The costs and benefits of microbial procrastination

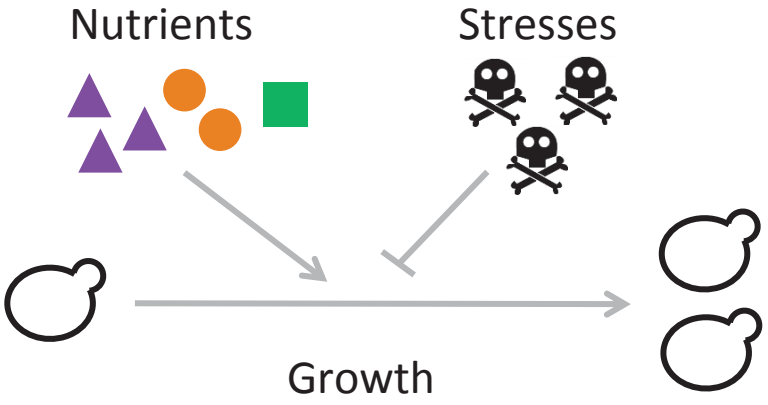
ICTP

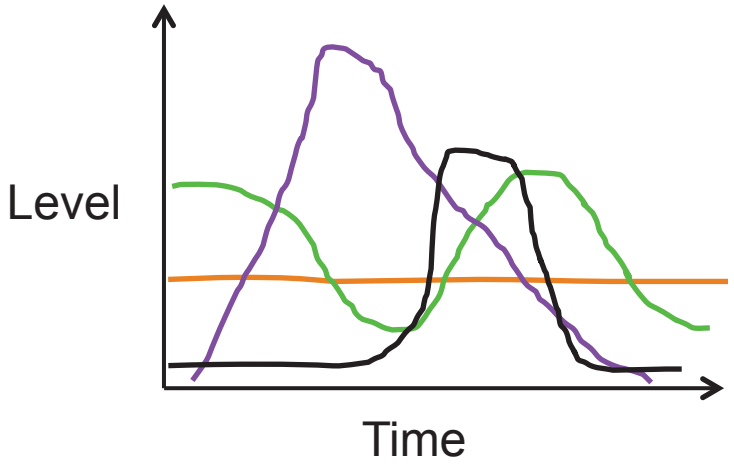
June 27th, 2014

Michael Springer

Harvard Medical School

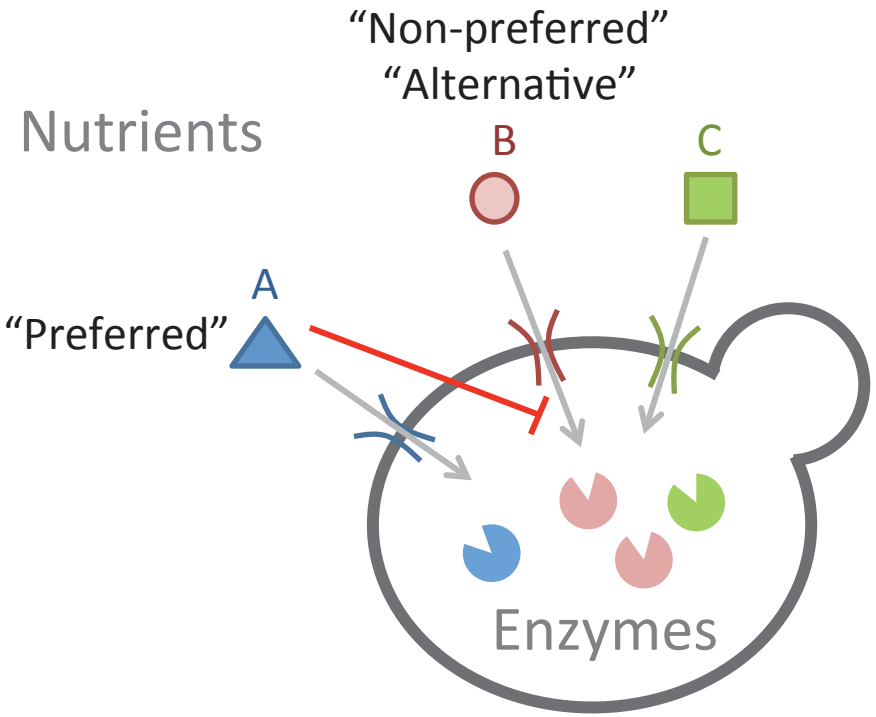
Cells live in mixed, dynamic environments.



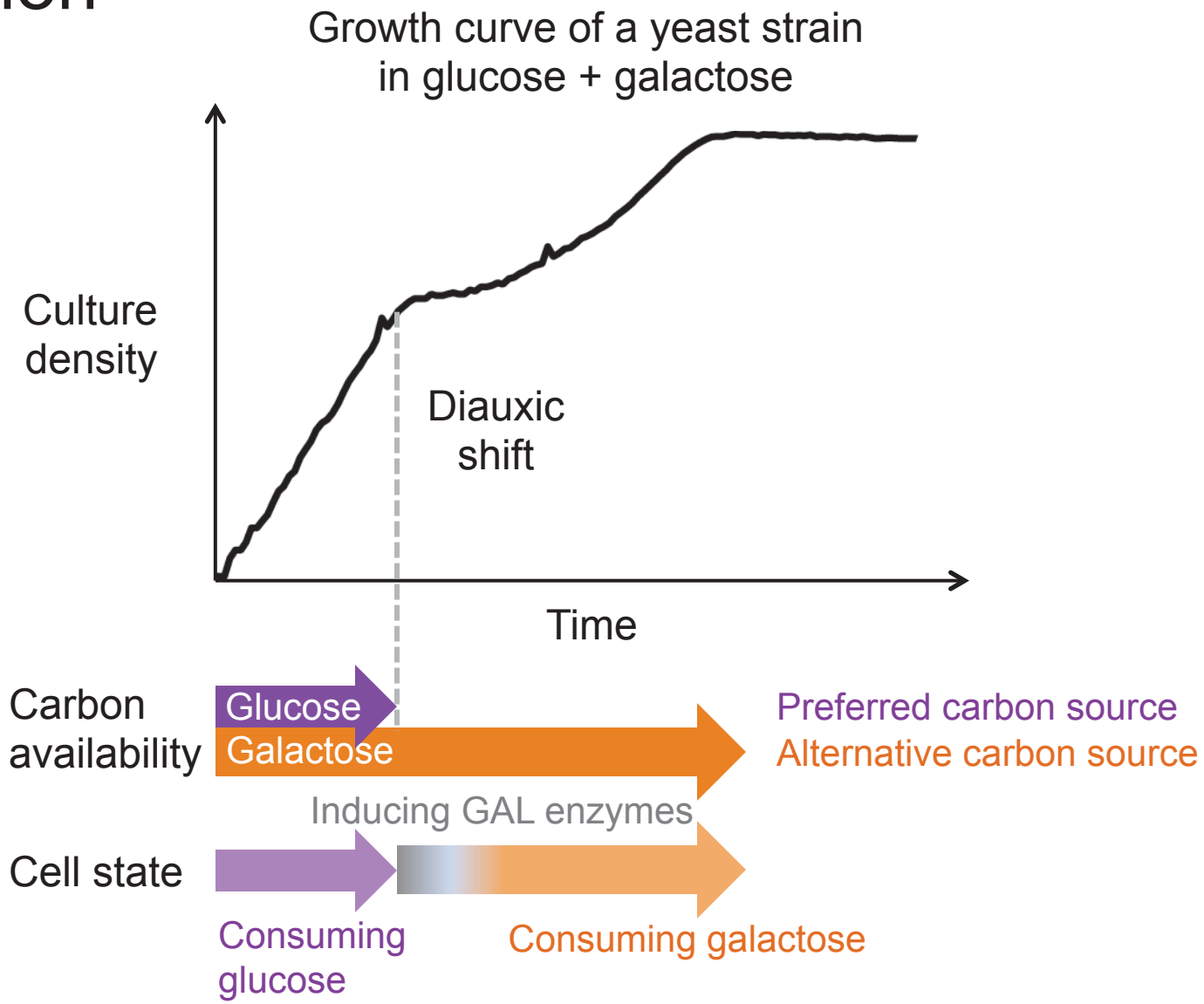


How do they deal with this?

Microbes use gene regulation to choose between nutrients



Diauxic growth: a classic example of gene regulation



Why don’t cells “prepare” for glucose depletion?

Examples of microbial preparation

- Simple biochemical circuits can predict environmental changes
  - E. coli: heat shock, low oxygen
  - Yeast: heat stress, oxidative stress
  - Many organism: circadian oscillators

Glucose -> Ethanol (derived from glucose)

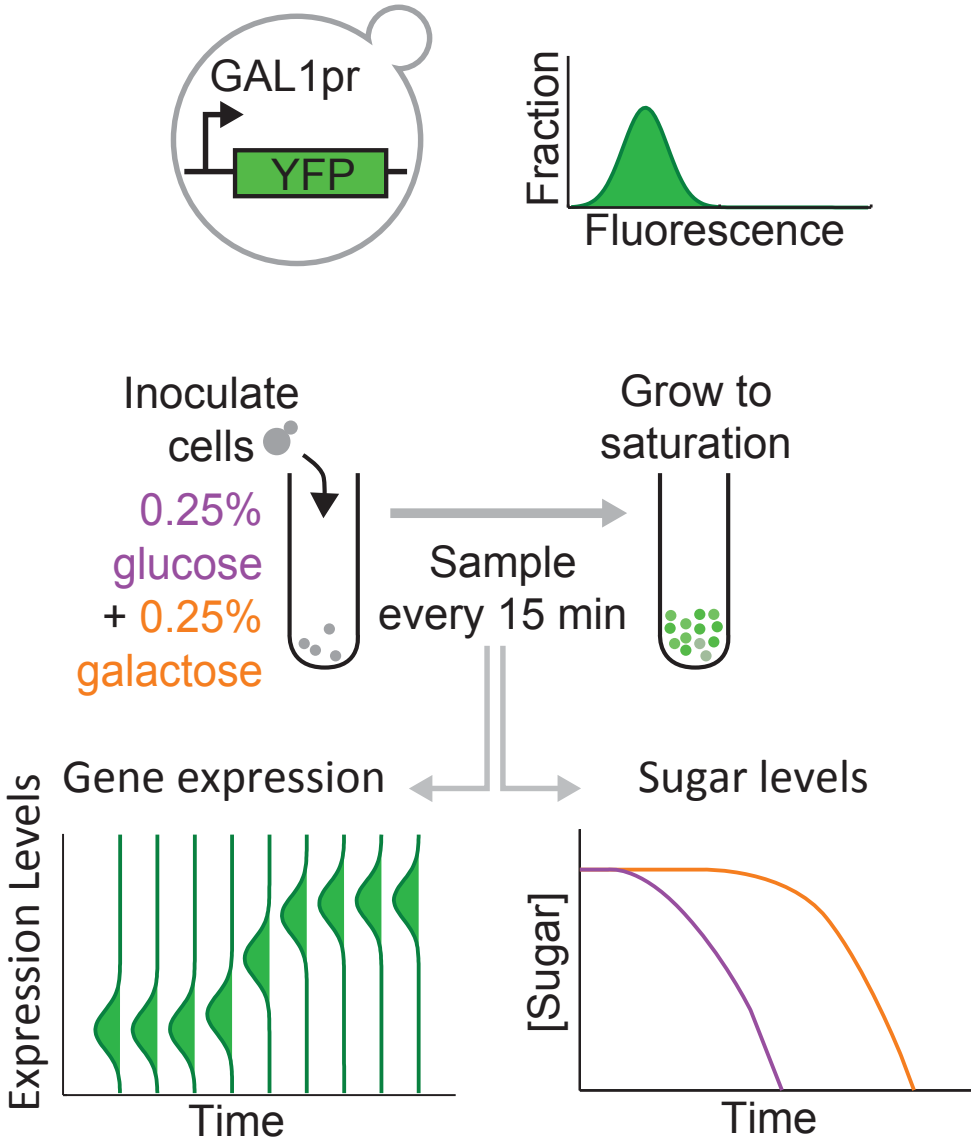
Glucose -> Galactose

Why don't cells prepare? Maybe they do?

Are difference in lag time due to difference in preparation?

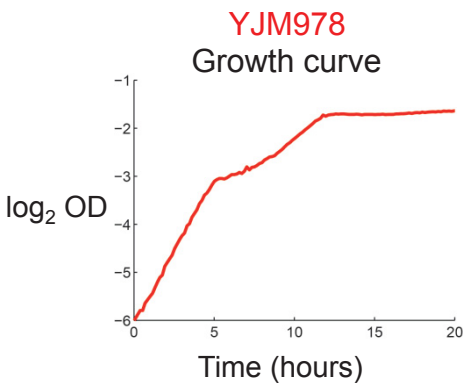
- New et al. (Verstrepen) –
  - Found variation in yeast.
  - Showed that they can evolve diauxic shift length
- Spencer et al. (Doebeli) –
  - E. coli diauxic length variability (Quick and slow responders)

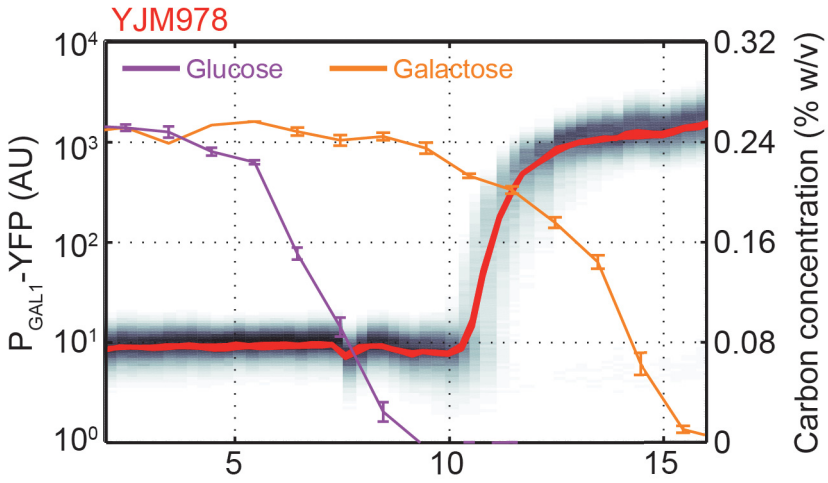
How to tell if cells are preparing for galactose utilization before glucose depletion?



YJM978 induces GAL genes *after* glucose depletion.

BC187 induces GAL genes *before* glucose depletion.





How should a cell decide whether to prepare?

Parameters	Costs	Benefits
Environmental	Sensing –	Length of benefit
	Ability to sense	
	Predictability of environment	Size of benefit
	Time scale of cellular response versus environment	
	Cost of false response	Direct
Organismal	Response –	
	Speed of response (execution time)	Indirect
	Beginning of response -	
	Time decision is made	
	Time decision is acted upon	
	Strength of response	
	Memory/Decay of response	

Let nature tell us what it cares about

Voltaire: “Observe that noses were made to wear spectacles …”

Differences in lag time:

New et al. (Verstrepen) –

Found variation in yeast.

Showed that they can evolve diauxic shift length

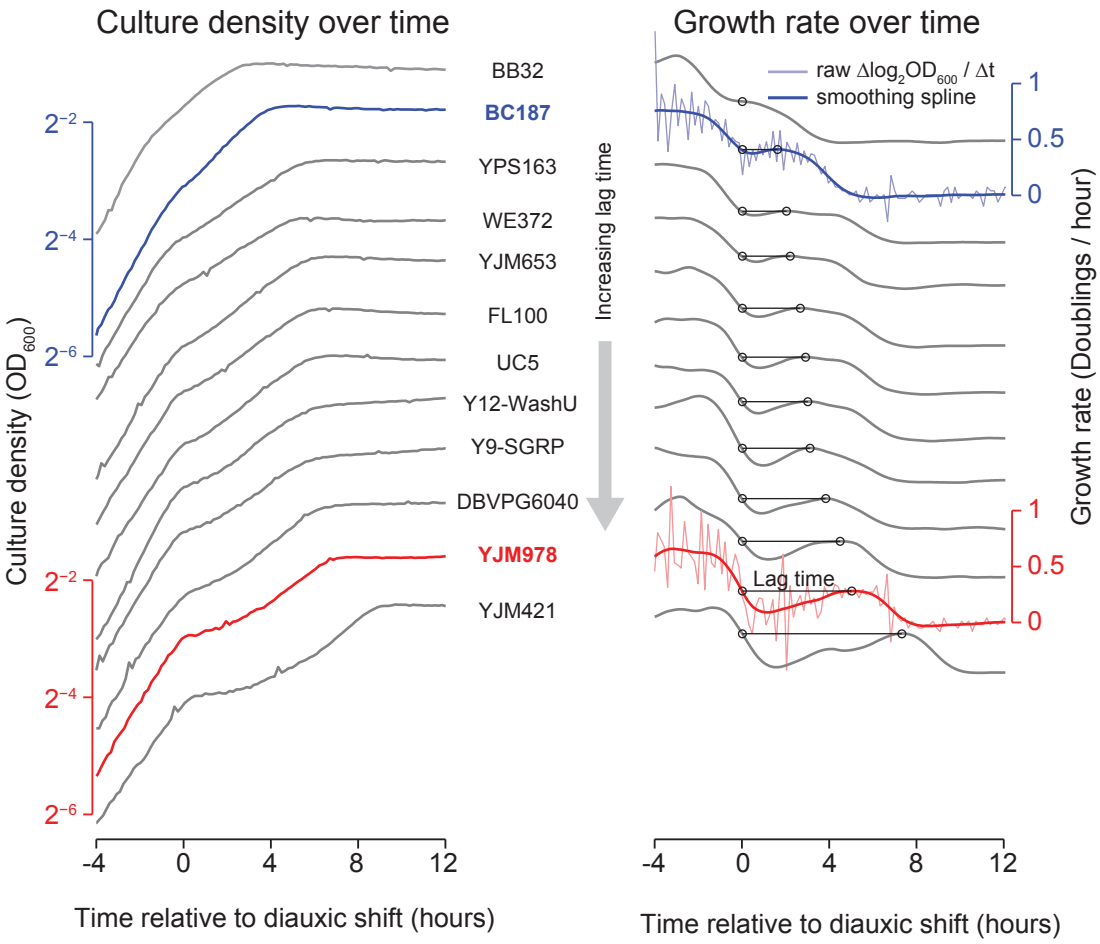
Spencer et al. (Doebeli) –

E. coli diauxic length variability (Quick and slow responders)

- From The Saccharomyces Genome Resequencing Project (Sanger Institute) and Justin Fay (Washington U.)

