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Hypothesis-Driven Specialization-based Analysis of Gene Expression Association Rules

by

Dharmesh Thakkar

A Thesis

Submitted to the Faculty

of the

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Abstract

During the development of many diseases such as cancer and diabetes, the pattern of gene expression within certain cells changes. A vital part of understanding these diseases will come from understanding the factors governing gene expression. This thesis work focused on mining association rules in the context of gene expression. We designed and developed a tool that enables domain experts to interactively analyze association rules that describe relationships in genetic data. Association rules in their native form deal with sets of items and associations among them. But domain experts hypothesize that additional factors like relative ordering and spacing of these items are important aspects governing gene expression.

We proposed hypothesis-based specializations of association rules to identify biologically significant relationships. Our approach also alleviates the limitations inherent in the conventional association rule mining that uses a support-confidence framework by providing filtering and reordering of association rules according to other measures of interestingness in addition to support and confidence. Our tool supports visualization of genetic data in the context of a rule, which facilitates rule analysis and rule specialization. The improvement in different measures of interestingness (e.g., confidence, lift, and p-value) enabled by our approach is used to evaluate the significance of the specialized rules.

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Chapter 1

Introduction

During the development of many diseases such as cancer and diabetes, the pattern of gene expression within certain cells changes. A vital part of understanding these diseases will come from understanding the factors governing gene expression. This thesis work focused on association rules mined in the context of gene expression. We designed and developed a tool that enables domain experts to interactively analyze association rules that describe relationships in genetic data. Association rules in their native form deal with sets of items and associations among them. But domain experts hypothesize that additional factors like relative ordering and spacing of these items are important aspects governing gene expression.

We proposed hypothesis-based specializations of association rules to identify biologically significant relationships. Our approach also alleviates the limitations inherent in the conventional association rule mining that uses a support-confidence framework by providing filtering and reordering of association rules according to other measures of interestingness in addition to support and confidence. Our tool supports visualization of genetic data in the context of a rule, which facilitates rule analysis and rule specialization. The improvement in different measures of inter-

estingness (e.g., confidence, lift, and p-value) enabled by our approach is used to evaluate the significance of the specialized rules.

1.1 Biological Motivation

One of the central questions in modern biology today is what controls gene expression. Deoxyribonucleic acid (DNA) is a complex molecule which encodes genetic information unique to an organism. Every cell in an organism contains the same set of instructions encoded in the DNA, and this information is arranged into regions called genes. Still, a brain cell is very different from a heart cell and performs an

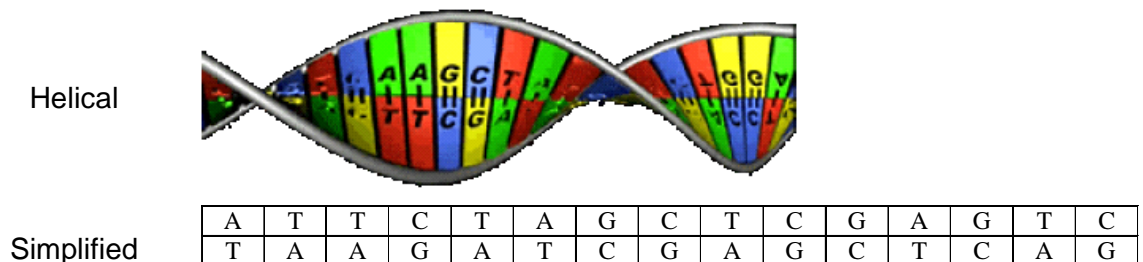


Figure 1.1: Structure of a DNA molecule and corresponding linear simplification.

Gene expression is the process by which the information encoded in a gene is copied (transcribed) into RNA which may further be translated into a protein. The promoter region of a gene is the portion of the DNA sequence upstream of the gene. The process of making an RNA copy of the relevant portion of the genes DNA is called transcription, and the point where the promoter region ends and the gene begins is called the start of transcription (SoT). RNA is chemically slightly different from DNA, but contains the relevant information for a particular gene, and it can move to a part of the cell where that information can be translated into protein.

Proteins are the basic chemicals that make up the structure of cells and direct their activities. Each protein has a specific function that is determined by the blueprint stored in DNA, specifically the gene.

Deeper understanding of gene expression would not only help in the functional classification of genes but would also be instrumental for developing cures to diseases where the gene expression patterns within a cell change. With technological advances more genetic data is being collected today than ever before. This is creating an increasing gap between the rate of data collection and the rate of data analysis.

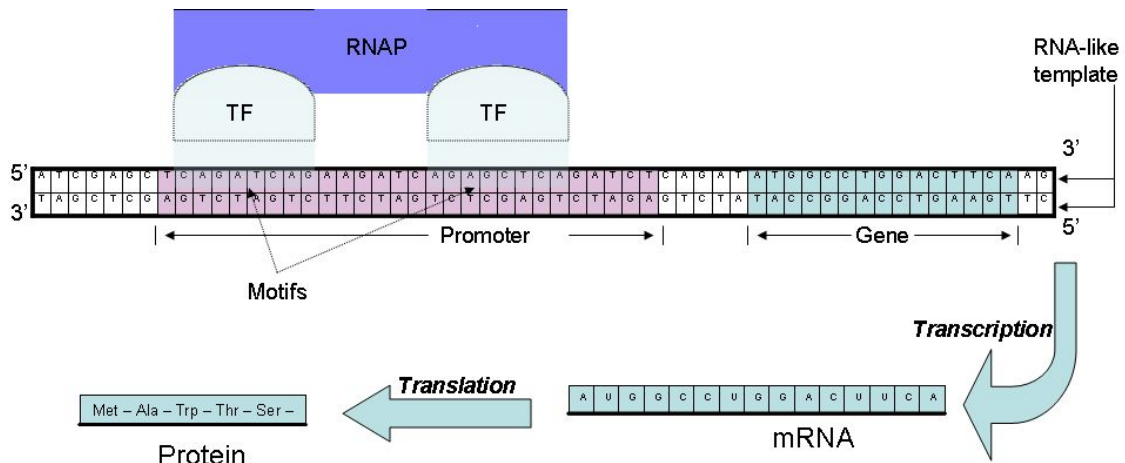


Figure 1.2: Central dogma of biology: DNA→RNA→Protein.

During transcription, RNA polymerase (RNAP) copies DNA to RNA using the template strand. The RNA transcribed is identical to the RNA-like strand except that U's are substituted for T's. A transcription protein (TP) binds to either enhance or repress transcription of a gene by assisting or blocking RNAP binding. During translation, the RNA encodes proteins.

