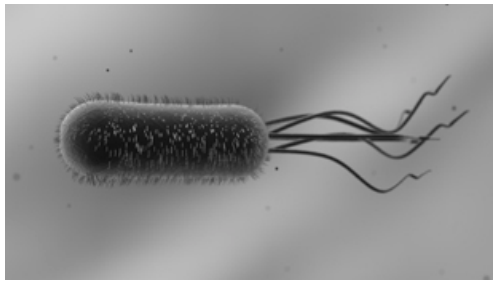
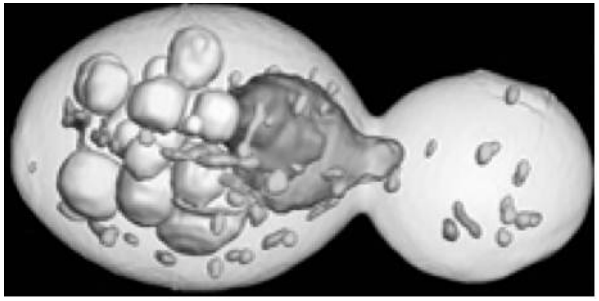
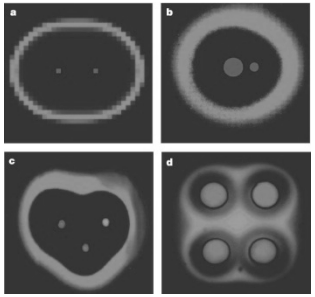
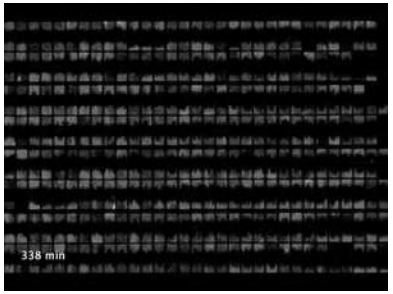
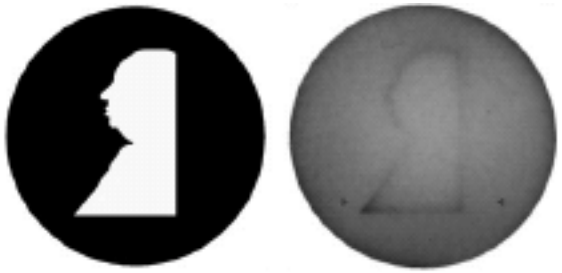
Recent applications of Synthetic Biology in *E. coli* and *S. cerevisiae*

