

Appendix "A"

MasterPure™ Yeast RNA Purification Kit from Epicentre Modified Version

(Done by: David Walker, Gary Lutz and Consuelo Alvarez, May 2005)

Modifications are shown in bold font. The original protocol can be seeing at Epicentre web page: www.EpiBio.com/

Part A. RNA Purification

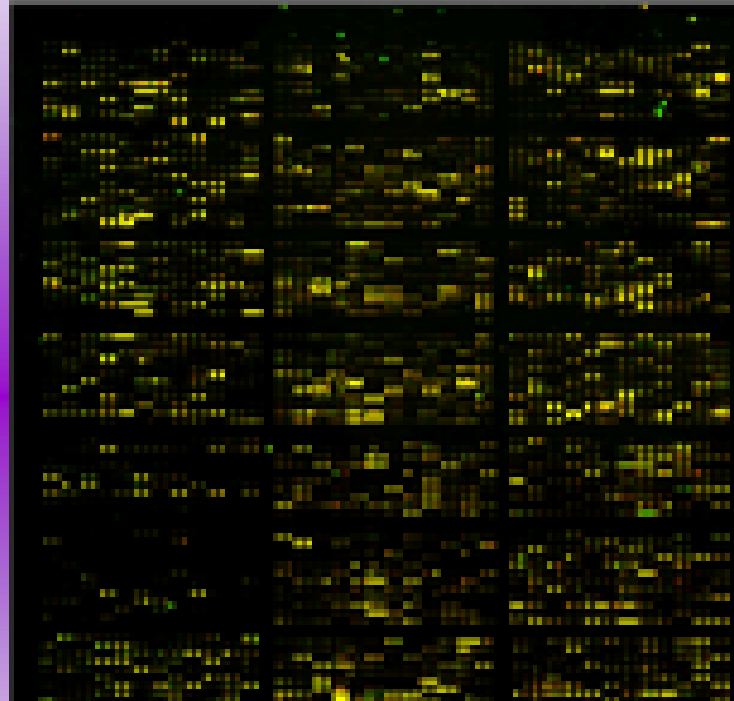
1. Dilute 1 ul of 50 ug/ul Proteinase K into 300 ul of Extraction Reagent for RNA for each sample. A premix may be prepared for multiple samples.
2. Pellet cells by centrifugation and discard the supernatant. The optimal number of cells varies with the species, but **2 ml** of a mid-log culture gives good results for *Saccharomyces cerevisiae*.
3. Vortex mix 10 seconds to loosen the cell pellet.
4. Add 300 ul of Extraction Reagent for RNA containing the Proteinase K and mix thoroughly by vortexing.
5. Incubate at 70°C for **30 minutes**; vortex mix every 5minutes.
6. Place the samples on ice for 3-5 minutes and add 175 ul of MPC Protein Precipitation Reagent to 300 ul lysed samples (solution may become cloudy). Vortex vigorously for 10 seconds.
7. Pellet the debris by centrifugation for 10 minutes at 4°C at $\geq 10,000 \times g$ in a microcentrifuge.
8. Transfer the supernatant fluid to a clean microcentrifuge tube and discard the pellet.
9. Add 500ul of isopropanol to the recovered supernatant fluid. Invert the tube 30-40 times.
10. Pellet the RNA by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
11. Carefully pour off or aspirate the isopropanol without dislodging the RNA pellet.
Since removal of contaminating DNA is required, proceed with the DNase I treatment in part B.

Part B. Removal of Contaminating DNA from RNA Preparations

1. Remove all of the residual isopropanol with a pipet.
2. Prepare 200ul of DNase I solution for each sample. Add 20 ul of 10X DNase Buffer to 175 ul deionized water, then add 5 ul of RNase-Free DNase I.
3. Completely resuspend the nucleic acid pellet in 200 ul of DNase I solution.
4. Incubate at 37°C for **30 minutes**.
5. Add 200ul of 2X T and C Lysis Solution; vortex for 5 seconds.
6. Add 200 ul of MPC Protein Precipitation Reagent (solution maybe be cloudy). Vortex for 10 seconds; place on ice 3-5 minutes.
7. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. **To obtain a completely clear supernatant, repeat step 7 and 8 at least one more time.**
10. Add 500 ul of isopropanol to the supernatant. Invert the tube 30-40 times.

11. Pellet the purified RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge at $\geq 10,000 \times g$.
12. Carefully pour off or aspirate the isopropanol without dislodging the RNA pellet.
13. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all the residual ethanol with a pipet.
- 14. Air-dry the pellet for a few minutes (3-5).**
- 15. Allow the cleaned RNA preparation to precipitate in ethanol at -20°C (Alvarez and Wise, 2001). The quality and concentration of the RNA sample should be determined before continuing with the cDNA preparation.**

Effects of Ultraviolet Radiation on *Saccharomyces cerevisiae* Using Microarray Analysis



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Research Components

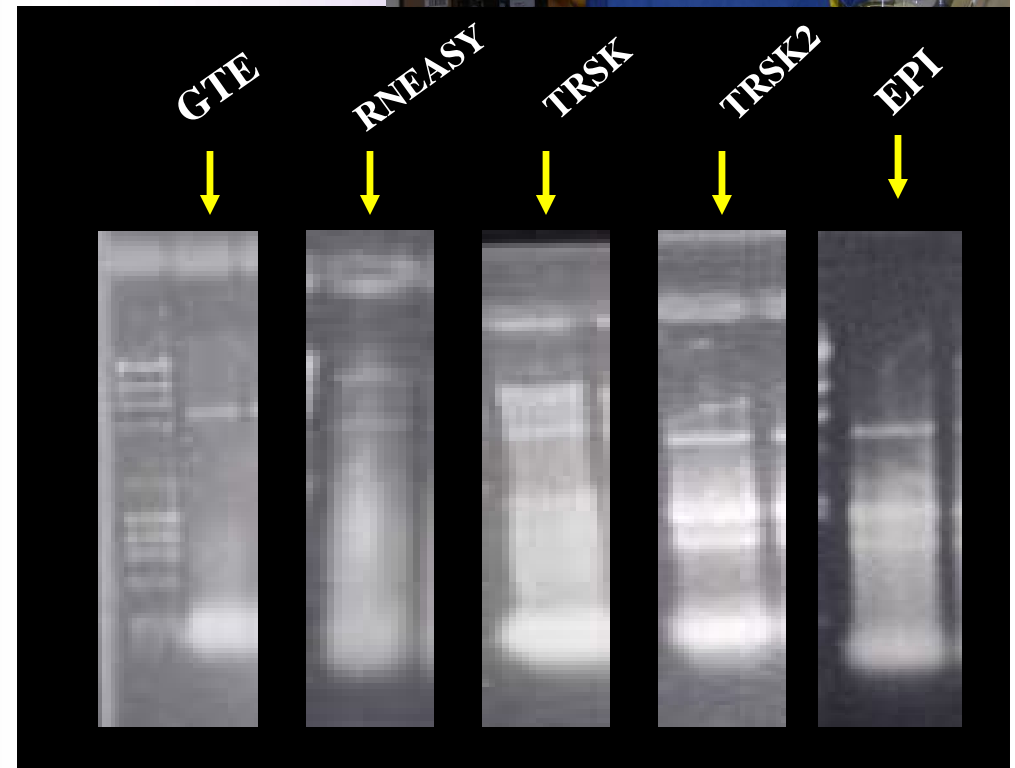
- Genetics: yeast, a model system of humans
- Stress Imposed: UV radiation
- Microarray: why?
- Goals
 - Examine the changes in gene expression to an environmental stress
 - Introduction to research and a novel technique



