New data analysis and development of new GRNsight functionality will aid researchers in discovering more about gene regulatory networks.

Blair V. Hamilton, Emma C. Tyrnauer, Katherine L. Wright, and Zachary S. Van Ysseldyke

BIOL/CMSI 367-01: Biological Databases

15 December 2017

Introduction

All organisms have certain mechanisms that allow them respond to and withstand environmental stressors such as high or low temperatures, chemicals, radiation, and disease. Multicellular organisms, such as humans, can deal with this stress in a myriad of different ways on several different biological system levels; humans can shiver, send signals via the endocrine system, digest food in the stomach, move muscles, and synthesize proteins, all in order to combat, for example, cold stress.

Cold stress affects nearly every living thing during its lifetime, and it is important that we learn as much as we can about it. Cold stress response in humans can be studied in a number of different ways, but is difficult to do so on the cellular level; stress response at the cellular level usually requires the use of living cells. Fortunately, cells of the model organism *Saccharomyces cerevisiae* (yeast) can be used by researchers to run experiments without the ethical concerns that come from experimentation with animal cells.

According to the SGD wiki, yeast is an ideal model organism for studying Eukaryotic cells. Yeast cells grow and multiply quickly; under the right conditions, the number of cells in a sample can double in 90 minutes. Yeast are similar to higher eukaryotes in cellular organization, and they can resist and respond to many different environmental stressors. In addition, since Yeast unicellular organisms, yeast are easy to manipulate in lab. Studying the way yeast deal with cold shock can help researchers understand how humans respond to cold shock on the cellular level. In particular, researchers would like to know more about changes in gene expression in response to cold shock

This goal can be achieved through the use of DNA microarray. DNA Microarray has a fascinating history; according to Lenoir & Giannella (2006), the development of new DNA microarray techniques was the result of several years of collaboration between public research universities, industrial firms, and Silicon Valley tech startups. This collaboration is the reason why microarray techniques have become increasingly inexpensive, and increasingly popular. The use of DNA microarray is very popular in the life sciences; according to the authors, the yearly number of research articles published citing microarray data has skyrocketed since 2000 (Lenoir & Giannella, 2006).

In this study, microarray data from the lab of Dr. Kam Dahlquist was analyzed and manipulated to create an additional gene regulatory network that can be viewed using GRNsight. DNA microarrays work by binding single-stranded DNA probes to specific places on a microarray chip. These probes will bind to specific, fluorescence labeled target DNA that they are complementary to. The expression of genes in a cell can be measured by extracting RNA from the cell and using reverse transcriptase to create cDNA. When the cDNA, and attaching fluorescent probes to the cDNA. If there are high levels of the target gene in a cell, the complementary DNA spot on the microarray chip will glow brightly.

The development of an online application, GRNsight, occurred when researchers at Loyola Marymount University wanted an easy way to visualize gene regulatory networks (Dahlquist et al., 2016). DNA Microarrays are one of the tools to measure gene regulatory networks, and after some analysis and manipulations of the data, it can be uploaded to GRNsight for viewing. Depending on the extent of data analysis, GRNsight will provide either an unweighted (figure 3) or weighted (figure 4) network. The unweighted network simply shows which genes in your data set are connected. The weighted network shows the direction and magnitude of these connections; the lines are pink or blue depending on whether a gene is up or down regulated, and the direction of the arrows indicate the direction of regulation. This network visualization tool provides an invaluable way for researchers to understand the gene regulatory networks that they are studying.

Constant innovation allows researchers to make more new discoveries at faster rates each year. The goal of GRNsight's is to make genetic research easier and faster. One way to achieve this would be adding the ability to learn about the genes in these networks at the touch of a button. Therefore, it was decided to add a new right-click functionality to GRNsight. The idea is that when a user right-clicks on a gene name in their gene network, a new tab will open populated with important information about the target gene. Making gene information readily available in this manner eliminates the need for extensive database searching; this project will make gene research more efficient for future researchers, thereby leading to even more discoveries about gene regulatory networks.

Methods, Results, and Discussion

Data Analyst

Data analysis for wild type microarray data began in week 8. Dr. Dahlquist provided an excel spreadsheet containing the log2 fold changes in gene expression for five time points: 15, 30, 60, 90, and 120 minutes. The yeast were exposed to cold shock at 13°C followed by a 60 minute recovery period at 30°C. An ANOVA test was performed in excel to determine if any genes had an expression change that was significantly different than 0 at any time point after cold shock (specifics of procedure for this analysis can be accessed on *Emmatyrnauer Week 8* individual assignment page) . As p-value values parameters for significance became more stringent, fewer genes were considered to have significantly changed gene expression following cold shock (Table 1). However, the Benjamini and Hochberg-corrected p<0.05 parameter revealed the second largest percentage of genes demonstrating change in expression following cold shock (Table 1).

Table 1 *Percentage of genes demonstrating significant changes in gene regulation decreased with stricter ANOVA p-values.*

ANOVA	***Wild type***
p<0.05	2,528 (40.85%)
p<0.01	1,652 (26.70%)
p<0.001	919 (14.85%)
p<0.0001	496 (8.01%)
Benjamini and Hochberg-corrected p<0.05	1,822 (29.44%)
Bonferroni-corrected p<0.05	248 (4.01%)

In week 10, STEM (Short Time-series Expression Miner) was utilized to cluster *wild type* genes into groups with similar responses to cold shock (detailed procedure can be accessed on *Emmatyrnauer Week 10* individual assignment page) (Fig. 1). These clusters were ordered based on significance (Fig. 1). While some clusters demonstrated clear downregulation of genes following cold shock (profiles 9 and 0), others demonstrated clear upregulation (profiles 45 and 48) (Fig. 1).