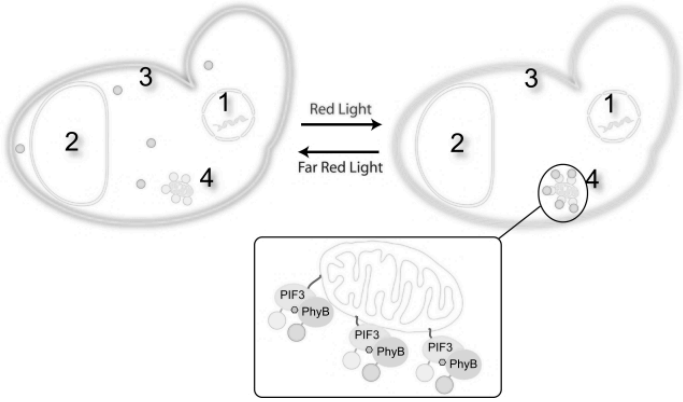




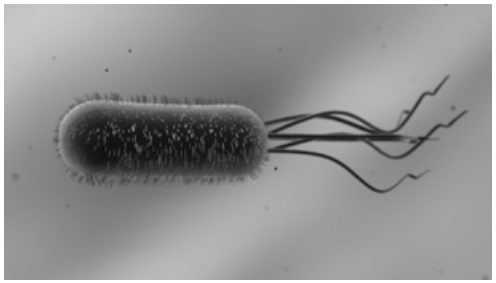


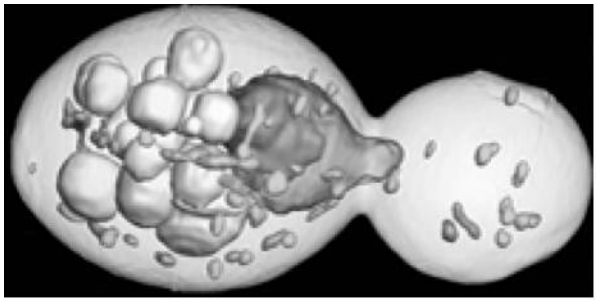






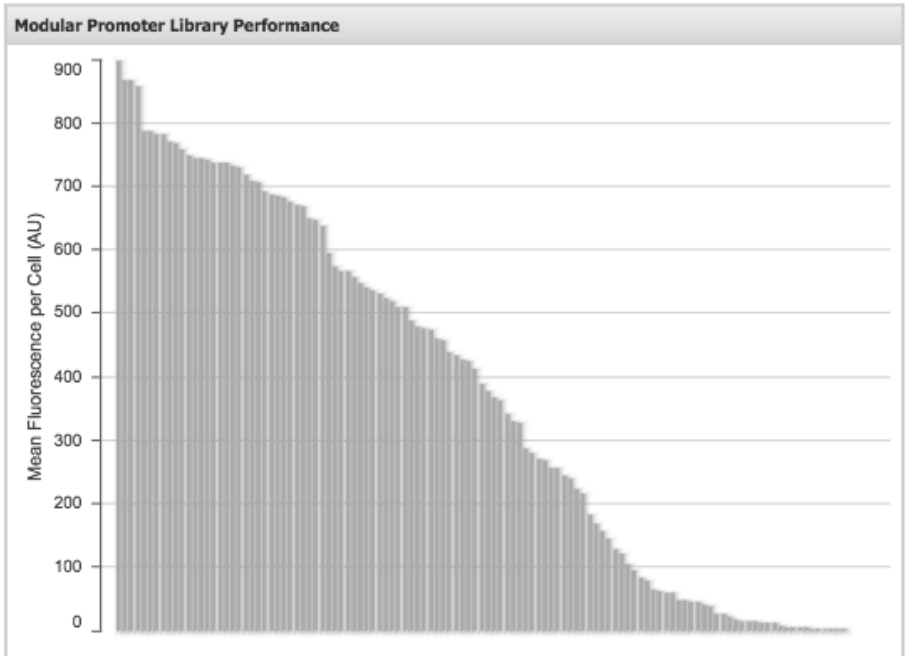
Different # of Parts for Synthetic Biology in *E. coli* and *S. cerevisiae*





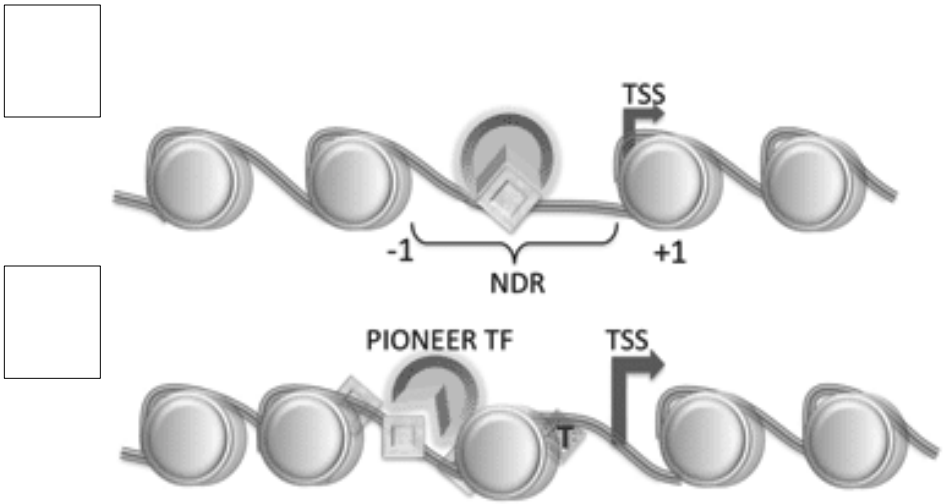
BioFab will soon publish 125 promoters of different

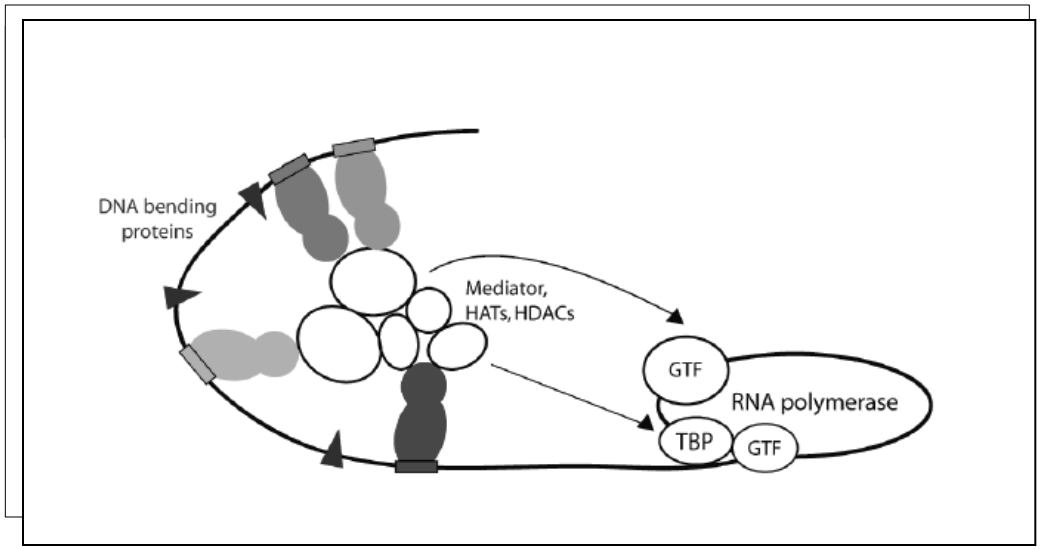
Registry of Standard Biological Parts Yeast



