A Yeast Synthetic Network for In Vivo Assessment of Reverse-Engineering and Modeling Approaches

Cantone, I., Marucci, L., Iorio, F., Ricci, M., Belcastro, V., Bansai, M., Santini, S., Bernardo, M., Bernardo, D., and Cosma, M.

Cell (2009) 137: 172-181

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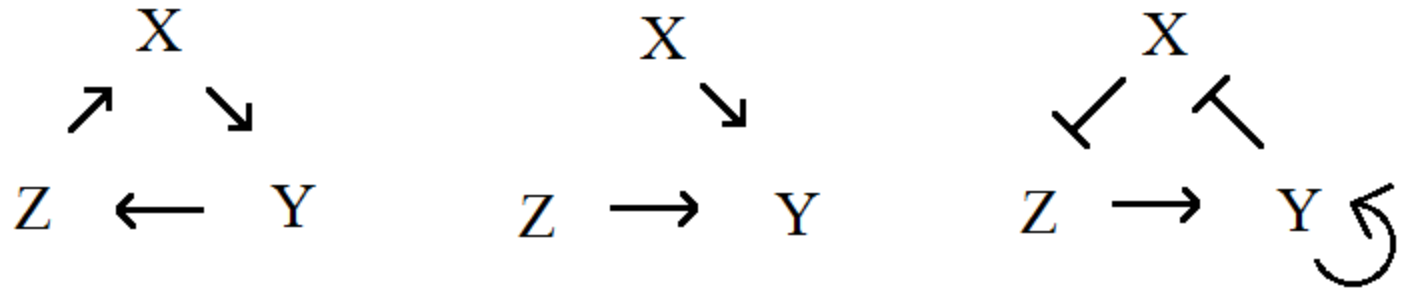
Problem: Determining Networks

- Elucidating and validating a gene regulatory network is hard and time consuming.



Solution: Need *in vivo* benchmarking tool

- **Design** a novel gene regulatory network
- **Characterize** the parameters of the network
- **Model** the network mathematically
- **Test** your reverse-engineering algorithm on the model

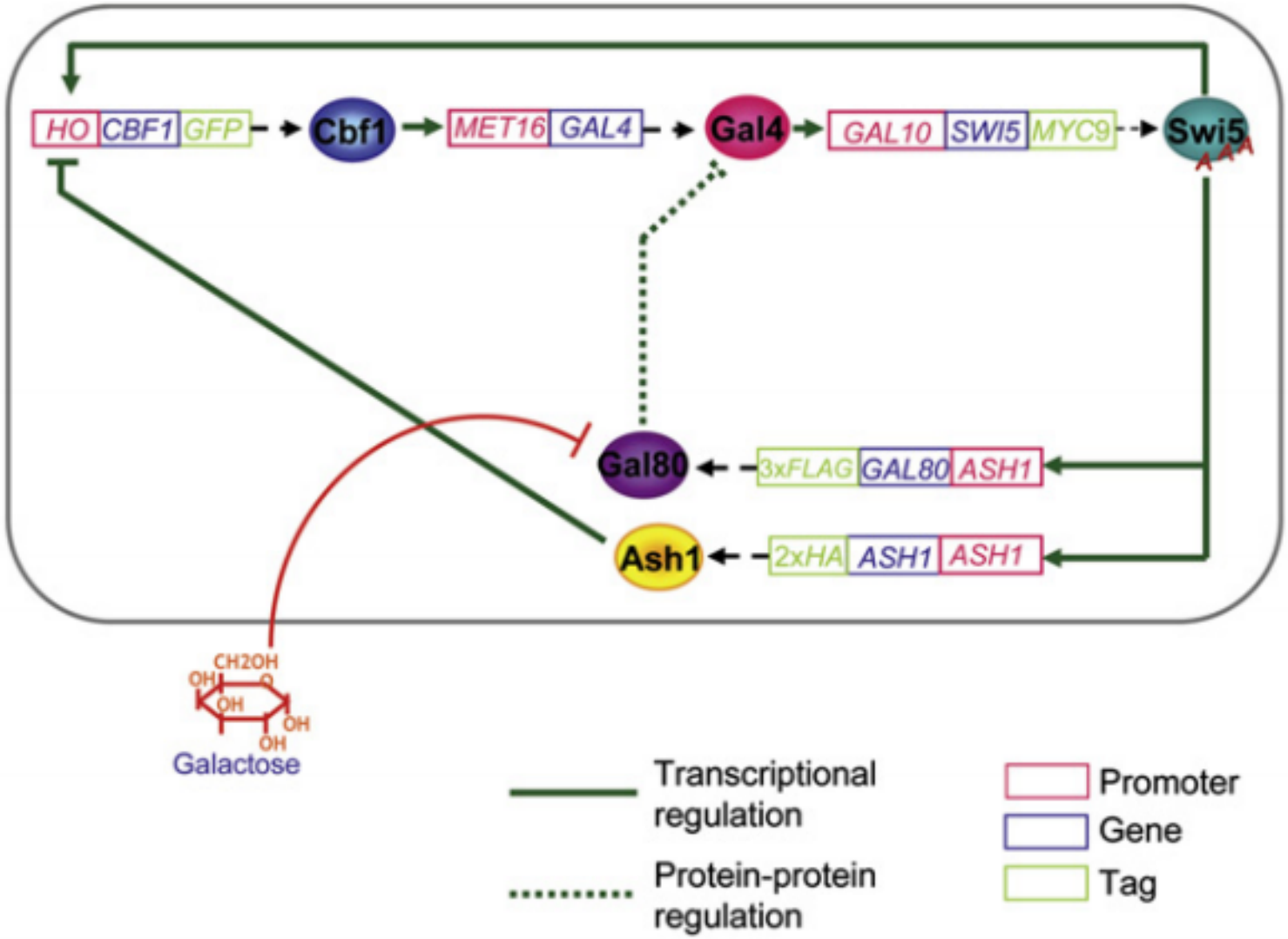
Design: A Novel Network

- Desired characteristics
  - Inducible
  - Orthogonal
  - Well characterized and nonessential parts