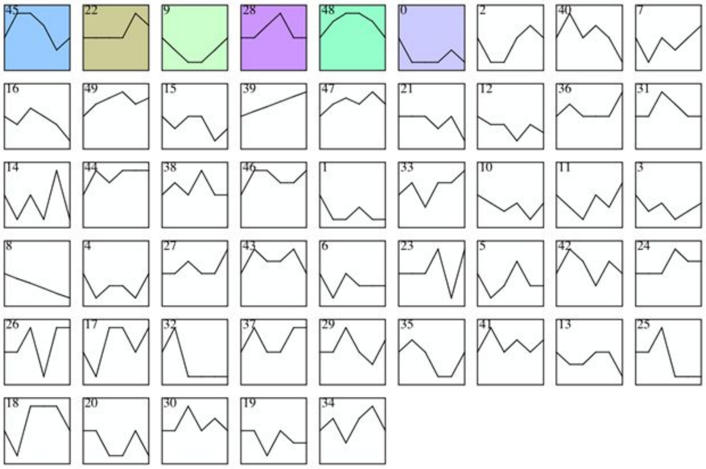


Figure 1. Gene clusters for *wild type* data demonstrating similar changes in gene expression following cold shock. Colored clusters are the most significant. Clustering was performed with STEM software.

Profile 45 cluster was selected for further analysis for various reasons; a large number of genes were assigned to this profile (549) and they demonstrated very clear upregulation following cold shock (Fig. 2). Specifically, genes were upregulated between 0m and 15m, maintained at a constant regulation between 15m and 60m, and then recovered to initial regulation starting at 60m (Fig. 2). Interestingly, some of the genes in this cluster were significantly downregulated following exposure to 30°C (Fig. 2). This suggests that the proteins that are used to maintain viability of the cell following cold shock are unnecessary upon exposure of the cells to warmer temperatures.

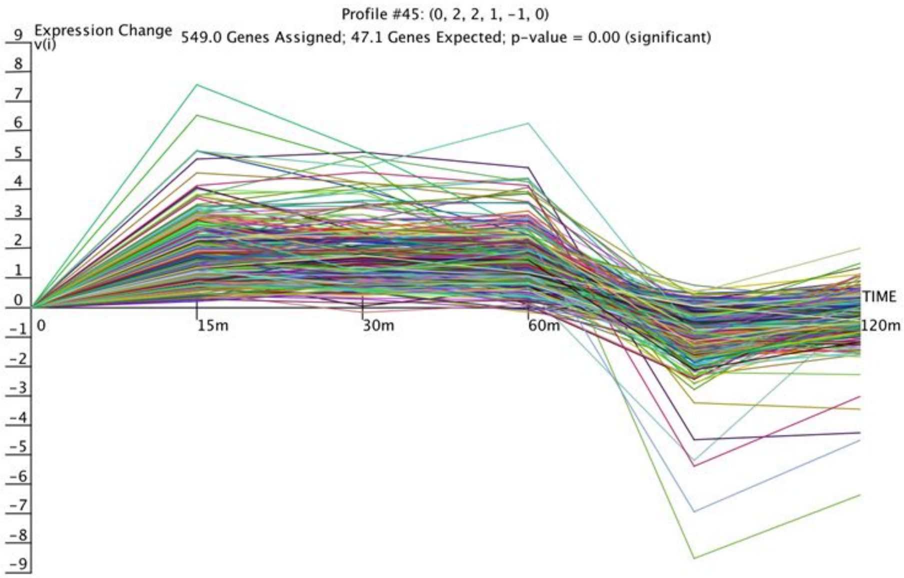


Figure 2. Expression changes for profile 45 genes over time. Yeast cells were exposed to 13°C at 0m and allowed to recover at 30°C starting 60m.

The function of the genes from profile 45 was examined further through analysis of the gene ontology terms associated with this cluster. Only three terms were selected for Table 2 because the list is very extensive. However, the complete file can be accessed from both *Emmatyrnauer Week 10* individual page as well as *Lights, Camera, InterACTION! Deliverables* page. All three terms in Table 2 have similar numbers of genes assigned to them. However, only “nuclear lumen” and “RNA binding” have associated p-values that are considered significant (Table 2). This suggests that while some genes that were upregulated in this profile were involved in cellular component organization, this change in expression was not significantly different compared to that prior to cold shock. On the other hand, “nuclear lumen” and “RNA binding” have very small associated p-values as well as corrected p-values. This is most likely due to the fact that these activities are extremely necessary for survival of the cell in extreme environmental conditions such as cold shock. This does not line up exactly with what Murata et al. (2006) suggest in *Genome-wide expression analysis of yeast response during exposure to 4 C*. These authors infer that many of the many heat shock proteins are utilized under cold shock due to the fact that they are both exposures to extreme environmental conditions (Murata et al. 2006).

Table 2. *Three gene ontology terms associated with Profile 45.*

Category name	**# Genes category**	**# Genes assigned**	**# Genes expected**	**# Genes enriched**	**P-value**	**Corrected p-value**	**Fold**
Cellular component organization (GO:0016043)	540	161	162.7	-1.7	0.6	1	1
Nuclear lumen (GO: 0031981)	327	183	98.5	84.5	2.40E-27	<0.001	1.9
RNA binding	215	116	64.8	51.2	6.30E-15	<0.001	1.8

The next steps of data analysis required examining which transcription factors were utilized in this cluster of genes that resulted in similar regulation. The Yeastract database was able to identify the most significant transcription factors (with the smallest p-values) involved in the expression of these genes. The initial network that was produced was too large and needed to be trimmed. This was completed by removing less significant transcription factors one by one and visualizing the network in GRNsight (to ensure that there were no free floating genes). The final group of transcription factors which were visualized in GRNsight and their associated p-values are represented in Table 3.