Domain experts attribute the selective activation of genes in any cell to:

1. The presence of a particular set of proteins controlling transcription called Transcription Proteins (TP) in a given cell.
2. The presence of certain repeated sequences of DNA (motifs) in the promoter

region, which is the section of the DNA sequence upstream of the gene. These motifs are bound by transcription proteins during transcription.

Throughout this thesis we focus on identifying control patterns, defined in terms of the presence of a motif, to model gene expression. Motifs control gene expression, as they are putative binding sites which bind the transcriptional proteins. Expression of each gene may require the presence of a combination of motifs. Domain experts also hypothesize that inter-motif distances and order of occurrence of motifs in the promoter region are additional factors that control this regulatory interplay of motifs and thus also influence gene expression.

1.2 Computational Motivation

Computational processes to identify and shortlist *interesting* relationships (associations) between motifs and gene expression, which could then be analyzed biologically in detail, are gaining importance, as these would help reduce the growing gap between data collection and analysis. Associations that are statistically significant may yield biologically valid connections between associated variables. Association rule mining, introduced in [AIS93], provides a useful mechanism for discovering relationships between variables in a dataset. Relationships are represented in an if-then format with statistical measures to indicate the strength of the relationship. Association rules are of the form:

$$Antecedent \Rightarrow Consequent[Support = S, Confidence = C] \text{ such that } S, C \in [0, 1]$$

A rule of this form in a market basket analysis of customer purchases could be:

$$Bread, Butter \Rightarrow Eggs[Support = 0.45, Confidence = 0.80] \tag{1.1}$$

The **Support** of the rule is the probability of finding both the antecedent (the *if* part) and consequent (the *then* part) of the rule in a data instance (i.e., a row in the dataset). For instance, in rule 1.1, the Support of the rule signifies that 45% of the customers in the database bought all three items, that is, Bread, Butter, and Eggs.

The **Confidence** of the rule is the conditional probability of the consequent given the antecedent. Again, in the context of sample rule 1.1, the Confidence of the rule signifies that 80% of the customers who bought Bread and Butter bought Eggs as well.

Previous work at WPI ([MPPT01], [BLT02], [BFG⁺03], [Ice03], [IRR03]) has provided the foundation for gene expression association rule mining. [BLT02] focused on creating a computational biology tool, CAGE, that built association rule based models to predict gene expression. [BFG⁺03] implemented a more elaborate methodology for discovering potential motifs and concentrated on improving the predictive accuracy of the models. However, none of these systems provide an interface that enhances the ability of a domain expert to analyze the resulting rules. This work focuses on facilitating visualization of the mined rules with respect to location of motifs on promoter regions of interest, since this is essential to interpretation of the rules by domain experts.

Besides setting the base methodology, [MPPT01] also attempted to address the biological hypothesis - “Does distance between motifs matter?”. [Ice03], [IRR03]

extended the idea and focused on incorporating distance information in the mining process itself. But they do not provide a way to verify alternative biological hypotheses. Consider the following scenario, which brings forth one of the shortcomings of incorporating more constraints in the mining process, and suggests why it might not be the best approach in an environment where we intend to perform exploratory analysis. A batch of gene sequences was mined for gene expression related association rules with a support and confidence of 0.5 or greater. If the user now wants to find out rules with support and confidence of 0.4 or greater, the only way to achieve this is to mine again, which is a very time consuming process. From an exploratory analysis perspective, it is imperative to facilitate visualization of data in the context of a rule in real-time. So one could mine for association rules with lowest bounds of support and confidence. Then this work lets the user visualize the data which may allow the user to quickly perceive a pattern in the data, suggesting a specialization that would greatly increase the confidence (or other measures of interestingness). This work also computes the different measures of interestingness of the specialization over the training data for each such identified specialization and thereby provides an instantaneous estimate on the statistical strength of the rule. This work provides the ability to test a few biological hypotheses, as such a provision is instrumental in the development of an effective data-mining algorithm for gene expression.

Moreover all the above mentioned efforts were based on the basic support-confidence framework of rule generation. Several other measures of interestingness have been proposed to measure the relative importance of association rules (e.g., gain [Mor98], chi-squared value [Alv03], and lift [BMS97]). But there is no one good measure that is applicable to all domains. This work provides a way to analyze important rules according to several measures and thereby observe the applicability

of each of these measures to this problem domain.

[PR05] introduced an algorithm to mine expressive positional relationships from complex sequential data. We adapted the data to define motifs as events and then utilized this algorithmic approach to mine for statistically significant association rules with positional relationships. This work also provides a facility to visualize genetic data in the context of such positional specializations.

1.3 Problem Statement

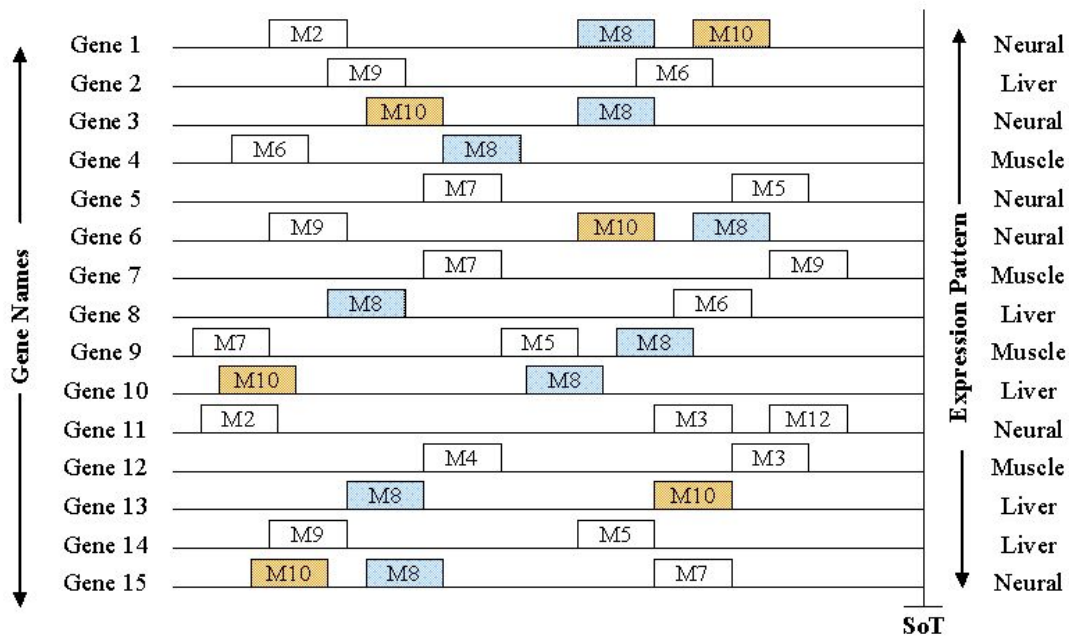


Figure 1.3: Hypothetical dataset of 15 genes and 10 motifs.

