Changes in gene expression in response to cold temperature in Saccharomyces cerevisiae grown in dextrose using microarrays

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Introduction

Saccharomyces cerevisiae is a model organism for eukaryotes. It is a favorable organism to study cellular responses to stress because it is unicellular and its genome has been sequenced. Microarrays are an excellent tool for identifying changes in gene expression in response to stress. Each yeast microarray contains 6,400 genes of distinct DNA sequences made with a simple robotic printing device (DeRisi, 1997). Previous studies using microarrays have examined the response of S. cerevisiae to cold shock at 4°C (Murata and other, 2005). In this research, we aimed to determine how gene expression changes in cells exposed to 10°C and to determine if there were differences in response to cold temperature between fermenting and respiring cells. S. cerevisiae cells were grown in dextrose, a media in which they ferment at 28°C. Control cells were collected and flash frozen in liquid nitrogen. Cold-treated cells were transferred into a 10°C shaking water bath and cells were harvested at 30 min., 2hr, and 8 hr. Gene expression was analyzed using Magic Tool software and compared to changes in gene expression in respiring cells grown in galactose. We hypothesized that S. cerevisiae grown in galactose at cold temperature would have higher levels of oxidative stress compared to cells grown in dextrose because oxygen is used in the electron transport chain during aerobic respiration. We concluded that yeast grown in dextrose and galactose have similar changes in gene expression in response to cold temperature.