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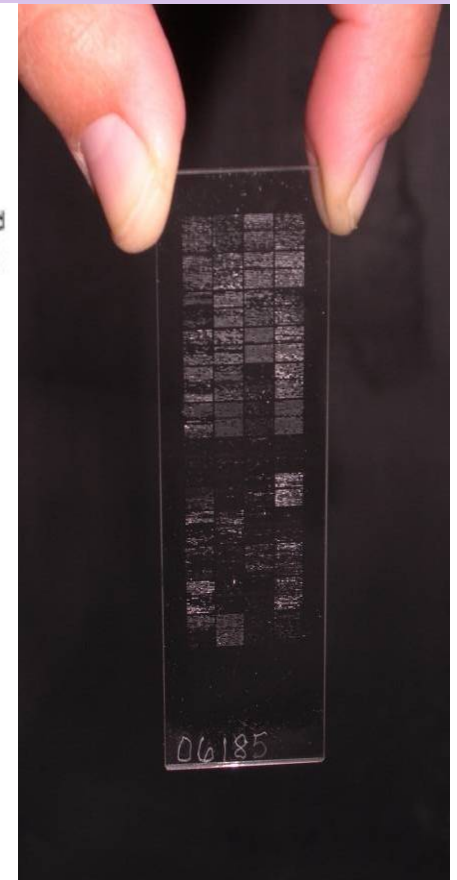
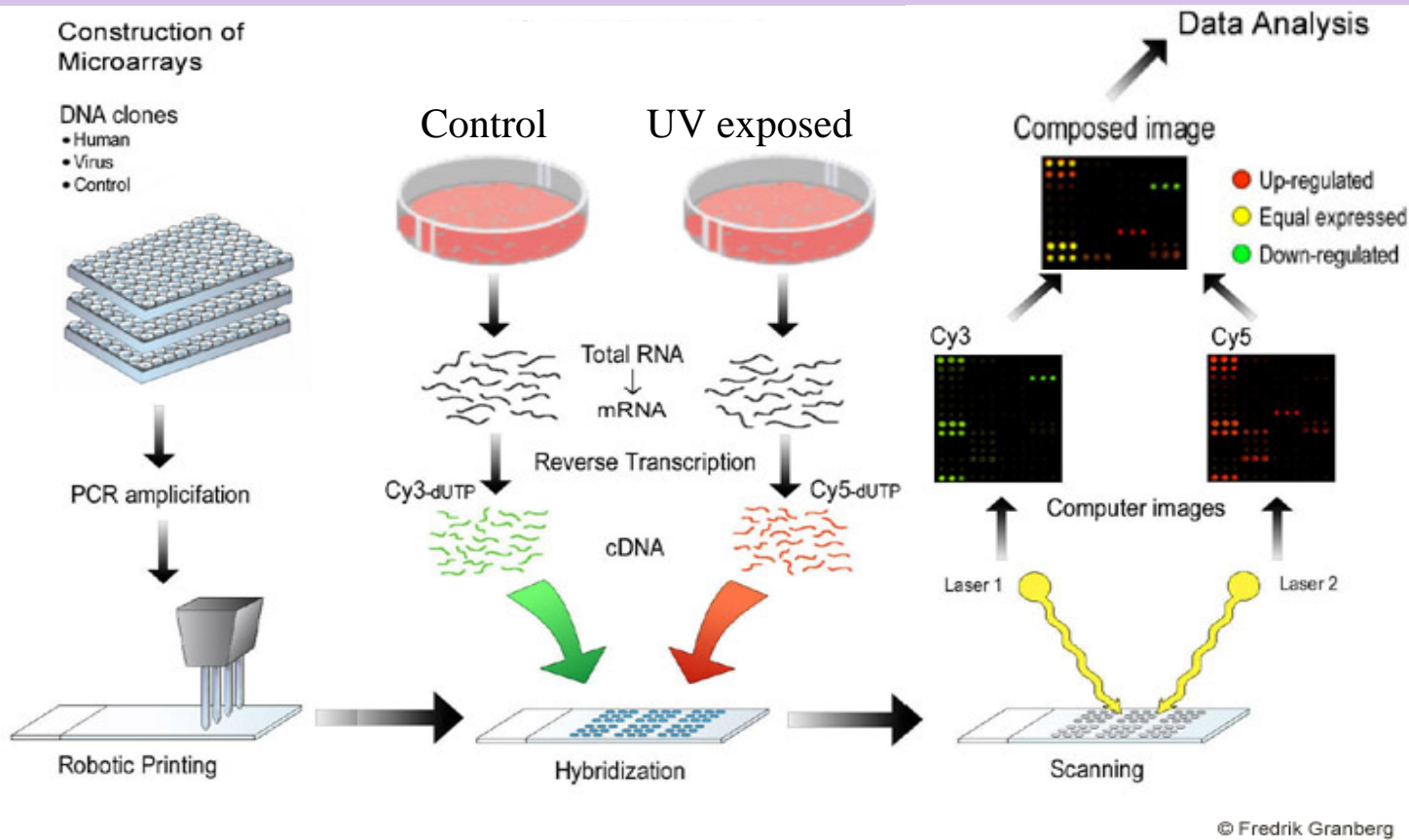
Procedure

- Cell growth
 - YPD media
 - Log phase
- Exposure of cells to UV light
 - UV-B region
- RNA prep
 - Isolation of mRNA
 - GTE Phenol-chloroform
 - Qiagen RNeasy
 - Total RNA Safekit
 - Epicenter MasterPure kit



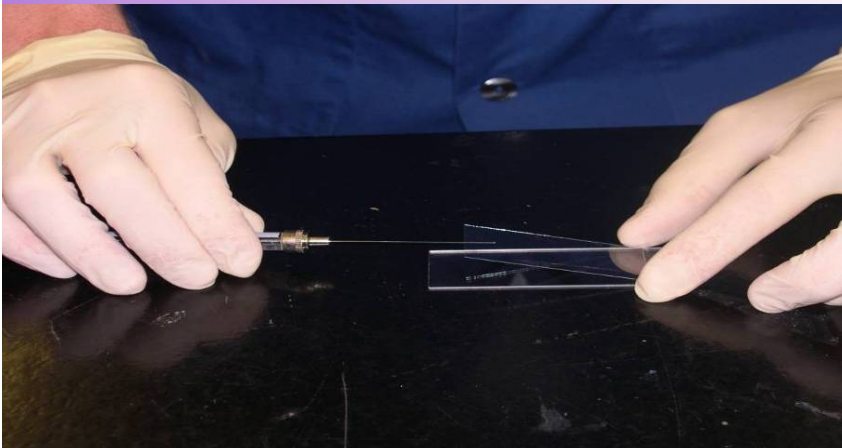
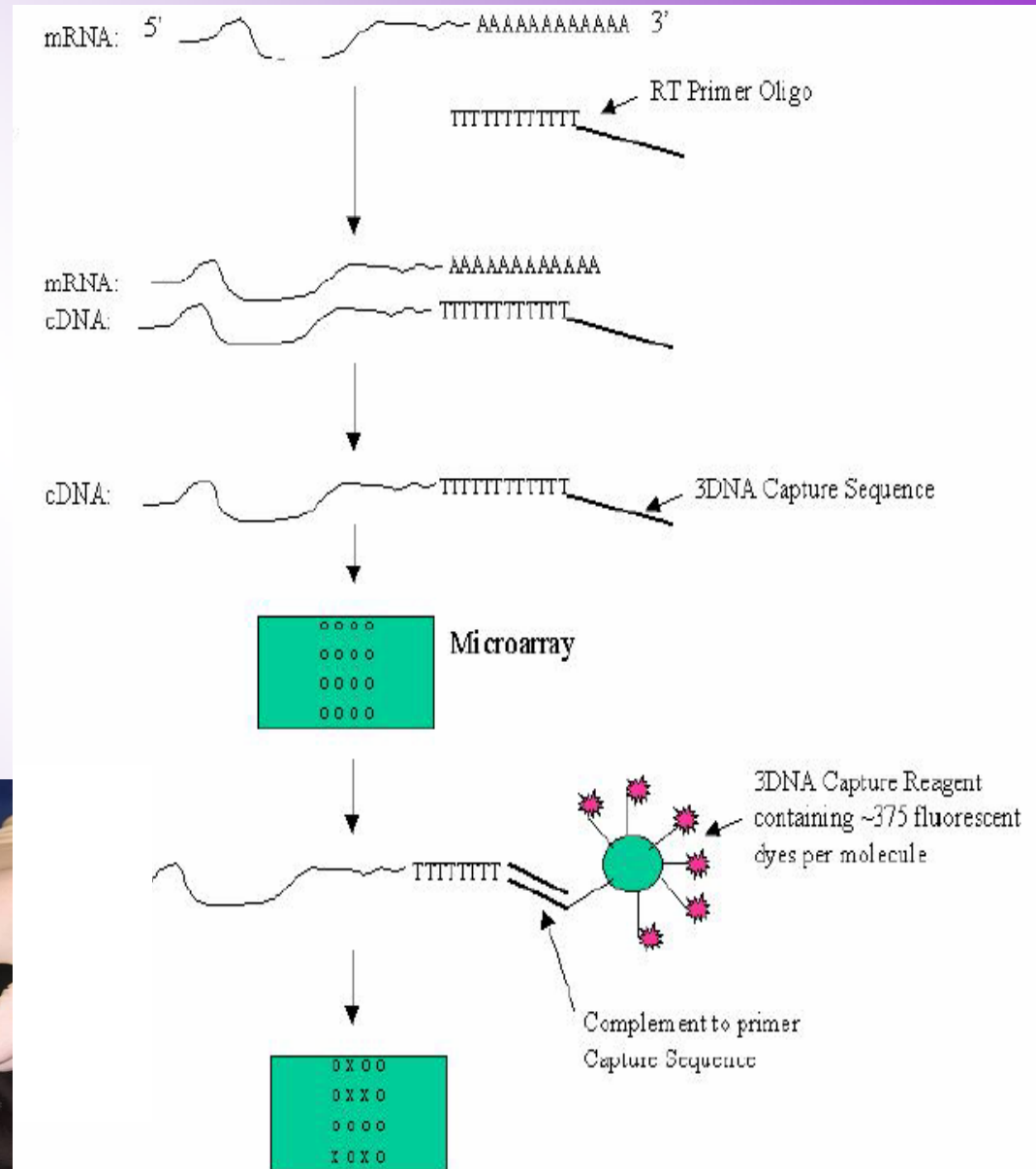
Procedure Continued