We designed and developed a tool to facilitate the post-mining analysis of rules for verifying biological hypotheses and also to aid the visualization of the rules generated. The tool has been integrated with the WPI-Weka system, a local version of the open source data mining tool. Mining of a hypothetical gene expression

dataset as shown in Figure 1.3 would produce association rules of the form:

$$M8 \ \&\& \ M10 \ \Rightarrow \ \text{neural} \ [Support = 0.27, Confidence = 0.67] \quad (1.2)$$

which states that the presence of Motif M8 and Motif M10 in the promoter region of a gene implies that there is a 67% likelihood that the gene is expressed in cells of type neural. Also, 27% of our data instances contain M8, M10 and are expressed in cells of type neural. The support and confidence statistics are computed from the hypothetical data in Figure 1.3.

This work will provide the necessary functionality for a domain expert to interactively analyze genetic data in the context of the following biological hypotheses:

1. *Inter-motif distance is important in characterizing gene expression.* DNA consists of linearly linked nucleotides. Subsequences of the DNA sequence, like a gene or a promoter region, can be represented for example as ATTTCCCGGT. By representing DNA as a sequence, the number of bases could be used to imply a notion of distance between motifs. In the sample sequence **ATTCGGGGGTAT** we could say that the Motif ATT is at a distance of 7 bases from the Motif TAT. Now consider the following specialized form of rule 1.3:

$$M8 \ (0-250) \ M10 \ \Rightarrow \ \text{neural} \ [Support = 0.20, Confidence = 1.0] \quad (1.3)$$

That is, the presence of Motif M8 within a distance of 250 bases from Motif M10 implies that the gene is likely to be expressed in cells of type neural. Again the support and confidence statistics are computed from the hypothetical data in Figure 1.3. Notice the change in the statistical measures with respect to (1.2). Every time a rule is specialized, it may be applicable to fewer data-

instances (genes) in the dataset. This explains the reduction in the Support value. The increase in Confidence is indicative of the classification accuracy. A value of 1.0 signifies that of the data instances (genes) to which the rule could be applied (i.e., those that match the antecedent of the rule), the expression type predicted by the consequent of the rule is correct (i.e., it matches the known expression type of the data instance).

2. *Distance of a motif from the start of transcription (SoT) affects gene expression.*

Since many of the known putative regulatory elements are found close to the Start of Transcription (SoT), we want to find out if the distance of the occurrence of a motif to the start of transcription has any effect on gene expression. This work facilitates visualization of gene sequences, along with the motifs involved and the start of transcription, in the context of the generic rule of the form (1.2). This enables the user to form and visualize specializations of the form:

$$\text{M8 (0-500) SoT \&\& M10} \Rightarrow \text{neural} [\textit{Supp} = 0.13, \textit{Conf} = 1.0] \quad (1.4)$$

where the presence of Motif M8 within a distance of 500 bases or less from the Start of Transcription (SoT) and presence of motif M10 anywhere in the promoter region imply that the gene is likely to be expressed in cells of type neural. Again the support and confidence statistics are computed from the hypothetical data in Figure 1.3. Observe again the alteration in support and confidence as compared to (1.2).

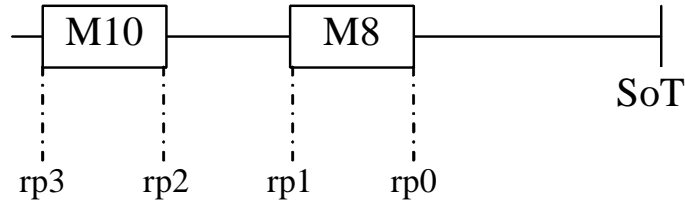


Figure 1.4: Syntax description of order of occurrence based specialized rules.

3. *The order of occurrence of motifs affects gene expression.* Knowledge of gene expression regulation is not complete. Domain experts hypothesize that the order of occurrence of motifs in the regulatory regions could also affect gene expression. This work provides the facility to visualize the gene sequences in the context of specializations of the following form which were either mined directly using the approach in [PR05] or visually observed and enhanced as a part of the exploratory analysis.

$$\text{M8 (rp0-rp1) M10 (rp2-rp3)} \Rightarrow \text{neural [Supp} = 0.20, \text{Conf} = 0.75] \quad (1.5)$$

This rule states that the presence of an occurrence of Motif M8 in the promoter region of a gene in between an occurrence of Motif M10 and the Start of Transcription (SoT) implies that the gene is likely to be expressed in cells of type neural. rp in the rule above refers to the relative position of the motif with respect to the Start of Transcription (Figure 1.4). Each motif has a begin point and an end point and hence requires both a begin index and an end index to capture the relative positioning of the motifs on the gene sequence. The support and confidence statistics are computed from the hypothetical data in Figure 1.3. Here again we notice that the specialization process produces an improvement in the confidence of the rule.

For each of the form of specializations discussed above, different measures of interestingness are computed to estimate if the hypothesis-based specialized rule better explains the underlying regulatory mechanism as compared to its generic counterpart.

1.4 Summary of the contributions of this work

This work focused on the development of a computational tool for exploratory specialization of association rules predicting gene expression in the context of the above mentioned biological hypotheses. Also it provides for filtering/sorting association rules based on measures of interestingness beyond the conventional measures of confidence and support.

The main contributions of this work are a framework and a tool that can:

1. Facilitate exploratory rule analysis (specialization) by providing a user-interface for rule visualization in the context of different biological hypotheses.
2. Select and present rules according to different interestingness metrics.
3. Test hypotheses relating the order of motifs, inter-motif distance and the distance of motifs from the start of transcription to gene expression control.
4. Provide updated genetic data, an important resource for any further research in the domain.
5. Be integrated seamlessly with the WPI Weka system. That is, gene expression association rules mined with the WPI-Weka system can be visualized and specialized using this work. Also, a model consisting of interesting rules and their specializations could be used by the WPI Weka to measure the model's classification accuracy over novel data.

Chapter 2

Background

2.1 Gene Expression

Simply put, a gene is a segment of DNA and is the physical and functional unit of heredity information. Gene expression is the process of using the information encoded in a gene to manufacture protein in the cell. Each cell of an organism, for instance neural or muscle, has the same DNA, but still performs completely different functions. This phenomenon is often referred to as the “The central dogma of biology” and is described in more detail in Section 1.1.

With technical advances in all fields, more and more data is being generated today than ever before, and the field of gene expression is no exception. Thus there is an imperative need for methods to analyze data at an equivalent rate.