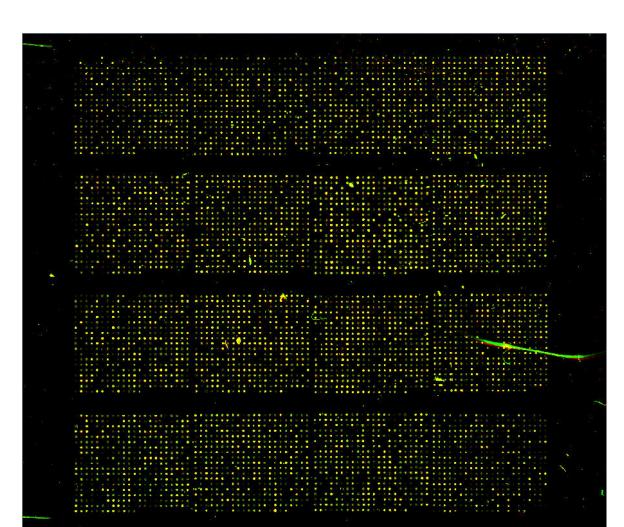
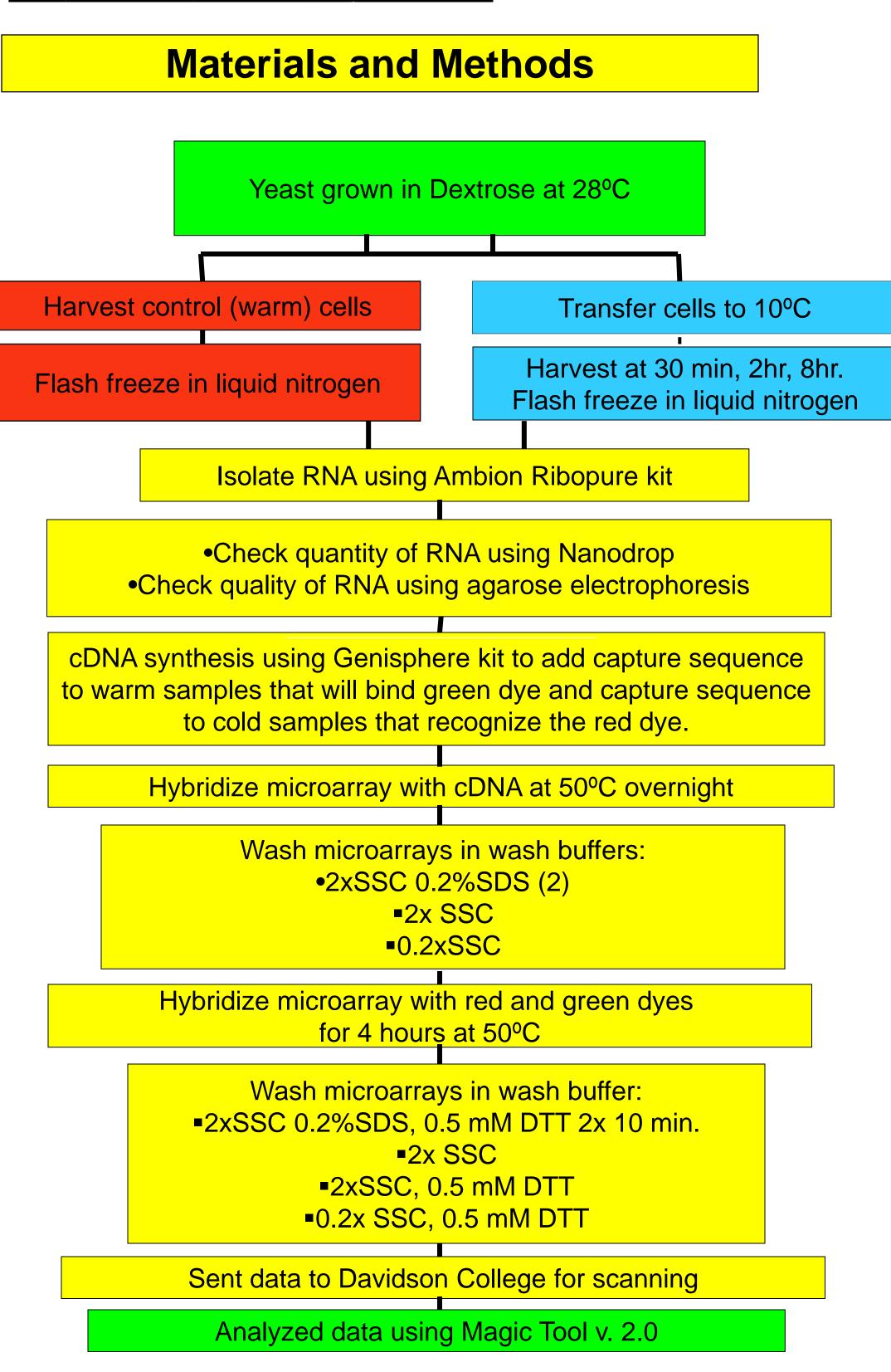
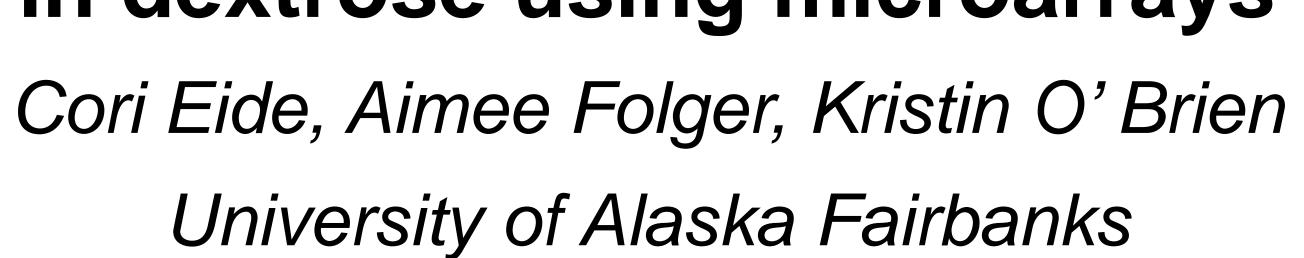


Fig. 1. Example of a microarray. Each spot represents a gene. **RED** indicates the gene has been induced. **GREEN** indicates it is repressed, and YELLOW indicates no change in expression in response to cold temperature exposure.