Table 3. *Most significant transcription factors for profile 45 with associated p-values.*

Transcription factor	**NDT80**	**YAP1**	**PDR3**	**UME6**	**MIG2**	**ZAP1**	**HAP4**	**CIN5**
P-value	9.74E-10	1.31E-09	2.82E-09	6.12E-09	1.96E08	2.99E-03	3.02E-02	0.806
Transcription factor	**YOX1**	**SFP1**	**YHP1**	**SUT1**	**STB5**	**ACE1**	**MSN2**	**GLN3**
P-value	0	0	0	7E-15	7E-15	1.9E-14	2.72E-13	9.15E-11

Transcription factors from Table 3 were utilized to generate a regulatory network with Yeastract; however, this network file was in table format. To better visualize the network, it was opened in GRNsight, which produced Figure 3. While this map allows for easier visualization of the network compared to Yeastract's table, it fails to detail the nature and magnitude of the interaction between the genes (i.e. whether or not the interaction is strong or weak, and induction or repression). As a result, Matalab was utilized to generate a weighted network output file, which when opened in GRNsight, produced Figure 4.

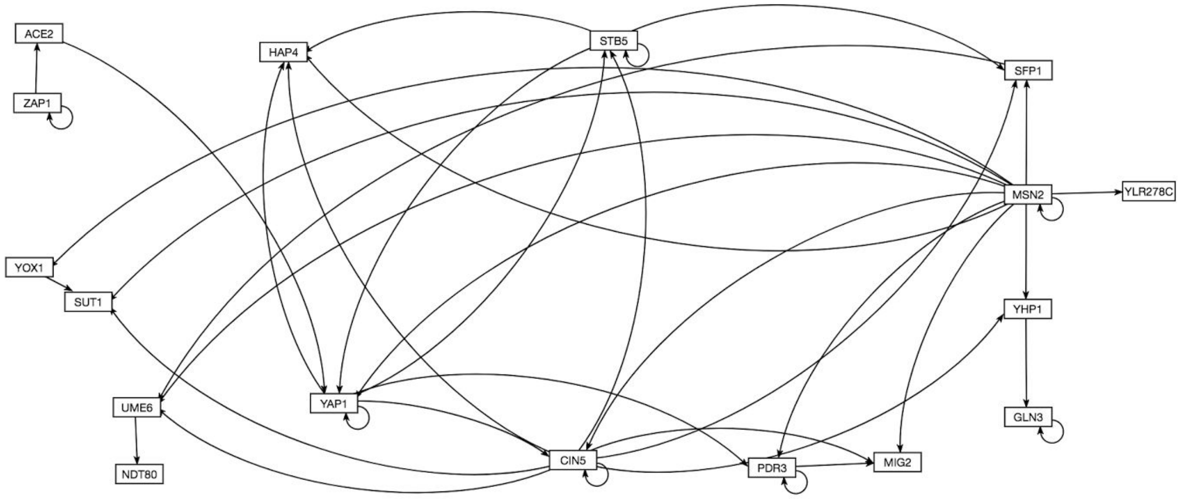


Figure 3. Unweighted gene regulatory network generated with GRNsight

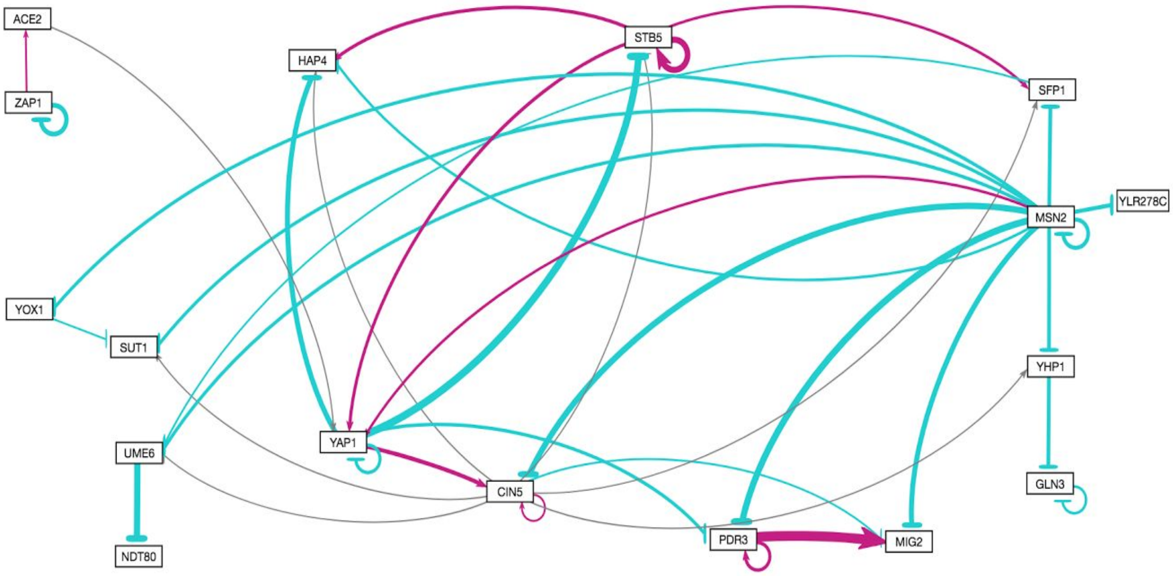


Figure 4. Weighted version gene regulatory network from Figure 3 generated with GRNsight. Weighs were determined using Matlab. Blue lines with terminating bars indicate repression of the gene at the end of the bar, while pink arrows indicate induction of genes.