-?-		Name	Description
	W	BBa_I766555	pCyc (Medium) Promoter
	W	BBa_I766556	pAdh (Strong) Promoter
	W	BBa_I766557	pSte5 (Weak) Promoter
1☆	W	BBa_J63005	yeast ADH1 promoter
1☆		BBa_K105027	cyc100 minimal promoter
1☆		BBa_K105028	cyc70 minimal promoter
1☆		BBa_K105029	cyc43 minimal promoter
1☆		BBa_K105030	cyc28 minimal promoter
1☆		BBa_K105031	cyc16 minimal promoter
A		BBa_K122000	pPGK1
		BBa_K124000	pCYC Yeast Promoter
	W	BBa_K124002	Yeast GPD (TDH3) Promoter
		BBa_M31201	Yeast CLB1 promoter region, G2/M cell cycle specific

Even textbooks show complex regulation of transcription in yeast





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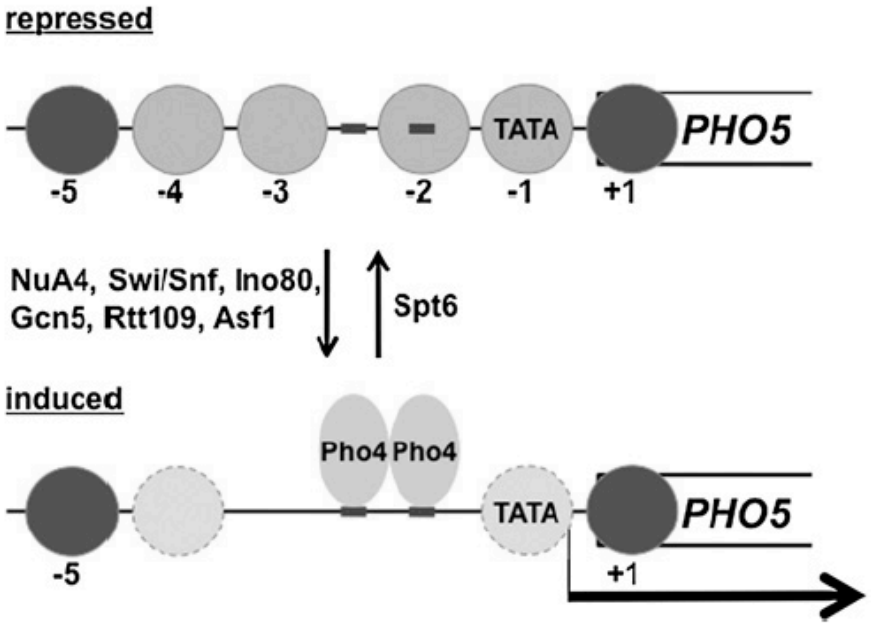
Dissertation
Derek Yung-Ho Chiang

Computational and Experimental Analyses of Promoter Architecture in Yeasts

Constructing a diverse pool of orthogonal promoters



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1. Start with a natural promoter
2. Diversify promoter strength
3. Rationally control output with logic gates
4. Deploy novel transcription factors to insulate regulation

Choosing a natural promoter to diversify

Microarray data

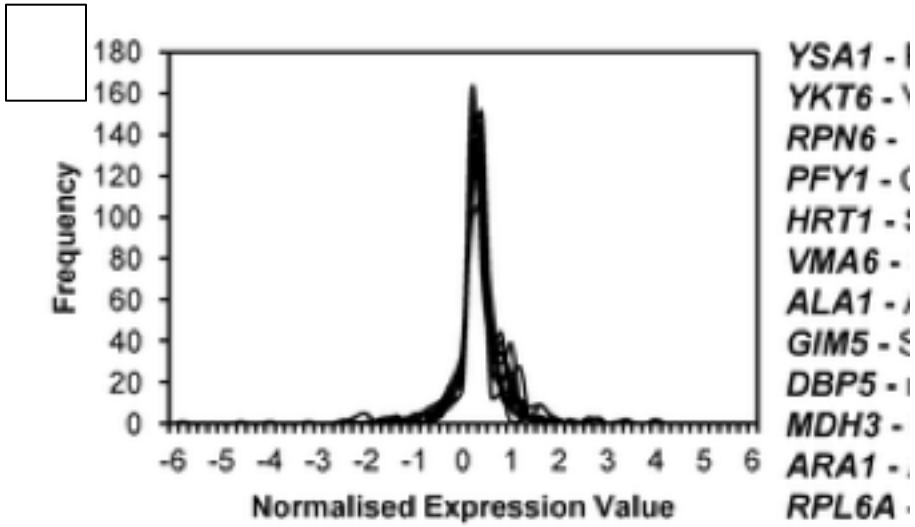


Fig. 1A

<u>Shortlisted a dozen genes</u>

- Not to low or too high
- Transcript of known function
- Lowest variations across carbon sources