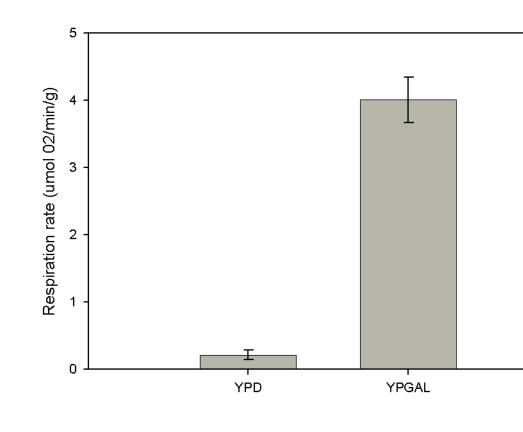






Results and Discussion

Respiration Rates in YPD & YPGAL



28 s

18s

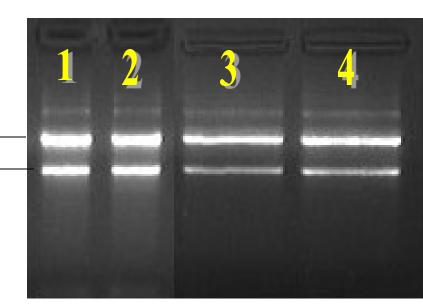


Fig. 2.Respiration rates of yeast cells grown in YPD (dextrose) & YPGAL (galactose). Respiration rates of cells grown in YPGAL were higher than cells grown in YPD. •Cells grown in YPGAL obtain energy through cellular respiration. Cells grown in YPD obtain energy through fermentation

Fig. 3. Agarose Gel 1. warm YPD 28°C 2. 30 min. YPD 10°C 3. 2hr YPD 10°C

4. 8hr YPD 10°C

• Quality check of RNA using agarose gel electrophoresis.

Table 1: Expression change of yeast genes at each time point in 10° C in YPD

A. Genes that are induced in response to cold temperature \geq 3-fold

Biological Process	Total # of genes	30 min	%	2hr	%	8hr	%
Biogenesis	229	22	10%	25	11%	0	0%
Cell Cycle	50	5	10%	5	10%	0	0%
Chaperone	84	9	11%	6	7%	0	0%
DNA Repair	53	2	4%	3	6%	0	0%
DNA Replication	42	4	10%	3	7%	0	0%
Folding	41	1	2%	1	2%	0	0%
Heat Shock	24	2	8%	3	13%	0	0%
Metabolism	206	16	8%	12	6%	0	0%
Modification	37	5	14%	7	19%	0	0%
Oxidative Phosphorylation	3	1	33%	1	33%	0	0%
Oxidative Stress	28	5	18%	3	11%	0	0%
Protein	770	70	9%	58	8%	0	0%
Respiration	48	3	6%	4	8%	0	0%
Signal	56	4	7%	2	4%	0	0%
Stress	142	17	12%	15	11%	0	0%
Transcription	289	27	9%	25	9%	0	0%
Translation	65	6	9%	4	6%	0	0%
Transport	396	39	10%	30	8%	0	0%
Unknown	2258	193	9%	181	8%	0	0%

•Genes involved in protein processes, metabolism, and stress were found to be significantly up regulated

B. Genes that are repressed \geq 3-fold in response to cold temperature

Biological Process	Total number of genes	30 min.	%	2 hr.	%	8 hr.	%
Biogenesis	229	3	1%	3	1%	0	0%
Cell Cycle	50	1	2%	0	0%	0	0%
Chaperone	84	3	4%	1	1%	0	0%
DNA Repair	53	1	2%	0	0%	0	0%
DNA Replication	42	3	7%	1	2%	0	0%
Folding	41	2	5%	0	0%	0	0%
Heat Shock	24	1	4%	1	4%	0	0%
Metabolism	206	4	2%	5	2%	0	0%
Modification	37	2	5%	0	0%	0	0%
Oxidative Phosphorylation	3	0	0%	0	0%	0	0%
Oxidative Stress	28	4	14%	0	0%	0	0%
Protein	770	19	2%	19	2%	0	0%
Respiration	48	2	4%	0	0%	0	0%
Signal	56	0	0%	0	0%	0	0%
Stress	142	7	5%	2	1%	0	0%
Transcription	289	11	4%	3	1%	0	0%
Translation	65	4	6%	0	0%	0	0%
Transport	396	4	1%	2	1%	0	0%
Unknown	2258	53	2%	37	2%	2	0%

•Fewer genes were repressed than induced during cold acclimation

Table 2: Metabolic genes that are induced in response to cold temperature \geq 3-fold