- 63 strains of *S. cerevisiae*
  - Genetically similar (can mate)
  - Ecologically and geographically diverse
  - Many phenotypic differences

We have strains with differences in diauxic lag



Why are some cells preparing?
Speed of response or timing of response?

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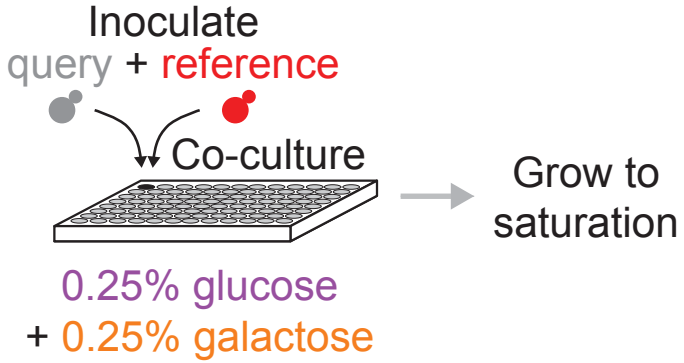
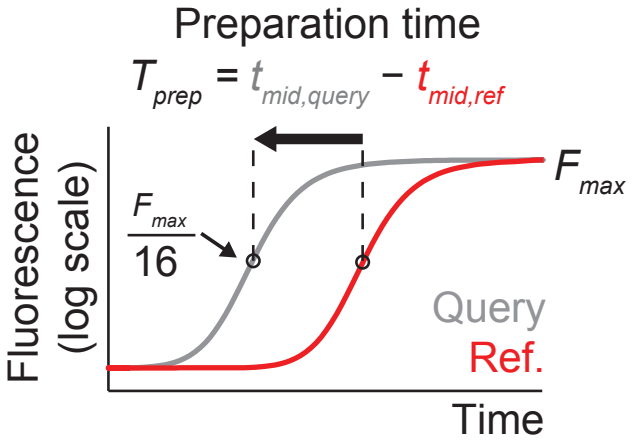
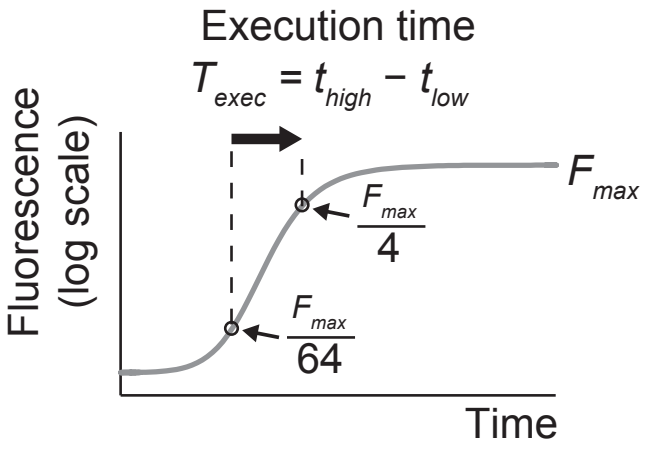
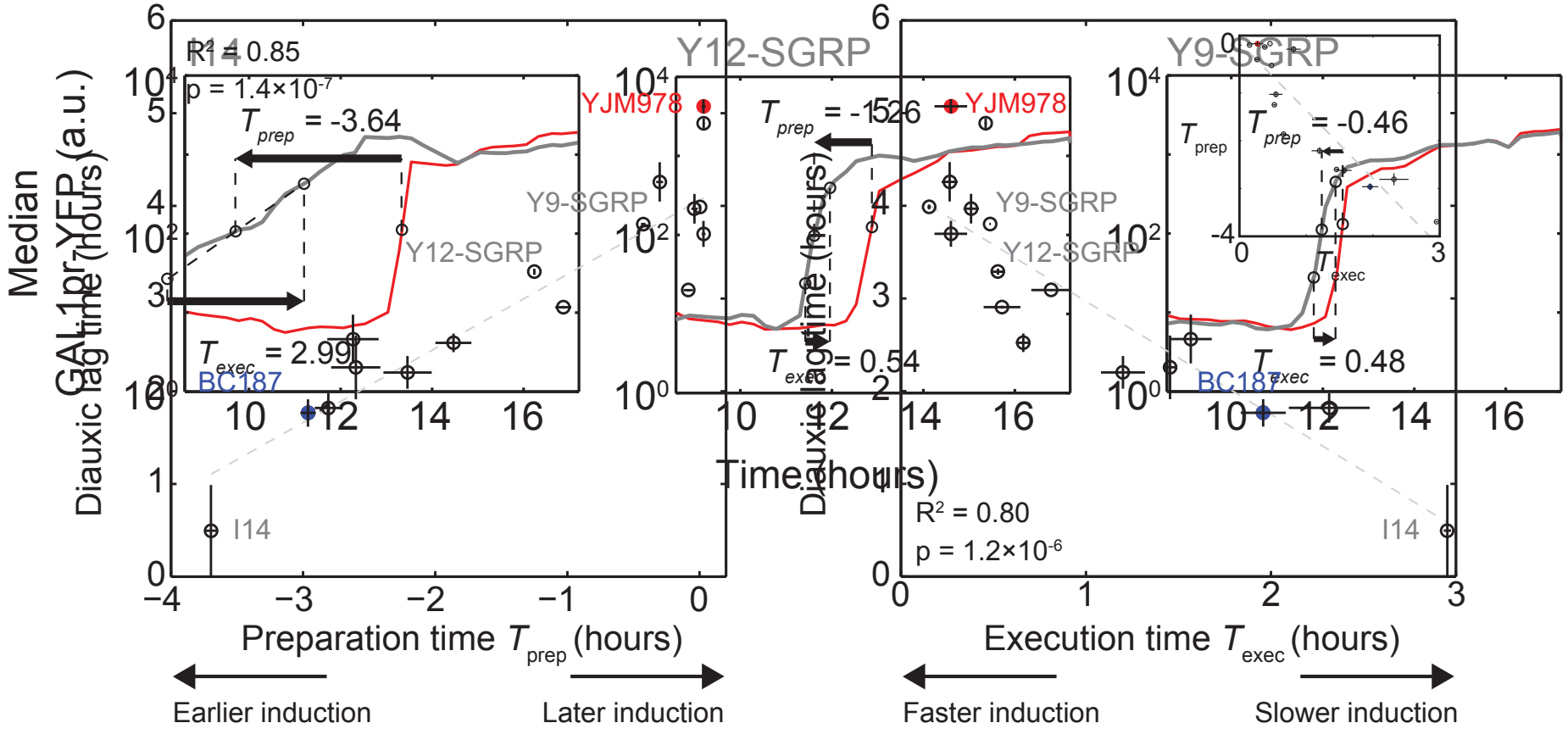
Schema and metrics to compare preparation and execution times







Preparation not execution time correlates with lag time



What controls the preparation time?

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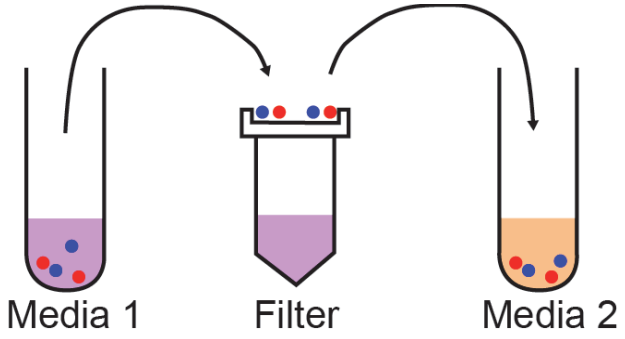
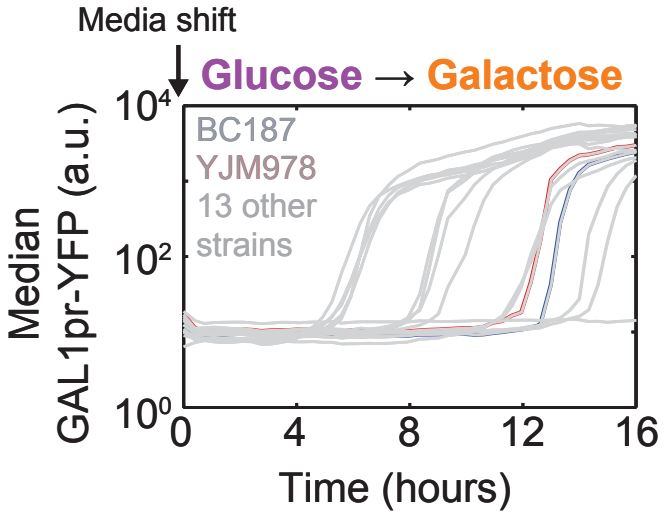
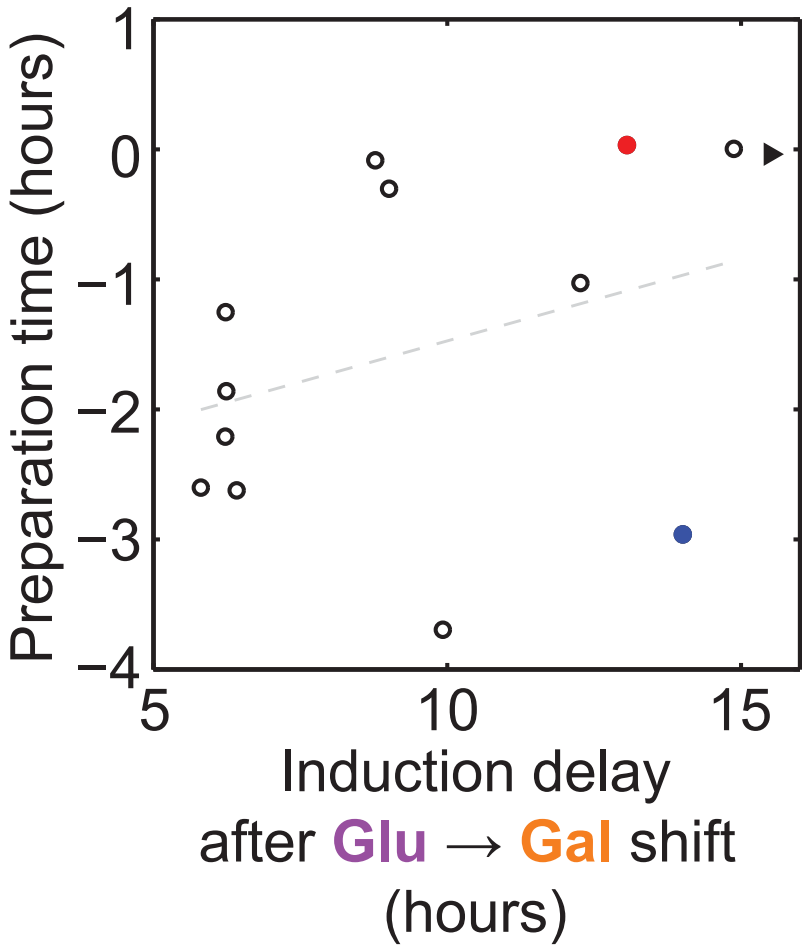
Differences between strains are not due to kinetics differences in delay before execution







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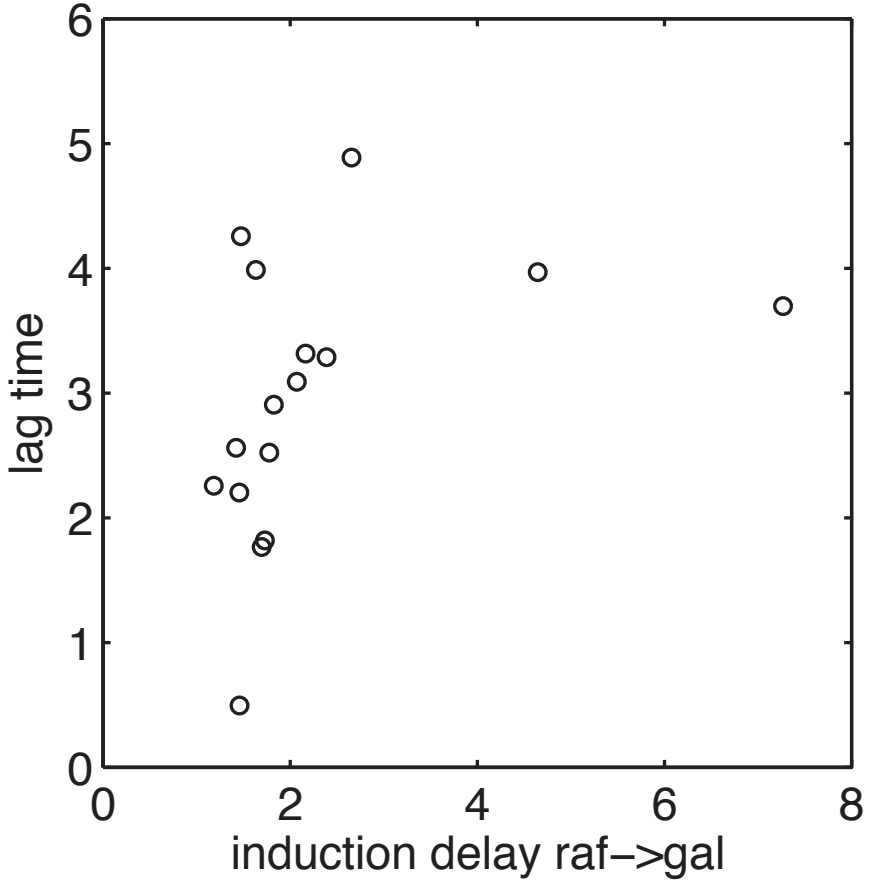
Do difference in time until a response explain the diauxic lag?



Is glucose and/or galactose sensed differently?