The focus of this work was to design and develop a tool that helps a biologist to visualize genetic data to explore interesting regulatory patterns governing gene expression. As discussed in Section 1.1, motifs (repeating DNA segments) control gene expression, as they are putative binding sites which bind the transcriptional proteins. Hence, it is central to our work to determine groups of motifs that are likely

to be real binding sites collectively controlling expression, which in turn is contingent upon the quality of the data used to discover (elicit) motifs. This was one of the prime reasons why we decided to collect data from scratch. We compiled a database using the Wormbase database [Wor] and RSA database [RSA] that consists of 164 genes, from nine different cell types with at least 30 genes known to be expressed in each cell type. Furthermore, we conducted a pilot experiment that elicited motifs from these sequences using both MEME [MEM] and GIBBS [JAC95] to observe the cost vs quality analysis of both algorithms. Since there was no perceived benefit in terms of quality of motifs we opted for lower cost (less time-consuming) elicitation algorithm MotifSampler (GIBBS). The data collection as well as the motif elicitation process is covered in detail in Section 3.

2.2 Association rules

Association rules were introduced in [AIS93]. Consider a database (D) in relational format where each record (data instance) consists of n boolean attributes. Association rules model relationships of the form: presence of a set A ($A \subset D$) implies the presence of the another set C ($C \subset D$) where the two sets A and C are disjoint (i.e., $A \cap C = \emptyset$). The most common statistical measures to estimate the strength of the rule are support and confidence and these have been covered in depth in Section 1.2.

Apriori is the traditional algorithm used to mine association rules [AS94]. Even a small dataset could yield a large number of rules and so the support-confidence framework is utilized to identify relationships which are statistically interesting. It follows an inductive approach to find *itemsets* (i.e., sets of items belonging to the data) that occur together *frequently*, within a dataset. An itemset is frequent if the

support of the itemset is greater than the minimum support, a threshold provided as an input parameter. The apriori principle basically states that an itemset is frequent only if all its subsets are frequent and this principle is utilized in the inductive approach to search for frequent itemset.

Even with the popularity of the support-confidence framework in the association rule mining literature there is no one good measure that is applicable to all domains. Several other measures of interestingness have been proposed to measure the relative importance of association rules. The lift value of an association rule is another measure to try to quantify the interestingness of the rule. It is defined as the ratio of the confidence of the rule and the support of the consequent of the rule. The p-value of the rule is the probability that the correlation between the antecedent and the consequent is due to chance by using the chi-square test.

2.3 Prior work at WPI

As discussed in Section 1.2 previous work at WPI has provided the foundation for gene expression association rule mining. *AprioriSetsAndSequences* [PR05] introduced an algorithm to mine expressive temporal relationships from complex sequential data in addition to the regular association rule mining and provides enhanced pruning mechanism, which is of significant value especially when mining large sequence databases. It takes preprocessed sequences, that is sequences of events (e.g., the price of a company's stock recorded every hour or the repeating patterns in a gene sequence) as input with a minimum support and confidence threshold and produces association rules with temporal relationships between events. [MPPT01] attempted to address the biological hypothesis - "Inter-motif distance influences gene expression". [Ice03], [IRR03] extended the idea and focused on incorporating

distance information in the mining process itself. Integrating this work with the WPI-Weka system has been a shared goal with [Rudss], a work in progress.

Chapter 3

Data: Motif Elicitation and Sequence Annotation

The data in the domain of interest (genetics) is sequential in nature. In this section we present the process followed in order to transform sequential genetic data to a relational format. That is, a format similar to the conventional database format that enables the use of existing data-mining algorithms to identify patterns of interest from a gene regulation perspective.

3.1 Data Collection

It cannot be stressed enough that no matter how good a mining algorithm may be, the information retrieved/discovered is only as good as the data. Adhering to this thought, we found an imperative need to collect genetic data from scratch.

C. elegans was our choice of organism. It is a well-studied organism often used as a model for genetic research because it is genetically tractable, that is, the entire *C. elegans* genome has been sequenced and the expression patterns of many genes are

known. More than 60% of human genes have homologs in the *C. elegans* genome. The facts that it is small and easy to culture are some of the secondary reasons why this nematode is popular amongst biologists.

As a first step we identified cell types to include in our study. The primary intent was to identify cell types in which there were at least 30 genes known to be expressed. We require them to be “high-density” cell types, because the data sample should be large enough to derive statistically significant information. Also, this would provide us with substantial data to break down into a training set and a test set.

We used the WormBase [Wor] database and the RSA Database [RSA] for identifying the cell types based on the conditions delineated above. The next step was to gather the actual data, that is, the promoter regions for each of the 30 or more genes per cell type. This data was downloaded from the sources listed above and manually cross verified using BLAST [BLA]. At the end we were able to identify nine cell types of interest to us, each of which had at least 30 known genes. We created nine batches of promoter sequences, one per cell type (Figure 3.1). These nine batches of gene sequences or promoter subgroups were also the initial input for the data transformation process as depicted in Figure 3.2, which provides the graphical overview of the contents of this chapter. Each subsequent step in the data transformation process corresponds to a subsequent section of this chapter.

Another important decision was with regard to the length of the promoter region for the data collected. Based on expert opinion, the length of promoter region included in the data was 5000 base pairs (bp) upstream (5' to the gene) of the gene. This choice was influenced by the fact that although the regulatory elements critical to gene expression are usually proximal to the initiation site, another type of regulatory elements called enhancers, that may influence expression, can be located