Microarray Protocol

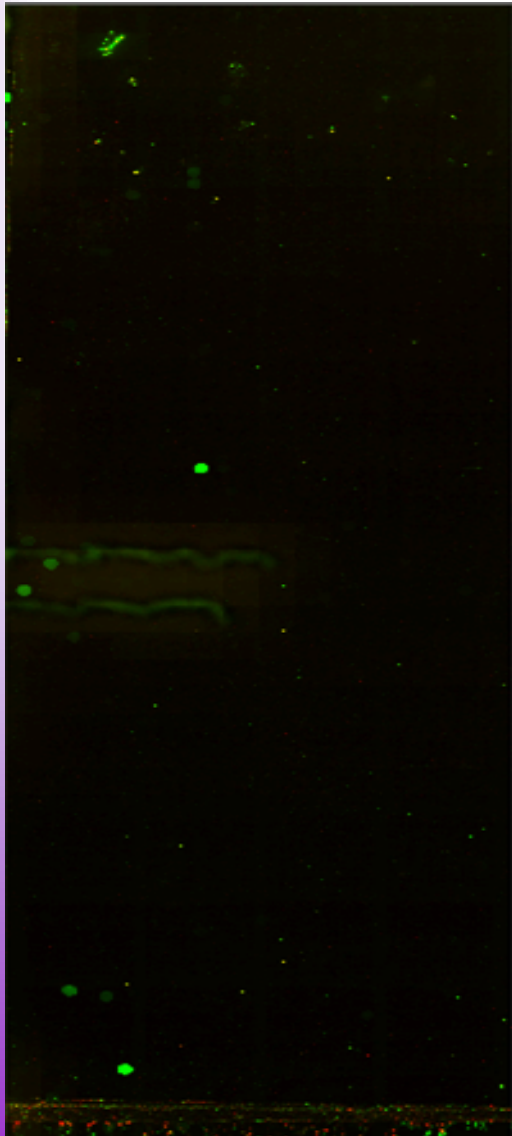


Microarray Protocol

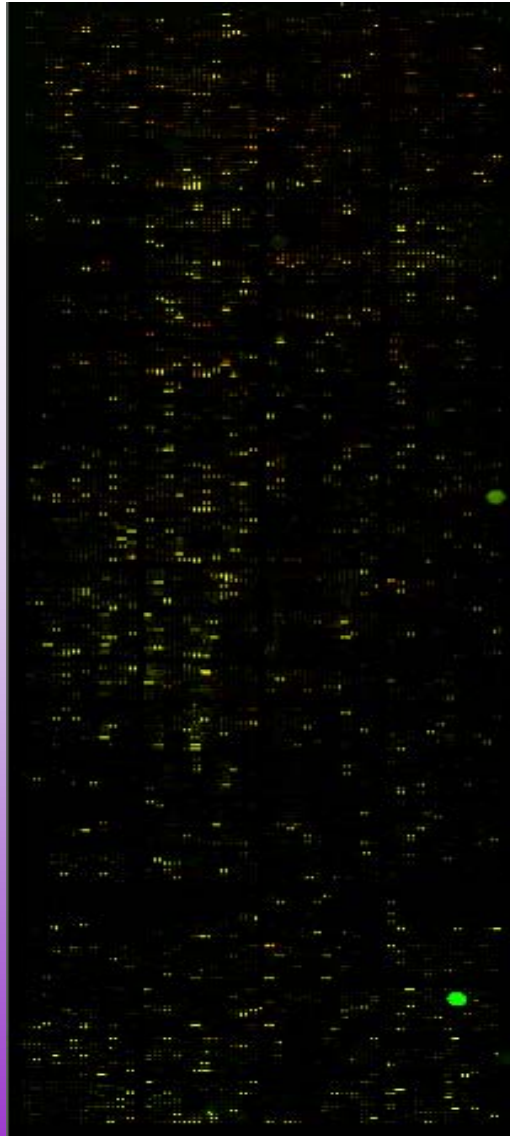
- Protocols Used:
 - Genisphere 3DNA Array 350
 - Use of dyes after cDNA hybridization
 - 10ug mRNA
 - ISB Protocol
 - Use of dyes during reverse transcription
 - 50ug of mRNA