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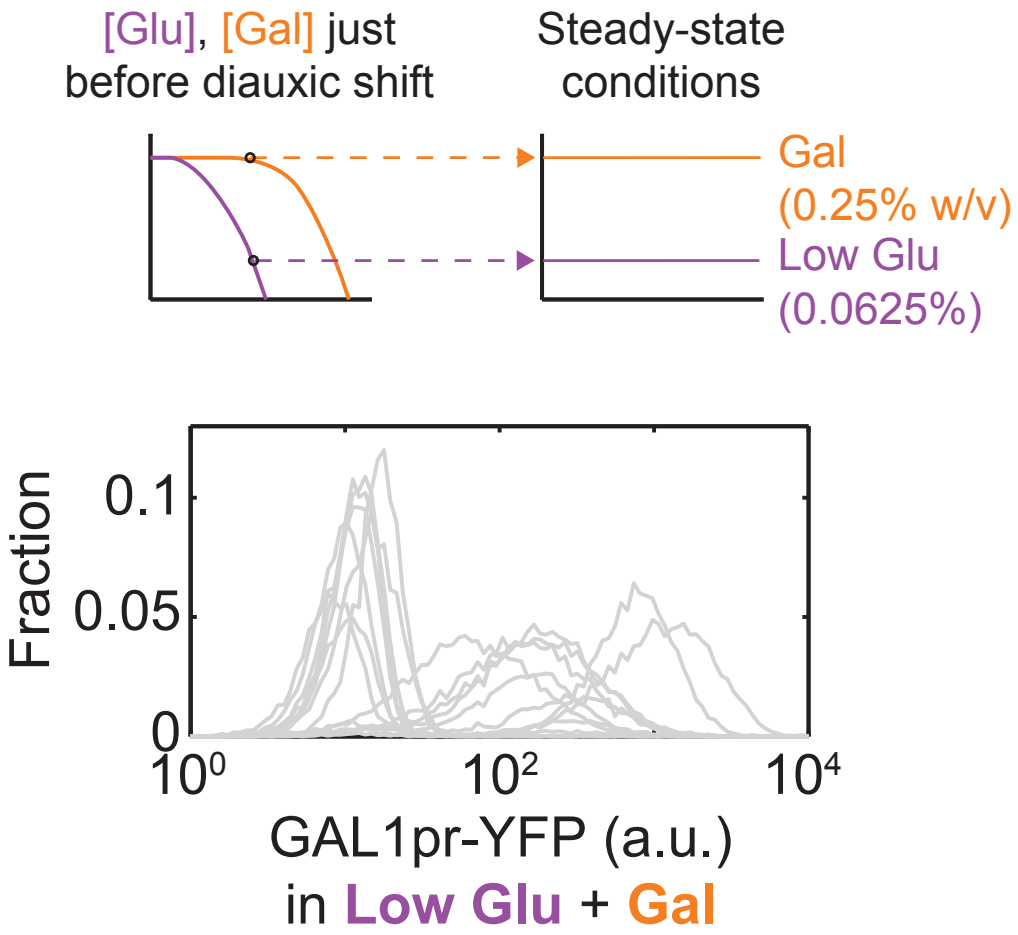
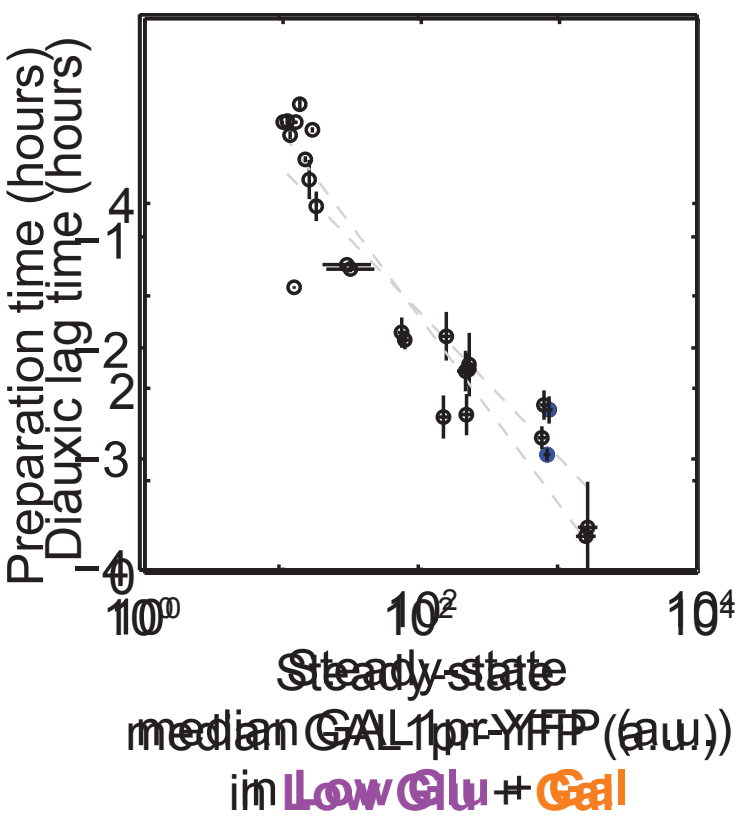
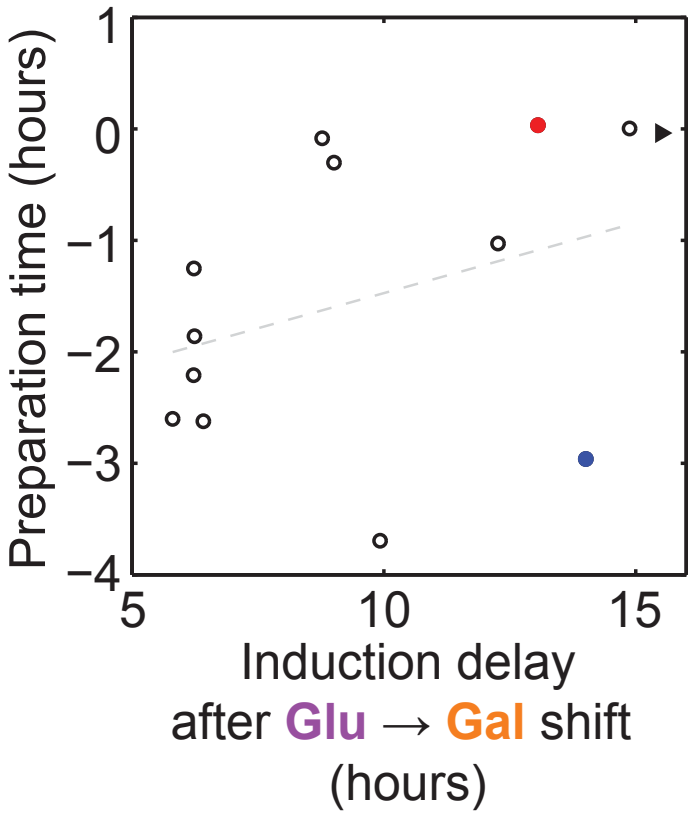
	Predictability of environment	Size of benefit
	Time scale of cellular response versus environment	
	Cost of false response	Direct
Organismal	Response –	
	~~Speed of response (execution time)~~	Indirect
	Preparation Time -	
	Time decision is made	
	~~Time decision is acted upon~~	
	Strength of response	
	Memory/Decay of response	

Measuring differences in response – steady-state difference at low glucose



Steady-state expression correlates with lag and preparation times





Do all strains prepare?

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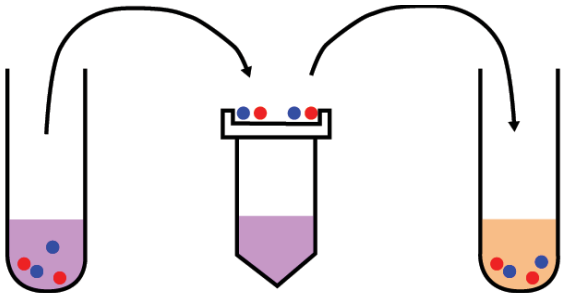
Do all strains prepare?

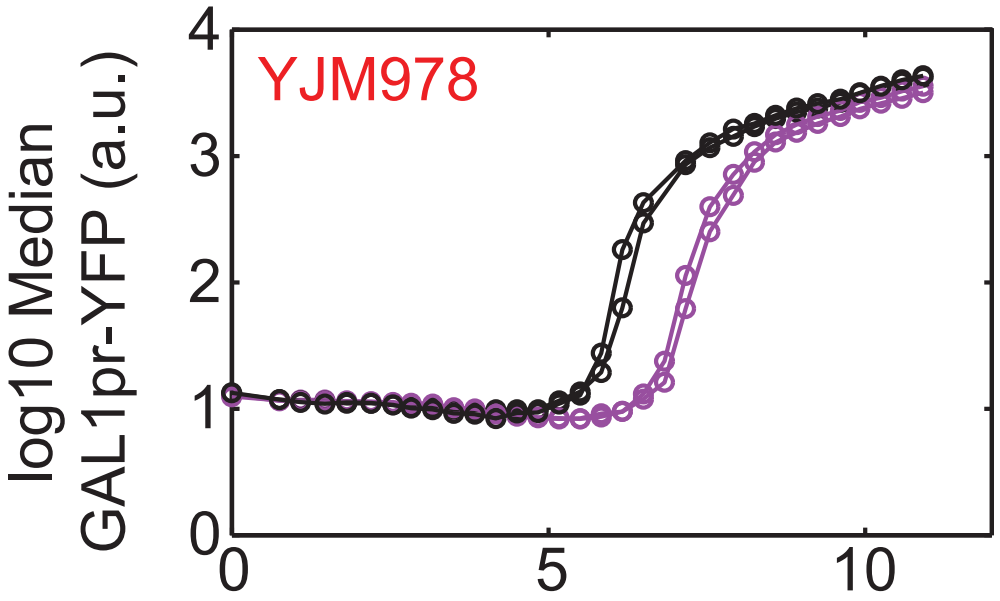


Low glucose + galactose

Low glucose

galactose

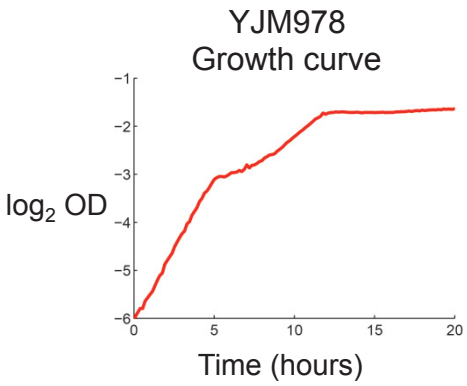
galactose

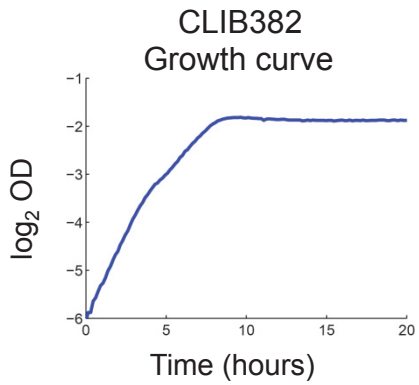


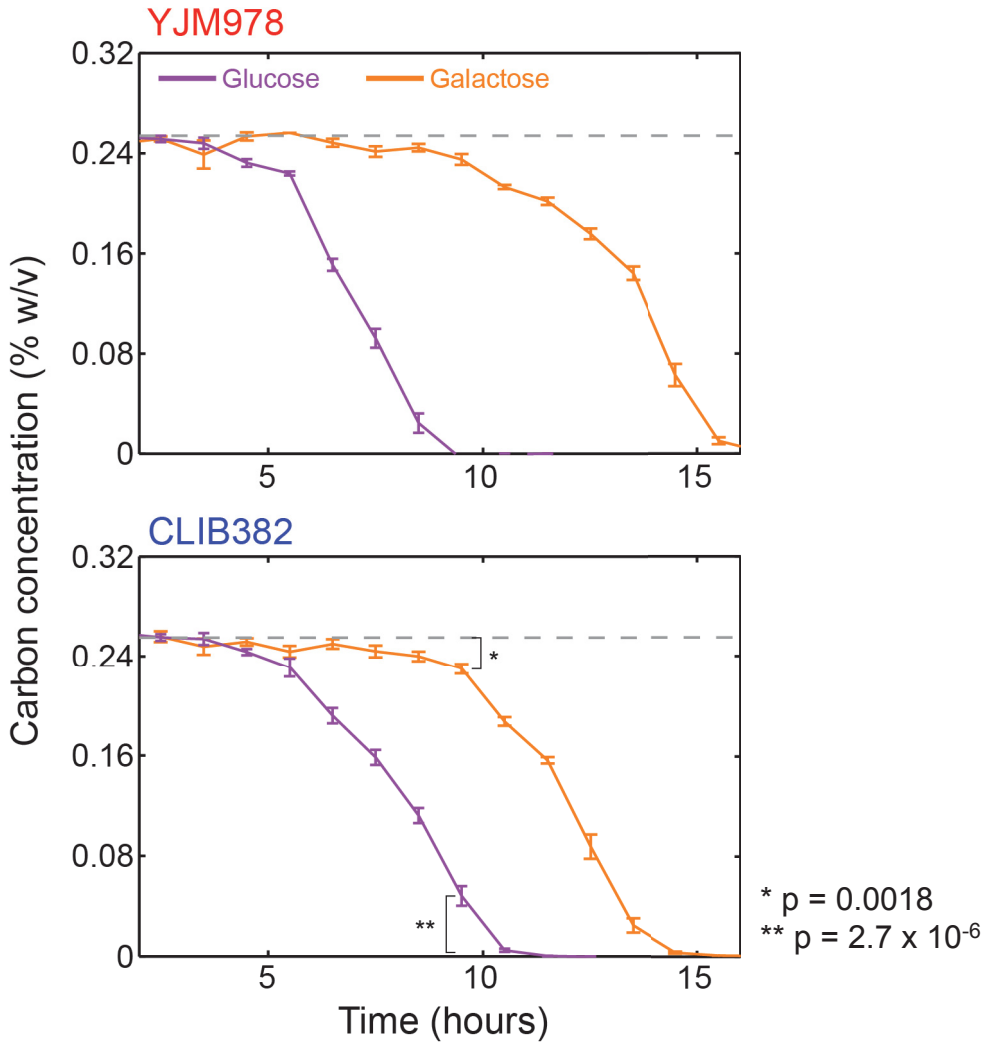
What are the cost and benefits of preparing?

Parameters	Costs	Benefits
Environmental	Sensing –	Length of benefit
	Ability to sense	
	Predictability of environment	Size of benefit
	Time scale of cellular response versus environment	
	Cost of false response	Direct
Organismal	Response –	
	~~Speed of response (execution time)~~	**Indirect**
	Preparation Time - **Time decision is made** ~~Time decision is acted upon~~	
	Strength of response	
	Memory/Decay of response	

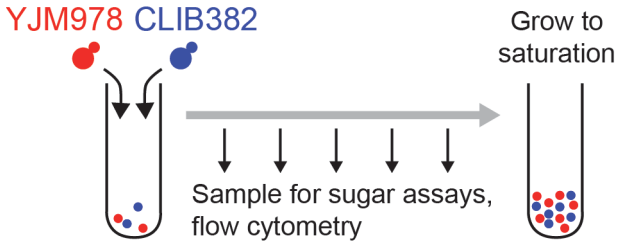
Short diauxic lag is associated with earlier galactose consumption

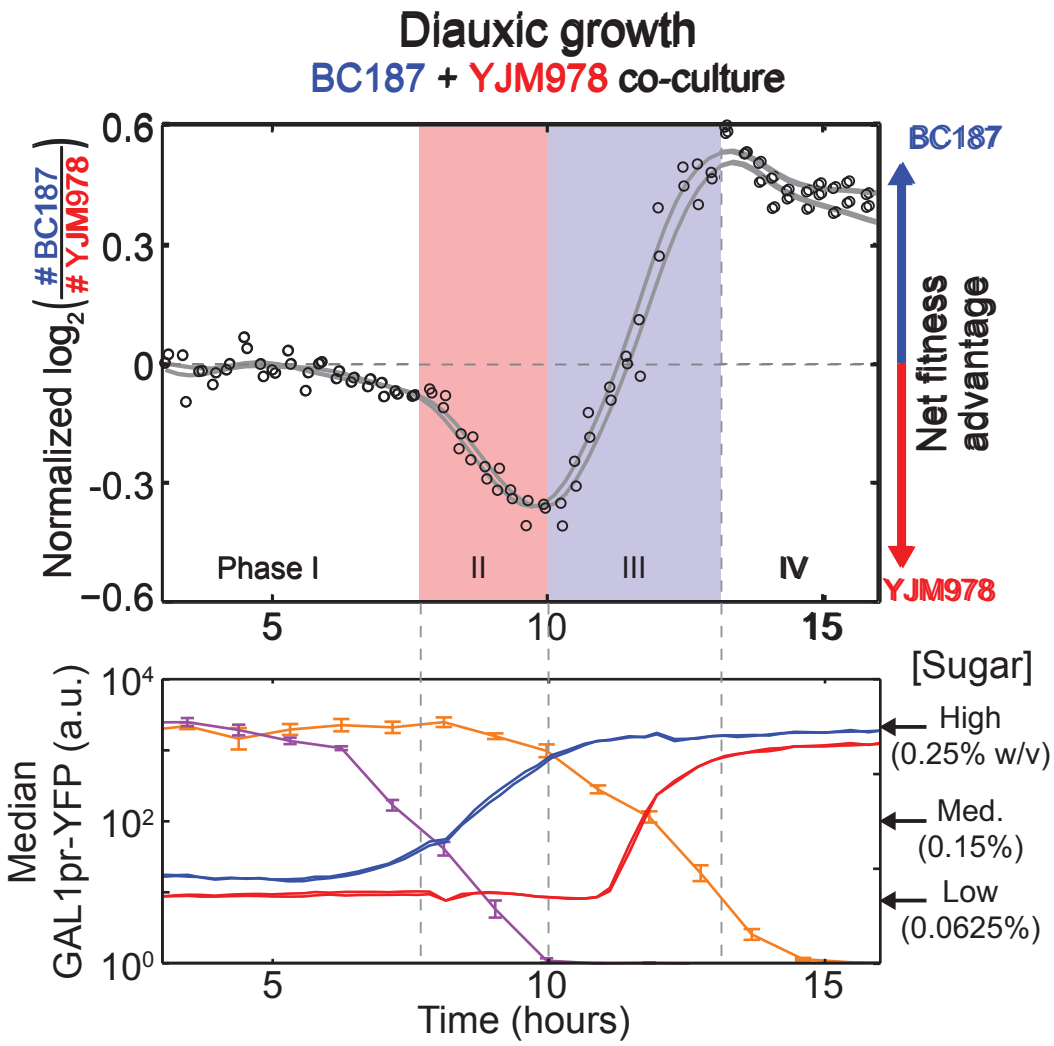






Measuring the cost and benefits of preparation





What is the nature of the cost and benefits?