In vivo Flow Cytometry data

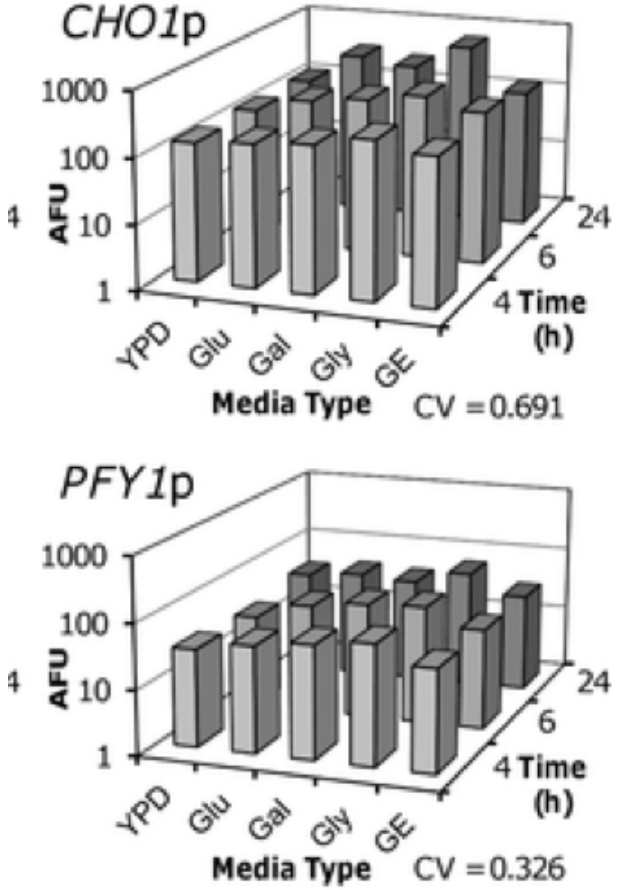
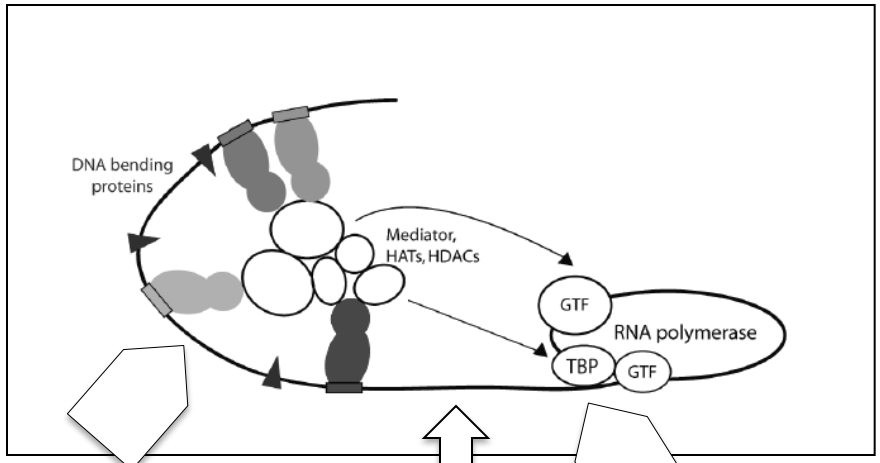


Fig. 2

Chose PFY1 based on low CV, consistent exp'n levels, but TATA-less!!

