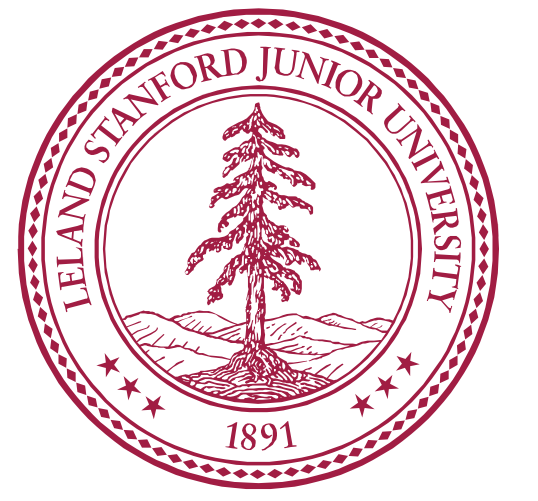


A single trait permits xylose utilization and transcription of the endogenous xylose pathway in natural *Saccharomyces* yeasts



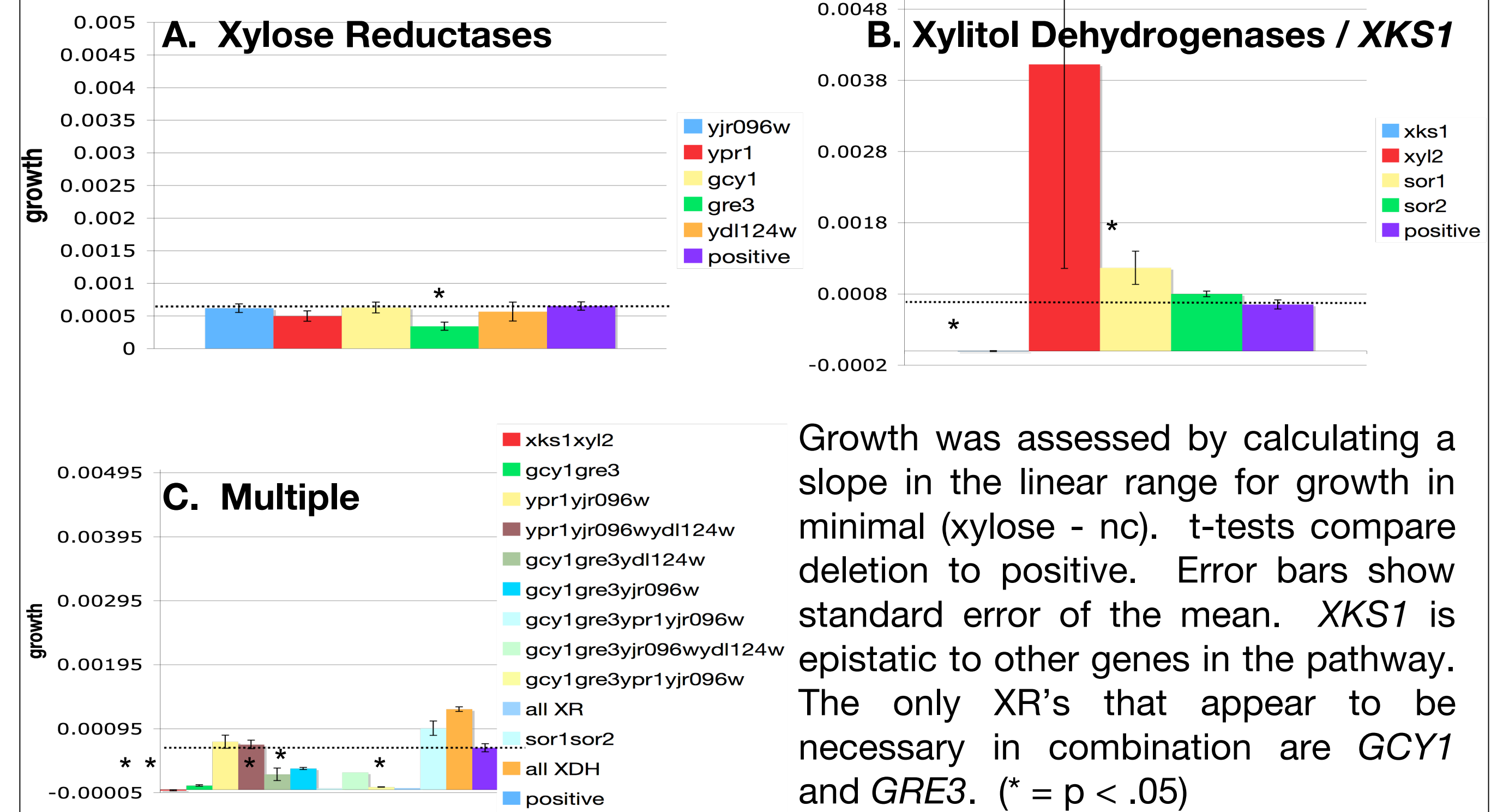
Jared Wenger, Katja Schwartz, and Gavin Sherlock
Department of Genetics, Stanford University