The gene regulatory network from Figure 4 reveals that most interactions involved repression of other genes. MSN2 represses 10 genes, including itself, indicating its importance in this regulatory network (Figure 4). On the other hand, STB5 is only involved in induction of 4 genes, including itself (Figure 4). While some genes are only repressed by genes belonging to the network, others are both induced and repressed; for example, CIN5 is induced by YAP1 but repressed by MSN2 (Figure 4). This may suggest a system of “checks and balances” so that genes are not overly expressed or underexpressed.

Project Manager and Quality Assurance

It was the job of the PM/QA to keep the team on track to finish their tasks, and to make sure that a satisfactory quality of work was maintained. One responsibility of the project managers was to decide which types of data should be retrieved from each of 5 major databases: JASPAR, NCBI, Ensembl, Uniprot and SGD. The conclusions can be found in tables 4-8.

Table 4 *Data to be retrieved from JASPAR*

Data	Reasoning
Class	This information is relevant to all genes, so it probably shouldn't have been retrieved from JASPAR. It is suggested that the gene page is re-coded to retrieve this data from a different database.
Family	
Matrix ID	JASPAR only contains information about genes that code proteins that act as transcription factors. This information is only relevant for transcription factors, so it is appropriate to use JASPAR for these elements. Matrix ID is unique to JASPAR, helps user find the gene on JASPAR.
Sequence Logo	
Frequency Matrix	

Table 5 *Data to be retrieved from NCBI*

Data	Reasoning
NCBI Gene ID	So users may easily find this gene in NCBI database.
Locus Tag	This information can be easily found in the summary section of a gene page within NCBI, which the project managers concluded would be easy for the coders to retrieve.
Also Known As	
Chromosome Sequence	NCBI has an extensive library of reference sequences. The project managers believed that this database would be most reliable in this regard.
Genomic Sequence	

Table 6 *Data to be retrieved from Ensembl*

Data	Reasoning
Gene ID	So users may easily find this gene in Ensembl database.
Description/function	We wanted descriptions from multiple databases
DNA sequence	Ensembl is a genome database, so genomic information is best found here.
Gene Location	
Gene Map	

Table 7 *Data to be retrieved from Uniprot*

Data	Reasoning
Uniprot Gene ID	So users may easily find this gene in Uniprot database.
Protein Sequence	Uniprot is known for its information on proteins, so the Project Managers decided that this database was the most appropriate choice.
Similar Protein	
Protein type/name	
Species	The species is easily found at the top of each protein page.

Table 8 *Data to be retrieved from SGD*

Data	Reasoning
SGD Gene ID, Standard Name, Systematic name	For easy reference
Regulation: Regulators & targets	Has in-depth information about these topics in yeast. Since SGD is a database that contains solely yeast data, it is suggested that this information is retrieved from another database if GRNsight incorporates data from other species.
Interaction: Total interactions, Physical interactions, Genetic interactions	
Gene ontology: Summary, Molecular function, Biological process, Cellular component	

An introduction to the coding methods, results, and discussion

It is the integration team's responsibility to uphold the integrity for other's code. Furthermore, through integration, all of the code would be represented on one consolidated webpage. Specifically, the integration team was tasked with coding a right click function and populating the page for the specific gene on the website. Before beginning, it was known that not all of the code would be in pristine condition the first few merges. With that in mind, it was still possible to construct a skeleton for the other team's code so it could easily be inserted into the skeletal framework.

Handling code from backend to front end demands consistency between IDs forcing careful code review. Then, the team is able to work on the right click function. The right click function bridged the existing GRNSight website with the new class designed webpage. Through communication with the page design team, the integration team effectively assimilated the code to represent where they wanted the data to go. Furthermore, the API team instantiated variables and functions which the integration team had to understand before manipulating the API calls.

Coders

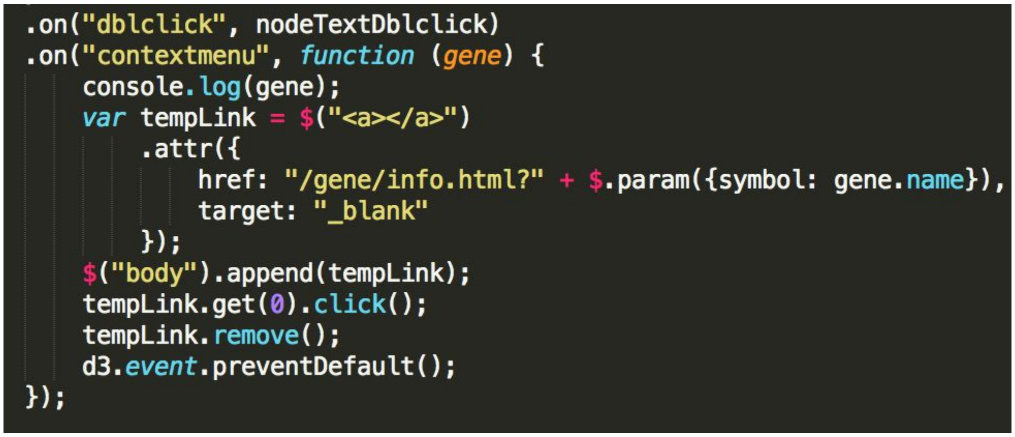
In order to make the GRNsight website more useful, Zach Van Ysseldyk and Blair Hamilton were placed in charge of the coding portion for the Interaction and Integration Team. Their work began Week 11 of the course with a journal club presentation on "The secret startup that saved the worst website in America" article written by Robinson Meyer. The article discusses the work of a small team in charge of fixing the Healthcare.gov website.