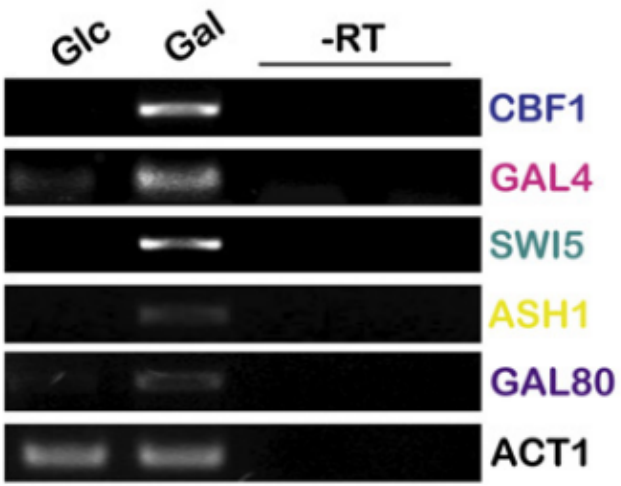
Design: A Novel Network

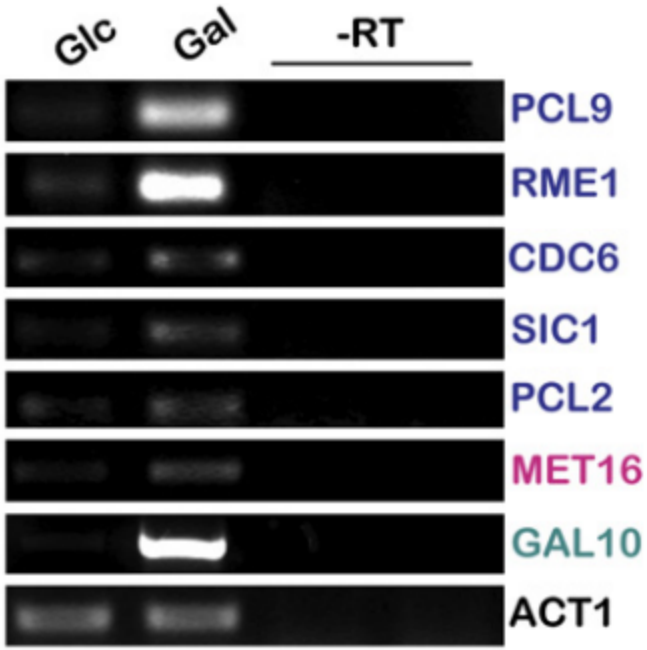
- Promoter/repressor/activator pairs
  - HO/$swi5_{AAA}$ and HO/Ash1D
  - MET16/CBF1
  - GAL1-10/GAL4
  - Ash1D/$swi5_{AAA}$
- One protein-protein interaction
  - GAL4/GAL80
- Chassis: YM4271 Yeast

Design: The IRMA Network

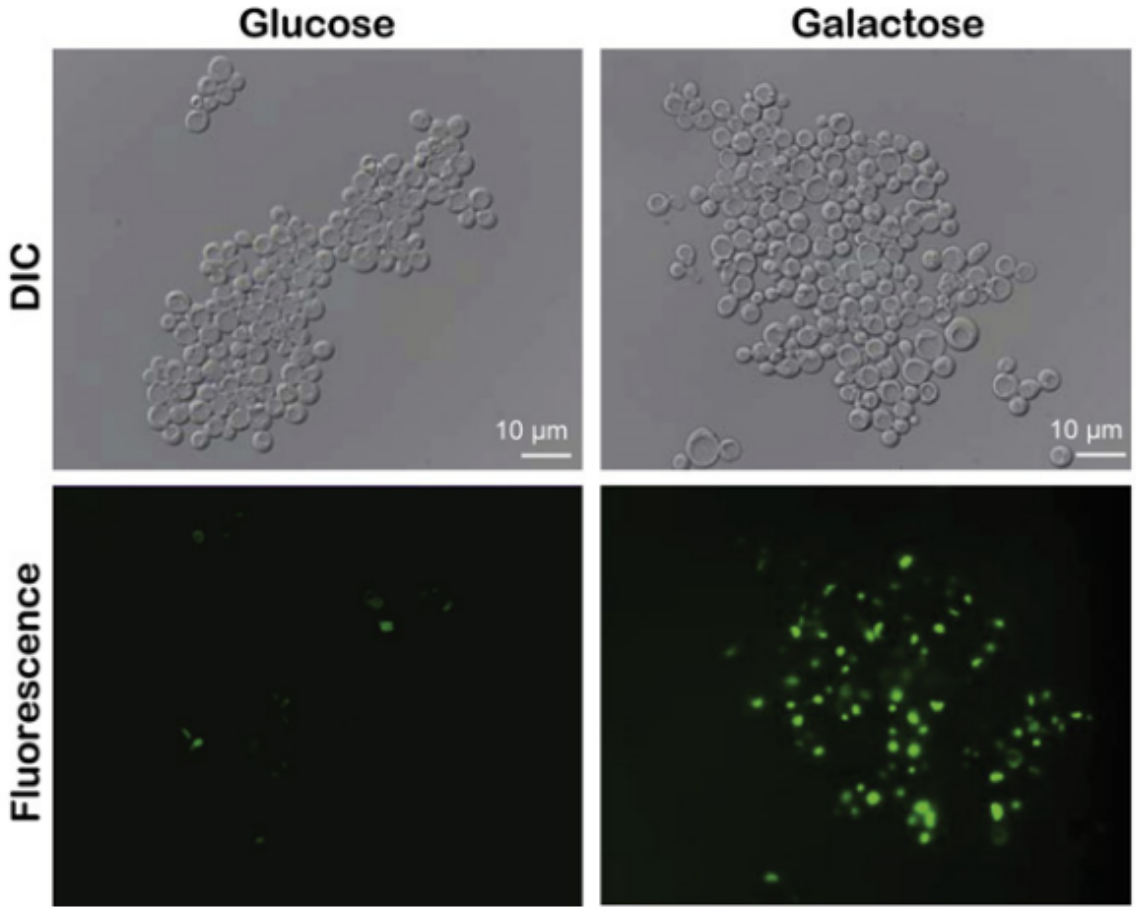


Design: The IRMA Network





Design: The IRMA Network



Model: A DE Approach

- Model network as a systems of differential equations (DE)
- Need to make assumptions!