Abstract

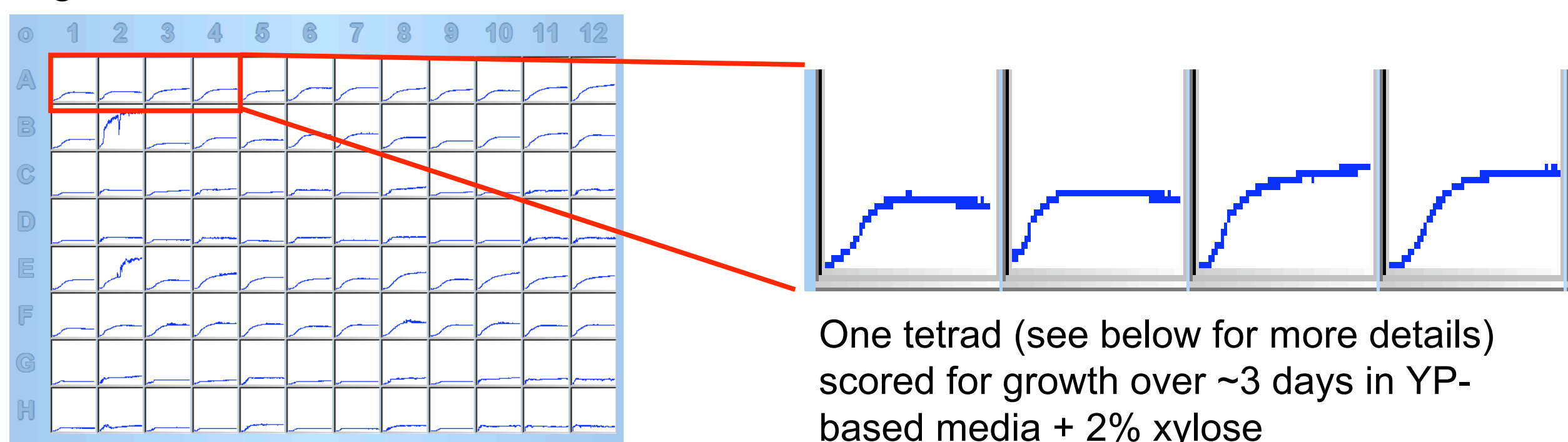
The production of renewable energy is of critical importance to the global economy and for the reduction of atmospheric accumulation of greenhouse gases. A key element of the renewable energy equation is fuel ethanol. Currently, fuel ethanol is produced by fermentation of hexoses found in sugar cane (Brazil), or corn (US), using *Saccharomyces cerevisiae*. However, current practices based on food production models do not maximize energy yield or green house gas benefits (because they use fossil fuels), have the potential to drive up food costs, and are not economically competitive with fossil fuels at today's energy prices. Production of ethanol from more abundant pentoses such as xylose, which is found in hemicellulosic biomass, would have a marked impact on the viability of ethanol as a renewable fuel source. To this end, we have identified naturally occurring xylose metabolic phenotypes in various *Saccharomyces sensu stricto* yeasts. In some of these strains, the xylose "positive" phenotype segregates as a single trait, the gene for which we have coarsely mapped, while in others it appears to be more complex. Gene expression microarray data show that these strains are capable of regulating components of the putative xylose pathway in response to xylose as the sole carbon source. Xylose negative strains do not show this transcriptional response. This suggests that these strains may be capable of using an endogenous, but normally non-functional, xylose metabolic pathway to shunt this sugar into the pentose phosphate pathway.