Healthcare.gov had many technical issues such as frequent crashes, and it was difficult for users to navigate.

During the journal club presentation held during Week 12, Zach and Blair discussed the relationship between this article and the hopes for the GRNsight project. One of the many takeaways from the article is the importance of communication with all stakeholders, in particular coders, data analysts, and project managers. The article mentions different communication pathways used, such as email and "HipChat," an instant messaging application for the work environment. This proved powerful: when we reached roadblocks, using multiple communication platforms allowed for easy access for questions and coding assistance both between Zach and Blair,and with other group members, coders and Drs. Dahlquist and Dionisio.

Following the Week 12 presentation, Zach and Blair created a fork off of the GRNsight github open source code and then collaborated with all other coders on their respective teams. Once collaboration forks were created Zach and Blair began work on creating a right-click functionality for the GRNsight page used for when a user clicks on a gene.

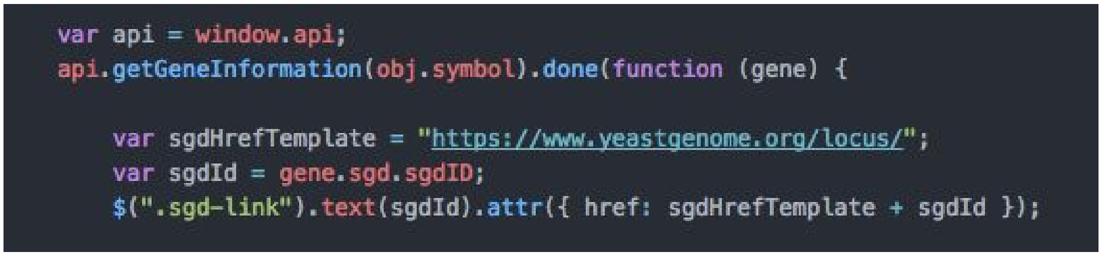
The right click function bridges the existing GRNSight website to the newly developed gene page. First, the user lands on a gene regulatory diagram. If the user desires more information on the gene, they may right click on the gene node to open up a new tab with the information about the gene. First, before even thinking about the gene page, one must code a function so that the right click opens up a new tab. Below one may find the code for the whole right click function.



```
.on("dblclick", nodeTextDblclick)
.on("contextmenu", function (gene) {
    console.log(gene);
    var tempLink = $("<a></a>")
        .attr({
            href: "/gene/info.html?" + $.param({symbol: gene.name}),
            target: "_blank"
        });
    $("body").append(tempLink);
    tempLink.get(0).click();
    tempLink.remove();
    d3.event.preventDefault();
});
```

The “`target: “_blank”;`” line is supposed to open up a new tab. However because of security reasons, the browsers denied this request to open up a new tab. With the help of Professor Dionisio, the integration team overcame this problem by creating a fake anchor. The fake anchor, denoted as: `var tempLink = $(“<a></a>”)` acts as a temporary landing page for our tab. Then, the page is able to land. After the page lands, the anchor is taken away denoted as: `tempLink.remove();`. The actual link that the user will see is noted as the “href.” A href consists of a database template URL which is then concatenated by tagging on the specific gene that the user clicks on.

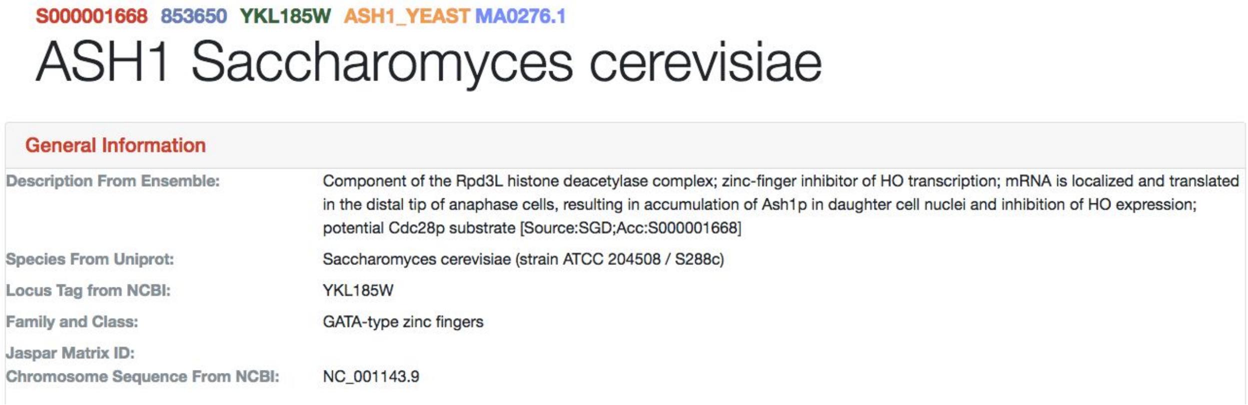
After the right-click function was working appropriately, the next milestone for the coders was to identify and integrate the NCBI, Ensembl, SGD, UniProt and JASPAR database data. Through the work of the gene database API and JASPAR teams, API functions were implemented to retrieve the desired data requested by the project managers and biologists in the class. This data needed a desirable layout and functionality to it which was implemented by the page design team to create an easy-to-use website for a central database data location. But these teams needed to come together in order to have a productive page, so this is where the interaction and integration team comes in. Zach and Blair were given two types of code, HTML and Javascript (js), in order to weld together the three groups' work. This was done through an info.js file that stored variable links between the data classes on the HTML page and the API calls set up by the API and JASPAR teams. Below is an example of the code that links these files:



```
var api = window.api;
api.getGeneInformation(obj.symbol).done(function (gene) {

    var sgdHrefTemplate = "https://www.yeastgenome.org/locus/";
    var sgdId = gene.sgd.sgdID;
    $(".sgd-link").text(sgdId).attr({ href: sgdHrefTemplate + sgdId });
```