Model: A DE Approach

$$[CBF1] \quad \frac{dx_1}{dt} = \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{{x_5}^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1$$

$$[GAL4] \quad \frac{dx_2}{dt} = \alpha_2 + v_2 \left(\frac{x_1^{h_3}}{k_3^{h_3} + x_1^{h_3}} \right) - (d_2 - \Delta(\beta_1))x_2$$

$$[SWI5] \quad \frac{dx_3}{dt} = \alpha_3 + \widehat{v_3} \left(\frac{x_2^{h_4}}{\widehat{k_4}^{h_4} + x_2^{h_4}(1 + \frac{x_4^4}{\widehat{\gamma}^4})} \right) - d_3 x_3$$

$$[GAL80] \quad \frac{dx_4}{dt} = \alpha_4 + v_4 \left(\frac{x_3^{h_5}}{k_5^{h_5} + x_3^{h_5}} \right) - (d_4 - \Delta(\beta_2))x_4$$

$$[ASH1] \quad \frac{dx_5}{dt} = \alpha_5 + v_5 \left(\frac{x_3^{h_6}}{k_6^{h_6} + x_3^{h_6}} \right) - d_5 x_5$$

Model: A DE Approach

$$
\begin{aligned}
[CBF1] \quad \frac{dx_1}{dt} &= \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{{x_5}^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1 \\
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\end{aligned}
$$

basal transcription rate

Model: A DE Approach

$$
\begin{aligned}
[CBF1] \quad \frac{dx_1}{dt} &= \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{{x_5}^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1 \\
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\end{aligned}
$$

MM kinetics

Model: A DE Approach

$$
\begin{aligned}
[CBF1] \quad \frac{dx_1}{dt} &= \alpha_1 + v_1 \left(\frac{x_3^{h_1}(t-\tau)}{(k_1^{h_1} + x_3^{h_1}(t-\tau)) \cdot \left(1 + \frac{{x_5}^{h_2}}{k_2^{h_2}}\right)} \right) - d_1 x_1 \\
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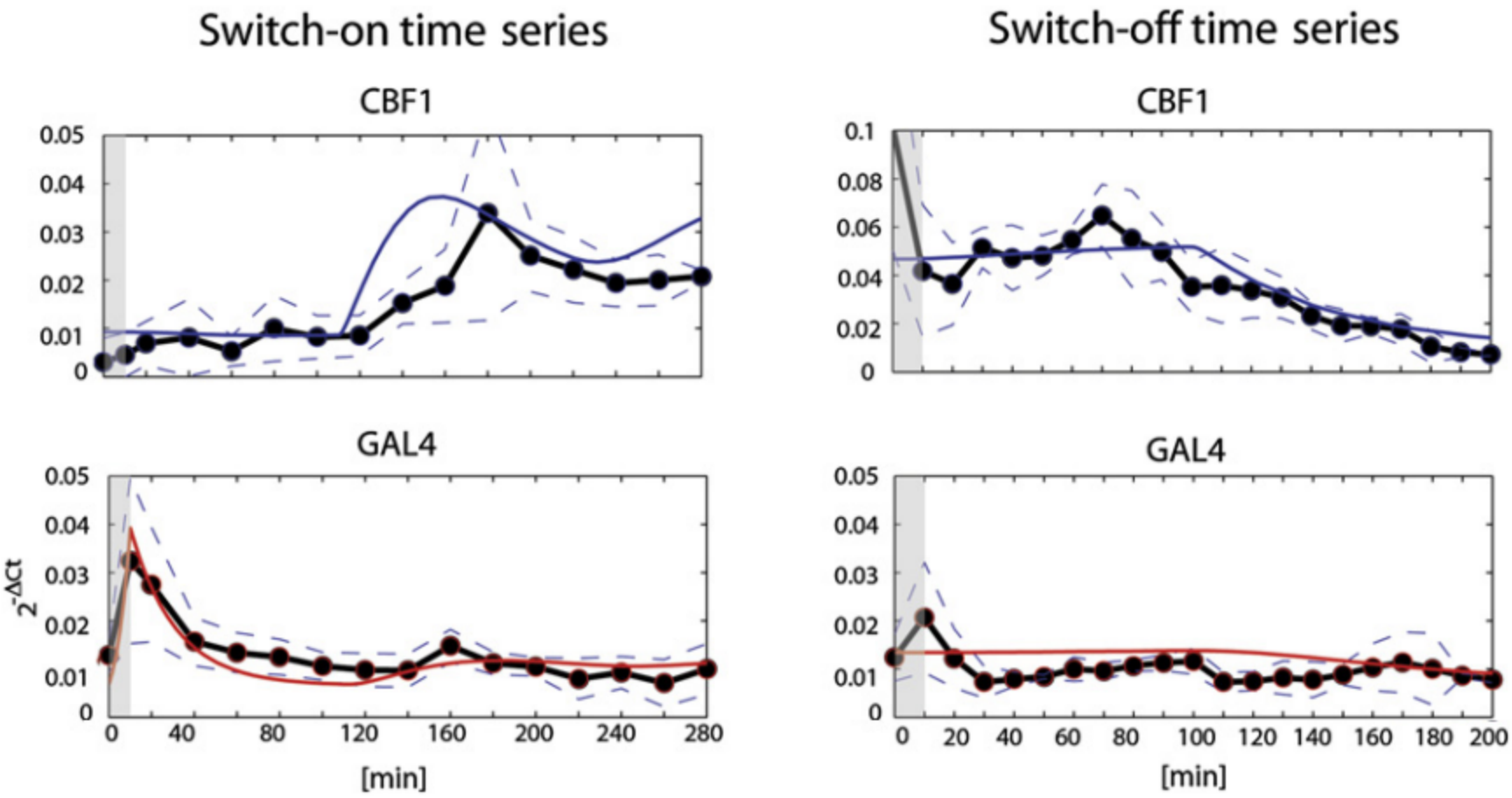
\end{aligned}
$$