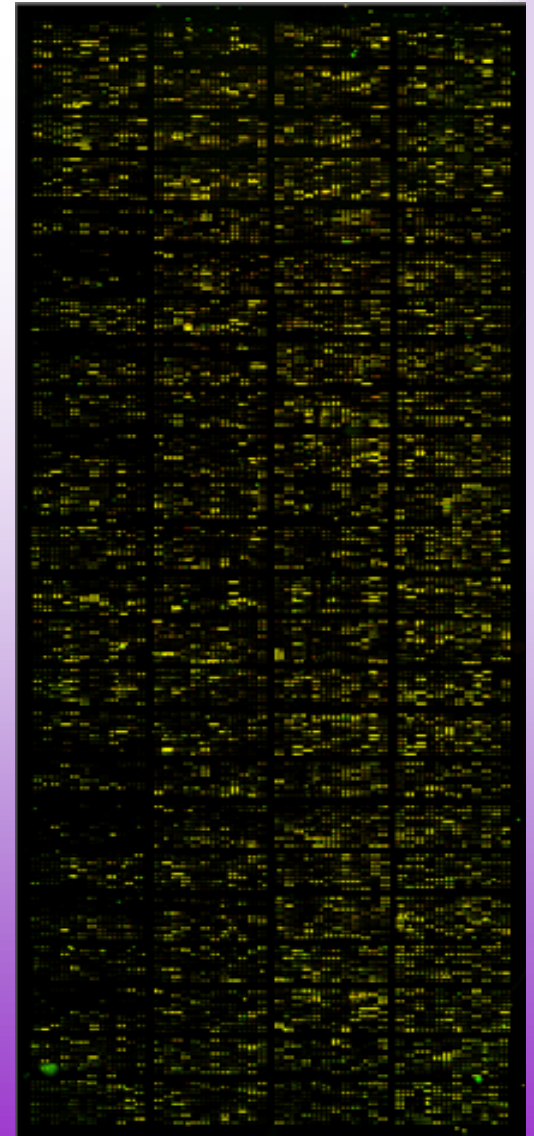
Results



Genisphere 2/4/05

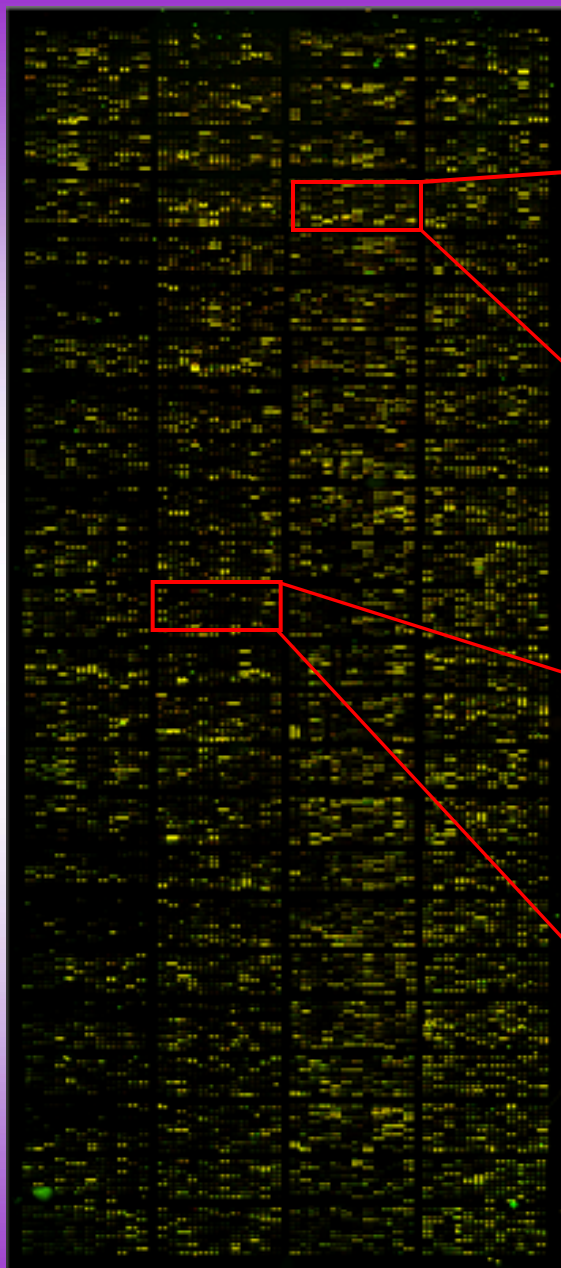


ISB 5/6/05

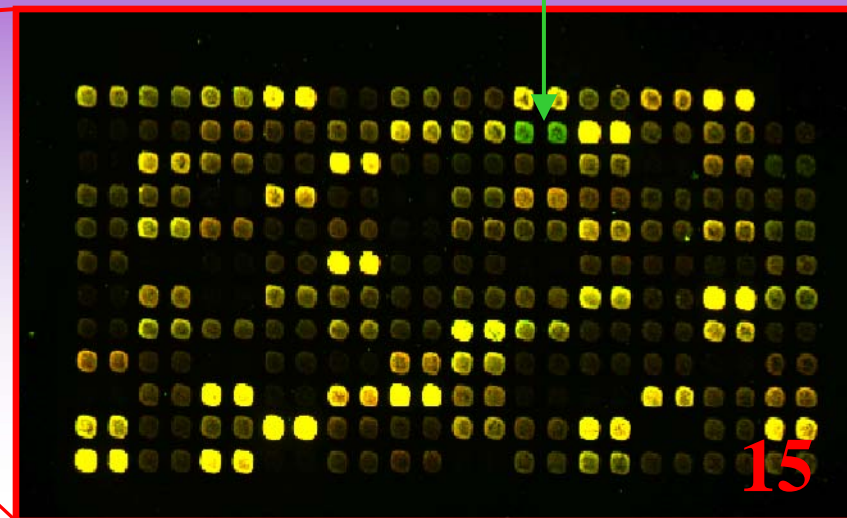


Genisphere 5/6/05

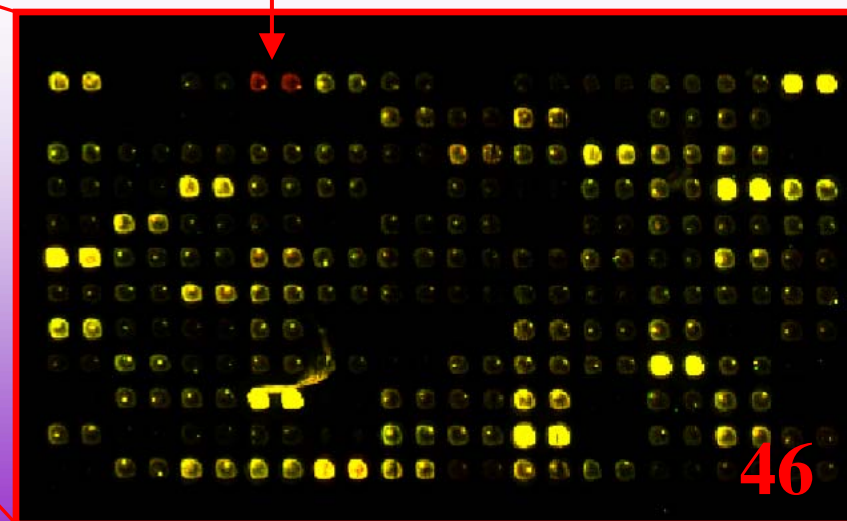
Results



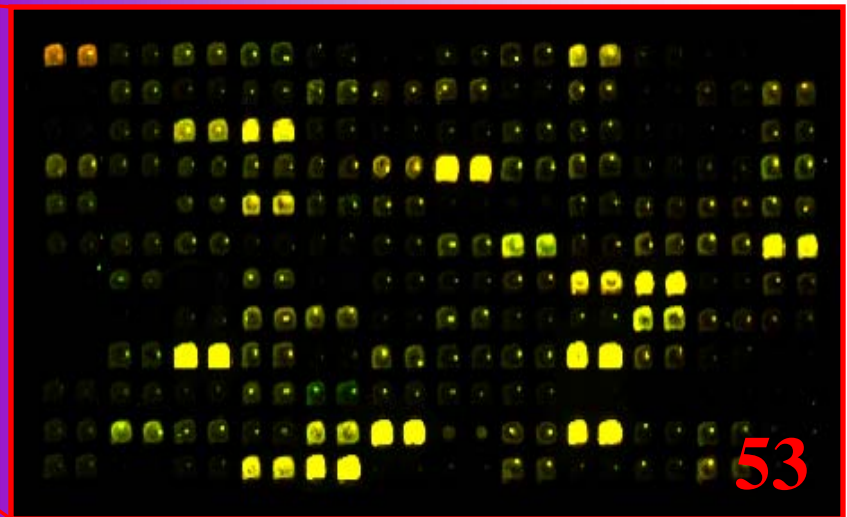
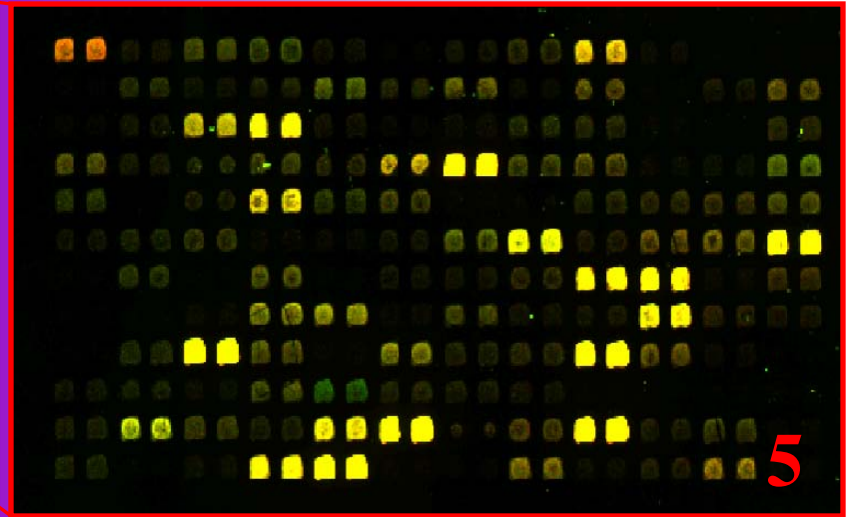
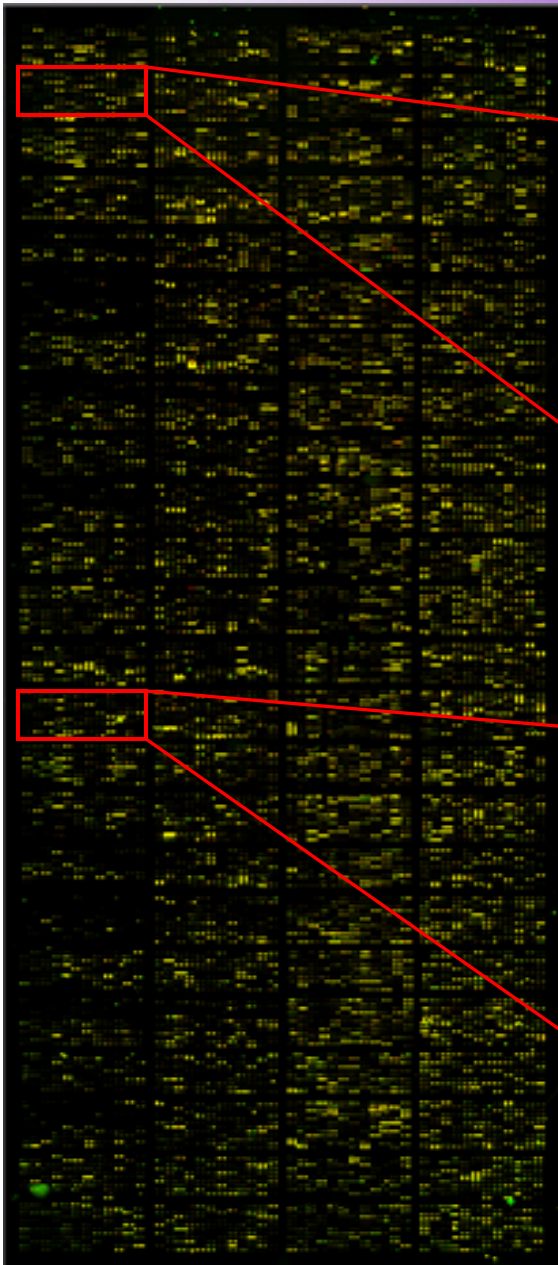
Strong Repression



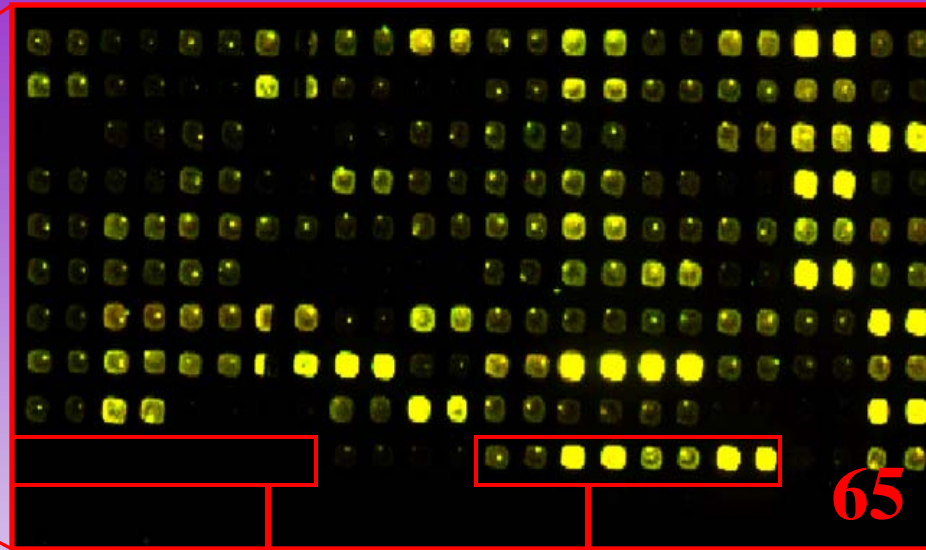
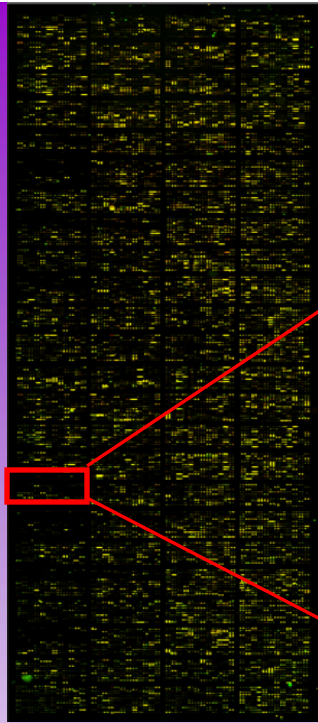
Strong Induction