The `var api` represents the overall api object that will contain all the necessary data points and links. The `getGeneInformation(obj.symbol)` represents the function the API teams made in order to retrieve the data from each desired database. Similar to the picture above, each database was given an HrefTemplate that contained the database's generic gene page URL. Once this template was set up almost every variable link utilized the template to reference that database's URL. For the picture above an `sgdID` was created to retrieve sgd specific numbering system, their "ID", for the gene that was selected and right-clicked. `Gene.sgd.sgdID` represents the pathway taken to retrieve the id from the overall gene object, then the sgd api calls, then finally the sgdID location named by the API team. Lastly, the third code portion: `$(".sgd-link").text(sgdId).attr({ href: sgdHrefTemplate + sgdId });`, represents the HTML class name set up by the page design team, next the data retrieved and finally the URL needed to reference the database plus the gene id. The picture below represents the website in its current form displaying the variable links, ids being shown and tabs with the desired data within it.



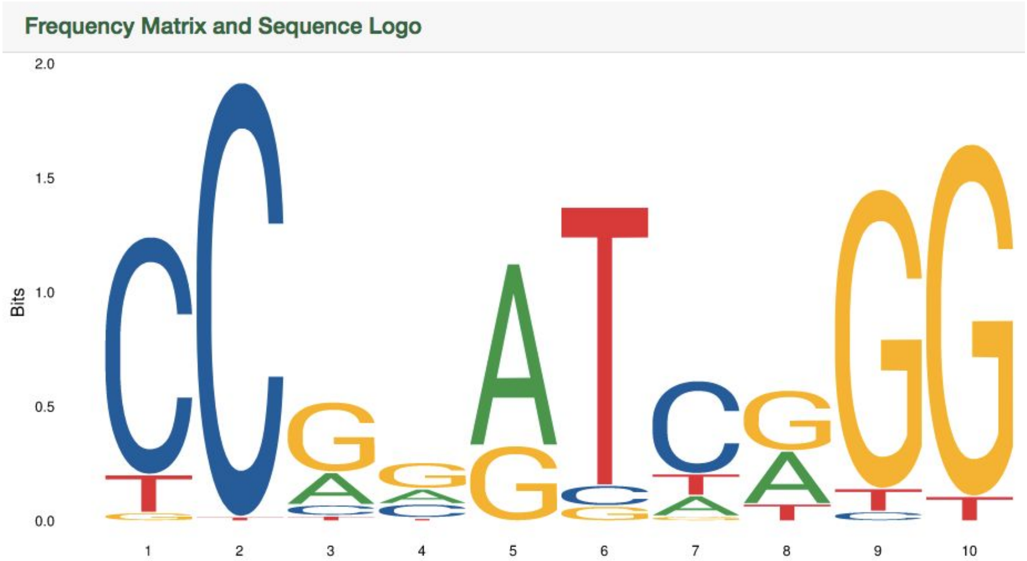
S000001668 853650 YKL185W ASH1_YEAST MA0276.1

ASH1 Saccharomyces cerevisiae

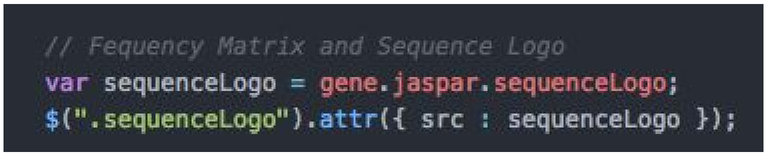
General Information

Description From Ensemble:	Component of the Rpd3L histone deacetylase complex; zinc-finger inhibitor of HO transcription; mRNA is localized and translated in the distal tip of anaphase cells, resulting in accumulation of Ash1p in daughter cell nuclei and inhibition of HO expression; potential Cdc28p substrate [Source:SGD;Acc:S000001668]
Species From Uniprot:	Saccharomyces cerevisiae (strain ATCC 204508 / S288c)
Locus Tag from NCBI:	YKL185W
Family and Class:	GATA-type zinc fingers
Jaspar Matrix ID:	
Chromosome Sequence From NCBI:	NC_001143.9

Every variable link has the above layout except for the sequence logo and the frequency matrix. The sequence logo required an img class on the info.html page in order to implement the image to appear on the website. The code to display this information and how it appears on the site is below:

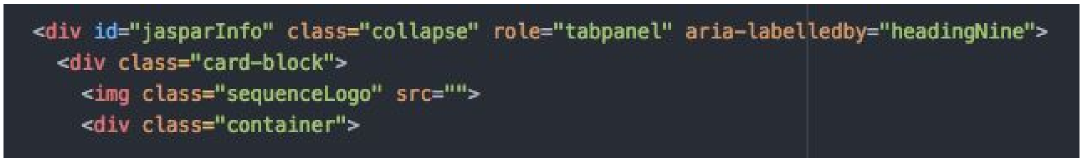


(sequence logo)



```
// Fequency Matrix and Sequence Logo
var sequenceLogo = gene.jaspar.sequenceLogo;
$(".sequenceLogo").attr({ src : sequenceLogo });
```