Cell Type	Genes Expressed
ASK	bra-1, cam-1, che-3, daf-11, eat-4, egl-4, gpa-14, gpa-15, gpa-3, ida-1, kin-29, kvs-1, nlp-10, nlp-14, nlp-8, odr-1, opt-3, osm-3, osm-6, osm-9, sra-7, sra-9, srg-2, srg-8, tax-2, tax-4, tax-6, unc-103, zig-4, zig-5
ASE	ceh-23, che-1, che-3, cog-1, csk-1, egl-2, egl-4, gcy-5, gcy-6, gcy-7, gpa-3, hen-1, kvs-1, lim-6, mps-1, ncs-1, nlp-14, nlp-3, nlp-7, npr-1, osm-3, osm-6, osm-9, src-1, tax-2, tax-4, tax-6, unc-5, nlp-1, flp-6
ASI	bra-1, cam-1, ceh-23, che-3, daf-11, daf-28, daf-7, gpa-1, gpa-10, gpa-14, gpa-3, gpa-4, gpa-5, gpa-6, gpc-1, ida-1, kal-1, kin-29, nlp-1, nlp-14, nlp-18, nlp-24, nlp-27, nlp-5, nlp-6, nlp-7, nlp-9, odr-1, opt-3, osm-10, osm-3, osm-6, osm-9, sra-6, srd-1, str-2, str-3, tax-2, tax-4, tax-6, ttx-3, unc-3, zig-3, zig-4
CAN	acy-1, acy-2, cam-1, ced-10, ceh-10, ceh-23, ceh-43, cle-1, ctl-2, dbl-1, ggr-2, goa-1, gpa-10, gpa-14, gpb-2, gsa-1, hbl-1, jkk-1, jnk-1, kal-1, lin-14, mig-2, nlp-10, nlp-15, pak-1, unc-129, unc-73, unc-76, vab-8, cat-1
HSN	cam-1, cdh-3, cha-1, clh-3, ctl-2, eat-16, egl-21, egl-3, egl-43, egl-44, egl-5, flt-1, gar-2, ggr-2, glr-5, goa-1, gpb-2, grd-6, gsa-1, ham-2, hbl-1, ida-1, inx-4, jkk-1, jnk-1, kal-1, mab-23, mec-6, mig-1, mig-2, nhx-5, nid-1, nlp-15, nlp-3, sax-3, sem-4, syg-1, tph-1, unc-103, unc-14, unc-17, unc-40, unc-51, unc-53, unc-73, unc-76, unc-8, unc-86, cat-1, lin-4
PHA	bra-1, ceh-14, che-2, che-3, egl-43, gcy-12, goa-1, gpa-1, gpa-13, gpa-14, gpa-15, gpa-2, gpa-3, ida-1, lin-11, ncs-1, nlp-14, nlp-7, npr-1, ocr-2, osm-10, osm-3, osm-6, osm-9, pkc-1, srg-13, tax-6, unc-103, flp-15, srb-6, tax-2
ADL	cam-1, ceh-23, ceh-32, che-3, cog-1, gpa-1, gpa-11, gpa-15, gpa-3, gpc-1, hlh-2, kvs-1, lin-11, nhr-79, nlp-10, nlp-7, nlp-8, ocr-1, ocr-2, opt-3, osm-3, osm-6, osm-9, qui-1, srb-6, sre-1, sro-1, tax-6, ttx-3, unc-103, ver-2
ASH	cam-1, ceh-23, che-1, che-3, eat-4, egl-3, egl-4, gpa-1, gpa-11, gpa-13, gpa-14, gpa-15, gpa-3, gpc-1, hlh-2, kin-29, kvs-1, mps-1, nhr-79, nlp-15, nlp-3, npr-1, ocr-2, odr-3, opt-3, osm-10, osm-3, osm-6, osm-9, qui-1, sra-6, srb-6, tax-6, unc-42, unc-8
ALM	cam-1, daf-1, deg-3, dyn-1, eat-4, egl-2, egl-21, egl-3, glr-8, goa-1, jkk-1, jnk-1, lin-14, mec-10, mec-2, mec-3, mec-4, mec-6, mec-7, mec-8, mig-2, mps-1, mtd-1, nid-1, pag-3, pat-4, pkc-1, ptl-1, tba-1, tol-1, unc-32, unc-73, unc-86, unc-97

Figure 3.1: List of high density cell types with the associated identified genes that are expressed in the worm's adult life stage.

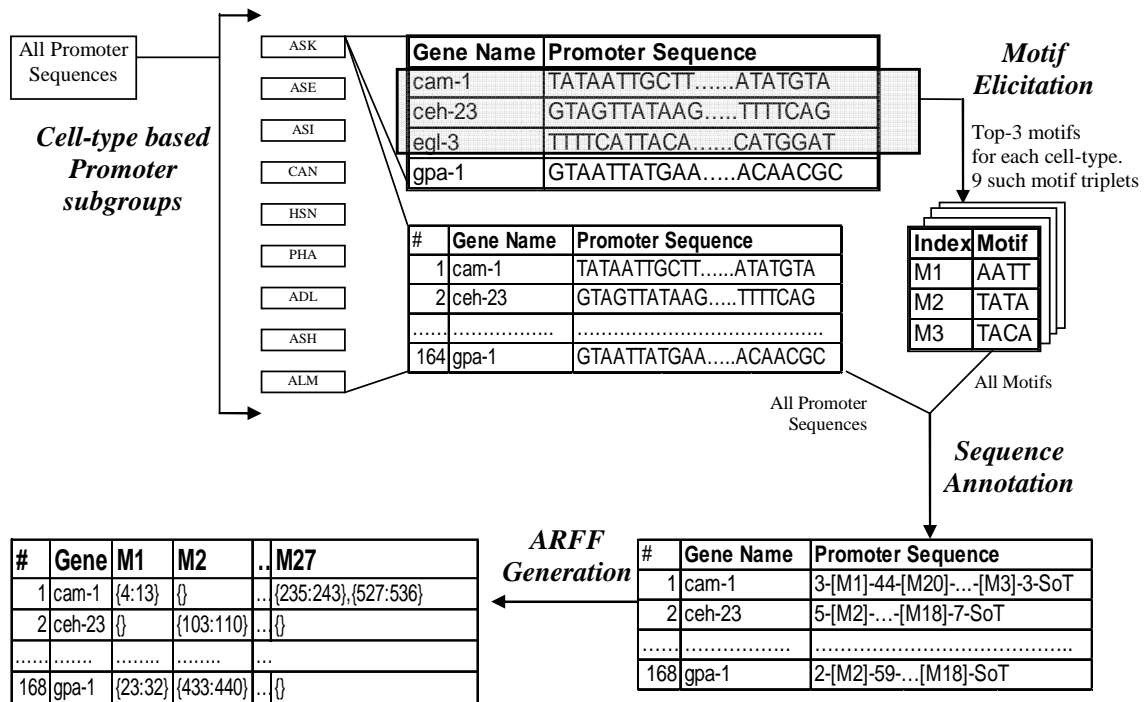


Figure 3.2: Overview of the process from data collection to the ARFF generation. Promoter regions were grouped based on the cell type in which their associated genes were expressed. DNA motifs common to promoters within a group were elicited for each group. All sequences were then annotated with all the elicited motifs. The gene expression information and the annotated sequences, that is, the sequences overlaid with the positional information of each motif, are transformed to ARFF format, Weka's input format.