Degradation

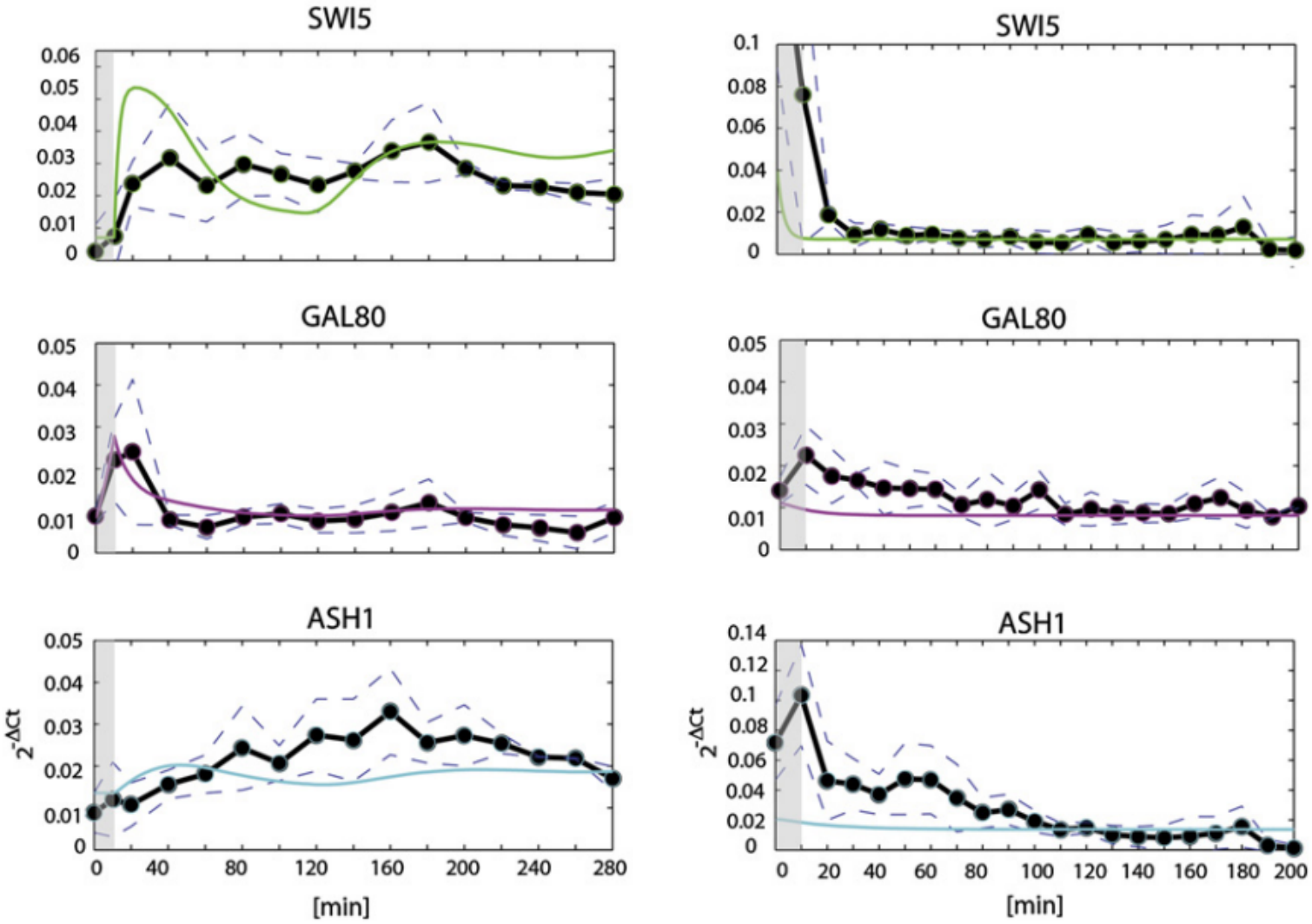
Characterize: Two Types of Perturbations

- Want to characterize the dynamics of the system
- Two types of perturbations
  - Single perturbation and measure mRNA levels at different time points
  - Multiple perturbations and measure steady-state mRNA levels