(info.js)

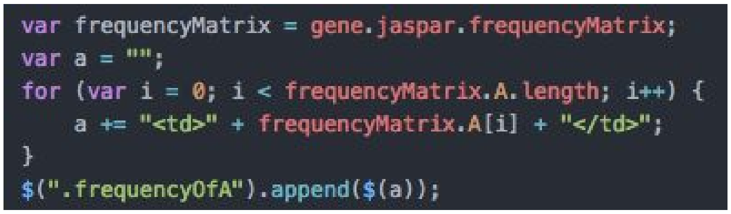


```
<div id="jasparInfo" class="collapse" role="tabpanel" aria-labelledby="headingNine">
  <div class="card-block">
    <img class="sequenceLogo" src="">
    <div class="container">
```

(info.html)

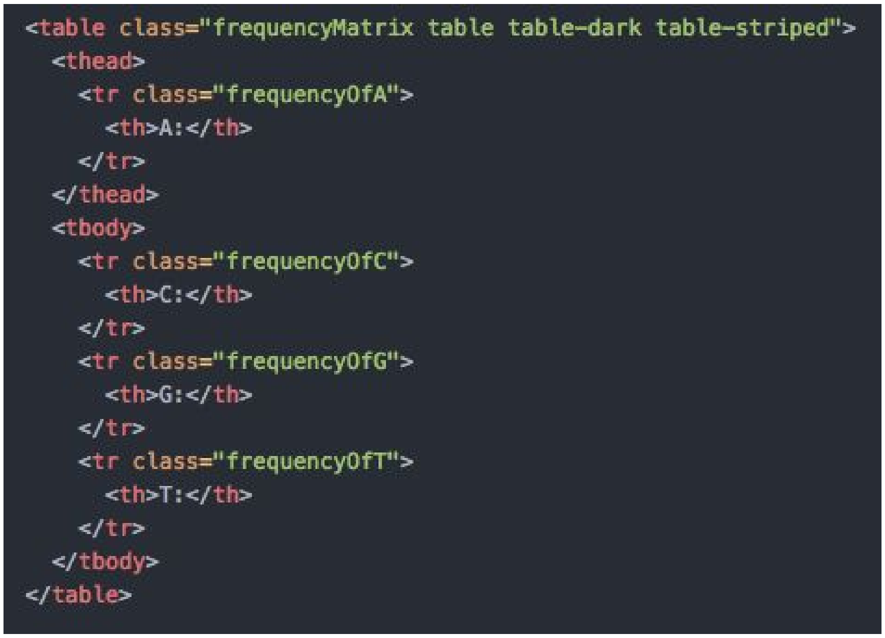
As described above the info.html file contains an img class that is different that the p classes for the gene ids and other variable links. The other difference revolves around the attr. Tag having a src call instead of an href. When talking with the JASPAR coding teams it was explained that their URL was already attached to their API call for the sequenceLogo so the only request needed was the name of the call.

The second variable link that is different is the frequency matrix which is in the form of a table. The code looks as follows:



```
var frequencyMatrix = gene.jaspar.frequencyMatrix;
var a = "";
for (var i = 0; i < frequencyMatrix.A.length; i++) {
    a += "<td>" + frequencyMatrix.A[i] + "</td>";
}
$(".frequencyOfA").append($(a));
```

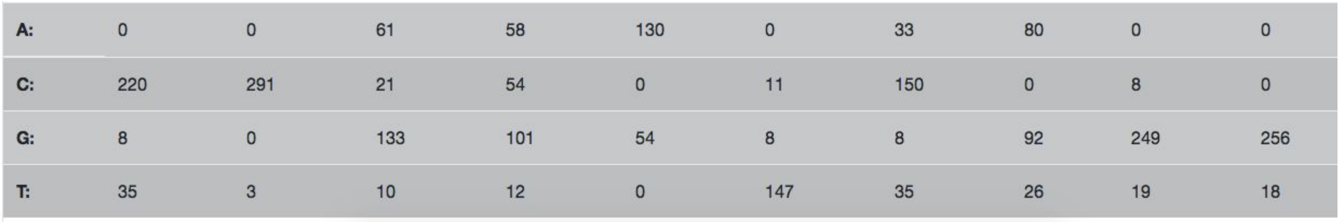
(info.js)



```
<table class="frequencyMatrix table table-dark table-striped">
  <thead>
    <tr class="frequencyOfA">
      <th>A:</th>
    </tr>
  </thead>
  <tbody>
    <tr class="frequencyOfC">
      <th>C:</th>
    </tr>
    <tr class="frequencyOfG">
      <th>G:</th>
    </tr>
    <tr class="frequencyOfT">
      <th>T:</th>
    </tr>
  </tbody>
</table>
```

(info.html)

In order to preserve the table look of the JASPAR site, the page design team and the interaction and integration team collaborated on this table layout. For the injo.js file the coders created separate for loops in order to parse through the frequency matrix data as it could range between 0-15 integer results. Once the for loops were created the coders created the link by appending to each A,C,G, and T class. The finished product looks as follows, this particular example is for the gene ASH1:



A:	0	0	61	58	130	0	33	80	0	0
C:	220	291	21	54	0	11	150	0	8	0
G:	8	0	133	101	54	8	8	92	249	256
T:	35	3	10	12	0	147	35	26	19	18

Conclusion

Cold shock response in wild-type yeast is a complicated process. Hundreds of genes are involved, coding many different classes of proteins. Many of these genes are responsible for activating or repressing other genes. A few of the GO terms in our regulatory network were Cellular component organization, Nuclear lumen, and RNA Binding. These terms are different from those found in a paper by Murata et al. in 2006. That paper claimed that proteins involved in energy use, metabolism, and protein synthesis were significantly regulated during cold shock. We are unaware if other papers corroborate our findings. However, we believe that our contribution of a new regulatory network to lead to more discoveries in this field.