Results



Results

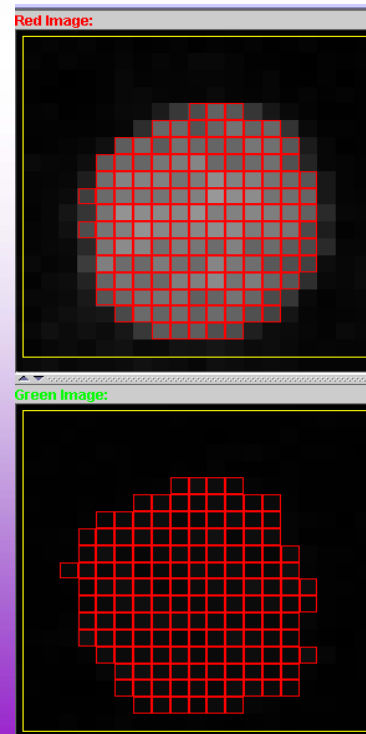
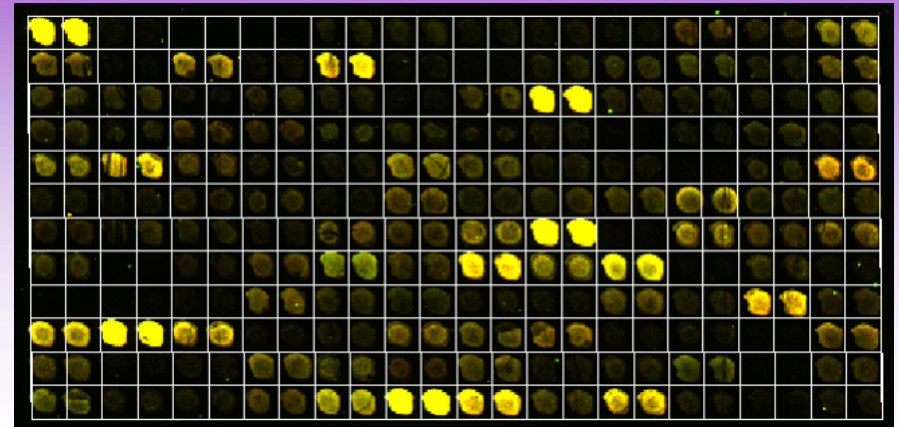


65

zoneRow	zoneCol	row	col	ref	gene_name	plate_384	external_ID	stranded	feature_size	feature_attachment
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10	3	10	2	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	3	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	4	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	5	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	6	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	7	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	8	EMPTY	EMPTY	YC-17	EMPTY	ssDNA	110um	covalent
10	3	10	9	YPR196W_	YPR196W	YC-17	YPR196W	ssDNA	110um	covalent
10	3	10	10	YPR196W_	YPR196W	YC-17	YPR196W	ssDNA	110um	covalent
10	3	10	11	YPR193C_HPA2	HPA2	YC-17	YPR193C	ssDNA	110um	covalent
10	3	10	12	YPR193C_HPA2	HPA2	YC-17	YPR193C	ssDNA	110um	covalent
10	3	10	13	YPR190C_RPC82	RPC82	YC-17	YPR190C	ssDNA	110um	covalent
10	3	10	14	YPR190C_RPC82	RPC82	YC-17	YPR190C	ssDNA	110um	covalent
10	3	10	15	YPR187W_RPO26	RPO26	YC-17	YPR187W	ssDNA	110um	covalent
10	3	10	16	YPR187W_RPO26	RPO26	YC-17	YPR187W	ssDNA	110um	covalent
10	3	10	17	YCR027C_RHB1	RHB1	YB-02	YCR027C	ssDNA	110um	covalent
10	3	10	18	YCR027C_RHB1	RHB1	YB-02	YCR027C	ssDNA	110um	covalent
10	3	10	19	YCR024C-A_PMP1	PMP1	YB-02	YCR024C-A	ssDNA	110um	covalent
10	3	10	20	YCR024C-A_PMP1	PMP1	YB-02	YCR024C-A	ssDNA	110um	covalent

Data Analysis

- Magic Tool
 - Load image files
 - Address and grid
 - Segmentation
 - Statistical analysis



Data For: YBR042C_rep1

Red FG Total: 15476

Red BG Total: 2156

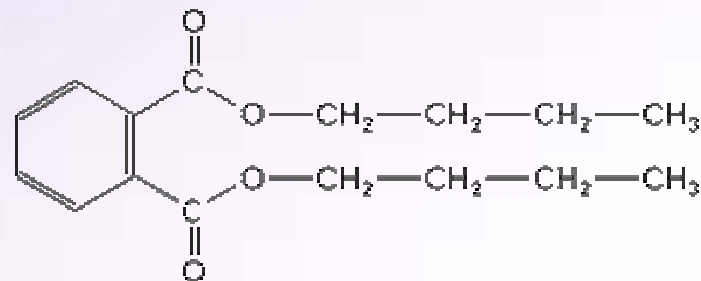
Green FG Total: 1639

Green BG Total: 359

Ratio: 9.4423

Future Work

- Replicate UV light experiment:
 - To prove reproducibility
 - Change experimental conditions
- Subject yeast to a chemical stress:
 - Plasticizers
 - Hormone mimics
 - n-Butyl Phthalate (Dibutyl Phthalate)



Acknowledgements

- Genomic Consortium for Active Teaching (GCAT @ Davidson College)
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 - Dr. Consuelo Alvarez
 - Dr. Michael Langham
 - Dr. Gary Lutz
 - Dr. Anthony Palombella
 - Student Funds

Microarray Analysis of *Saccharomyces cerevisiae* cells under chemical and environmental stress

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Chemical Stress: Dibutyl phthalate

Background

Dibutyl phthalate is a common plasticizer used in a wide variety of polymers. It is a colorless, odorless, and tasteless liquid. It is used in a wide variety of products, including paints, coatings, and plastics. It is also used in the production of synthetic fibers and in the manufacture of dyes and pigments. Dibutyl phthalate is also used in the production of perfumes and cosmetics. It is a common environmental pollutant and is known to be toxic to aquatic life. It is also known to be a carcinogen and to cause reproductive and developmental effects in humans and animals.



Figure 1: Chemical structure of dibutyl phthalate.



Figure 2: Chemical structure of dibutyl phthalate.



Figure 3: Chemical structure of dibutyl phthalate.

Research Question

What genes are upregulated in *S. cerevisiae* cells exposed to dibutyl phthalate stress?

Methods

The yeast cells were grown in YEA medium. Cells were exposed to dibutyl phthalate for 24 hours. Total RNA was extracted and labeled with a fluorescent dye. The labeled RNA was hybridized to a microarray chip containing DNA probes for all genes in the yeast genome. The array was scanned and the resulting image was analyzed using software to identify differentially expressed genes.

Results

Gene Name	Function	Log2 Fold Change	Significance
YEA001	Yeast cell wall protein	1.5	0.001
YEA002	Yeast cell wall protein	1.2	0.01
YEA003	Yeast cell wall protein	1.8	0.0001
YEA004	Yeast cell wall protein	1.1	0.05
YEA005	Yeast cell wall protein	1.3	0.005
YEA006	Yeast cell wall protein	1.4	0.002
YEA007	Yeast cell wall protein	1.6	0.0005
YEA008	Yeast cell wall protein	1.7	0.0001
YEA009	Yeast cell wall protein	1.9	0.00001
YEA010	Yeast cell wall protein	2.0	0.000001

Figure 4: Table of differentially expressed genes.

Future work

Future work will focus on identifying the specific mechanisms by which dibutyl phthalate stress leads to the upregulation of these genes. This may involve studying the signaling pathways involved in the response to chemical stress.

References

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Walker, D.E., Lutz, G.P., Alvarez, C.J. (2019) The role of the yeast cell wall in response to chemical stress. *Journal of Cell Biology*, 231(1), 1-10.

Environmental Stress: Ultraviolet light

Background

Ultraviolet light is a form of electromagnetic radiation with a wavelength shorter than visible light, but longer than X-rays. It is divided into three categories: UVA, UVB, and UVC. UVA is the least harmful, but can still cause skin aging and some skin cancer. UVB is more harmful and can cause sunburn and skin cancer. UVC is the most harmful and is used for sterilization. Ultraviolet light is a common environmental stressor for many organisms, including plants and animals.



Figure 1: Ultraviolet light source.

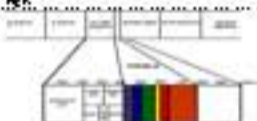


Figure 2: Diagram of the microarray technique.



Figure 3: DNA double helix structure.

Research Question

What genes are upregulated in *S. cerevisiae* cells exposed to ultraviolet light stress?

Methods

The yeast cells were grown in YEA medium. Cells were exposed to ultraviolet light for 24 hours. Total RNA was extracted and labeled with a fluorescent dye. The labeled RNA was hybridized to a microarray chip containing DNA probes for all genes in the yeast genome. The array was scanned and the resulting image was analyzed using software to identify differentially expressed genes.