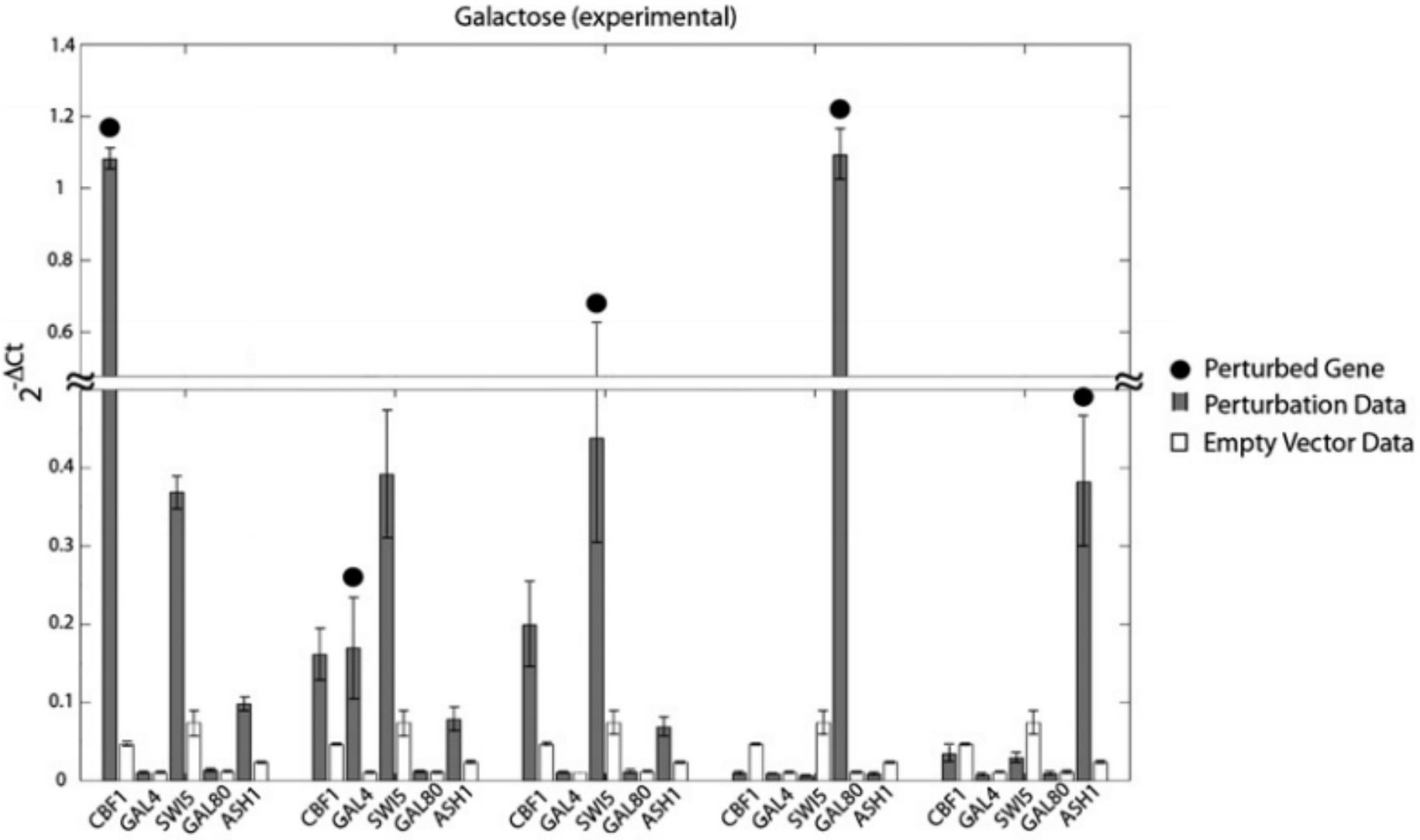
Characterize: A Single Perturbation



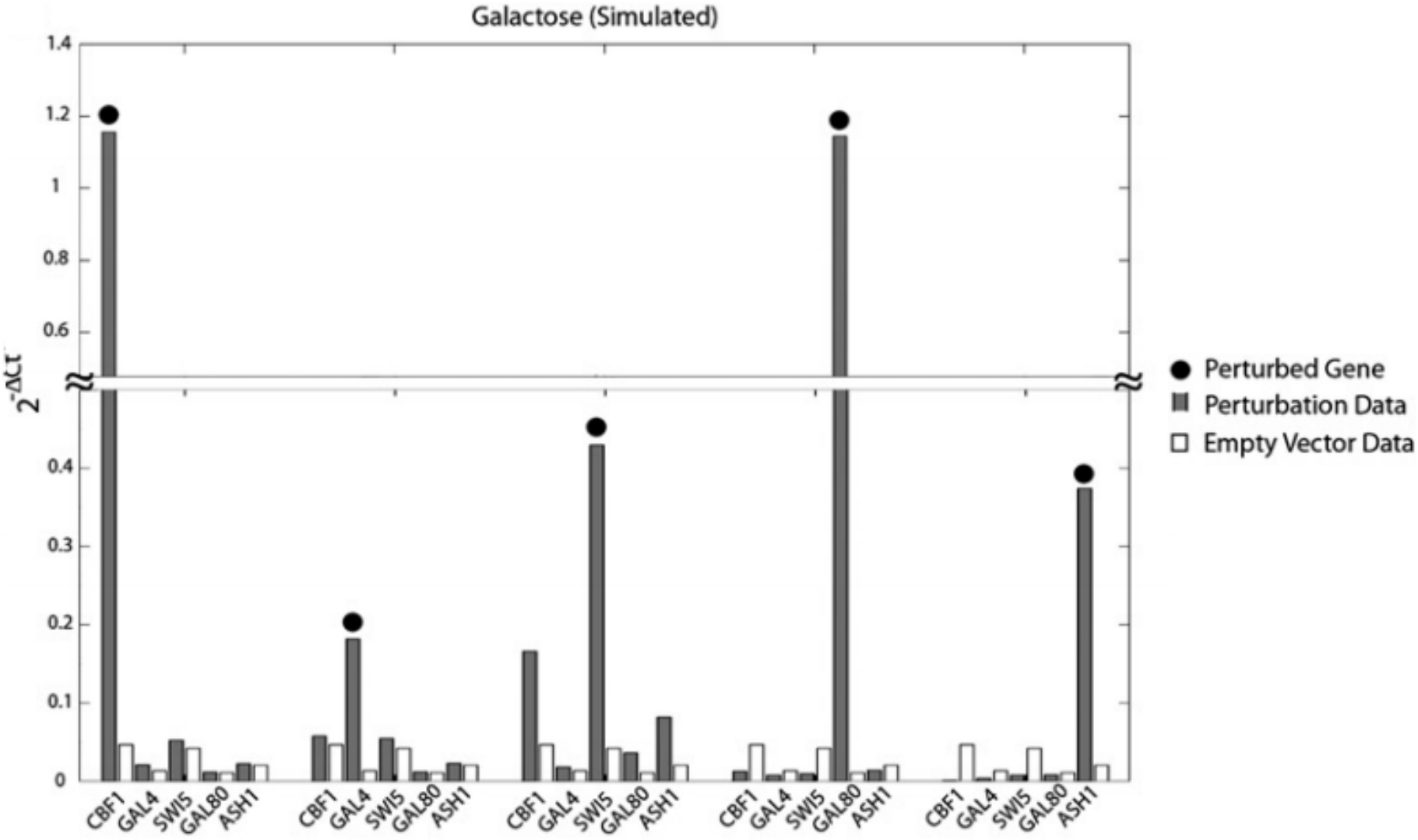
Characterize: A Single Perturbation



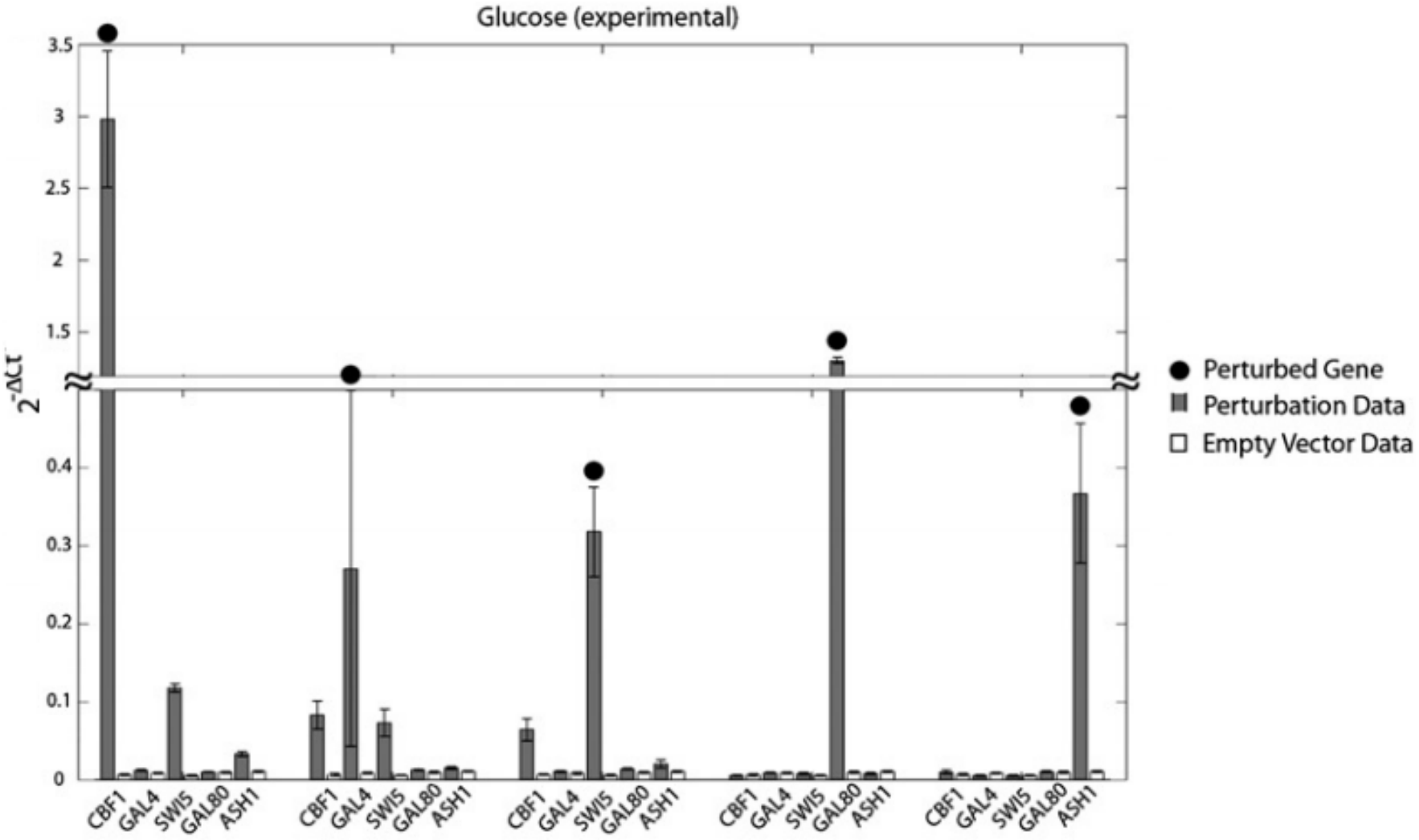
Characterize: Multiple Perturbations



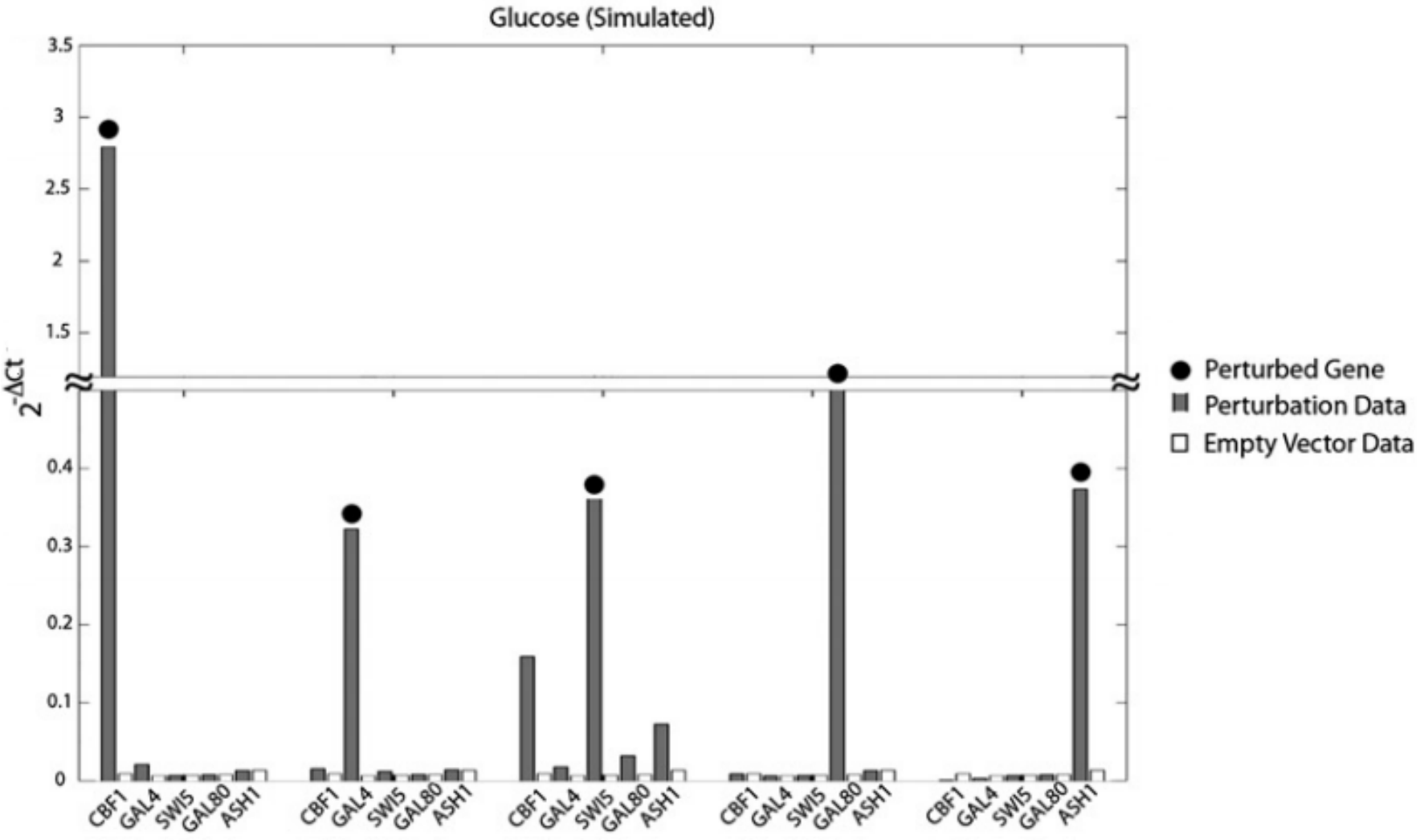