Sample Promoter Sequence in FASTA format
<pre> >osm-6 25148409 upstream sequence, from -425 to -1, size 425 TTTTATAATTGCTTATATGTAGTAGTTATATTTTCAGTTTTTCATTACATTTTCATGGGTAT TTATTTATTA ACTATAATCTTGTATAAGACGATGTAATTATGAAACAACGATTTCCACACT TCCGGTTTTTCATGTAAAATTTTTTCGTTCCAAATAAATTGTTATAAAAATTAATTACATC TTTCATCAAACCTTCAAAAATGAAATTGCATTTTTTAATAATTAGGAGTCTATTACGGAATT CATTAATTTTCAGAAAACAAAGTAACTATATATTTCTCTAGTAGTTCCTTTCCAGGAG ACCCTTCCAAGATTTGTATCCACATGTTACCATAGTAACCACTCATTGCTTCTCGCTCAC ATTGTCTGCTCCCTCTCTTGGGGCTTATATCTCTTTCAAGCTATTACCTTCATTAGTATA CATCT </pre>

Figure 3.3: Sample promoter sequence from the data collected.

several thousand base-pairs from the gene. In case another gene was found within the 5000 bp upstream sequence of the promoter sequence, the length of the promoter being considered was truncated at the start of this gene.

The data collected consists of 164 promoter sequences and is a valuable resource for future work. The collected sequences were represented in the FASTA format for further processing by motif discovery and annotation tools. A sample sequence is shown in Figure 3.3.

3.2 Motif Elicitation

The next step in the data transformation process is motif elicitation(Figure 3.2). A motif is a sequence pattern that occurs repeatedly (ideally at least once per gene) in a group of related promoter sequences. Motif elicitation is the process of discovering significant motifs from a group of sequences. There are several tools available that use different statistical modeling techniques to discover significant motifs. The basic premise governing the elicitation process is that the regulatory controls governing the expression of genes in the cells of the same cell type might be in common to at

least few of these sequences. The idea was to use motif search programs to identify cell type specific motifs. To avoid over fitting we decided to include only 90% of the sequences as input to motif elicitation programs and the remaining 10% of the sequence for testing.

Two different motif elicitation programs (algorithms), MotifSampler [Mot] and MEME [MEM] were used in the preliminary tests on the same dataset. MotifSampler is a motif finding algorithm that uses GIBBS sampling [JAC95] to find the position probability matrix that represents the motif. MEME discovers one or more motifs in a collection of sequences by using the technique of expectation maximization [BE94]. Based on the similarity of the outputs from both algorithms and considerably less computational time exhibited by MotifSampler, GIBBS was our choice of algorithm to be used for the motif elicitation stage. It is worth noting, however, that the preliminary tests were in no way a detailed comparative study of the two methods.

Based on the input from the domain expert it was decided to look for motifs of size 8, 10, or 12 base pairs. Thus, motif elicitation was performed by executing MotifSampler individually on each of the nine batches of promoter sequences (Figure 3.2), once for each size. The output of this process was in the form of a set of position probability matrices (Figure 3.4) each representing a motif. For each combination of a size and batch, the three best scoring matrices (motifs) were selected for future use. As a result we ended up with 81 motifs; nine motifs for each cell type.

3.3 Sequence annotation

Motifs identified by MotifSampler are in the form of a probability-matrix. The next step was to annotate all promoter sequences with the elicited motifs. Annotation

Sample motif represented as a position probability matrix				
#	Width = 8			
#	Consensus = ATAACTAG			
#	A	C	G	T
	0.996545	0.000890	0.000840	0.001726
	0.001520	0.000890	0.000840	0.996750
	0.499033	0.000890	0.000840	0.499238
	0.499033	0.000890	0.000840	0.499238
	0.001520	0.498402	0.498352	0.001726
	0.001520	0.000890	0.000840	0.996750
	0.996545	0.000890	0.000840	0.001726
	0.001520	0.000890	0.498352	0.499238

Figure 3.4: Sample motif represented as a position probability matrix. The commented row titled width provides the length of the motif. The consensus sequence of the motif is a sample occurrence of the motif built by using the most common base at each position. Each column of the position probability matrix corresponds to the bases A, C, G and T respectively. Each row corresponds to a position of a base within the motif. For instance a position probability matrix for a motif with width 8 would consist of 8 rows. The value at row i column j in the matrix is the probability of finding the base j at position i in the motif.

is the process of finding matches of a given motif(s) in a given set of sequences and also quantifies how good each match is. We used Motif Alignment and Search Tool (MAST) [MAS] for the annotation process. A PERL script (Appendix B) was developed to convert probability-matrices from MotifSampler output to corresponding MAST friendly format(log-odds matrices as shown in Figure 3.6). A master motif file was created which consisted of all 81 identified motifs irrespective of the cell type. A master gene sequences file was created which consisted of promoter sequences for all high-density genes listed in Figure 3.1. The master gene sequences file and the master motif file were fed to MAST as input to annotate all promoter sequences with all elicited motifs. The MAST output file is a HTML file consisting

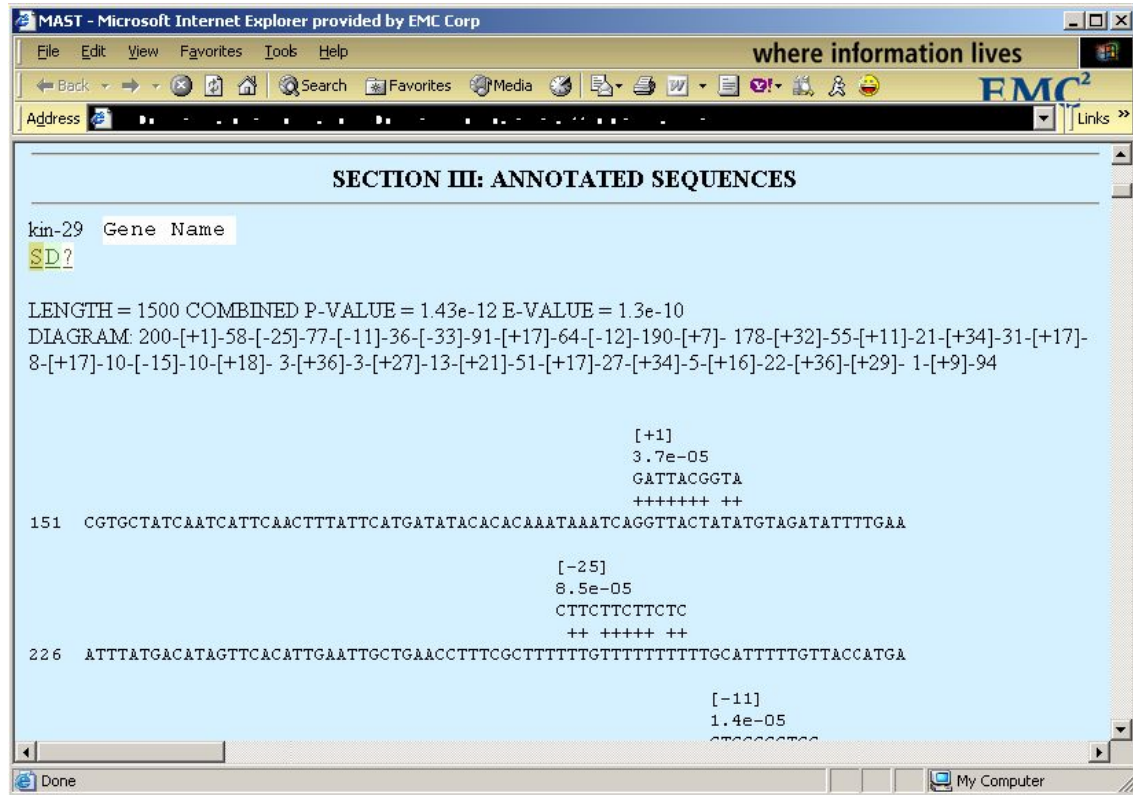


Figure 3.5: MAST annotated sequence sample.