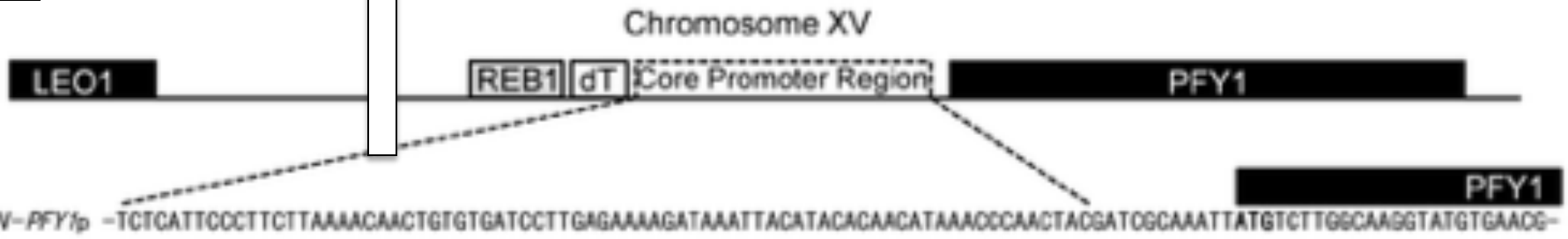
Diversifying the PFY1p promoter



REB1p binding sites
Poly dT stretches

TATA-less

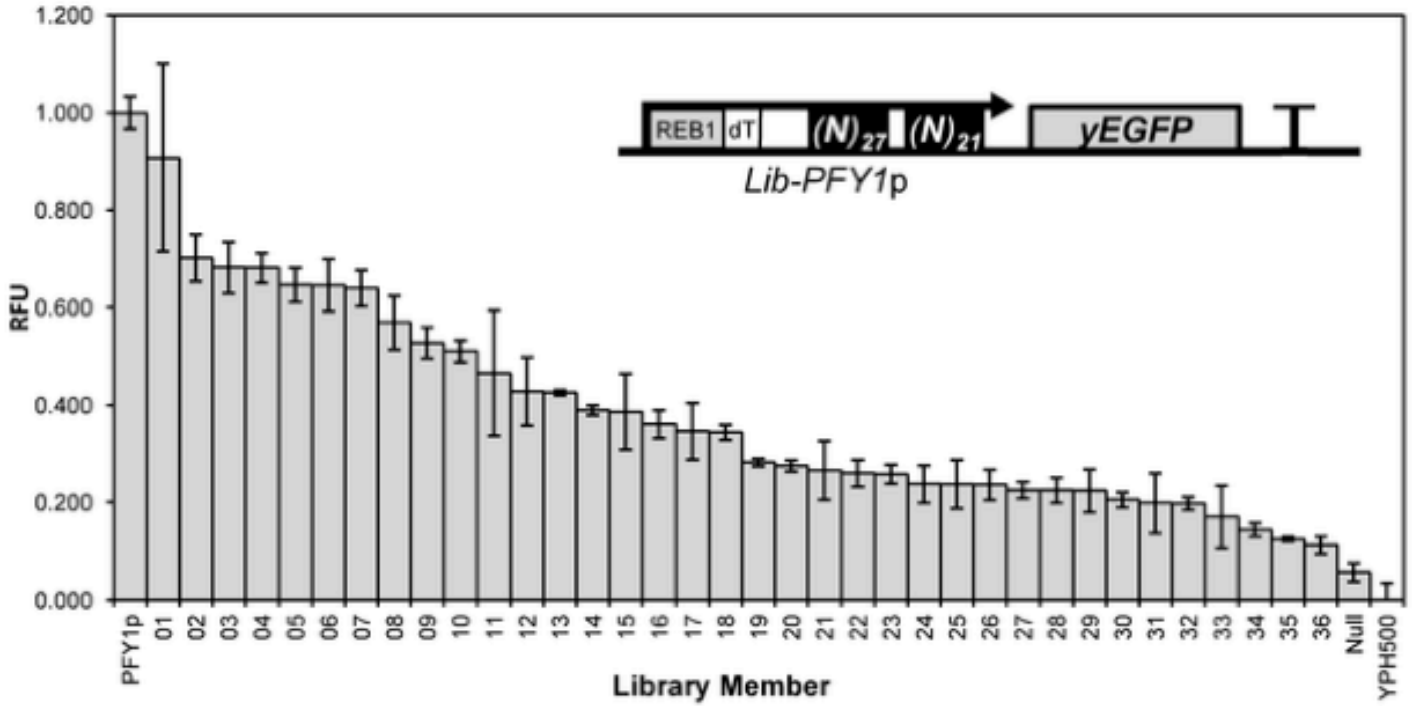
- "CPEC" cloning method
- pSV-PFY1p: GFP into URA3
- Flow cytometry to measure
- Chose 37 with range of exp'n