Results

Gene Name	Function	Log2 Fold Change	Significance
YEA011	Yeast cell wall protein	1.5	0.001
YEA012	Yeast cell wall protein	1.2	0.01
YEA013	Yeast cell wall protein	1.8	0.0001
YEA014	Yeast cell wall protein	1.1	0.05
YEA015	Yeast cell wall protein	1.3	0.005
YEA016	Yeast cell wall protein	1.4	0.002
YEA017	Yeast cell wall protein	1.6	0.0005
YEA018	Yeast cell wall protein	1.7	0.0001
YEA019	Yeast cell wall protein	1.9	0.00001
YEA020	Yeast cell wall protein	2.0	0.000001

Figure 4: Table of differentially expressed genes.

Future work

Future work will focus on identifying the specific mechanisms by which ultraviolet light stress leads to the upregulation of these genes. This may involve studying the signaling pathways involved in the response to environmental stress.

Acknowledgements

We thank the following individuals for their assistance in the laboratory: Dr. John Doe, Dr. Jane Smith, and Dr. Bob Johnson. We also thank the following organizations for their support: the National Science Foundation, the American Cancer Society, and the National Institutes of Health.

The Microarray Technique

The microarray technique is a powerful tool for studying gene expression. It involves the simultaneous measurement of the expression levels of thousands of genes. The technique is based on the hybridization of labeled RNA to DNA probes on a solid support. The resulting image is analyzed using software to identify differentially expressed genes.

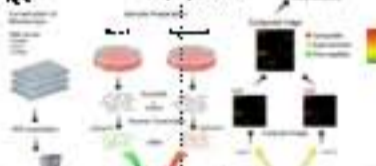


Figure 5: Diagram of the microarray technique.

Data Analysis

Data analysis is a critical step in the microarray process. It involves the identification of differentially expressed genes and the interpretation of the resulting data. This may involve the use of statistical methods and bioinformatics tools.



Figure 6: Diagram of data analysis.



Figure 7: Microarray image showing gene expression patterns.

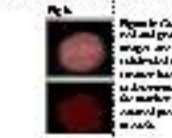


Figure 8: Microarray image showing gene expression patterns.



Figure 9: Microarray image showing gene expression patterns.

- 1) The microarray data was analyzed using software to identify differentially expressed genes.
- 2) The results were compared to a database of gene expression data to identify potential targets.
- 3) The identified genes were validated using quantitative PCR and Western blotting.
- 4) The expression levels of the identified genes were measured in response to stress.
- 5) The results were used to develop a model of the signaling pathway involved in the response to stress.

GENE EXPRESSION OF *SACCHAROMYCES CEREVISIAE* EXPOSED TO COMMERCIAL WOOD PRESERVATIVES BY MICROARRAY ANALYSIS

Madison M. Stevens (Consuelo J. Alvarez¹, Gary P. Lutz²) ¹Department of Biological and Environmental Sciences, ²Department of Chemistry and Physics, Longwood University, 201 High Street, Farmville, VA, 23909

Pentachlorophenol (PCP) and Creosote are two commercial wood preservatives that are regulated by the EPA because of their toxicity to wildlife and humans. To observe changes in gene expression in organisms exposed to these compounds, a model system such as *Saccharomyces cerevisiae* (baker's yeast) was used. *S. cerevisiae* cells were exposed to creosote concentrations of 30ng/ml or 50ng/ml and to a PCP concentration of 50 μ M. Since creosote and PCP were suspended in methylene chloride and ethanol, respectively, yeast cells were also exposed separately to the solvents as controls. cDNA was prepared from a total RNA extraction of exposed and non-exposed *S. cerevisiae* cells and was hybridized onto microarray chips containing the entire yeast genome using Genisphere Array Kit procedures. Analysis of the microarray data using Magic Tool software shows interesting trends in gene expression. In the creosote data, ~10% of genes were induced at 30ng/ml, while ~82% were induced at 50ng/ml. In the PCP data, ~7% of genes were induced. Particular genes of interest from the data include TIP and CIS3 which are responsible for cell wall organization and biogenesis. In addition TRX3 and ATX1, which are responsible for response to oxidative stress, proved interesting. The data is reported as the log₂ of the ratios of expression. In the future, the genes of interest will be selected for RT-PCR (real time PCR) to validate that their expression was correctly measured in the original microarray.

DATA ANALYSIS OF A GENE EXPRESSION EXPERIMENT FOR *SACCHAROMYCES CEREVISIAE* EXPOSED TO COMMERCIAL WOOD PRESERVATIVES BY MICROARRAY ANALYSIS

Ashley M. Swandby (M. Leigh Lunsford¹, Madison M. Stevens², Consuelo J. Alvarez², Gary P. Lutz³)¹ Department of Mathematics and Computer Science, ²Department of Biological and Environmental Sciences, ³Department of Chemistry and Physics, Longwood University, 201 High Street, Farmville, VA, 23909

DNA Microarray experiments produce a large amount of data that must be evaluated in order to draw biological conclusions. Because of the nature of experimentation, many processes can contribute to variation in the data. Microarray data is sensitive to this variation and can lead to incorrect conclusions being drawn. By using statistical methods, we evaluate the data and determine if there are any biases or other unexpected occurrences that need to be investigated. By performing normalization of the data, we can eliminate discrepancies between the data and create a large set of consistent data. We are not attempting to alter the data, but we simply want to eliminate the presence of influences that affect the actual experiment being observed. Once the data has been normalized, we will apply various statistical methods to determine the outcome of the experimental processes being performed by the Biology Department.

	A	B	C	D	E	F	G	H	I	J
5536	YBL092W	12	21	14	YBL092W	Ribosomal protein L32,II				
5537	YPL016W	12	21	15	YPL016W	Zinc-finger transcription factor,XVI				
5538	YJL052W	12	21	16	YJL052W	Glyceraldehyde-3-phosphate dehydrogenase 1,X				
5539	Empty	12	21	17	Empty	Empty				
5540	Empty	12	21	18	Empty	Empty				
5541	Empty	12	21	19	Empty	Empty				
5542	Empty	12	21	20	Empty	Empty				
5543	Empty	12	21	21	Empty	Empty				
5544	Empty	12	21	22	Empty	Empty				
5545	YBL068W	13	1	1	YBL068W	ribose-phosphate pyrophosphokinase 4,II				
5546	YBL070C	13	1	2	YBL070C	Ybl070cp,II				
5547	YBL072C	13	1	3	YBL072C	Ribosomal protein S8A (S14A) (rp19) (YS9),II				
5548	YBL074C	13	1	4	YBL074C	MATa1-mRNA splicing factor,II				
5549	YBL076C	13	1	5	YBL076C	cytoplasmic isoleucyl-tRNA synthetase,II				
5550	YBL078C	13	1	6	YBL078C	Aut7p has homology to LC3, a microtubule-associated protein				
5551	YBL091C-A	13	1	7	YBL091C-	Ybl091c-ap,II				
5552	YBL093C	13	1	8	YBL093C	RNA polymerase II holoenzyme/mediator subunit,II				
5553	YBL095W	13	1	9	YBL095W	Ybl095wp,II				
5554	YBL097W	13	1	10	YBL097W	involved in chromosome maintenance; similar to Drosophila				
5555	YBL099W	13	1	11	YBL099W	mitochondrial F1F0-ATPase alpha subunit,II				
5556	YBL101C	13	1	12	YBL101C	involved in cell wall biogenesis,II				
5557	YBL113C	13	1	13	YBL113C	Ybl113cp,II				
5558	YBR002C	13	1	14	YBR002C	cis-prenyltransferase,II				
5559	YBR004C	13	1	15	YBR004C	Ybr004cp,II				
5560	YBR006W	13	1	16	YBR006W	succinate semialdehyde dehydrogenase,II				
5561	YBR008C	13	1	17	YBR008C	Major Facilitator Transporter,II				
5562	YBR010W	13	1	18	YBR010W	Histone H3 (HHT1 and HHT2 code for identical proteins),II				
5563	YBR022W	13	1	19	YBR022W	Ybr022wp,II				
5564	YBR024W	13	1	20	YBR024W	SCO1 protein homolog (<i>S. cerevisiae</i>),II				
5565	YBR026C	13	1	21	YBR026C	Nuclear protein that binds to T-rich strand of core consensus				
5566	YBR028C	13	1	22	YBR028C	Probable ser/thr-specific protein kinase, homolog to YKR2				
5567	YBR030W	13	2	1	YBR030W	Ybr030wp,II				
5568	YBR032W	13	2	2	YBR032W	Ybr032wp,II				
5569	YCL032W	13	2	3	YCL032W	STE50,III				
5570	YCL034W	13	2	4	YCL034W	Ycl034wp,III				
5571	YCL036W	13	2	5	YCL036W	Ycl036wp,III				
5572	YCL038C	13	2	6	YCL038C	Membrane transporter,III				
5573	YCL040W	13	2	7	YCL040W	Glucokinase,III				
5574	YCL042W	13	2	8	YCL042W	Ycl042wp,III				
5575	YCL057W	13	2	9	YCL057W	Saccharolysin (oligopeptidase yscD),III				
5576	YCL059C	13	2	10	YCL059C	involved in cell division and spore germination,III				
5577	YCL063W	13	2	11	YCL063W	Ycl063wp,III				
5578	YCL065W	13	2	12	YCL065W	Ycl065wp,III				
5579	YCL067C	13	2	13	YCL067C	Mating type protein alpha-2,III				
5580	YCL069W	13	2	14	YCL069W	Membrane transporter,III				