Characterize: Multiple Perturbations



Characterize: Multiple Perturbations



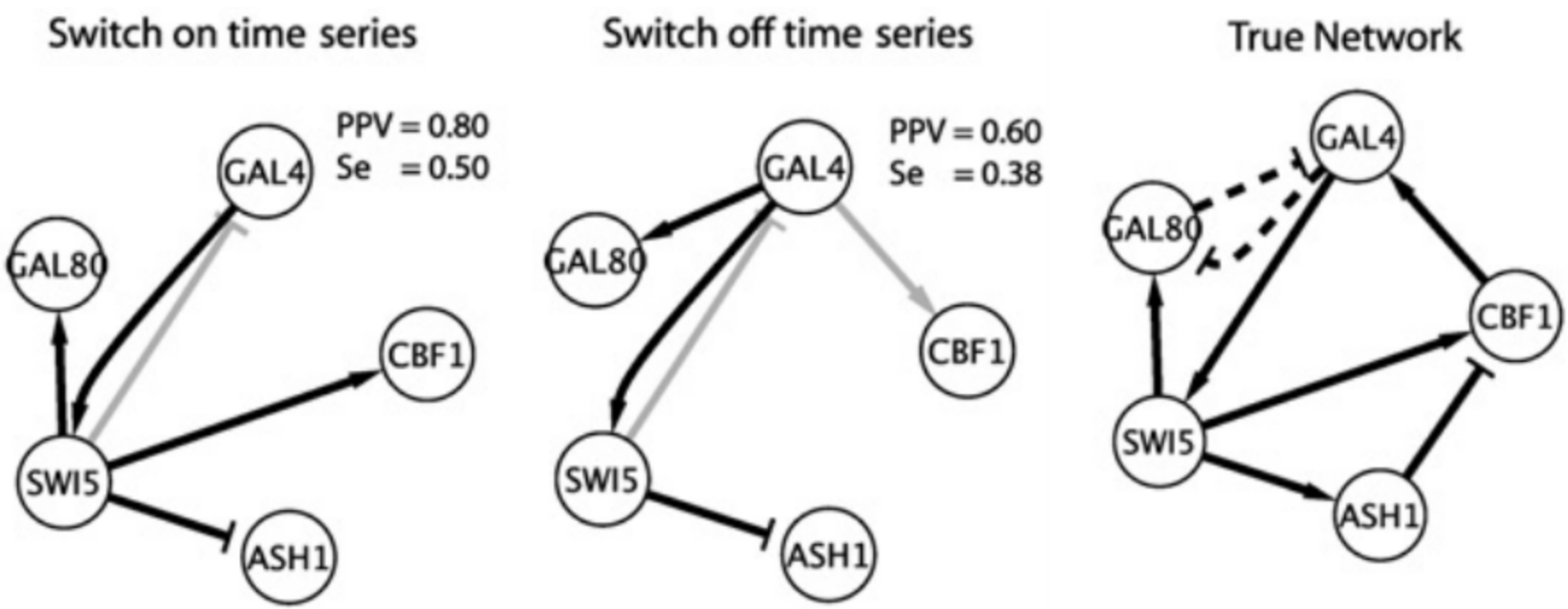
Characterize: Multiple Perturbations



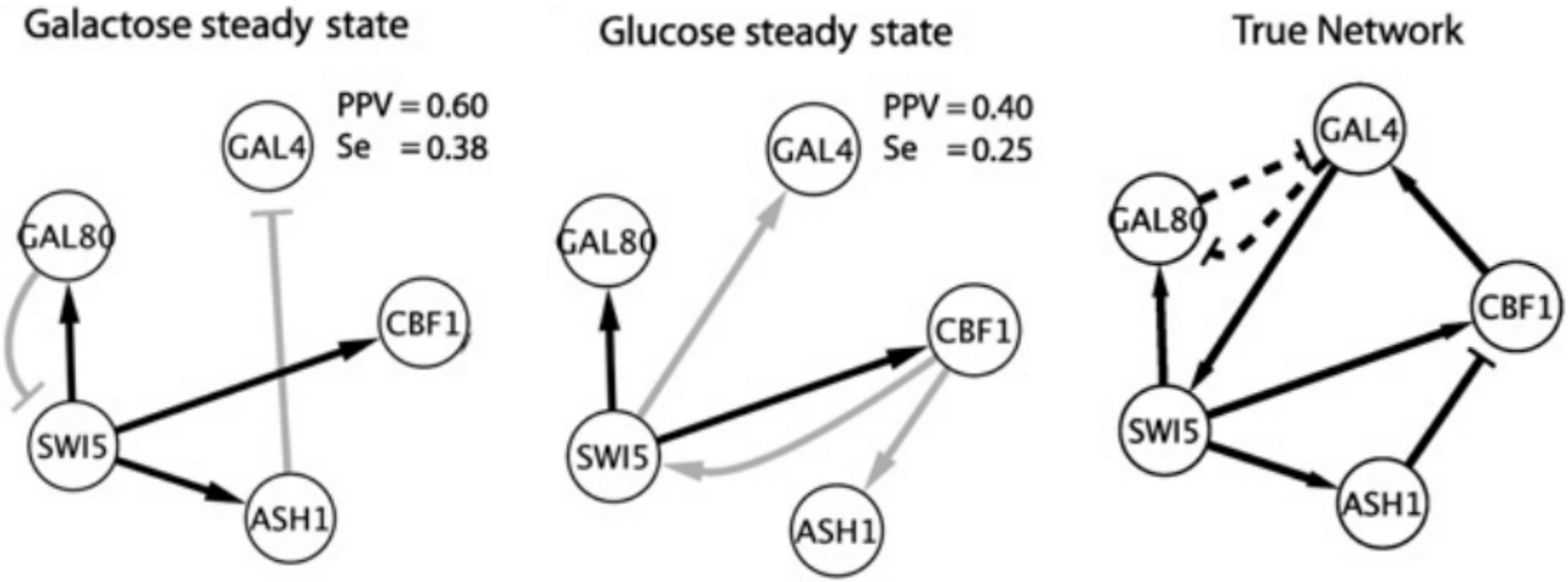
Test: Reverse Engineering

- From model parameters, test to see which algorithms can derive a network closest to the true network
- TSNI: Time Series Network Identification
- NIR: Network Inference by Regression

Test: TSNI


Switch on time series
PPV = 0.80
Se = 0.50
GAL4
GAL80
CBF1
SWI5
ASH1
Switch off time series
PPV = 0.60
Se = 0.38
GAL4
GAL80
CBF1
SWI5
ASH1
True Network
GAL4
GAL80
CBF1
SWI5
ASH1