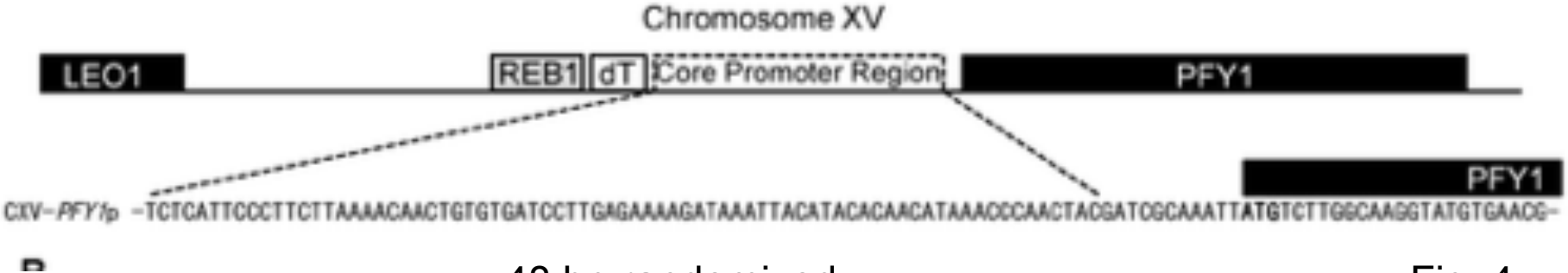


48 bp randomized

Fig. 4

Diversifying the PFY1p promoter



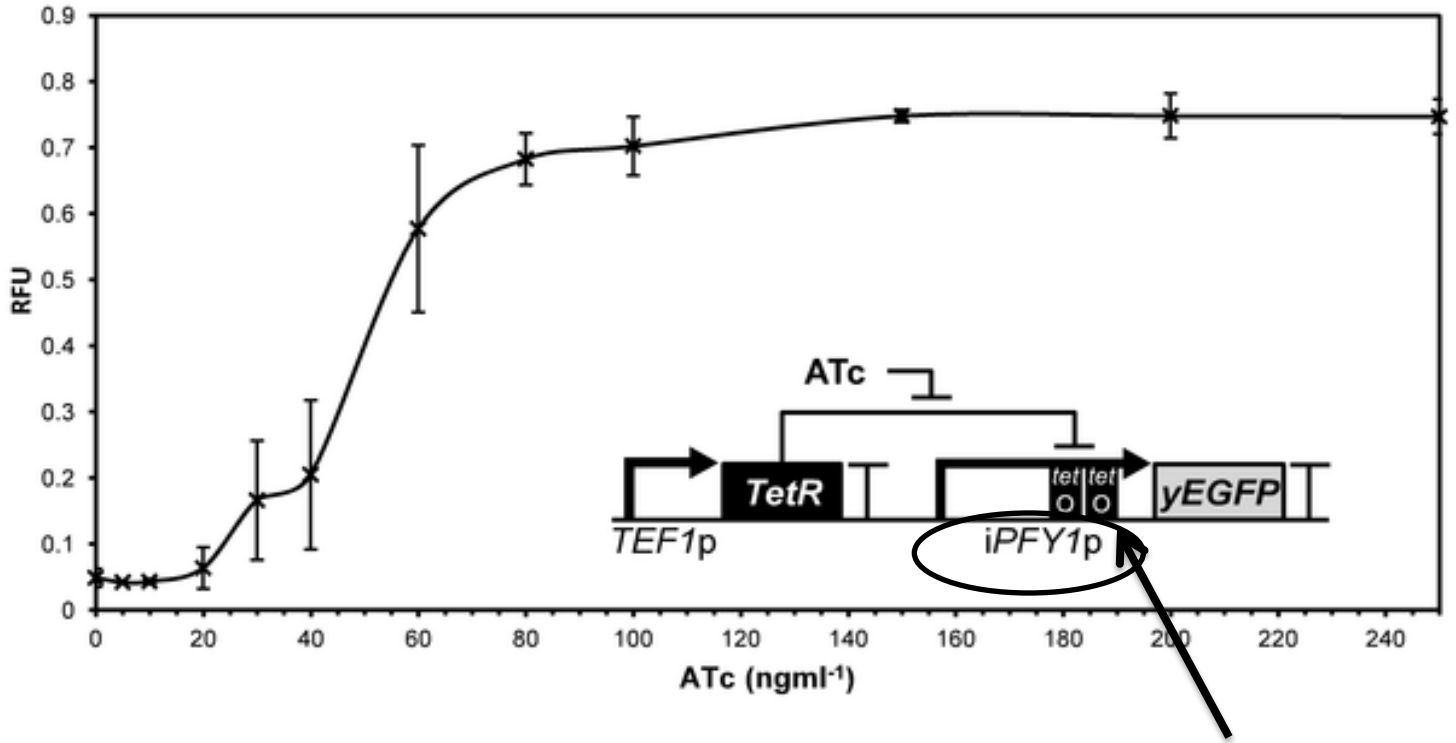


48 bp randomized

Fig. 4

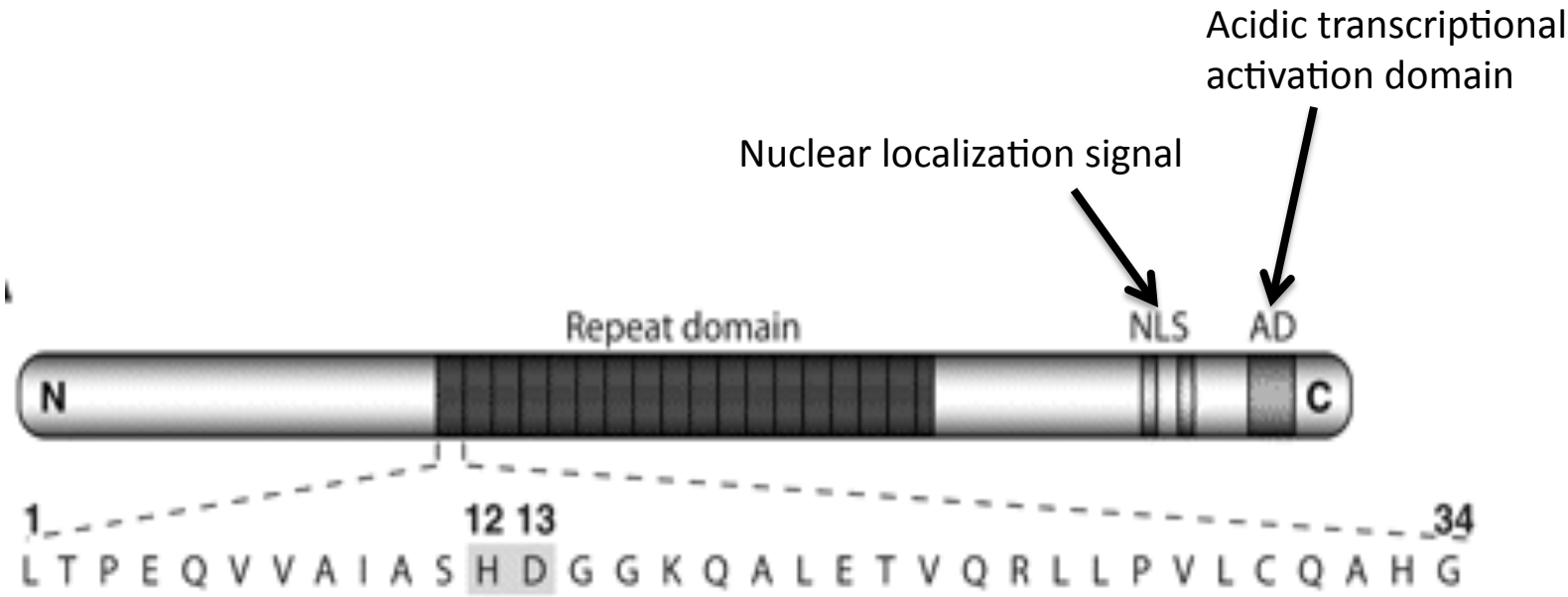
Dose Response TetR-Mediated Repression