Parameters	**Costs**	**Benefits**
Environmental	Sensing –	Length of benefit
	Ability to sense	
	Predictability of environment	Size of benefit
	Time scale of cellular response versus environment	
	Cost of false response	Direct
Organismal	Response –	
	~~Speed of response (execution time)~~	**Indirect**
	Preparation Time - **Time decision is made** ~~Time decision is acted upon~~	
	Strength of response	
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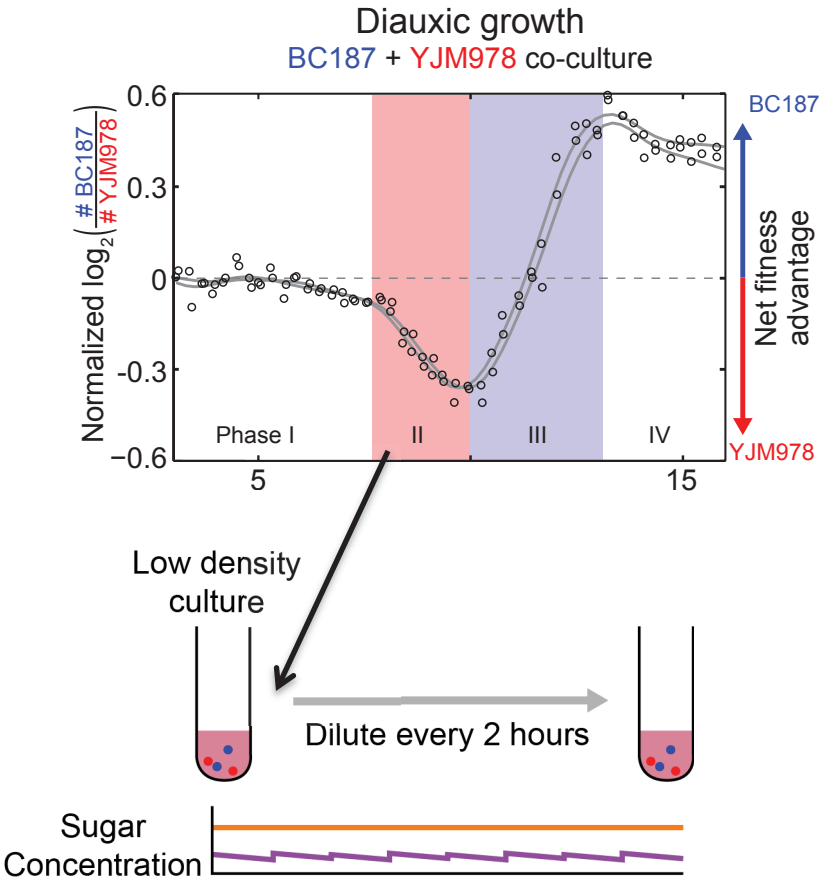
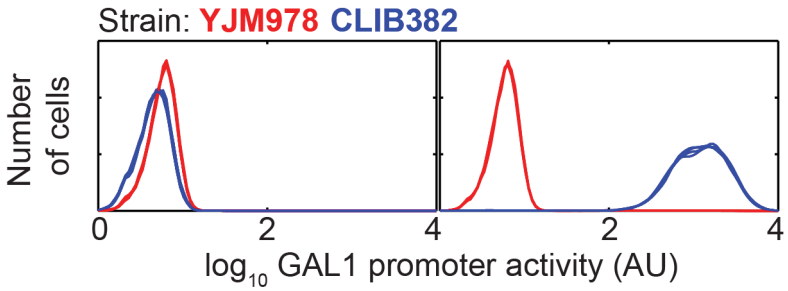
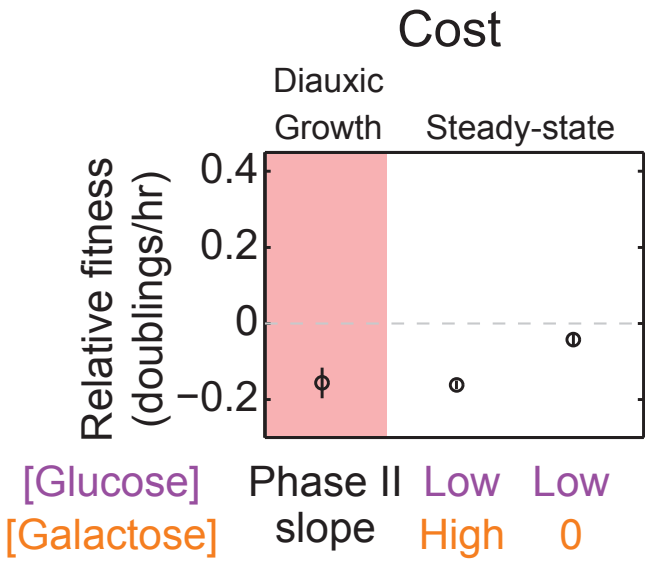
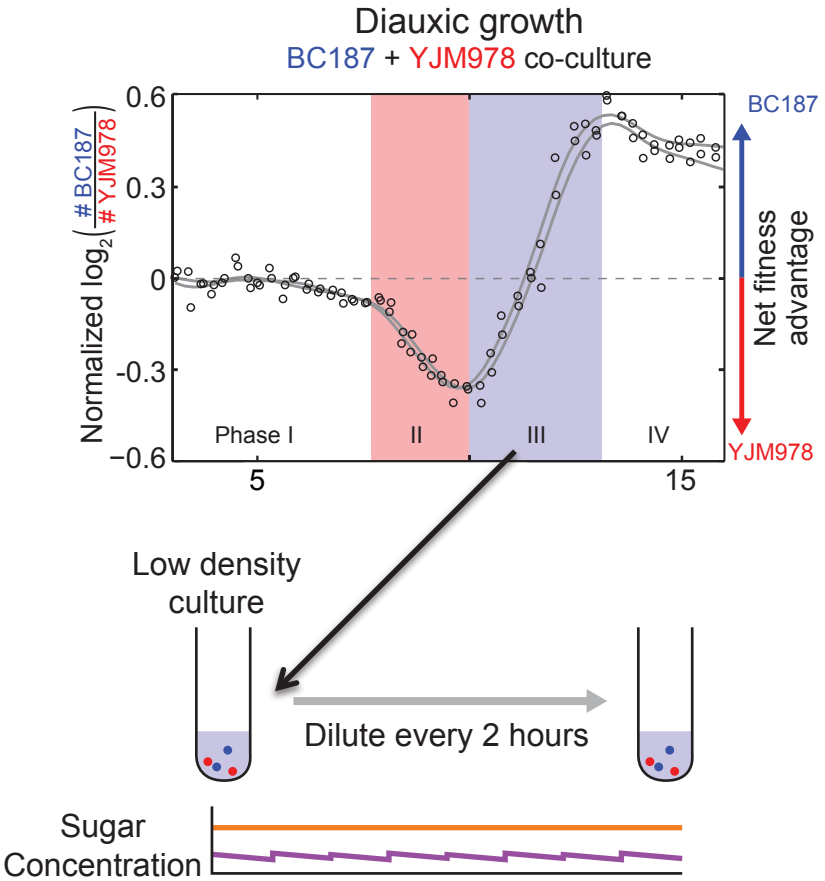
Is the cost steady-state or kinetic?

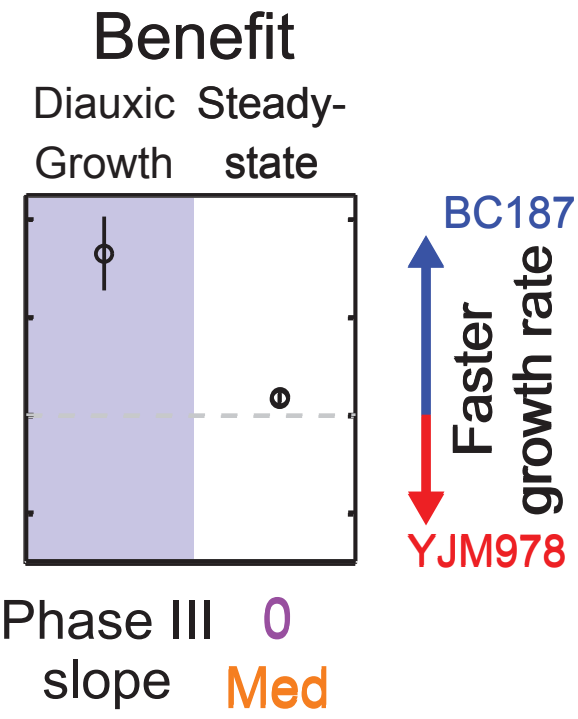






Is the benefit steady-state or kinetic?





Is the decision to response limited by the cost and benefits we measure?

Parameters	**Costs – steady-state**	**Benefits - kinetc**
Environmental	Sensing –	**Length of benefit**
	Ability to sense	
	Predictability of environment	**Size of benefit**
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	Cost of false response	**Direct**
Organismal	Response –	
	~~Speed of response (execution time)~~	**Indirect**
	Preparation Time - **Time decision is made** ~~Time decision is acted upon~~	
	Strength of response	
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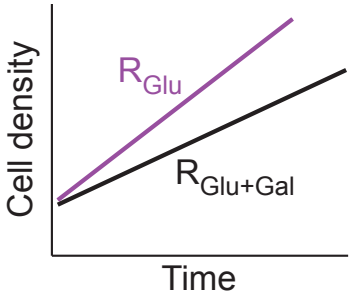
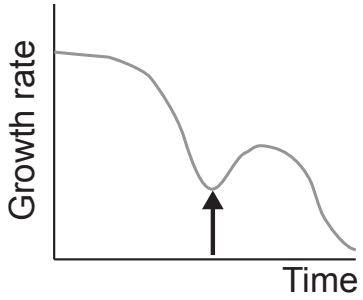
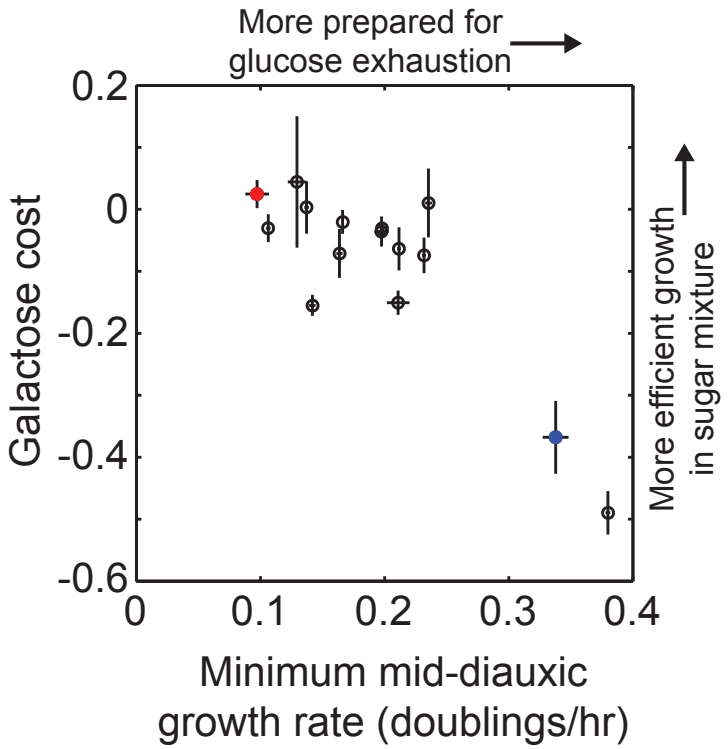
Trade-off between costs and benefits

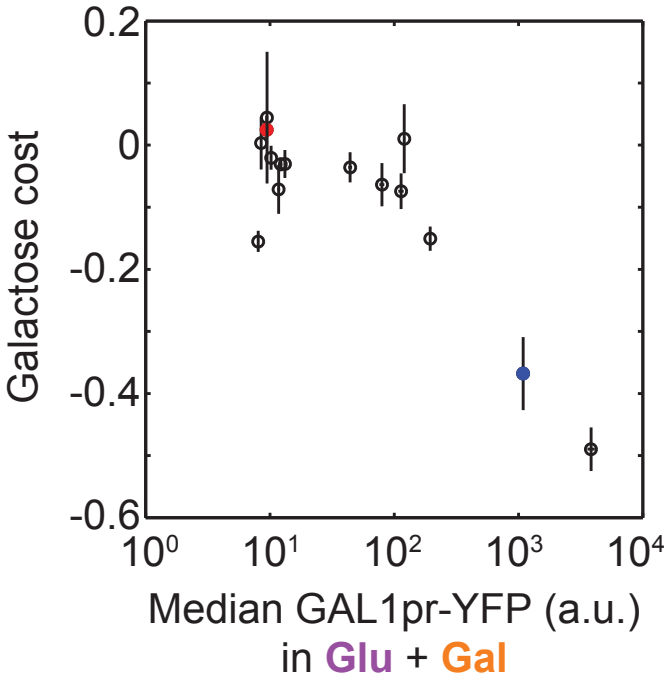


Preparation cost = $R_{Glu+Gal} - R_{Glu}$



Minimum mid-diauxic growth rate





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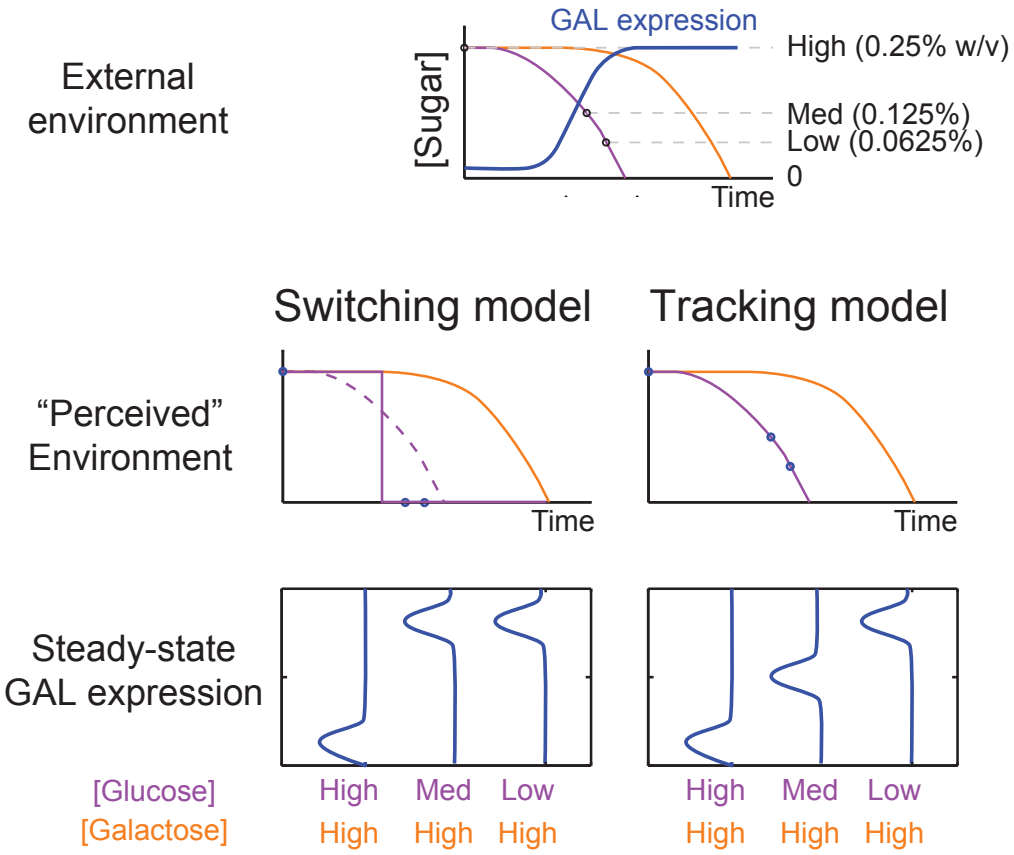
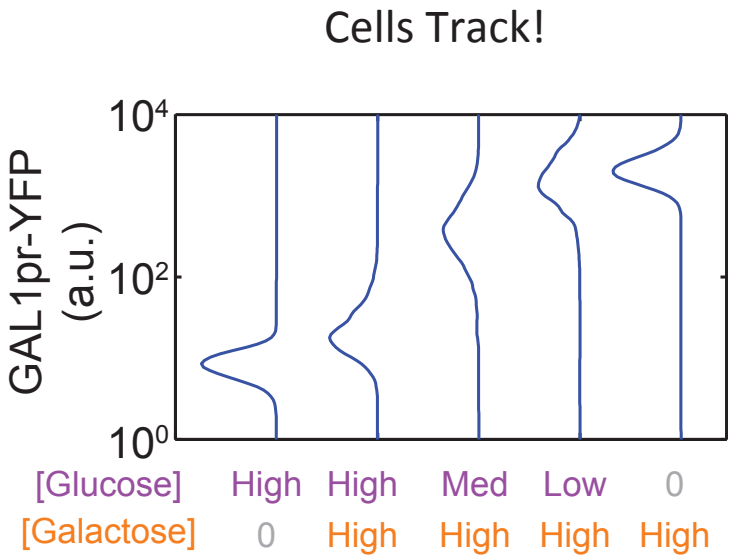
Does glucose modulate galactose response - do cell switch or track?