Gene Name	Alias	Biological Process	Molecular Function
30 Minutes			
YAL044C	GCV3	one-carbon compound metabolism	glycine dehydrogenase (decarboxylating) activity
YPL273W	SAM4	sulfur amino acid metabolism	homocysteine S-methyltransferase activity
YOL011W	PLB3	phosphoinositide metabolism*	lysophospholipase activity
YER091C_rep2	MET6	methionine metabolism	5-methyltetrahydropteroyltriglutamate- homocysteine S-methyltransferase activity
YPL038W	MET31	sulfur amino acid metabolism	specific RNA polymerase II transcription factor activity
YDR204W	COQ4	ubiquinone metabolism	molecular_function unknown
YJR139C	HOM6	methionine metabolism*	homoserine dehydrogenase activity
YER024W	YAT2	alcohol metabolism*	carnitine O-acetyltransferase activity
YJR010W	MET3	methionine metabolism*	sulfate adenylyltransferase (ATP) activity
YDR287W		myo-inositol metabolism	myo-inositol-1(or 4)-monophosphatase activity
YKR080W	MTD1	one-carbon compound metabolism*	methylenetetrahydrofolate dehydrogenase (NAD+) activity
YDR502C	SAM2	methionine metabolism	methionine adenosyltransferase activity
YOR125C	CAT5	ubiquinone metabolism	molecular_function unknown
YKL038W	RGT1	glucose metabolism	DNA binding activity*
YDL109C		lipid metabolism	lipase activity
YMR170C	ALD2	aldehyde metabolism*	aldehyde dehydrogenase activity
2 hr Up			
YPL170W	DAP1	sterol metabolism	molecular_function unknown
YOL011W	PLB3	phosphoinositide metabolism*	lysophospholipase activity

•Genes involved in amino acid biosynthesis and lipid metabolism were up regulated in response to the cold

Table 4: Genes involved in stress that are induced in response to cold temperature \geq 3-fold

Gene Name	Alias	Biological Process	Molecular Function
30 minutes			
YMR175W	SIP18	response to osmotic stress*	phospholipid binding activity
YBL075C	SSA3	response to stress*	heat shock protein activity
YLL039C_rep11	UBI4	response to stress*	protein tagging activity*
YML100W_rep2	TSL1	response to stress*	enzyme regulator activity
YIR037W	HYR1	response to oxidative stress	glutathione peroxidase activity
YLL039C_rep14	UBI4	response to stress*	protein tagging activity*
YER103W_rep1 7	SSA4	response to stress*	chaperone activity*
YDL166C	FAP7	response to oxidative stress	molecular_function unknown
YLL026W_rep15	HSP10 4	response to stress*	heat shock protein activity*
YKL062W	MSN4	response to stress*	transcription factor activity*
YDR258C	HSP78	response to stress*	chaperone activity*
YPL091W	GLR1	response to oxidative stress	glutathione reductase (NADPH) activity
YLL039C_rep15	UBI4	response to stress*	protein tagging activity*
YLL039C_rep16	UBI4	response to stress*	protein tagging activity*
YGR209C	TRX2	response to oxidative stress*	thiol-disulfide exchange intermediate activity
YBR244W	GPX2	response to oxidative stress	glutathione peroxidase activity
YLL039C_rep17	UBI4	response to stress*	protein tagging activity*

•Cold temperature induces the expression of chaperones

Conclusions

•Overall, cells grown in YPD and YPGAL had similar changes in gene expression in response to cold temperature. Thus, our data do not support our hypothesis that cells grown in YPGAL would experience more oxidative stress compared to cells grown in YPD.

•Our analysis shows that in response to cold temperature, there are more genes induced than repressed.

•For more definitive conclusions the experiment should be repeated.

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Literature Cited

•DeRisi, J.L, Vishwanath R. L and P.O Brown. 1997. Exploring the metabolic control of gene expression on a genomic scale. SCIENCE volume# 278: 680-686.

•Murata, Yoshinori, and others. 2005. Genome-wide expression analysis of yeast response during exposure to 4°C. EXTREMOPHILES. Volume#10: 117-128.

Table 3: Genes involved in protein processes that are induced in response to cold temperature \geq 3-fold