- Engineer regulation: insert TEF1p-tetR-ADH1 terminator upstream
- Dose response to ATc resemble Hill function indicating cooperative binding



TetR operator sequences

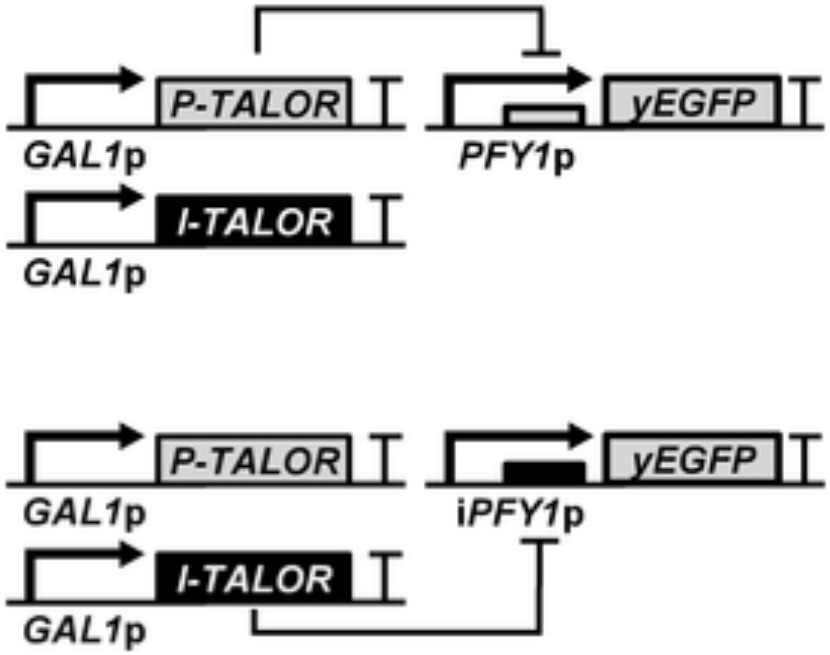
Transcription Activator-Like Effectors (TALEs)