Cells are preparing. How do they decide when and how to respond?

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Why do we see multiple strategies in nature?

High Glucose
High Galactose

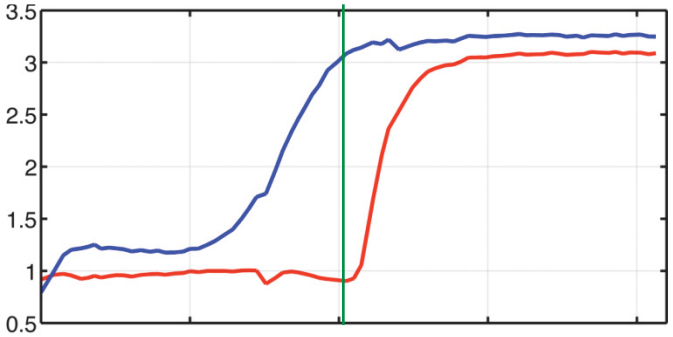
Low Glucose
High Galactose

No Glucose
High Galacatose

Gal not induced → Gal induced

3 hours

But what if a different nutrient becomes limiting and it cannot be predicted?



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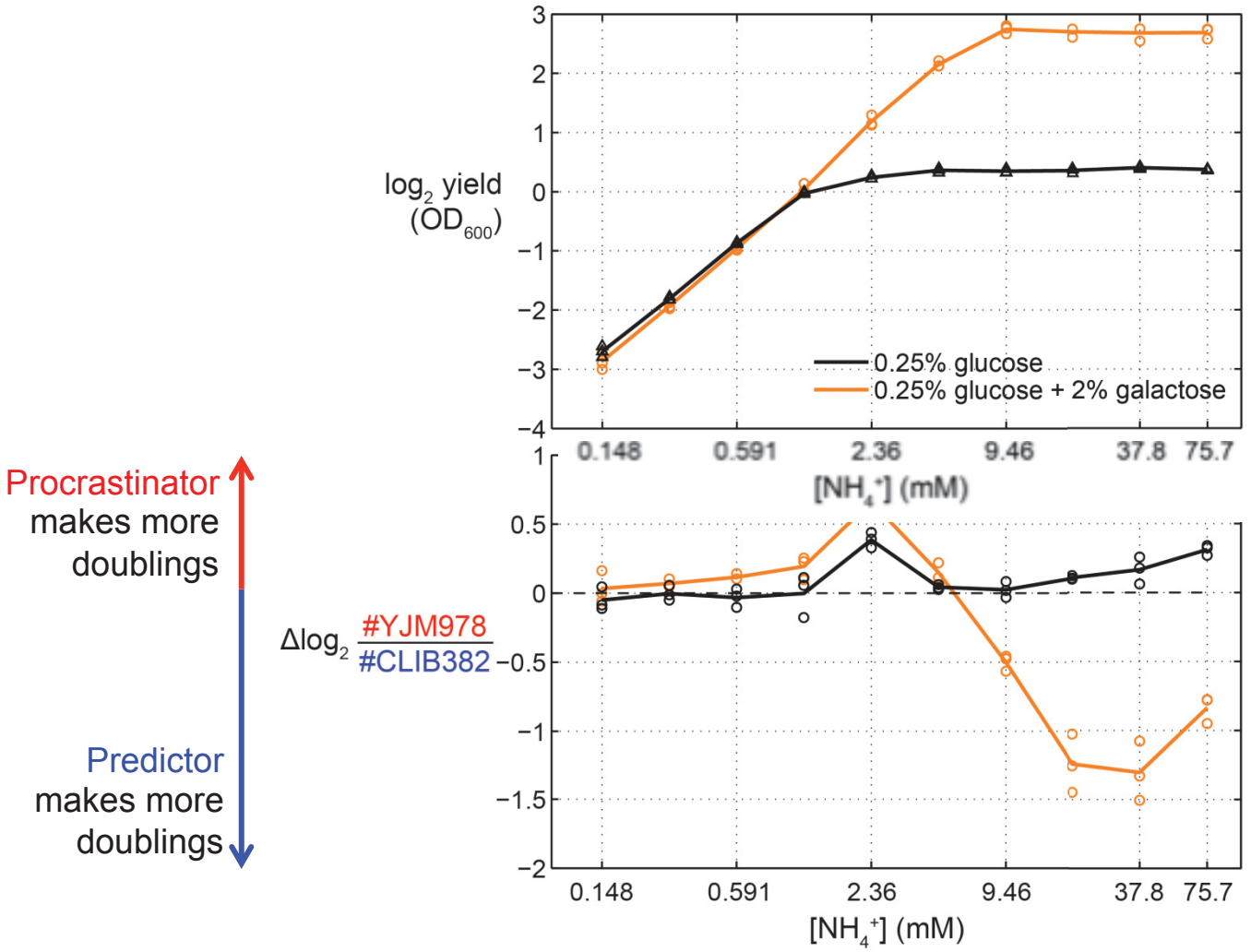
If there are not enough non-carbon nutrients to finish consuming carbon, it can “freeze time”.



Presence of galactose selects against pre-emptive utilization of galactose.

Cells are preparing. How do they decide when and how to respond?

Parameters

Environmental

Organismal

Costs – steady-state

Sensing –

Ability to sense

Predictability of environment

Time scale of cellular response

versus environment

Cost of false response

Response –

~~Speed of response (execution time)~~

Preparation Time -

Time decision is made

~~Time decision is acted upon~~

Strength of response

Memory/Decay of response

Benefits - kinetc

Length of benefit

Size of benefit

Direct

Indirect

Acknowledgements

Ratio Sensing

Renan Escalante

Yonatin Savir

John Ingraham

Chris Marx

Sean Carroll

Anticipation

Jue Wang

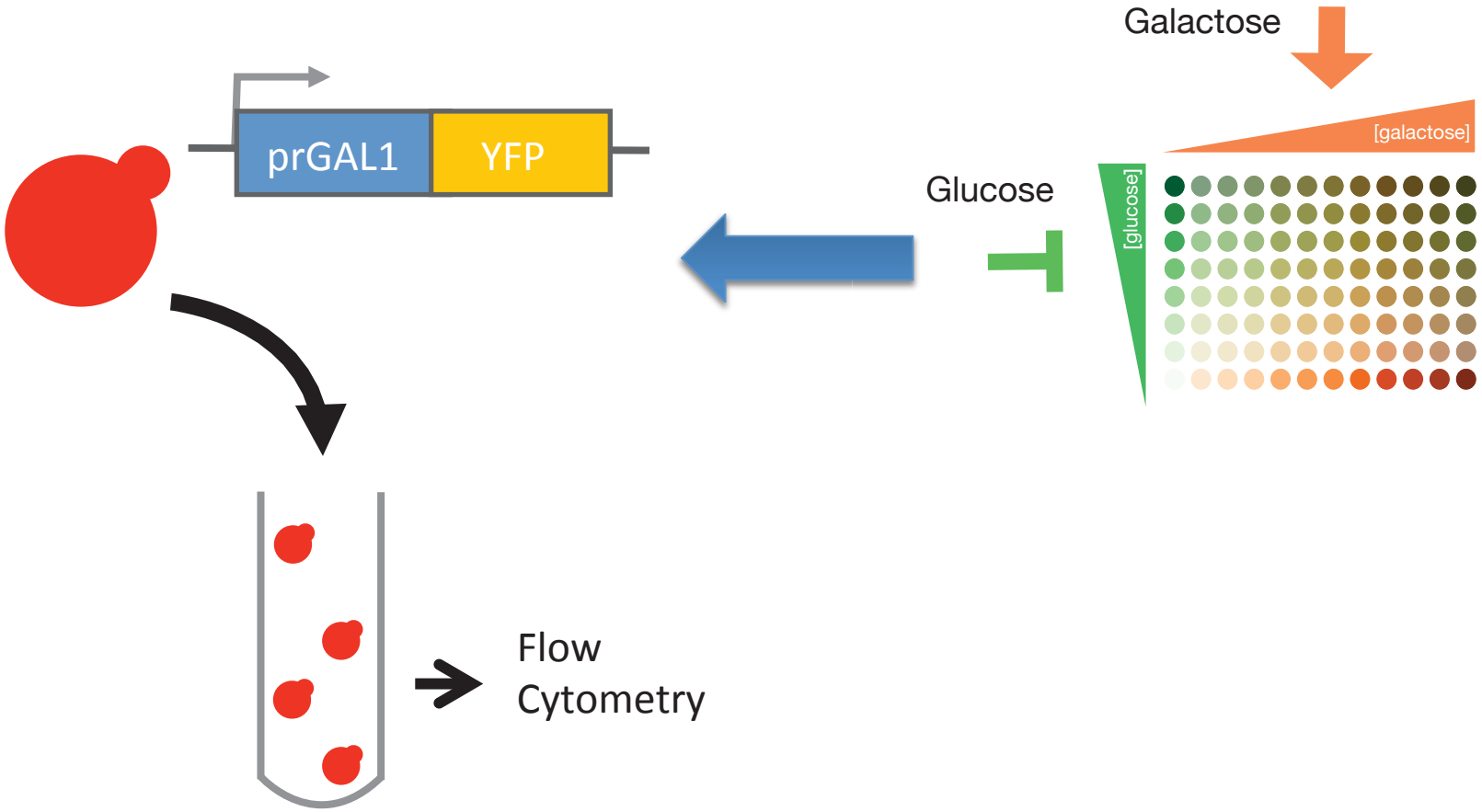
Esha Atolia

Bo Hua

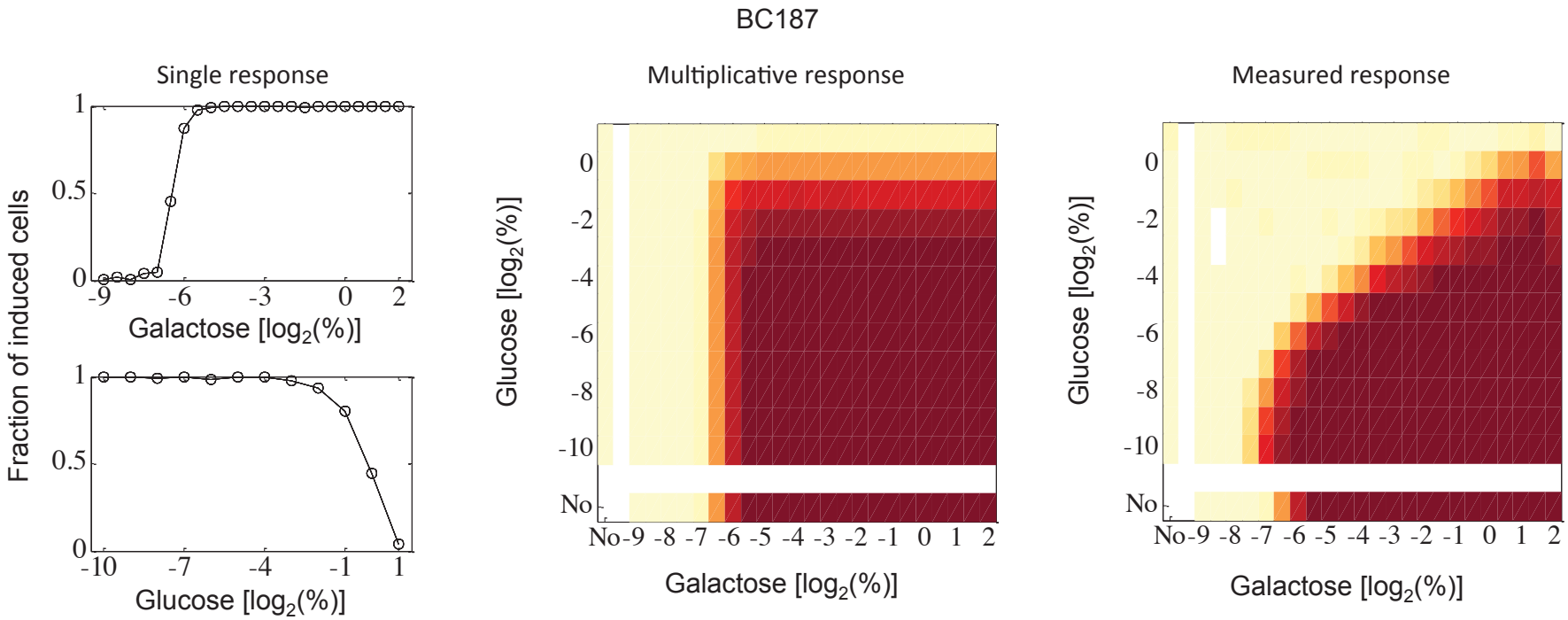
Yonatin Savir

Renan Escalante

How do cells respond to galactose in the presence of glucose?