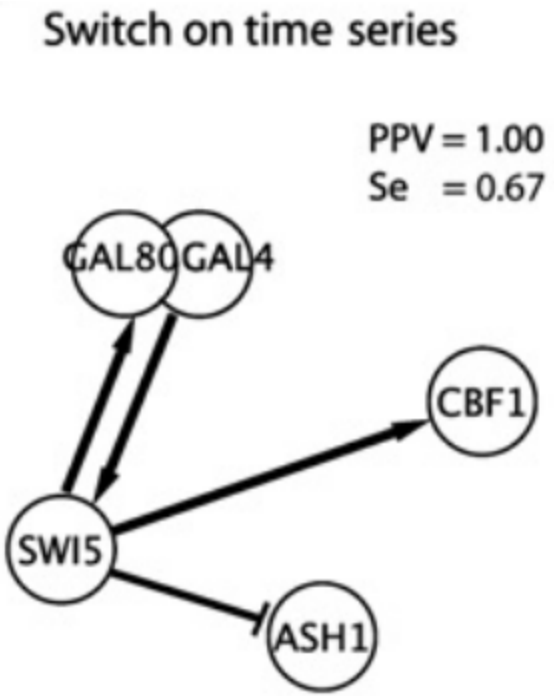
Test: NIR

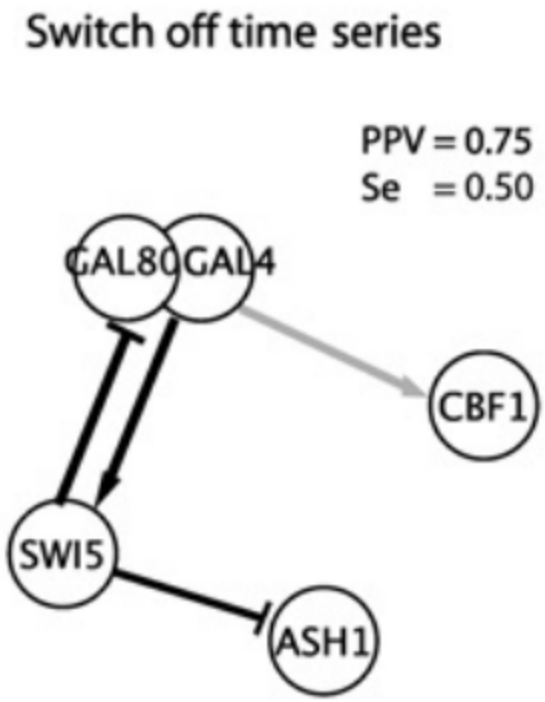

Galactose steady state
PPV = 0.60
Se = 0.38
GAL4
GAL80
CBF1
SWI5
ASH1
Glucose steady state
PPV = 0.40
Se = 0.25
GAL4
GAL80
CBF1
SWI5
ASH1
True Network
GAL4
GAL80
CBF1
SWI5
ASH1

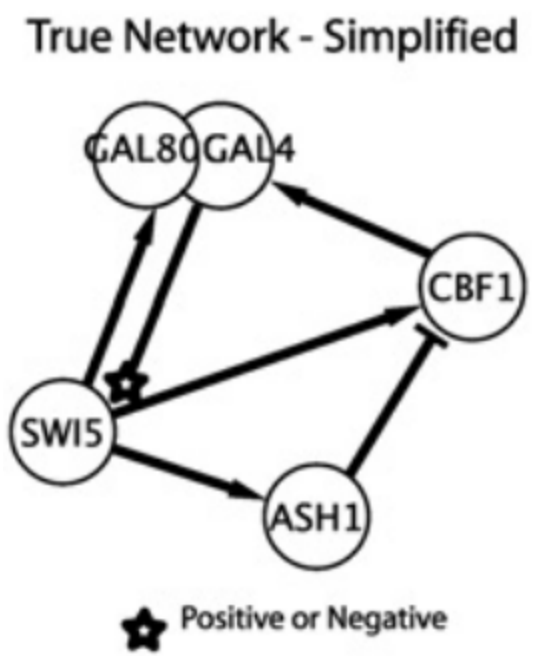
Test: Simplified Network

- Most false interactions involve the protein regulators GAL4 and GAL80.
- Simplify the true network by eliminating the protein-protein interaction, leaving only transcriptional regulation

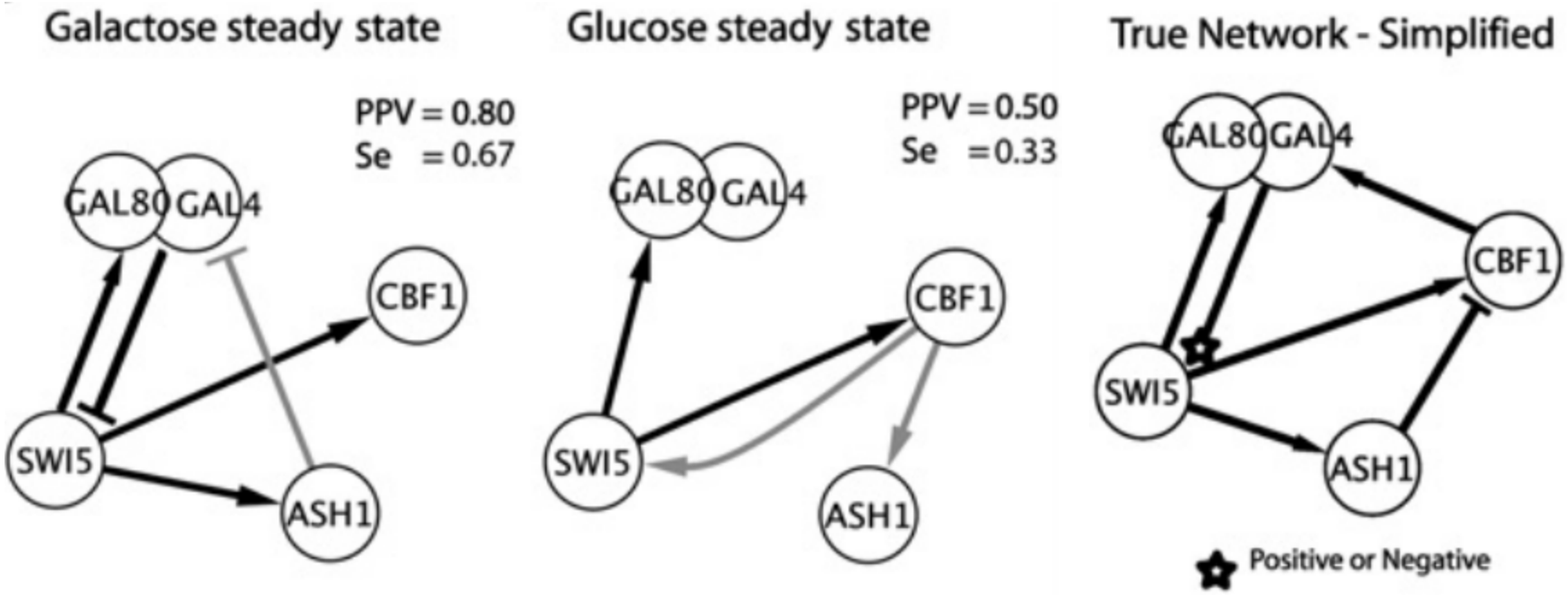
Test: TNSI


Switch on time series
PPV = 1.00
Se = 0.67
GAL80
GAL4
CBF1
SWI5
ASH1


Switch off time series
PPV = 0.75
Se = 0.50
GAL80
GAL4
CBF1
SWI5
ASH1


True Network - Simplified
GAL80
GAL4
CBF1
SWI5
ASH1
Positive or Negative

Test: NIR


Galactose steady state
PPV = 0.80
Se = 0.67
GAL80
GAL4
CBF1
SWI5
ASH1
Glucose steady state
PPV = 0.50
Se = 0.33
GAL80
GAL4
CBF1
SWI5
ASH1
True Network - Simplified
GAL80
GAL4
CBF1
SWI5
ASH1
Positive or Negative

Conclusions

- Developed and characterized a novel synthetic system

- Can be used to assess different models and network prediction algorithms

Significance

- Computational approaches help elucidate regulatory interactions in biological processes.

- Offers a tool to test and improve computational models and network algorithms

Concerns

- Mathematical model could use additional refining

- Made claims that didn't necessarily emerge from the data.

Questions?