Individual deletions and combinations reveal the endogenous genes contributing to the functionality of the xylose pathway



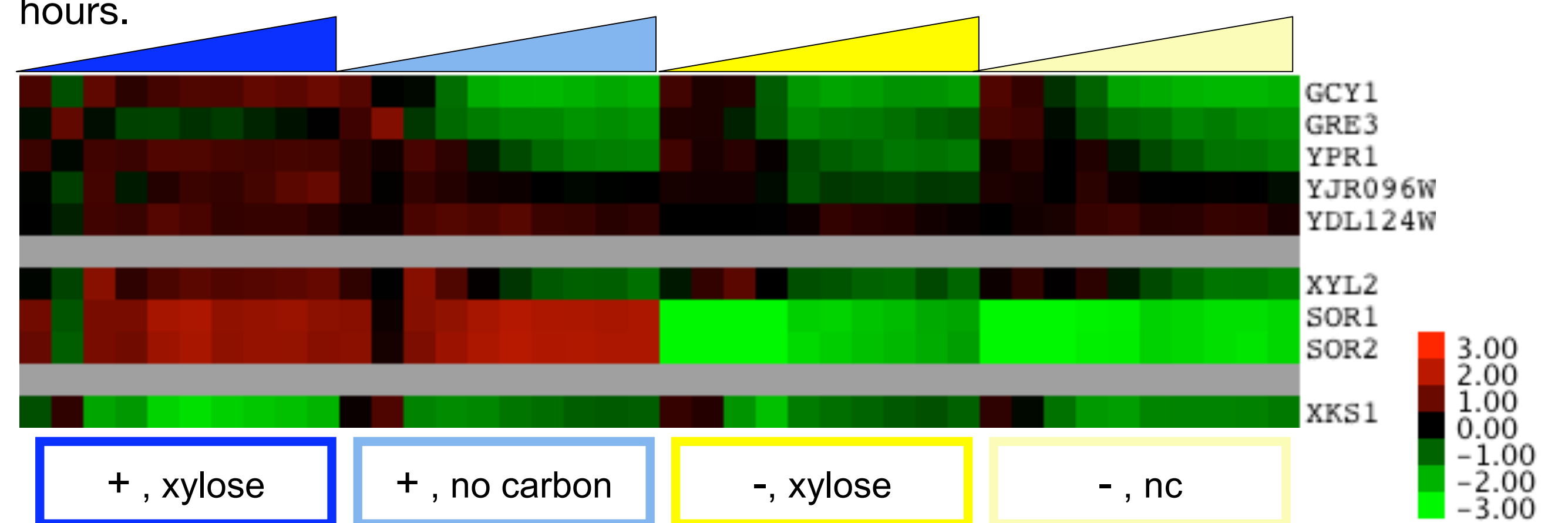
Identification of naturally occurring xylose metabolic phenotypes

The Sherlock Lab's strain collection, which includes many *Saccharomyces sensu stricto* yeasts from vineyards, breweries, and bakeries around the world, was screened for ability to grow on xylose as the sole carbon source. Our screen (done by Katja Schwartz) assays for growth over ~3 days (increase in optical density) in minimal or YP-based media with 2% xylose. We can screen a large number of strains at once using a TECAN 96-well plate reader. The phenotypes we have identified are very modest, but are reproducible both in the TECAN and in larger batch cultures.