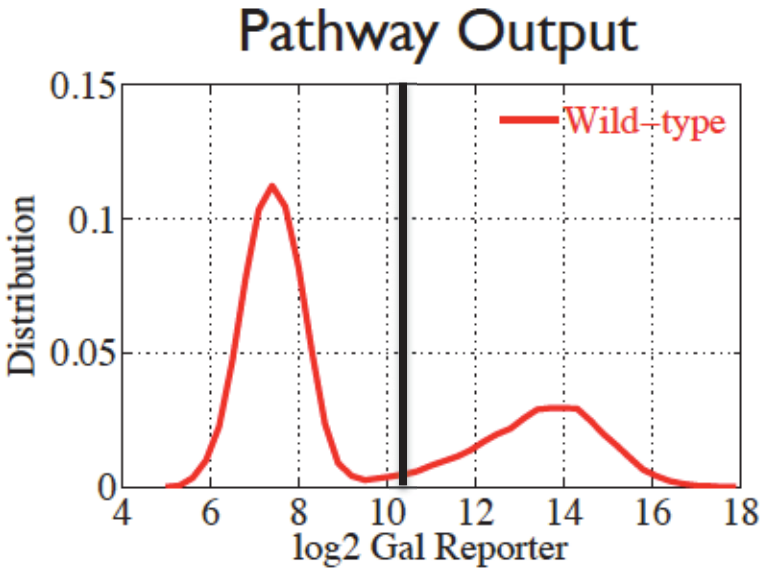


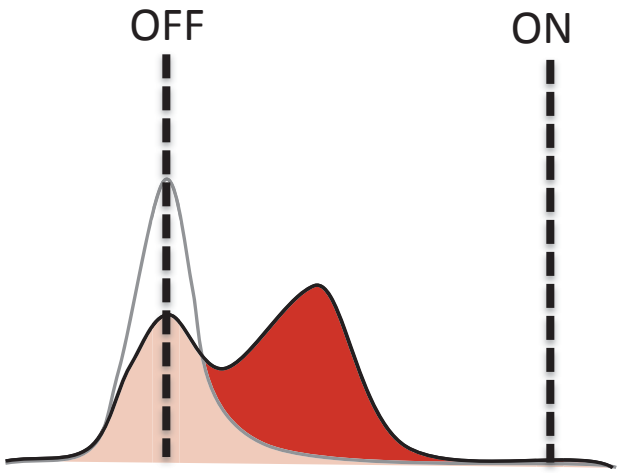
Response to glucose and galactose



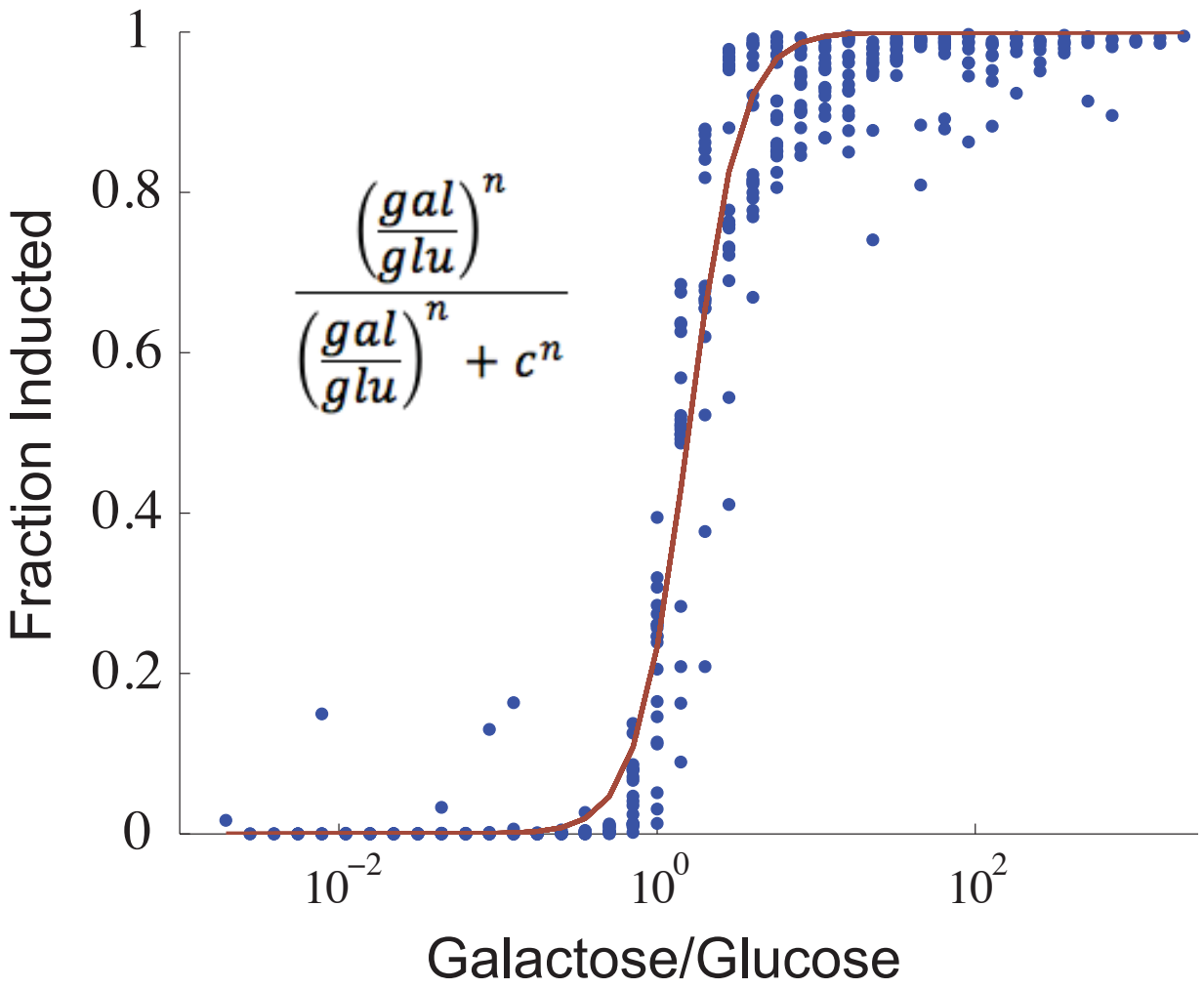
Decision versus output

Output = Decision

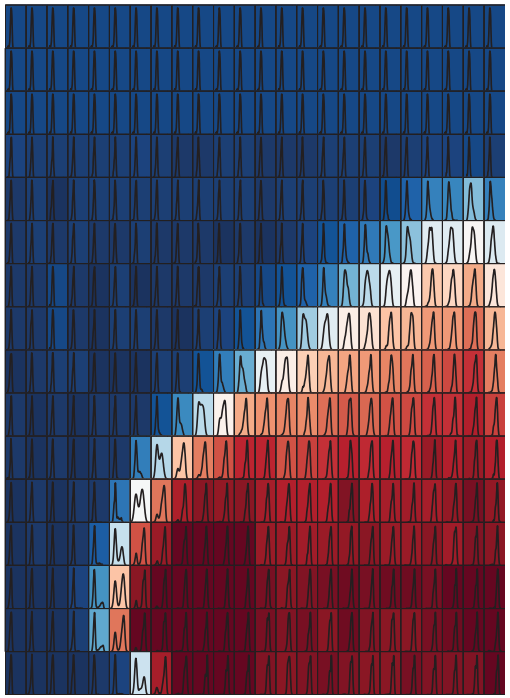




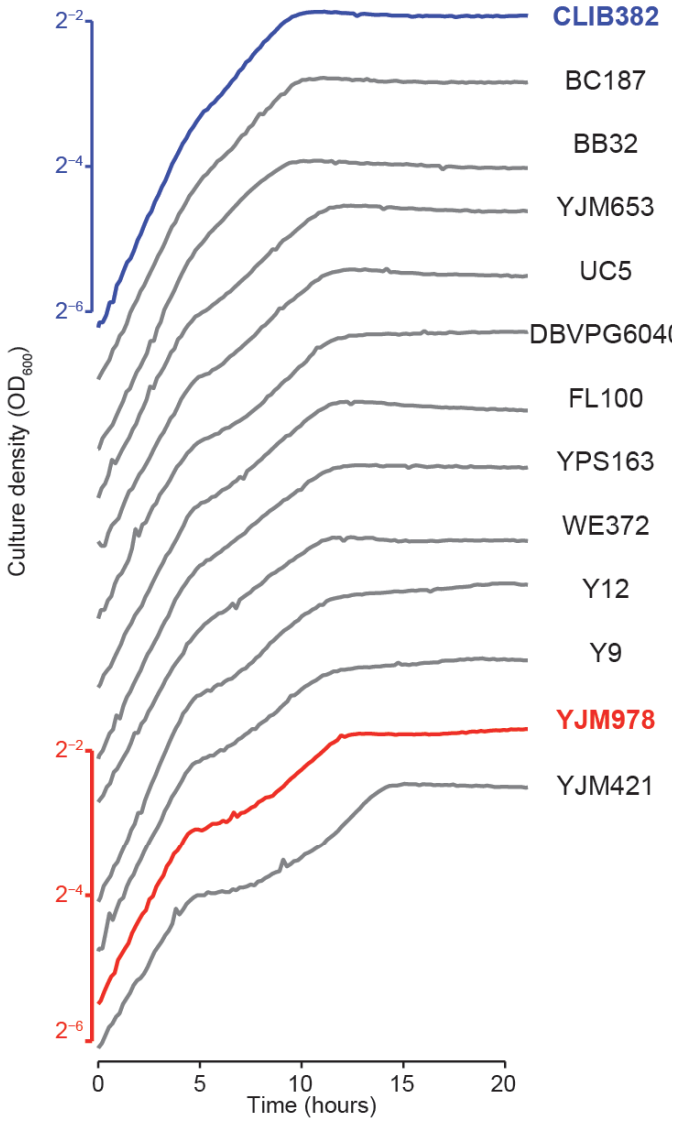
Response is one dimensional

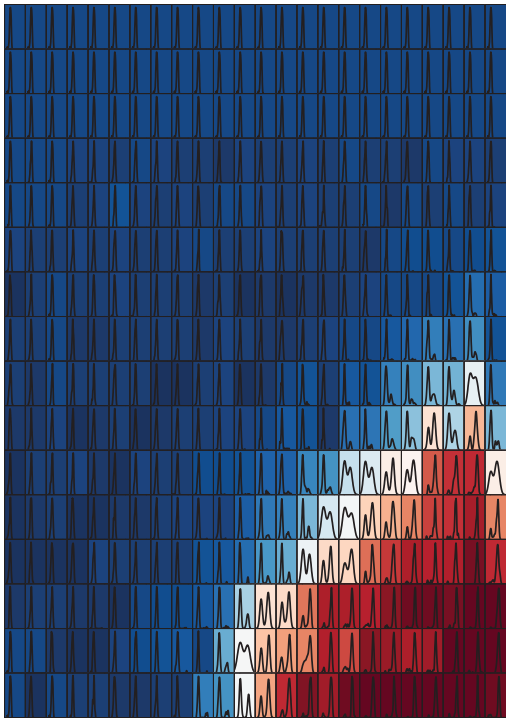


Diauxic lag duration varies across strains with similar growth rates in galactose



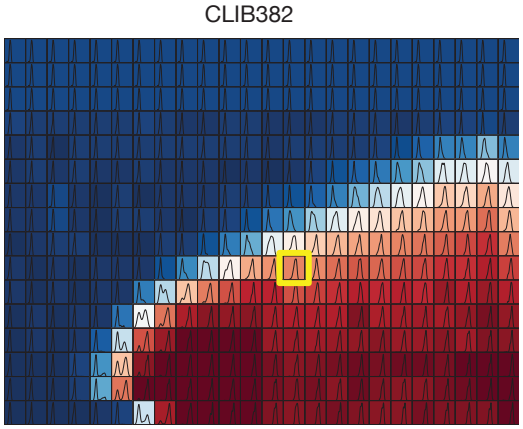
CLIB382

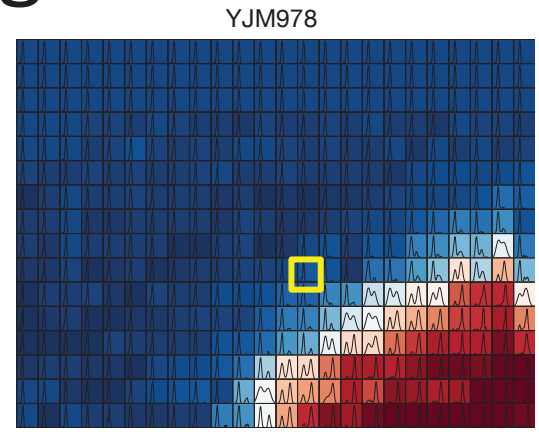


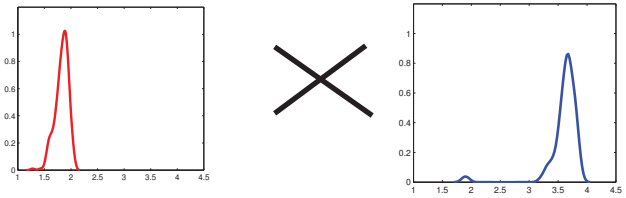


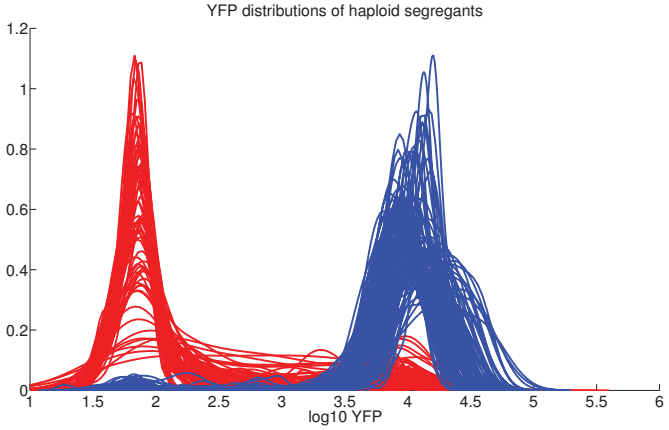
YJM978

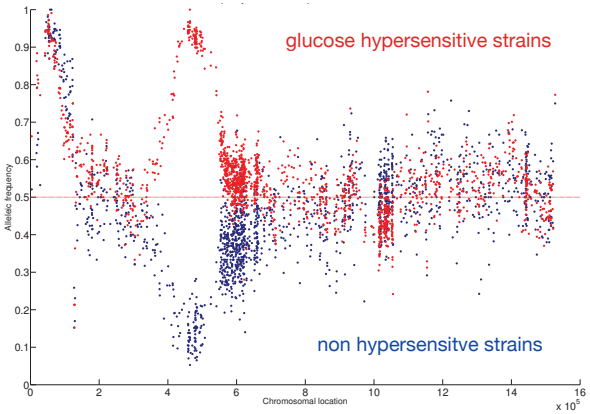
How many genes?