During annotation each supplied sequence is searched for matches with each supplied motif. The four lines above each motif occurrence contain, respectively, the motif number of the occurrence, the position p-value (i.e., a measure of the match quality, lower is better) of the occurrence, the consensus sequence of the motif, and a plus sign ('+') above each letter in the occurrence that has a positive match score to the motif. MAST can automatically generate the reverse complement strand for each supplied sequence and search for motif occurrences on either the given strand or its reverse complement. The ('+') or ('-') sign alongside the motif number is used to distinguish whether the match occurred on the given strand or the reverse complement respectively.

Sample motif represented as a log odds matrix				
# Width = 8				
# Consensus =	ATAACTAG			
# A	C	G	T	
166	-766	-757	-764	
-770	-766	-757	154	
66	-766	-757	54	
66	-766	-757	54	
-770	146	164	-764	
-770	-766	-757	154	
166	-766	-757	-764	
-770	-766	164	54	

Figure 3.6: Sample motif represented as a Log-odds matrix.

This matrix is a log-odds matrix calculated by taking the log (base 2) of the ratio p/f at each position in the motif where p is the probability of a particular letter at that position in the motif, and f is the average frequency of that letter in the training set.

of all annotated gene sequences. A relevant section of the output file is shown in Figure 3.5.

3.4 ARFF Generation

A Java module was developed to transform the union of annotated promoter sequences from the MAST output and the known gene expression information, that is, all of the cells in which the gene is expressed, to Attribute-Relation File Format (ARFF) format dataset. The ARFF format is a format similar to the relational database format. Each gene occurs as a tuple in the relation. Each motif is an attribute of the gene tuple. A set-value consisting of the known gene expression pattern is also an attribute of the gene. A sample of the same has been included in Appendix A. As mentioned in section 3.2, to avoid mining and exploring relationships that overfit the data, the sequences were divided into a training set (90% of the sequences) and a test set (10% of the sequence). Thus the ARFF generation was performed twice, one to create a training set ARFF file and the other for a test set ARFF file.

Chapter 4

Data Visualization and Mining

4.1 Mining Process

The sequence annotation process described in Chapter 3, transformed the gene sequences into a relational format. This provides us the ability to utilize algorithms from the WPI-Weka system to discover relationships or patterns that describe gene expression. These relationships are rules like “Genes whose promoter regions contain the motifs M10 and M16 are often expressed in Neural cells”. The ability to uncover such relationships in an automated fashion is of prime importance, as they provide the domain experts with a relevant view of the data that demands further exploration.

[Ice03] investigated the problem of incorporating hypothesis based information into the mining process. We instead decided to follow a post mining exploratory approach for hypothesis testing, that is, to specialize (post-process) *interesting* rules produced by conventional association rule mining algorithms. The reasons that influenced this choice were:

1. It is very difficult, if not impossible, to determine in advance the right distance-

related and position-related mining parameters so that the mined rules will capture the desired patterns. This is due to the fact that the appropriate distance and position values vary for each subset of motifs in the context of each cell type under consideration. For instance, the appropriate values for the distance and relative position of motifs M10 and M16 in neural cells might be very different to those in muscle cells. Furthermore, these values might vary in the presence of other motifs. That is, the distance and relative position of motifs M10 and M16 in neural cells might vary even for the same cell type once that say motif M20 is added to the mix. Since mining association rules is expensive in terms of execution time, a trial and error approach in which possible values of the input parameters are guessed would require multiple executions of the mining algorithm, which would be too time consuming. Another alternative is to perform an automatic search for the right values of those parameters within the mining algorithm, but the time complexity of an exhaustive search is exponential in the size of the input data and would make the runtime of the mining algorithm prohibitive. It is unclear that good heuristics to prune the search are possible. Our selected post mining exploratory analysis approach is much faster since it is performed on one rule (i.e., one subset of motifs under one cell type) at a time, and only on such rules that are deemed interesting by the domain expert user.

2. Exploratory analysis often combines visualization with data mining tools that provides a *simple* sequence of steps(work flows) to identify and isolate *relevant* information. *Simplicity* is important because we need to bridge the gap between the domain experts and the tools usage. Equipped with an easy to use tool, the domain experts could utilize their knowledge to analyze and define the data patterns displayed by the tool in an intuitive manner. The tool is

not limiting but instead banks on the experts, and it could easily evolve to accommodate newer hypotheses.

The association rule mining modules ([Sho01] and [PR05]) of the WPI-Weka system [WPI] were used to mine for basic gene expression patterns in the annotated sequences. The visualization system that we develop here facilitates exploratory analysis to specialize these mined patterns. [Rudss] contributions to WPI Weka helped integrate the visualization modules into the WPI Weka in a transparent fashion. The user now has the choice to either save the results of the mining for perusal at a later point in time or could directly invoke the visualization modules from the mining modules.

4.2 Visualization and Specialization Module

Some of the prime contributions of this work are the Visualization and Specialization Module (VSM) which is an extension to the WPI Weka system. This module enables visualization of the annotated promoter sequences in the context of a specific rule or a set of motifs. The primary interface of the VSM is the Analysis frame, which is the first screen to be displayed when VSM is invoked is depicted in Figure 4.1. We explain the Analysis frame below.