Boch et al. "Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors", *Science*, 2009

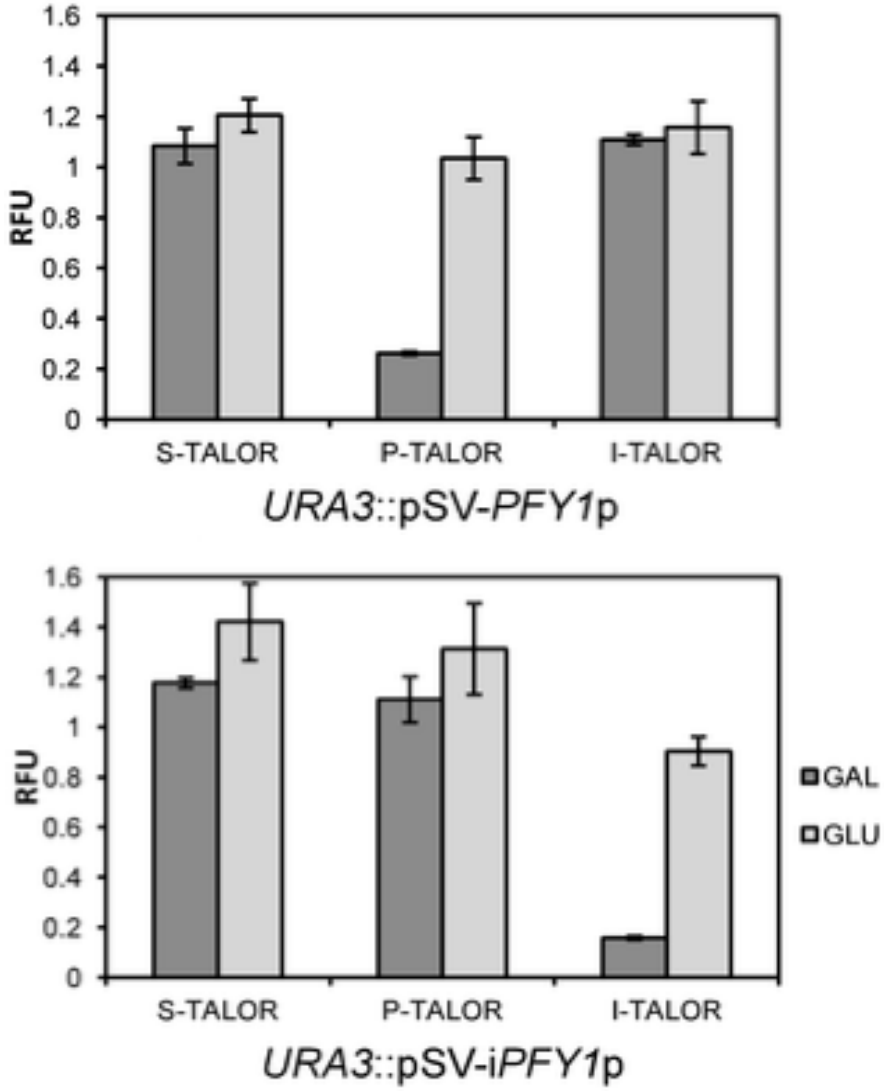
Using dTALE for regulation

- TAL proteins designed for PFY1p and iPFY1p
- Tested for dTALEs target binding score and off-target binding scores
- 3rd TAL protein created by scrambling targeting sequence to further measure orthogonality
- TAL protein regulated by addition of galactose



Using dTALE for regulation

- Shows repression of targeted promoter
- Output repression:
  - P-TALOR: 74%
  - I-TALOR: 84%
  - S-TALOR: no affect



Assumptions

- The development of synthetic biology in yeast is limited by the promoters known
- Promoters can be placed in different locations without altering its function.
- ATc and galactose only affect the regions indicated in the experiments
- Fluorescence is a good reference for promoter strength

Limitations

- Maintaining regulation without affecting native cellular processes
- Galactose regulation still only gives one point of regulation
- Reliance on whole cell gene expression data
- No definite levels of fluorescence

Significance of the work??

Professor Richard Kitney, Co-Director of the Centre for Synthetic Biology and Innovation at the College, adds: "The work by Dr Ellis and the team at the Centre really takes us closer to developing much more complex biological machines with yeast, which may help to usher in a new age where biological machines could help to improve our health, the way we work, play and live."

http://www.eurekalert.org/pub_releases/2012-03/icl-sdt031912.php

Significance and Future Developments

- Potential to regulate individual genes with dTALE proteins
- Enhance repression efficiency by designing 2 TAYLORs per promoter
- Use additional criteria in determining target sequences to decrease off target binding

Discussion Questions

- What are some other types of controls that can be used in the place of galactose or tetracycline?
- How could multiple TALORs be included to work cooperatively in regulation?