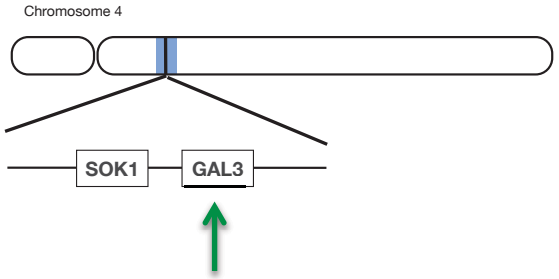




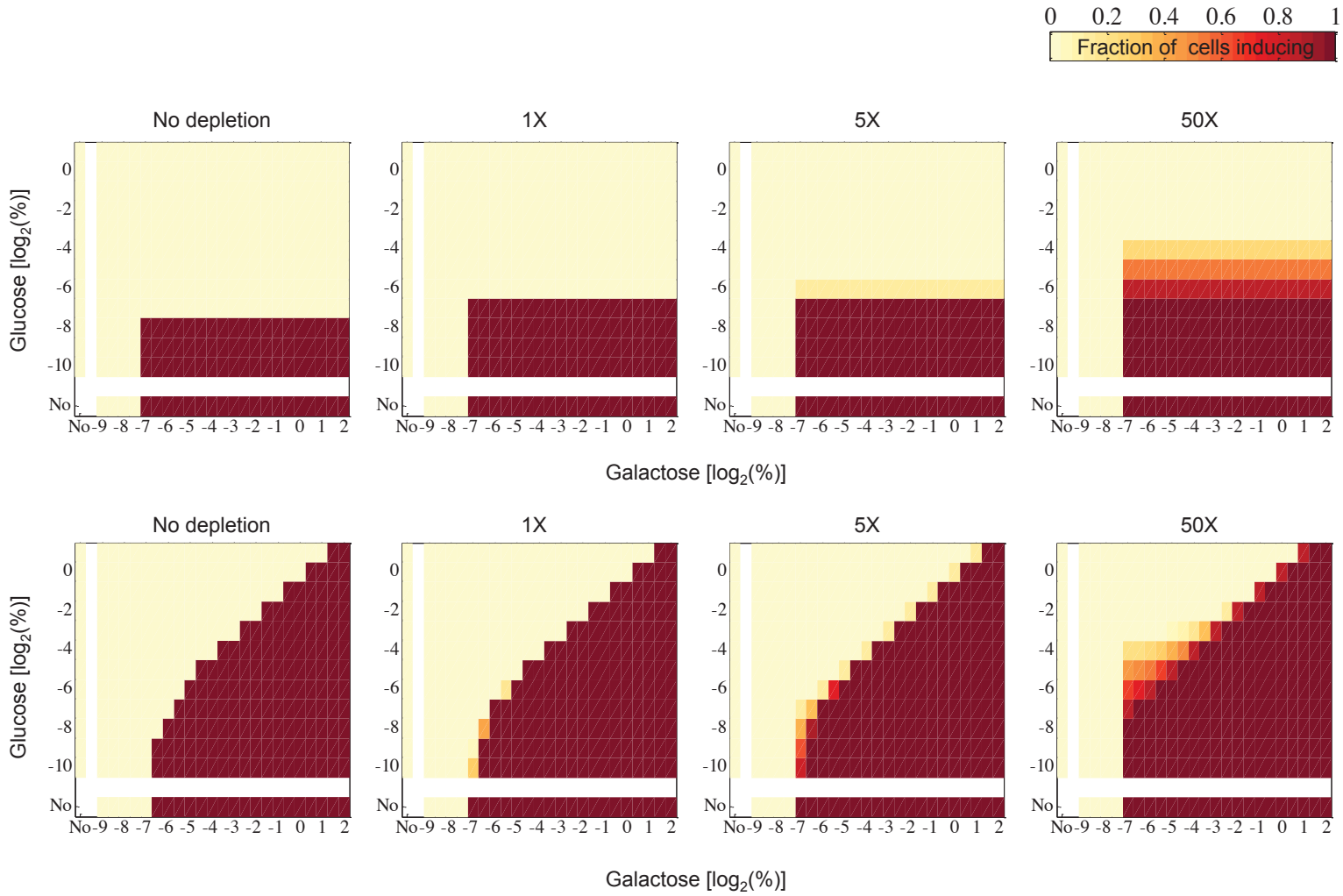




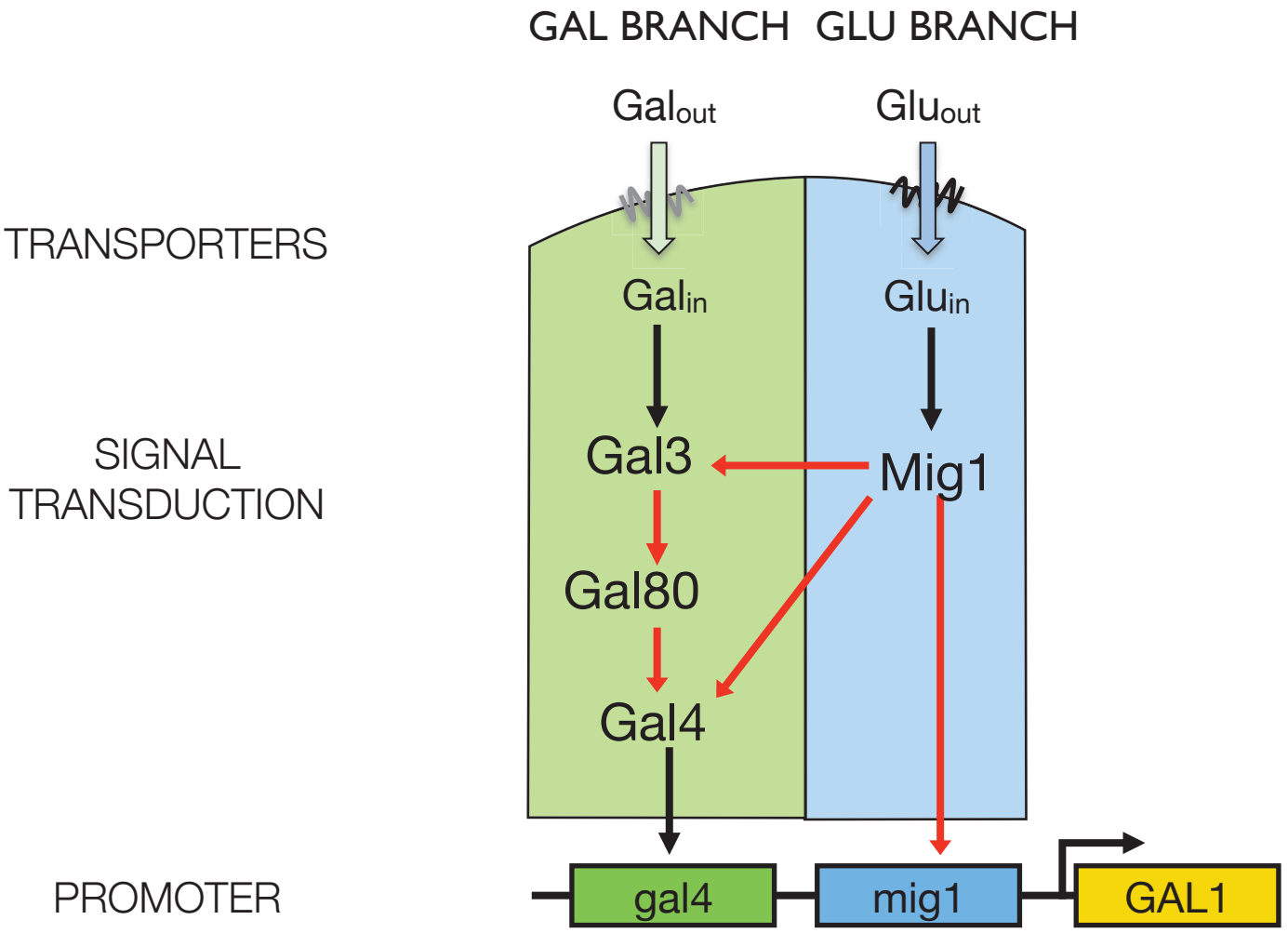




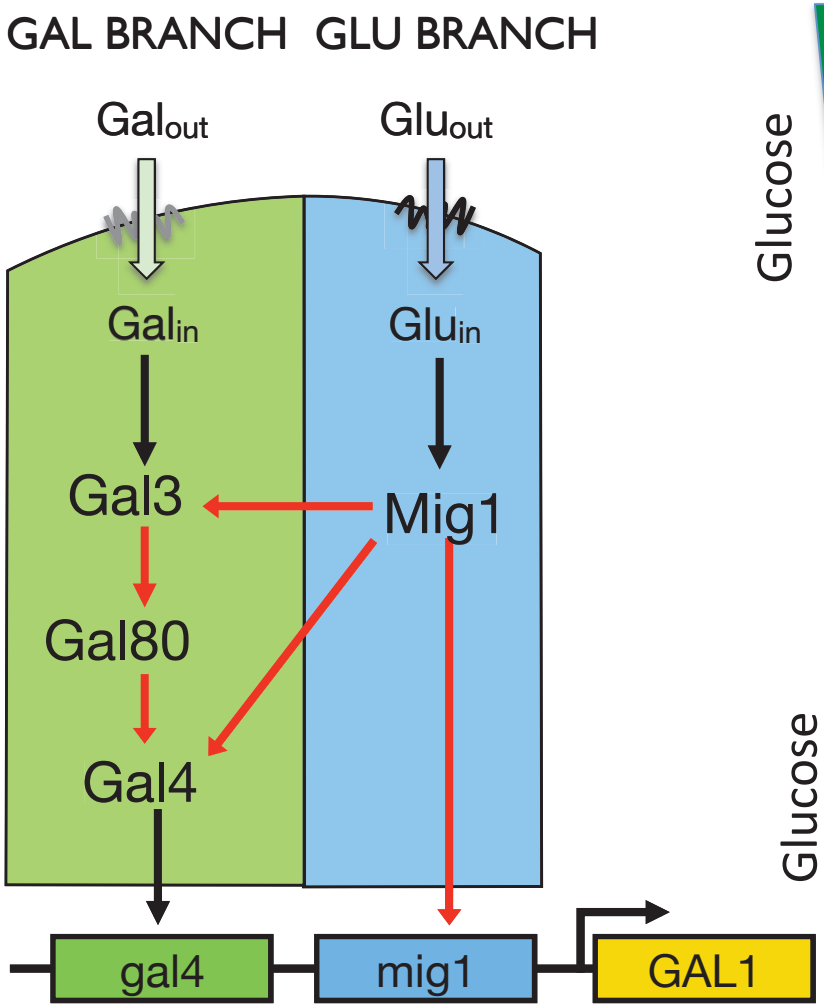
Ratio sensing is not an artifact of depletion

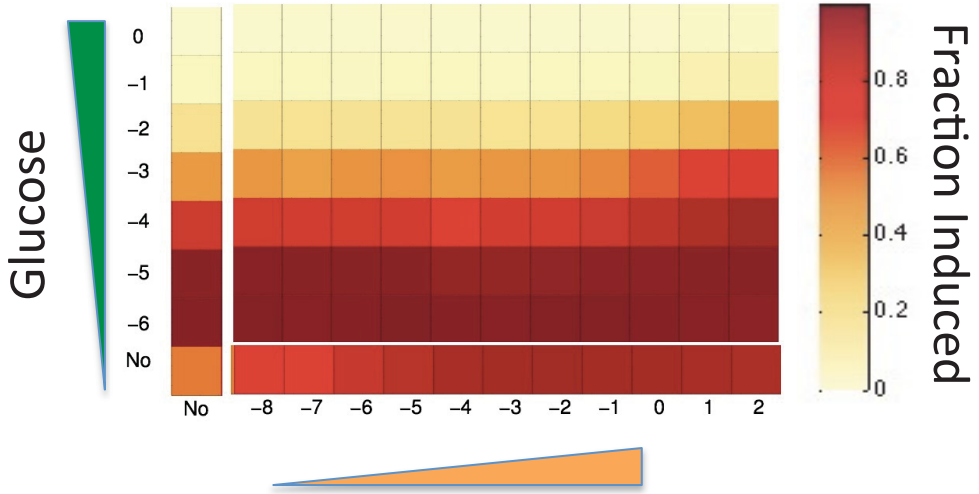


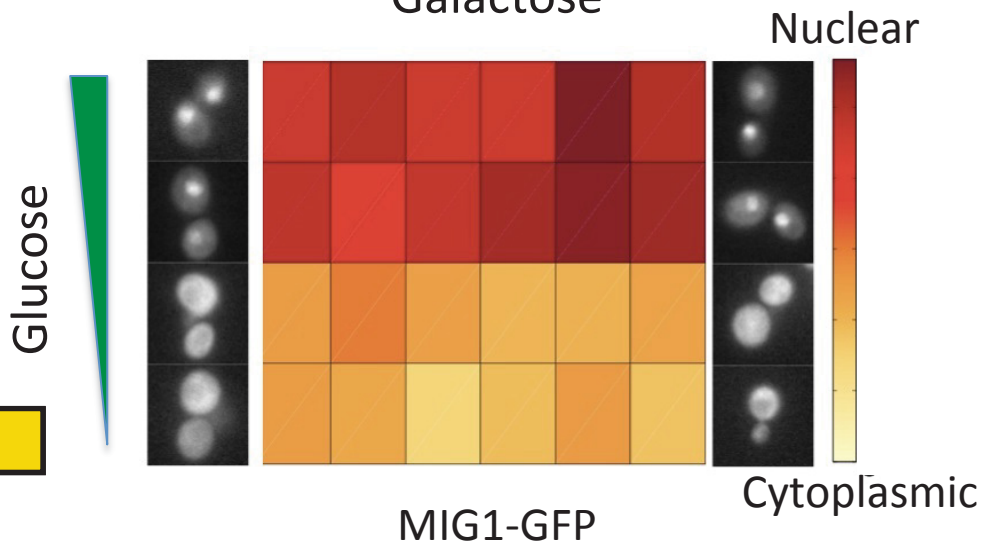
Where is glucose and galactose ratiod?



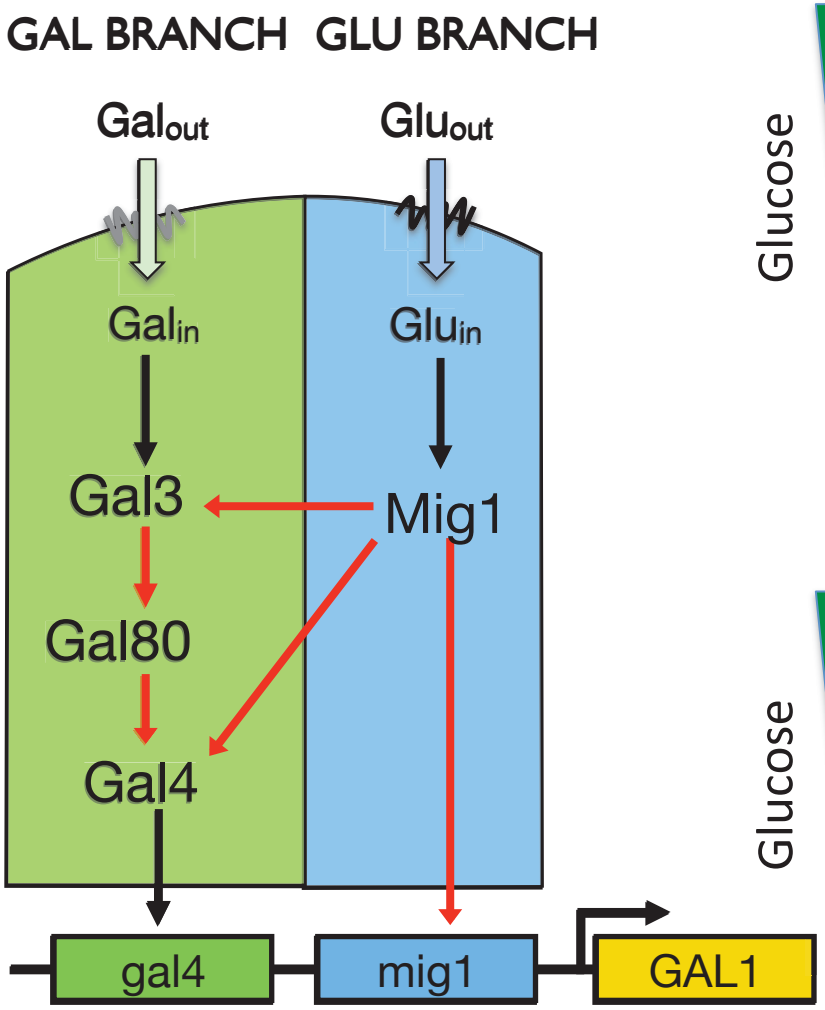
Is the glucose branch really galactose independent?

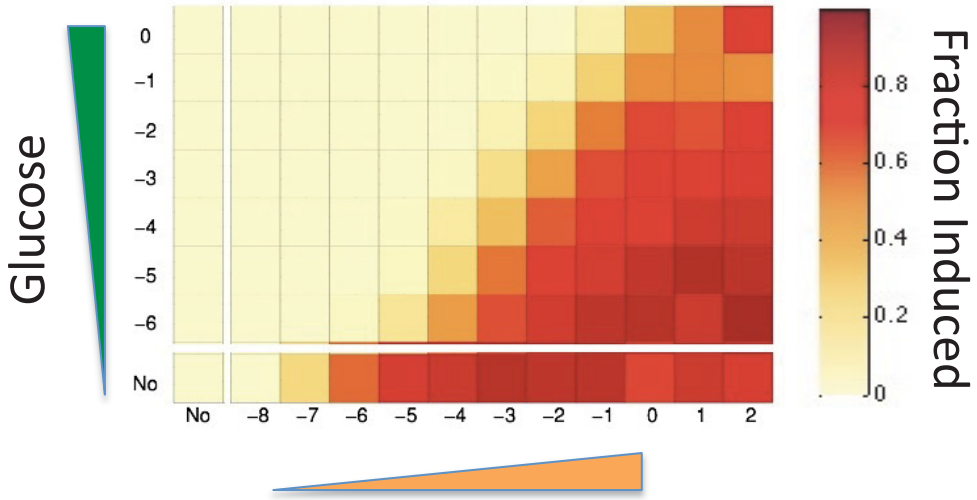


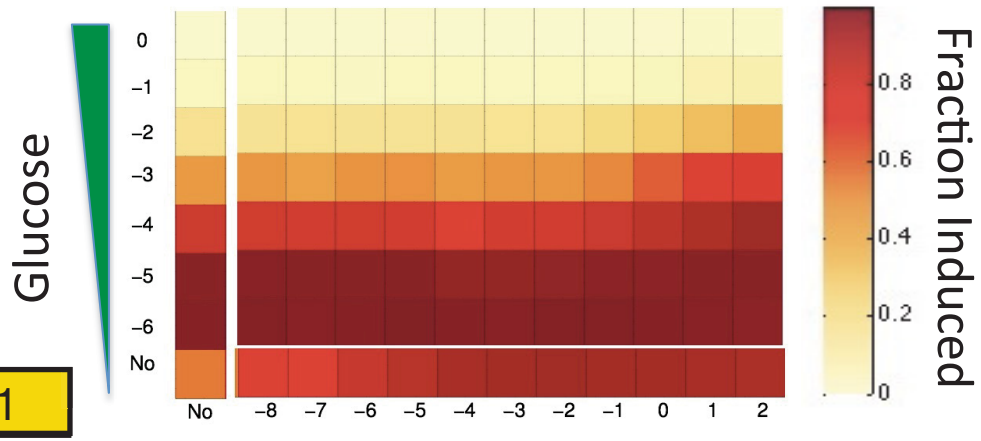




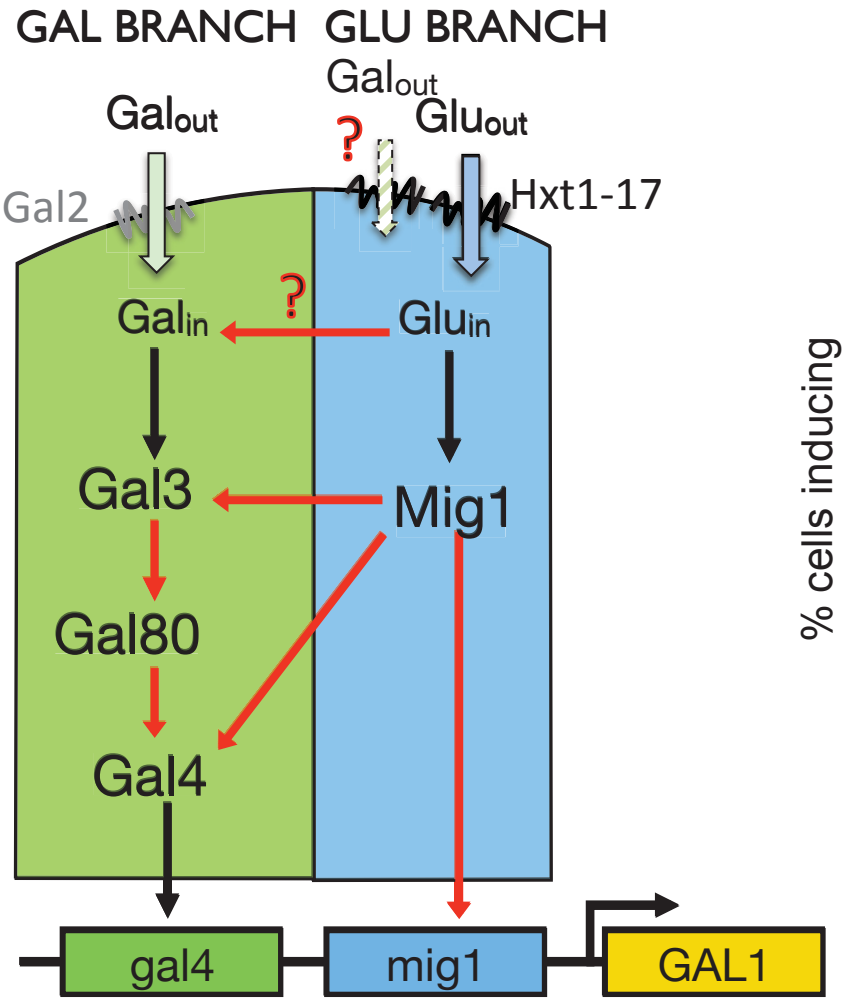
Is the ratio dependent of the ‘glucose branch’?