	A	B	C
5103	YER103W_rep8	0.870967742	
5104	YLL039C_rep9	0.898089172	
5105	YBL092W_rep9	1.42595769	
5106	YPL016W_rep9	1.142857143	
5107	YJL052W_rep9	1.251714882	
5108	YBL068W_rep1	1.685990338	
5109	YBL070C_rep1	999	
5110	YBL072C_rep1	1.230916844	
5111	YBL074C_rep1	6.666666667	
5112	YBL076C_rep1	1.276621787	
5113	YBL078C_rep1	0.66557377	
5114	YBL091C-A_rep1	1.04368932	
5115	YBL093C_rep1	1.193103448	
5116	YBL095W_rep1	1.030612245	
5117	YBL097W_rep1	3	
5118	YBL099W_rep1	1.016784452	
5119	YBL101C_rep1	1.606299213	
5120	YBL113C_rep1	1.113246753	
5121	YBR002C_rep1	1.328244275	
5122	YBR004C_rep1	0.936046512	
5123	YBR006W_rep1	0.615384615	
5124	YBR008C_rep1	1.454545455	
5125	YBR010W_rep1	1.130877508	
5126	YBR022W_rep1	1.072164948	
5127	YBR024W_rep1	0.9375	
5128	YBR026C_rep1	1.066176471	
5129	YBR028C_rep1	1.623655914	
5130	YBR030W_rep1	0.52293578	
5131	YBR032W_rep1	999	
5132	YCL032W_rep1	1.010526316	
5133	YCL034W_rep1	1.076470588	
5134	YCL036W_rep1	4	
5135	YCL038C_rep1	1.134615385	
5136	YCL040W_rep1	0.541343079	
5137	YCL042W_rep1	0.633763838	
5138	YCL057W_rep1	1.371052632	
5139	YCL059C_rep1	1.167676768	
5140	YCL063W_rep1	3.044444444	
5141	YCL065W_rep1	999	
5142	YCL067C_rep1	1.207165109	
5143	YCL069W_rep1	999	
5144	YCR010C_rep1	4.5	
5145	YCR012W_rep1	1.326435675	
5146	YCR014C_rep1	1.962962963	
5147	YCR016W_rep1	1.635135135	
5148	YCR018C_rep1	1.583333333	

	A	B	C
5477	YPL187W_rep1	1.442169132	
5478	YPL189W_rep1	999	
5479	YPL191C_rep1	1.619047619	
5480	YPL205C_rep1	999	
5481	YPL207W_rep1	1.445820433	
5482	YPL209C_rep1	2.066666667	
5483	YPL211W_rep1	1.323920266	
5484	YPL213W_rep1	1.571428571	
5485	YPL215W_rep1	1.039215686	
5486	YPL229W_rep1	1.169811321	
5487	YPL231W_rep1	1.283918282	
5488	YPL233W_rep1	1.346153846	
5489	YPL235W_rep1	1.334872979	
5490	YPL237W_rep1	1.42524377	
5491	YPL239W_rep1	1.517808219	
5492	YCONTROL57_rep1	999	
5493	YCONTROL63_rep1	999	
5494	YCONTROL91_rep1	999	
5495	YCONTROL02_rep1	999	
5496	YCONTROL97_rep1	999	
5497	YCONTROL99_rep1	0.142857143	
5498	YCONTROL57_rep	1	
5499	YCONTROL63_rep	999	
5500	YCONTROL91_rep	999	
5501	YCONTROL02_rep	999	
5502	YCONTROL97_rep	999	
5503	YCONTROL99_rep	999	
5504	YCONTROL57_rep	999	
5505	YCONTROL63_rep	999	
5506	YCONTROL91_rep	3.5	
5507	YCONTROL02_rep	999	
5508	YCONTROL97_rep	999	
5509	YCONTROL99_rep	999	
5510	YCONTROL57_rep	3	
5511	YCONTROL63_rep	999	
5512	YCONTROL91_rep	999	
5513	YCONTROL02_rep	999	
5514	YCONTROL97_rep	999	
5515	YCONTROL99_rep	999	
5516	YAR019C_rep1	1.225352113	
5517	YAR023C_rep1	1.416666667	
5518	YAR028W_rep1	1.162037037	
5519	YAR030C_rep1	11	
5520	YAR033W_rep1	1.285714286	
5521	YAR042W_rep1	1.428571429	
5522	YAR073W_rep1	1.188449848	

	A	B	C	D	E	F	G	H	I	J
1	ORF	RedFGtot	RedBGtot	GrnFGtot	GrnBGtot	RedFGavg	RedBGavg	GrnFGavg	GrnBGavg	
2	YBR045C_rep1	24	3	9	59	0.161074	0.014151	0.060403	0.278302	
3	YBR047W_rep1	125	5	107	0	0.838926	0.023585	0.718121	0	
4	YBR049C_rep1	380	4	231	0	2.550336	0.018868	1.550336	0	
5	YBR051W_rep1	8	1	3	0	0.053691	0.004717	0.020134	0	
6	YBR053C_rep1	540	94	490	698	3.624161	0.443396	3.288591	3.292453	
7	YBR055C_rep1	117	37	154	197	0.785235	0.174528	1.033557	0.929245	
8	YBR069C_rep1	795	12	476	0	5.33557	0.056604	3.194631	0	
9	YBR071W_rep1	70	3	45	1	0.469799	0.014151	0.302013	0.004717	
10	YBR073W_rep1	337	2	232	0	2.261745	0.009434	1.557047	0	
11	YBR075W_rep1	353	5	280	0	2.369128	0.023585	1.879195	0	
12	YBR077C_rep1	257	6	210	0	1.724832	0.028302	1.409396	0	
13	YBR079C_rep1	1186	13	867	0	7.959732	0.061321	5.818792	0	
14	YBR091C_rep1	237	4	147	0	1.590604	0.018868	0.986577	0	
15	YBR093C_rep1	2107	206	1328	105	14.14094	0.971698	8.912752	0.495283	
16	YBR095C_rep1	172	20	110	9	1.154362	0.09434	0.738255	0.042453	
17	YBR097W_rep1	84	20	49	9	0.563758	0.09434	0.328859	0.042453	
18	YBR099C_rep1	44	13	46	9	0.295302	0.061321	0.308725	0.042453	
19	YBR101C_rep1	415	32	273	22	2.785235	0.150943	1.832215	0.103774	
20	YBR115C_rep1	292	106	211	70	1.959732	0.5	1.416107	0.330189	
21	YBR117C_rep1	11	16	2	5	0.073826	0.075472	0.013423	0.023585	
22	YBR119W_rep1	71	8	69	2	0.47651	0.037736	0.463087	0.009434	
23	YBR121C_rep1	882	489	641	319	5.919463	2.306604	4.302013	1.504717	
24	YBR123C_rep1	133	3	96	11	0.892617	0.014151	0.644295	0.051887	
25	YBR125C_rep1	189	5	161	0	1.268456	0.023585	1.080537	0	
26	YCR053W_rep1	1808	2	1147	0	12.13423	0.009434	7.697987	0	
27	YCR057C_rep1	427	2	215	0	2.865772	0.009434	1.442953	0	
28	YCR060W_rep1	531	24	400	164	3.563758	0.113208	2.684564	0.773585	
29	YCR062W_rep1	456	8	220	0	3.060403	0.037736	1.47651	0	
30	YCR064C_rep1	4	4	79	0	0.026846	0.018868	0.530201	0	
31	YCR066W_rep1	40	6	40	10	0.268456	0.028302	0.268456	0.04717	
32	YCR083W_rep1	354	2	346	1	2.375839	0.009434	2.322148	0.004717	
33	YCR085W_rep1	17	6	0	0	0.114094	0.028302	0	0	
34	YCR087C-A_rep1	433	9	313	0	2.90604	0.042453	2.100671	0	
35	YCR088W_rep1	1333	34	1044	21	8.946309	0.160377	7.006711	0.099057	
36	YCR090C_rep1	325	110	270	173	2.181208	0.518868	1.812081	0.816038	
37	YCR092C_rep1	147	11	73	0	0.986577	0.051887	0.489933	0	
38	YCR105W_rep1	6	2	1	0	0.040268	0.009434	0.006711	0	
39	YCR107W_rep1	72	7	63	4	0.483221	0.033019	0.422819	0.018868	
40	YDL002C_rep1	349	35	277	19	2.342282	0.165094	1.85906	0.089623	
41	YDL004W_rep1	351	46	303	37	2.355705	0.216981	2.033557	0.174528	
42	YDL006W_rep1	157	40	114	26	1.053691	0.188679	0.765101	0.122642	
43	YDL008W_rep1	152	29	97	21	1.020134	0.136792	0.651007	0.099057	
44	YDL022W_rep1	989	37	1557	37	6.637584	0.174528	10.44966	0.174528	
45	YDL024C_rep1	5	12	3	1	0.033557	0.056604	0.020134	0.004717	
46	YDL026W_rep1	6	0	1	0	0.040268	0	0.006711	0	