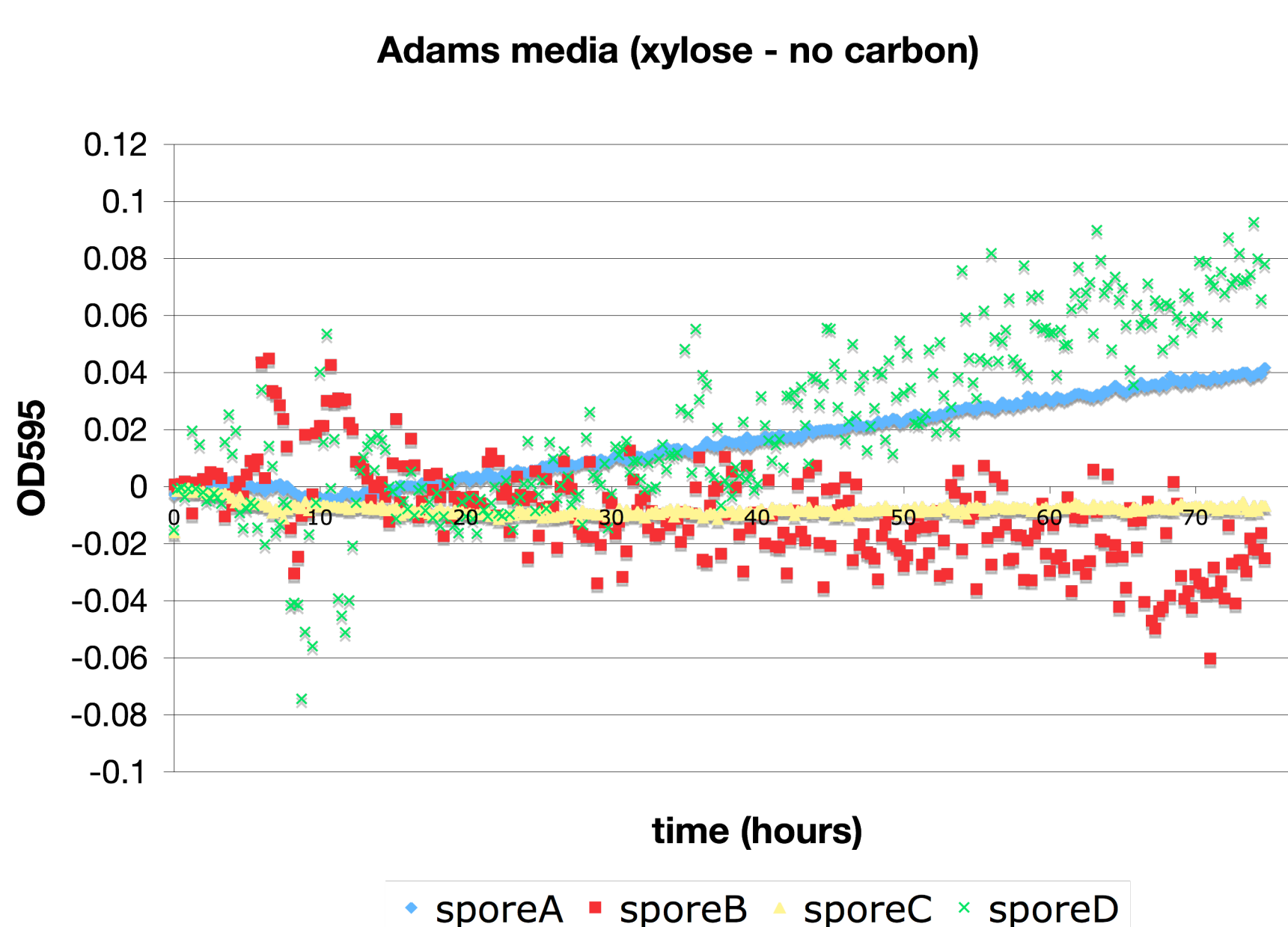
72 hour microarray time course to determine transcriptional response of "xylose positive" (and negative)

Positive and negative strains (in triplicate) were grown in minimal media with or without xylose for 3 days, and microarrays were done on samples taken every 8 hours.



A single locus is responsible for xylose utilization in a Simi Valley wine yeast

A Simi Valley wine strain was one of a few strains identified in a screen of over 400 *Saccharomyces* isolates that can utilize xylose. The phenotype segregates as a single genetic trait, but the locus responsible remains unidentified. The same locus appears to be responsible for xylose utilization in unrelated yeasts.