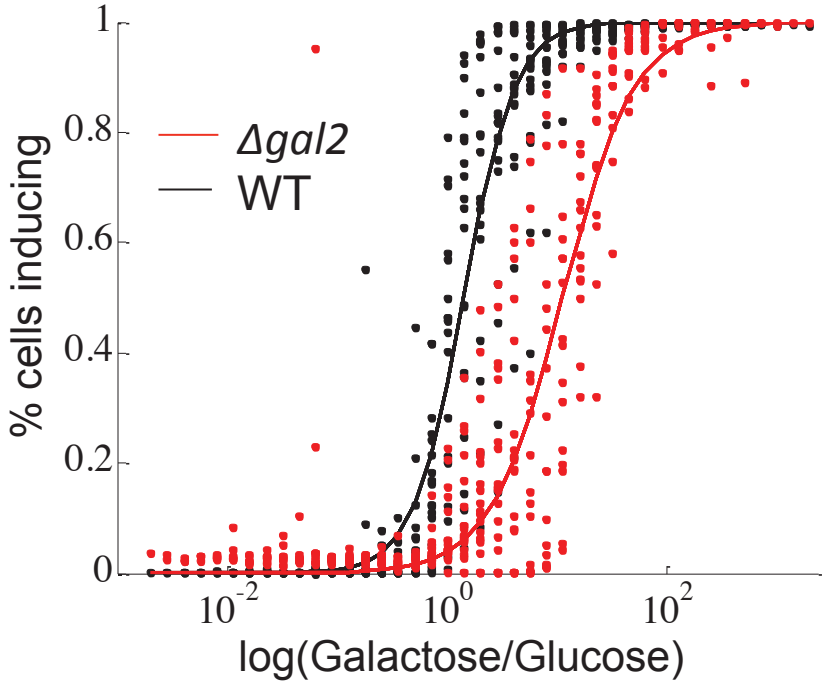




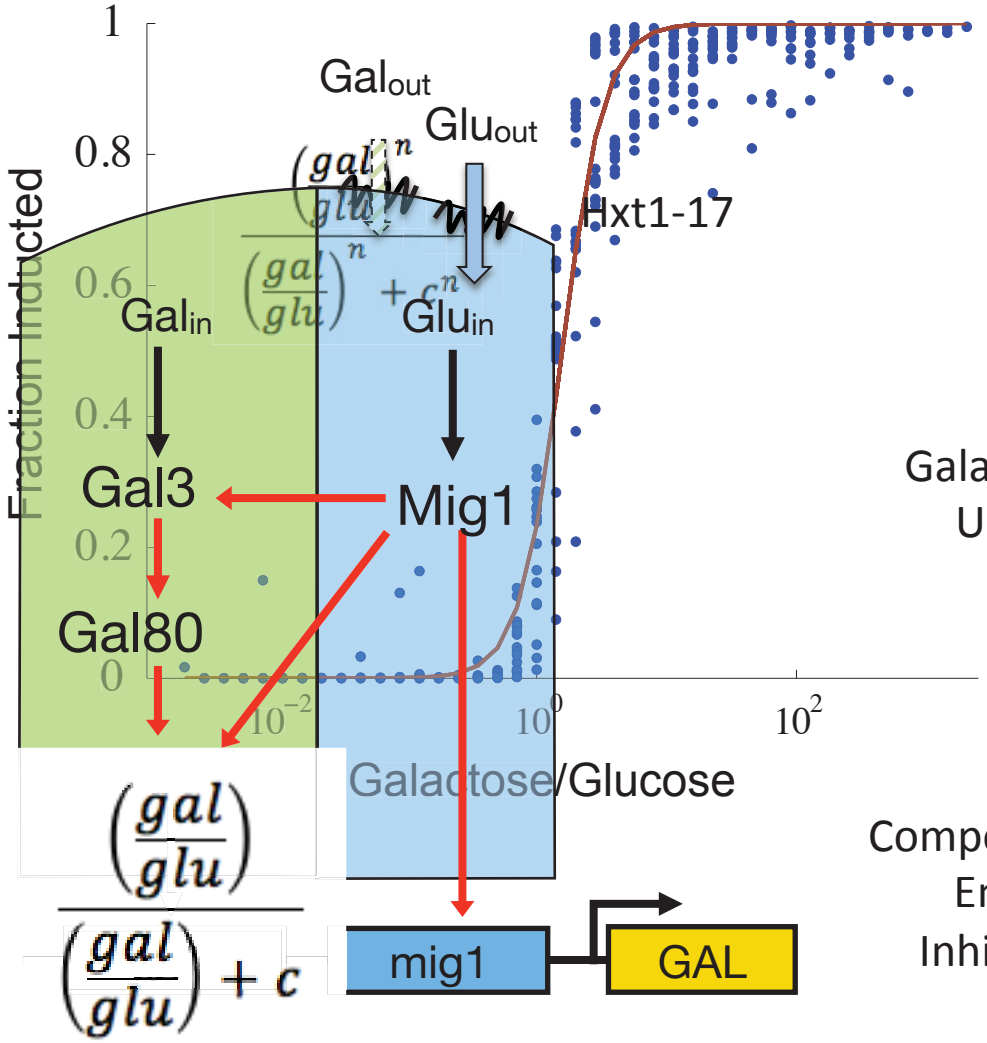


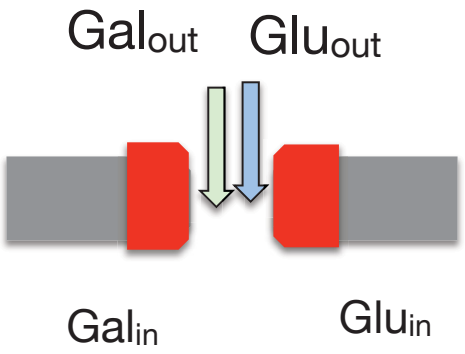
Is Gal2 needed for ratio sensing?





Competition through transport?





Galactose Uptake Rate

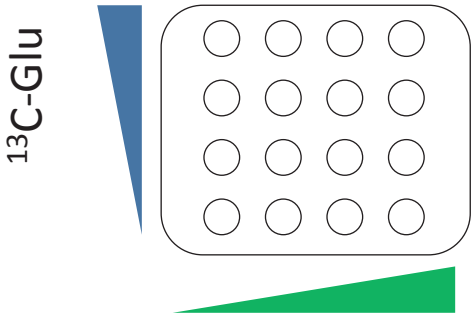
$$\frac{A_{gal}gal}{A_{gal}gal + A_{glu}glu + 1}$$

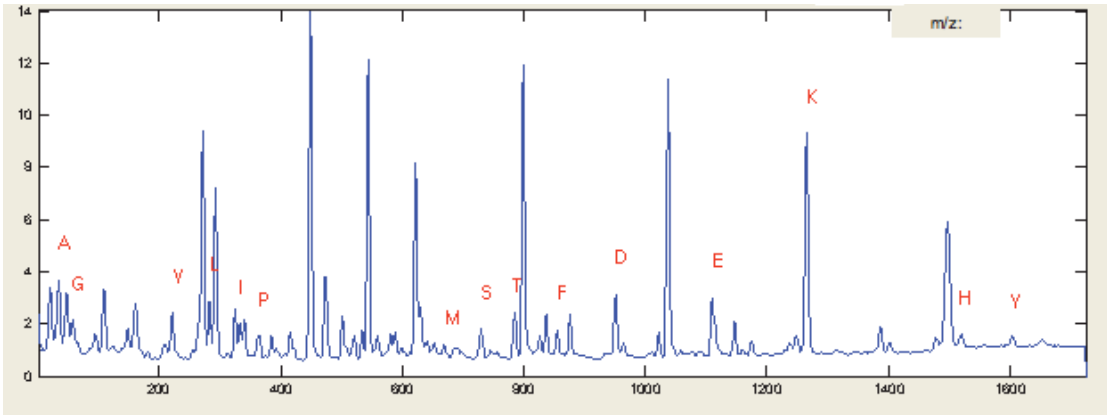
At saturation

$$\frac{A_{gal}gal}{A_{gal}gal + A_{glu}glu}$$

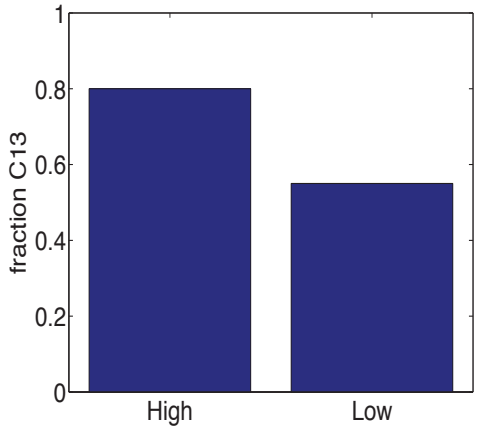
Competitive Enzyme Inhibition

Does gal uptake depend on the ratio of gal and glu?

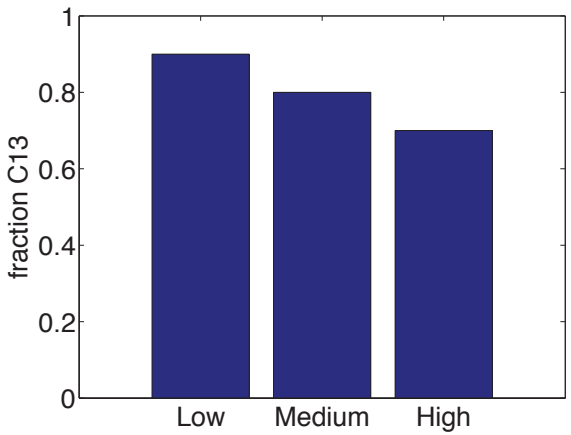




Measure C13/C12 Amino Acids by GC-MS

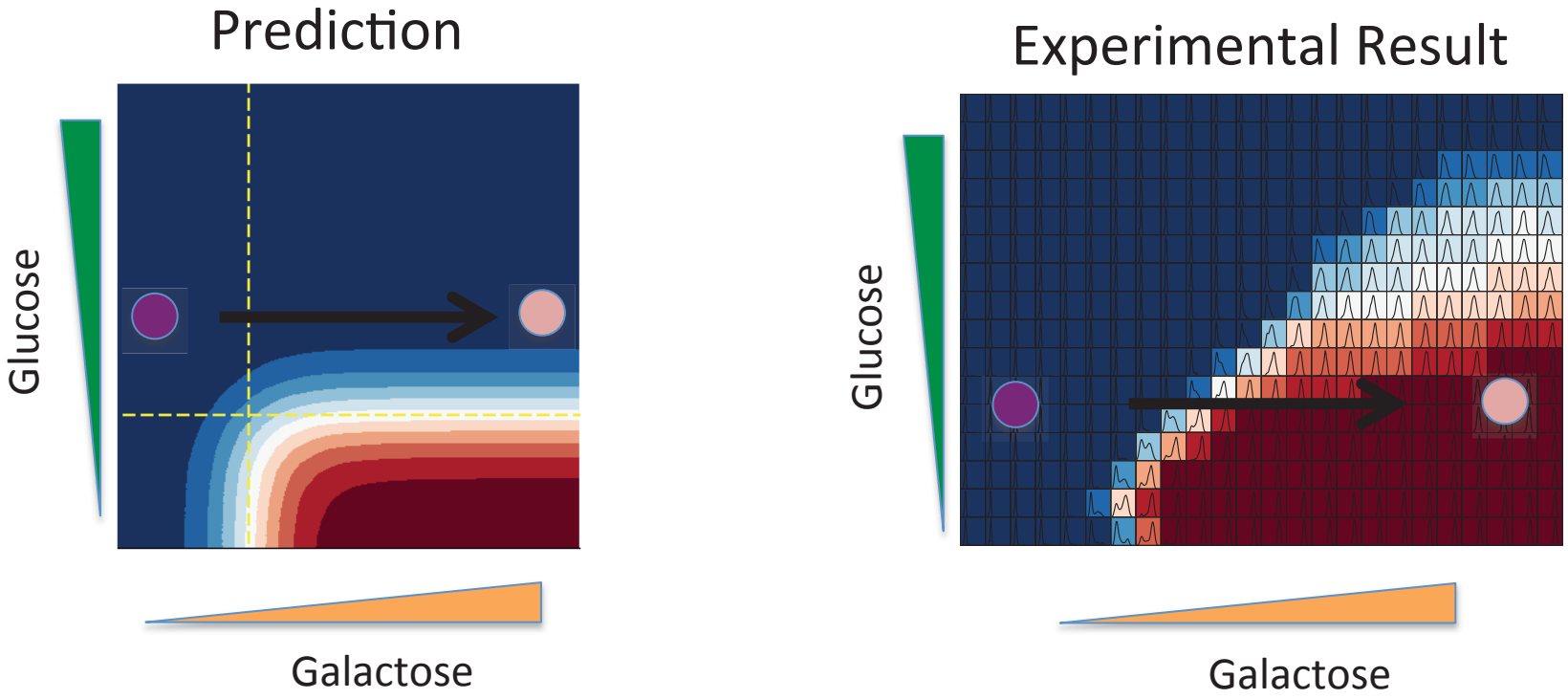


Glucose

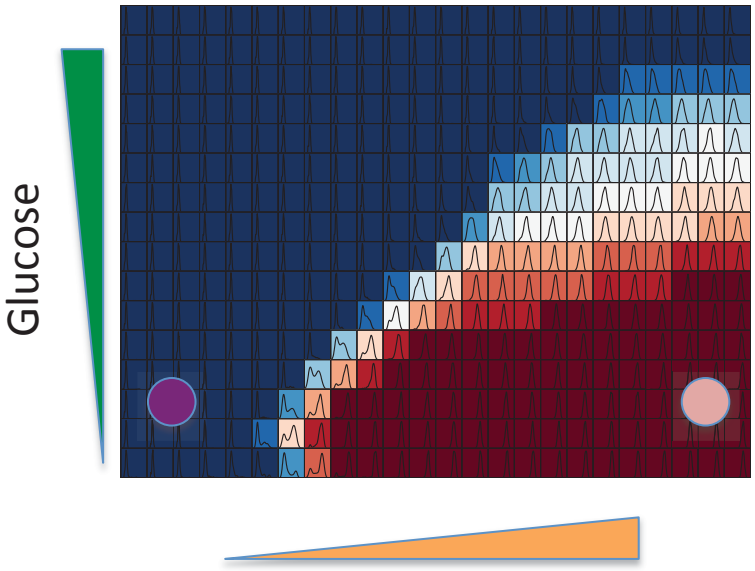


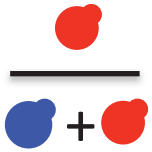
Galactose

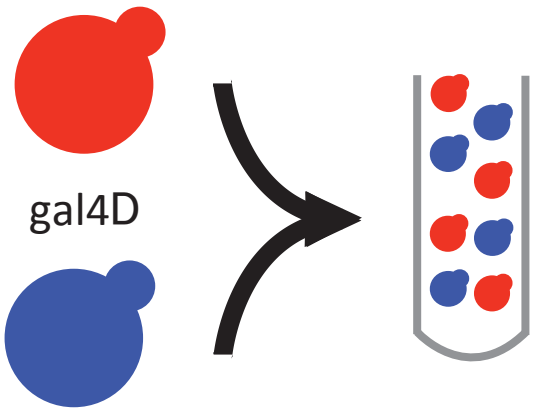
Is there an advantage to ratio sensing?

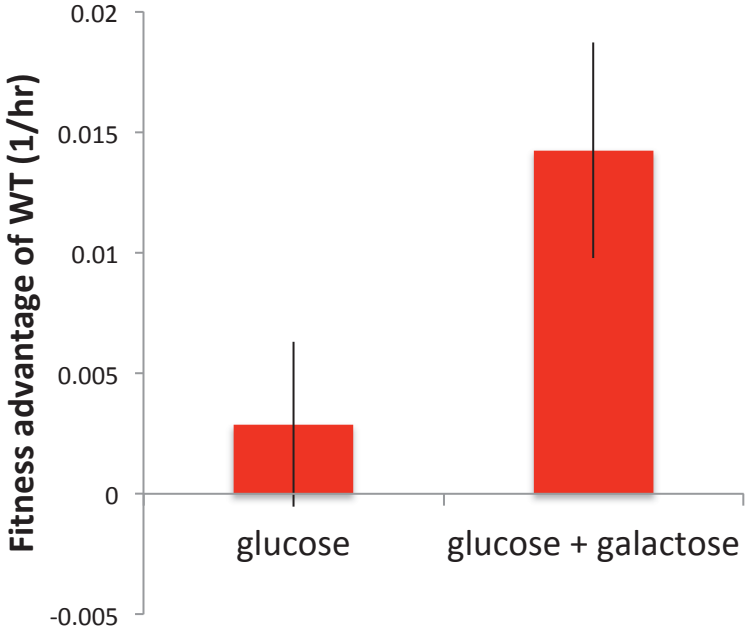


Can ratio sensing be advantageous?









Ratio sensors could be everywhere

Promoter binding $\frac{K_A A}{K_A A + K_R R + \partial K_R K_A A R + 1}$

Saturation $\partial \rightarrow \infty$ ↓

$$\frac{\left(\frac{gal}{glu}\right)}{\left(\frac{gal}{glu}\right) + c}$$

$$\frac{\left(\frac{K}{P}\right)}{\left(\frac{K}{P}\right) + \propto}$$

$$\frac{\left(\frac{A}{R}\right)}{\left(\frac{A}{R}\right) + \propto}$$

Gal Ratio Sensor

Competitive Transport

Reversible Modification

Kinase Phosphatase

HAT HDAC

Exclusion

Competitive Binding

Acknowledgements

Ratio Sensing

Renan Escalante

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Bo Hua

Yonatin Savir

Renan Escalante