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D5150 Ratio					
	A	B	C	D	E
5150	ORF	RedFGtot	GrnFGtot	Ratio	high 200
5151	YIL120W_rep1	81	70	1.157143	
5152	YIL134W_rep1	105	76	1.381579	
5153	YIL136W_rep1	152	306	0.496732	good
5154	YIL138C_rep1	891	686	1.298834	good
5155	YIL140W_rep1	201	150	1.34	good
5156	YIL142W_rep1	749	594	1.260943	good
5157	YIL144W_rep1	81	58	1.396552	
5158	YJR001W_rep1	416	163	2.552147	good
5159	YJR003C_rep1	150	80	1.875	good
5160	YJR005W_rep1	206	156	1.320513	good
5161	YJR007W_rep1	972	692	1.404624	good
5162	YJR009C_rep17	11225	10858	1.0338	good
5163	YJR010W_rep1	248	67	3.701493	good
5164	YJR024C_rep1	350	295	1.186441	good
5165	YJR026W_rep1	1706	1123	1.519145	good
5166	YJR028W_rep1	1355	907	1.493936	good
5167	YJR030C_rep1	99	65	1.523077	
5168	YJR032W_rep1	203	161	1.26087	good
5169	YJR034W_rep1	233	240	0.970833	good
5170	YJR048W_rep1	590	468	1.260684	good
5171	YJR050W_rep1	115	103	1.116505	good
5172	YJR052W_rep1	95	89	1.067416	
5173	YJR054W_rep1	167	96	1.739583	good
5174	YJR056C_rep1	115	98	1.173469	good
5175	YJR058C_rep1	474	459	1.03268	good
5176	YJR072C_rep1	462	383	1.206266	good
5177	YJR074W_rep1	251	201	1.248756	good
5178	YJR076C_rep1	394	305	1.291803	good
5179	YJR078W_rep1	12	4	3	
5180	YJR080C_rep1	202	168	1.202381	good
5181	YJR083C_rep1	86	65	1.323077	
5182	YKL217W_rep1	17	3	5.666667	
5183	YKL219W_rep1	121	87	1.390805	good
5184	YKL221W_rep1	13	3	4.333333	

F1 Induction

	A	B	C	D	E	F	G
1	ORF	RedFGI	GmFGI	Ratio	high 20	Inducti	Repress
29	YCR062W_rep1	456	220	2.072727	good	ind	
37	YCR092C_rep1	147	73	2.013699	good	ind	
76	YDR449C_rep1	202	80	2.525	good	ind	
102	YFL002W-B_rep1	331	92	3.597826	good	ind	
117	YFL064C_rep1	295	87	3.390805	good	ind	
190	YJL011C_rep1	264	122	2.163934	good	ind	
228	YLL012W_rep1	157	69	2.275362	good	ind	
346	YOR296W_rep1	158	72	2.194444	good	ind	
349	YOR301W_rep1	164	77	2.12987	good	ind	
359	YOR343C-A_rep1	252	82	3.073171	good	ind	
457	YDL127W_rep1	147	69	2.130435	good	ind	
496	YEL045C_rep1	1622	579	2.801382	good	ind	
522	YGL014W_rep1	591	288	2.052083	good	ind	
561	YHR122W_rep1	160	75	2.133333	good	ind	
570	YHR149C_rep1	198	92	2.152174	good	ind	
574	YHR169W_rep1	236	114	2.070175	good	ind	
657	YLR409C_rep1	347	168	2.065476	good	ind	
658	YLR410W-A_rep1	170	60	2.833333	good	ind	
722	YOL151W_rep1	333	133	2.503759	good	ind	
839	YCR047C_rep1	260	115	2.26087	good	ind	
864	YDR075W_rep1	223	111	2.009009	good	ind	
870	YDR098C-A_rep1	1728	799	2.162703	good	ind	
919	YFL014W_rep1	566	262	2.160305	good	ind	
958	YHL049C_rep1	226	95	2.378947	good	ind	
976	YHR066W_rep1	436	176	2.477273	good	ind	
1070	YLR347C_rep1	377	176	2.142045	good	ind	
1074	YLR355C_rep1	4084	1771	2.306042	good	ind	
1108	YNL110C_rep1	1396	647	2.157651	good	ind	
1162	YOR306C_rep1	307	142	2.161972	good	ind	
1181	YPL267W_rep1	178	83	2.144578	good	ind	
1205	YLR212C_rep2	243	120	2.025	good	ind	
1211	YLR212C_rep3	268	120	2.233333	good	ind	
1306	YDR210W-A_rep1	241	114	2.114035	good	ind	
1415	YLL025W_rep1	153	73	2.09589	good	ind	

original \noC,G,R \noC,G,I/

1	ORF	RedFG	GrnFG	Ratio	high 20	Inducti	Repress
129	YGR008C_rep1	966	2304	0.419271	good		rep
218	YKR086W_rep1	823	1934	0.425543	good		rep
280	YMR104C_rep1	68	141	0.48227	good		rep
431	YBR216C_rep1	73	199	0.366834	good		rep
758	YPL014W_rep1	192	430	0.446512	good		rep
1066	YLR327C_rep1	260	667	0.389805	good		rep
1324	YEL011W_rep1	179	618	0.289644	good		rep
1333	YEL039C_rep1	179	560	0.319643	good		rep
1392	YHR092C_rep1	1251	2512	0.49801	good		rep
1633	YBR233W-A_rep1	384	1006	0.38171	good		rep
2054	YPL230W_rep1	64	168	0.380952	good		rep
2319	YLR177W_rep1	113	305	0.370492	good		rep
2604	YGR249W_rep1	58	168	0.345238	good		rep
2621	YHL021C_rep1	287	724	0.396409	good		rep
2697	YKR058W_rep1	129	301	0.428571	good		rep
2719	YLR258W_rep1	513	1039	0.493744	good		rep
2911	YBR298C_rep1	84	210	0.4	good		rep
2948	YDR277C_rep1	301	690	0.436232	good		rep
2984	YER067W_rep1	523	3086	0.169475	good		rep
3039	YHR185C_rep1	21	190	0.110526	good		rep
3066	YJL142C_rep1	293	591	0.49577	good		rep
3570	YMR103C_rep1	122	288	0.423611	good		rep
3571	YMR105C_rep1	568	1256	0.452229	good		rep
3726	YDL048C_rep1	83	331	0.250755	good		rep
4152	YDR070C_rep1	91	209	0.435407	good		rep
4154	YDR074W_rep1	400	1156	0.346021	good		rep
4319	YKR075C_rep1	79	191	0.413613	good		rep
4418	YOL052C-A_rep1	193	453	0.426049	good		rep
4466	YPL247C_rep1	160	321	0.498442	good		rep
4551	YBR183W_rep1	114	230	0.495652	good		rep
4588	YDR171W_rep1	684	1537	0.445023	good		rep
4635	YFR015C_rep1	361	1539	0.234568	good		rep
4636	YFR017C_rep1	240	883	0.271801	good		rep

Filter Mode

Q #1	Q #2	Q #3	Q #4	Q #5	Q #6	Q #7	Q #8	Q #9	Q #10	Q #11	Q #12	Q #13	Q #14	Q #15	Q #16	Q #17	Q #18	Q #19	Q #20	Student status	GPA
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	JR	3
3	3	3	3	3	4	3	2	3	3	4	4	3	3	4	3	4	3	3	3	SR	3.23
3	3	3	3	3	4	4	3	3	3	4	4	4	3	3	3	3	3	4	4	JR	2.2
3	3	3	3	3	1	3	1	1	1	1	1	1	2	2	0	1	3	4	0	JR	2.99
4	4	4	3	3	0	3	4	4	2	3	3		2	4	4	3	2	4	4	JR	3.5
3	3	3	4	4	0	0	0	1	0	3	3	3	3	0	0	2	3	4	3	SO	2.92
3	3	4	1	3	1	3	0	0	0	3	4	4	0	0	0	3	3	3	3	SO	2.6
4	4	4	5	4	3	4	3	3	4	3	4	3	4	4	4	5	5	5	4	JR	3
3	3	3	4	2	0	0	0	0	3	3	3	3	3	0	0	0	2	4	2	SO	3.5
3	4	4	5	5	4	5	5	4	4	5	4	4	5	4	4	4	5	5	5	JR	2.6
5	5	2	3	5	1	3	1	1	1	2	1	5	5	1	5	5	5	5	2	SO	2.7
4	3	2	3	3	4	3	1	3	4	3	3	3	4	3	3	3	4	5	4	SO	2.6
0	2	2	3	2	2	2	3	3	2	3	3	2	3	3	2	2	2	3	3	JR	2.7
2	3	3	3	3	2	3	2	3	2	3	2	3	3	2	3	2	3	2	3	JR	3.3
3	2	2	3	2	3	3	2	3	2	1	3	3	4	2	2	3	4	4	3	SO	2.9
5	5	5	5	5	3	4	4	4	3	5	5	4	5	5	5	5	5	5	5	SO	3
3	3	2	2	3	3	3	1	0	2	3	3	4	4	2	3	3	0	3	3	SO	3
3	3	5	4	4	2	4	3	4	0	4	0	0	3	4	2	3	3	4	0	SO	3.4
3	3	2	4	3	0	3	3	3	4	4	4	4	4	4	3	3	2	3	4	SO	2.29