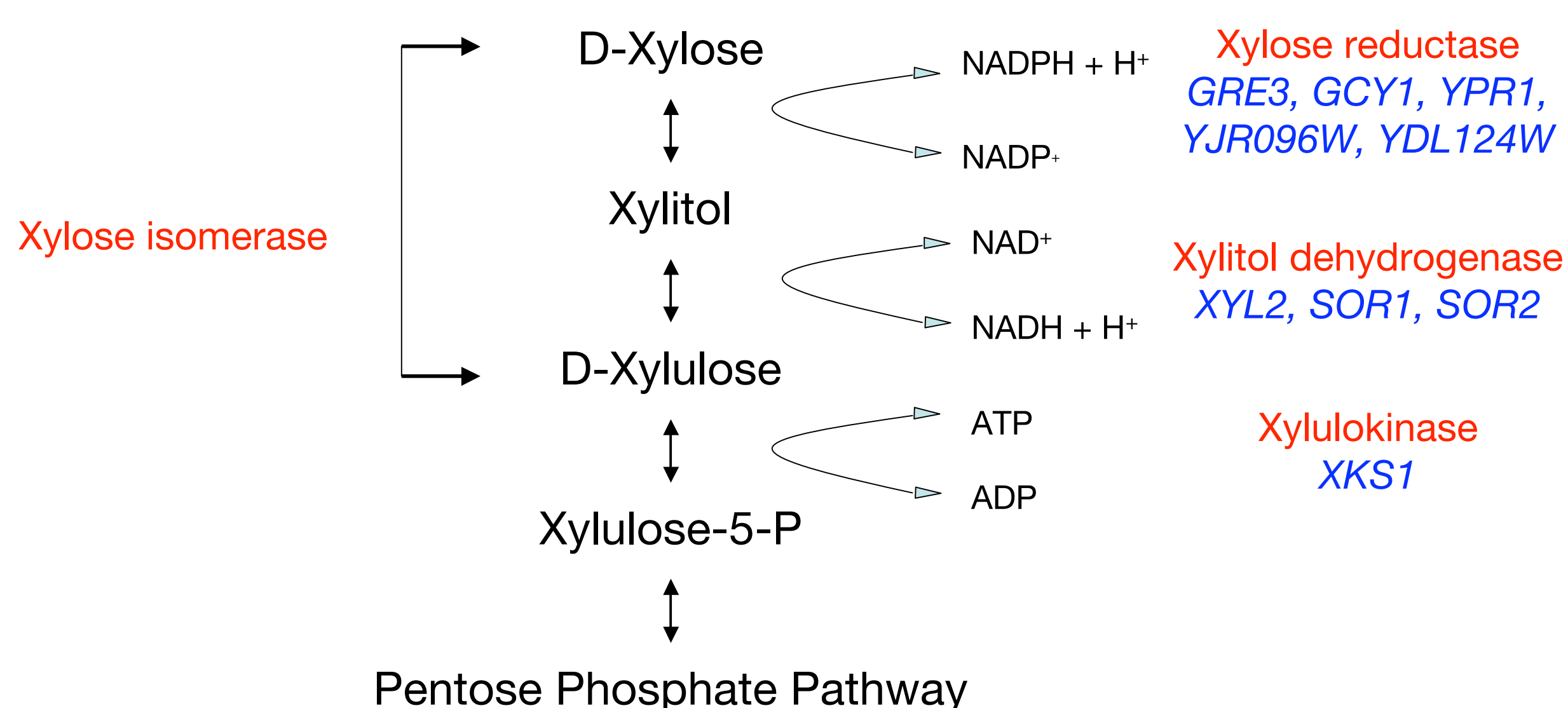


Carbohydrate transport and glycolysis identified as up regulated by iterative Group Analysis (iGA) in the Positive strain (in xylose)

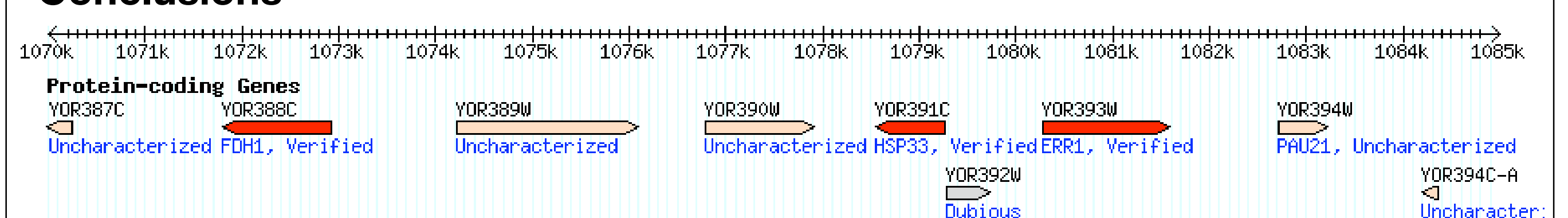
	Positive (xylose timecourse)	Positive (xylose vs. nc)
Up regulated groups	carbohydrate transport (<i>HXT10, 14-17</i>)	Glycolysis (G-3-P dehydrogenase activity)
	biotin biosynthesis	small ribosomal subunit
	pyruvate hydratase (<i>ERR1-3</i>)	
Down regulated groups	cell wall	mRNA processing
	cytochrome-c oxidase activity	spliceosome

iGA uses a ranked list of each feature on the array to determine which groups are significantly represented in the list. Significance analysis was used to give each gene a "slope" across the timecourse, or simply a score in a 2 class comparison of xylose vs. no carbon. (iGA - *BMC Bioinformatics*. 2004; 5: 34. SAM - *PNAS*. 2001; 98: 5116-5121)

Canonical xylose pathway and putative *Saccharomyces* enzymes



Conclusions



We have identified naturally occurring yeast strains that are capable of utilizing their endogenous xylose metabolic pathway to grow slowly in the presence of xylose as the sole carbon source. The trait which is responsible for this phenotype is, currently, not known, but we have mapped it to the right arm of chromosome 15 and we are working to clone it.

I am using genetic and genomic approaches to understand how a yeast strain, in the presence of this trait, is otherwise capable of utilizing xylose.

Funding: Stanford GCEP (Global Climate and Energy Project), NIH Training Grant