Gene Name 30 Minutes	Alias	Biological Process	Molecular Function
YGL038C	OCH1	N-linked glycoprotein	alpha-1,6-mannosyltransferase activity*
YLR106C	MDN1	maturation protein complex	ATPase activity
YDR394W	RPT3	assembly ubiquitin-dependent	ATPase activity*
YOR117W	RPT5	protein catabolism ubiquitin-dependent	ATPase activity*
		protein catabolism	
YHR135C	YCK1	protein amino acid phosphorylation*	casein kinase I activity
YGL095C	VPS45	protein complex assembly*	chaperone activity
YOR056C	NOB1	ubiquitin-dependent protein catabolism*	chaperone activity
YDL229W	SSB1	protein biosynthesis	chaperone activity*
YNL064C	YDJ1	protein-mitochondrial targeting	chaperone regulator activity*
YPL106C	SSE1	protein folding	co-chaperone activity
YML112W	СТКЗ	protein amino acid phosphorylation*	cyclin-dependent protein kinase activity
YBR160W	CDC28	protein amino acid phosphorylation*	cyclin-dependent protein kinase activity
YOR085W	OST3	protein complex	dolichyl-diphospho-oligosaccharide-prote
YPR054W	SMK1	assembly* protein amino acid	glycosyltransferase activity MAP kinase activity
YOR231W	MKK1	phosphorylation*	MAP kinase kinase activity
		phosphorylation*	
YPL140C	MKK2	protein amino acid phosphorylation*	MAP kinase kinase activity
YLR362W	STE11	protein amino acid phosphorylation*	MAP kinase kinase kinase activity
YPL084W	BRO1	ubiquitin-dependent protein catabolism*	molecular_function unknown
YAL058W	CNE1	ER-associated protein	molecular_function unknown
YLR119W	SRN2	catabolism protein-vacuolar	molecular_function unknown
YNL297C	MON2	targeting* protein-vacuolar	molecular_function unknown
		targeting*	
YJL053W YGL236C	PEP8 MTO1	protein-Golgi retention*	molecular_function unknown molecular_function unknown
YIL147C	SLN1	protein amino acid	osmosensor activity*
YOR253W	NAT5	phosphorylation*	peptide alpha-N-acetyltransferase activity
		acetylation	
YMR264W	CUE1	ER-associated protein catabolism*	protein binding activity
YPR185W	APG13	protein-vacuolar targeting*	protein binding activity
YNL298W	CLA4	protein amino acid phosphorylation*	protein serine/threonine kinase activity
YLR248W	RCK2	protein amino acid	protein serine/threonine kinase activity
YOR351C	MEK1	phosphorylation* protein amino acid	protein serine/threonine kinase activity
YPL203W	TPK2	phosphorylation*	protein serine/threonine kinase activity*
		phosphorylation*	
YKL122C YKR014C	SRP21 YPT52	protein-ER targeting protein-vacuolar	Protein signal sequence binding activityRAB small monomeric GTPase activity
YLR417W	VPS36	targeting* protein-vacuolar	regulator of G-protein signaling activity
		targeting*	
YBR181C_rep15 YBR181C_rep16	RPS6B RPS6B	protein biosynthesis protein biosynthesis	structural constituent of ribosome
YBR181C_rep17	RPS6B	protein biosynthesis	structural constituent of ribosome
YLR325C	RPL38	protein biosynthesis	structural constituent of ribosome
YPL143W	RPL33A	protein biosynthesis	structural constituent of ribosome
YMR142C YMR242C	RPL13B RPL20A	protein biosynthesis protein biosynthesis	structural constituent of ribosome
YMR242C YPL079W	RPL20A RPL21B	protein biosynthesis	structural constituent of ribosome
YGL147C	RPL9A	protein biosynthesis	structural constituent of ribosome
YOR312C	RPL20B	protein biosynthesis	structural constituent of ribosome
YDR500C YBL092W_rep16	RPL37B RPL32	protein biosynthesis protein biosynthesis	structural constituent of ribosome
YER117W	RPL32 RPL23B	protein biosynthesis	structural constituent of ribosome
YGL076C	RPL7A	protein biosynthesis	structural constituent of ribosome
YNL162W_rep2	RPL42A	protein biosynthesis	structural constituent of ribosome
YLR406C	RPL31B	protein biosynthesis	structural constituent of ribosome
YDR064W	RPS13	protein biosynthesis	structural constituent of ribosome
YLR048W YOL121C	RPS0B RPS19A	protein biosynthesis*	structural constituent of ribosome
YOR182C	RPS19A RPS30B	protein biosynthesis	structural constituent of ribosome
YHL015W	RPS20	protein biosynthesis	structural constituent of ribosome
YJR123W	RPS5	protein biosynthesis	structural constituent of ribosome
YLR333C	RPS25B	protein biosynthesis	structural constituent of ribosome
YNL185C	MRPL19	protein biosynthesis	structural constituent of ribosome

•In response to the cold temperature genes involved in protein synthesis are induced

Acknowledgements