Additionally, the GRNsight page now possesses a right-click function that brings the user a new tab that contains gene information. The gene page contains data from the following sources: NCBI, SGD, Ensembl, UniProt and JASPAR. This data has been organized and carefully chosen by each Biological Database team, and all necessary code files and information has been pushed to the GRNsight github page. The new gene page will facilitate data analysis by allowing researchers to learn more about associated genes in a regulatory network with no need for extensive database searching.

Finally, we used some of the lessons learned from Meyer's 2015 paper while working on our own project. We made it a goal to communicate early and often with our teammates. We also tried to work on the project, even when there seemed to be roadblocks or holdups. We learned a lot of hard skills over the course of this project, from data analysis to coding, but we also learned many lessons about time management and teamwork.

In future work, we would like GRNsight to be able to handle gene regulatory networks from multiple species, since it is currently restricted to *Saccharomyces cerevisiae*. On the gene page, we would like to add a function to alert the user that the page is loading or that data are being pulled. We would also like to see on the gene page a prediction of other genes which may appear in regulatory networks with the selected gene. We believe these new directions would greatly enhance GRNsight and the future of gene regulatory research.

Acknowledgements

We would first like to thank our professors, Dr. Kam D. Dahlquist and Dr. John David N. Dionisio, for their help and support throughout this project; we learned much about databases, coding, research best practices, and much more.

We would also like to thank all of our fellow students in the BIOL/CMSI 367-01: Biological Databases course. We worked with each member of the class at some point during this semester, and we learned from each other in ways which impacted our final project. We extend particular thanks to the following students: Eddie Azinge and Eddie Bachoura, for their coding expertise; Hayden Hinsch, Quinn Lanners, and Corinne Wong, for their support of our own Project Manager.

References

Dahlquist, K. D., Dionisio, J. D. N., Fitzpatrick, B. G., Anguiano, N. A., Varshneya, A., Southwick, B. J., & Samdarshi, M. (2016). GRNsight: a web application and service for visualizing models of small-to medium-scale gene regulatory networks. *PeerJ Computer Science*, *2*, e85. https://doi.org/10.7717/peerj-cs.85

Gene Ontology Consortium. (2017). The Gene Ontology. Retrieved November 19, 2017, from http://geneontology.org

Get url parameter jquery Or How to Get Query String Values In js. Retrieved December 04, 2017, From https://stackoverflow.com/questions/19491336/get-url-parameter-jquery-or-how-to-get-query-string-values-in-js

GRNsight (2017) Retrieved December 4, 2017, from http://dondi.github.io/GRNsight/

How to retrieve GET parameters from javascript? Retrieved December 04, 2017, from https://stackoverflow.com/questions/5448545/how-to-retrieve-get-parameters-from-javascript

JavaScript Window Location. Retrieved December 04, 2017, from https://www.w3schools.com/js/js_window_location.asp

Lenoir, T., & Giannella, E. (2006). The emergence and diffusion of DNA microarray technology. *Journal of biomedical discovery and collaboration*, *1*(1), 11.

LMU BioDB 2017. (2017). Coder. Retrieved November 28, 2017, from https://xmlpipedb.cs.lmu.edu/biodb/fall2017/index.php/Coder

LMU BioDB 2017. (2017). Week 8. Retrieved October 23, 2017, from https://xmlpipedb.cs.lmu.edu/biodb/fall2017/index.php/Week_8

LMU BioDB 2017. (2017). Week 9. Retrieved November 19, 2017, from https://xmlpipedb.cs.lmu.edu/biodb/fall2017/index.php/Week_9

LMU BioDB 2017. (2017). Week 10. Retrieved December 4, 2017, from https://xmlpipedb.cs.lmu.edu/biodb/fall2017/index.php/Week_10

Matlab. Retrieved December 7, 2017, from LMU's computer lab https://www.mathworks.com /products/matlab.html

Meyer, R. (2015, July 09). The Secret Startup That Saved the Worst Website in America. Retrieved November 7, 2017, from https://www.theatlantic.com/technology/archive/ 2015/07/the-secret-startup-saved-healthcare-gov-the-worst-website-in-america/397784/

Murata, Y., Homma, T., Kitagawa, E., Momose, Y., Sato, M. S., Odani, M., ... & Fujita, K. (2006). Genome-wide expression analysis of yeast response during exposure to 4 C. *Extremophiles*, 10(2), 117-128.

Short Time-series Expression Miner (STEM). (2006). Retrieved November 19, 2017, from http://www.cs.cmu.edu/~jernst/stem/

What are yeast? (last update 3 October 2016) Retrieved December 15, 2017, from the SGD Wiki:

http://wiki.yeastgenome.org/index.php/What_are_yeast%3F

Window.location. Retrieved December 04, 2017, from

https://developer.mozilla.org/en-US/docs/Web/API/Window/location

YEASTRACT. Retrieved December 4, 2017, from

http://www.yeastract.com/formgenerateregulationmatrix.php