4.2.1 Analysis Frame

The analysis frame is the focal point for using visualization extensions to WPI Weka. The analysis frame loads with two sections, the **Rules** area and the **Commands** area (Figure 4.1). We explain the Rules area below and we explain each of the options in the Commands area in subsequent subsections. The Rules area is used to display base association rules along with the corresponding values for certain

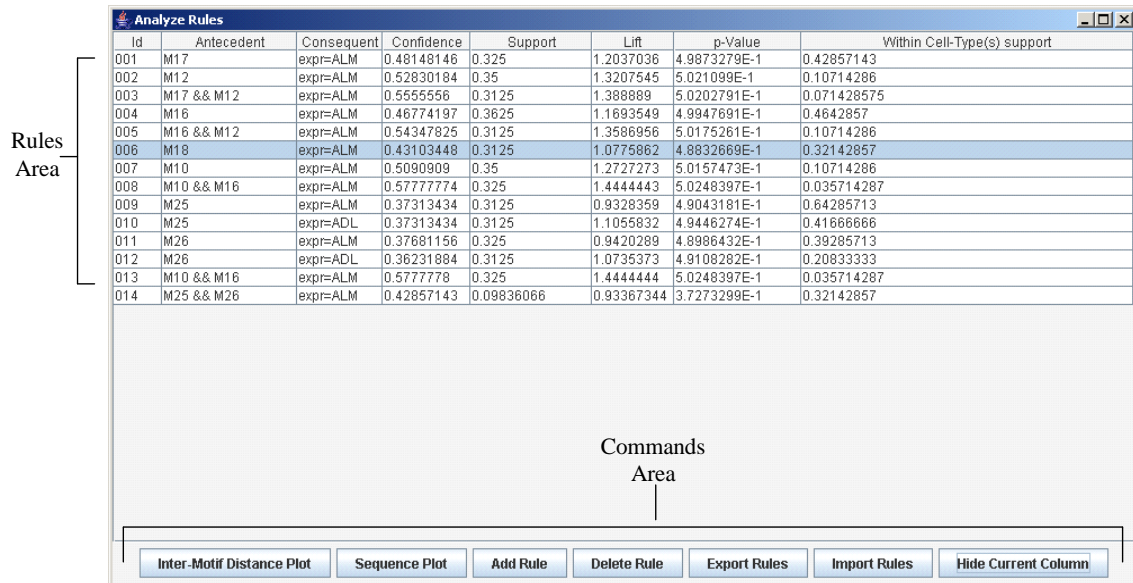


Figure 4.1: Sample Analysis Frame.

Analysis Frame can either be invoked from the mining interface of the WPI-Weka system or could be invoked as a standalone application using an exported set of mined association rules, the associated MAST results (HTML format), and a list of gene names alongside the known expression patterns. A sample of the gene expression information file is presented in Figure 4.2.

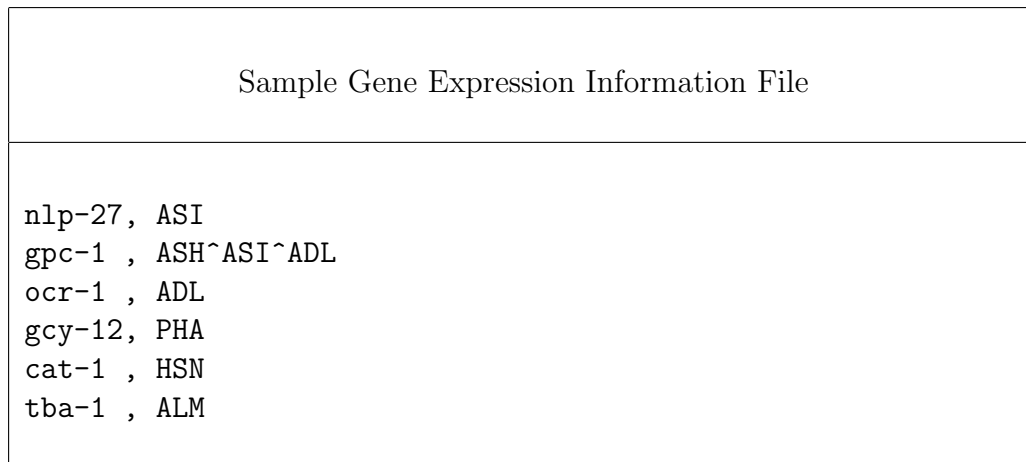


Figure 4.2: A sample gene expression information file.

The file is in CSV (Comma Separated Value) format. First value in each row is the gene name (e.g., nlp-27). The next value contains a set of cell-types the gene is known to be expressed in. Elements of the set are separated using the ^ symbol.

measures of interestingness. The design is extensible; that is, new measures of interest could be added in the future with minimal code changes. In the current state, a Rule tuple consists of the following items:

- **Id** - this is a unique id assigned to the rule. The usability of this field increases once the user starts to generate specializations from the rule, as the Id column helps us trace the history or the specialization path of new rules.
- **Antecedent** - The left-hand side of the rule. It contains the motifs present in the rule.
- **Consequent** - The right-hand side of the rule. It contains the cell-types predicted by the rule.
- **Support** - As discussed in Section 1.2, the support of a rule is the relative frequency with which the antecedent and consequent appear together in the data. That is, $P(\text{Antecedent} \ \& \ \text{Consequent})$.
- **Confidence** - As discussed in Section 1.2, the confidence is the likelihood that the consequent appears in a data instance that contains the antecedent. That is, $P(\text{Consequent}|\text{Antecedent})$.
- **Lift** - The lift value of an association rule is another measure to try to quantify the interestingness of the rule. It is defined as the ratio of the confidence of the rule and the support of the consequent of the rule [BMS97]. In other words

$$lift(rule) = p(consequent|antecedent) / p(consequent)$$

- **p-Value** - The p-value of the rule is the probability that the antecedent and the consequent would be as highly correlated as they are, just by chance according

to a Chi square test of independence. We calculate the p-value of an association rule using the approach in [Alv03].

- **Within Cell-Type Support** - Provides the support of the rule among only those instances of the data that contain the consequent of the rule. This metric is very important in the context of this work because we expect to see different motifs and rules for different cell types, so we are primarily interested in the support of the rule within each cell type.

The Commands area of the Analysis frame provides buttons to perform a range of functions. The following subsections describe each of these functions provided by the visualization extensions via the analysis frame. It is important to note that most of these functions are invoked in the context of a specific rule and so it is necessary to select a rule in the rules area of the analysis frame before invoking a command.

4.2.2 Inter-Motif Distance Plot

Selecting a Rule in the rules area and then invoking the inter-motif distance plot via the button with the same label lets a user visualize the data in the context of the rule from an inter-motif distance perspective (Figure 4.3). This action enables a user to perform exploratory analysis in the context of the hypothesis - “Inter-motif distance influences gene expression”.

On invoking this command a new frame with the pairwise inter-motif distance plot(s) is displayed. It displays one graph for each pair of motifs in the rule (selected in the Analysis Frame). For the sake of simplicity we start with a rule with only two motifs. We revisit plots originating from rules consisting of more than two motifs later in the section.

Notice that an inter-motif distance plot (Figure 4.3) is sliced into 2 parts by a

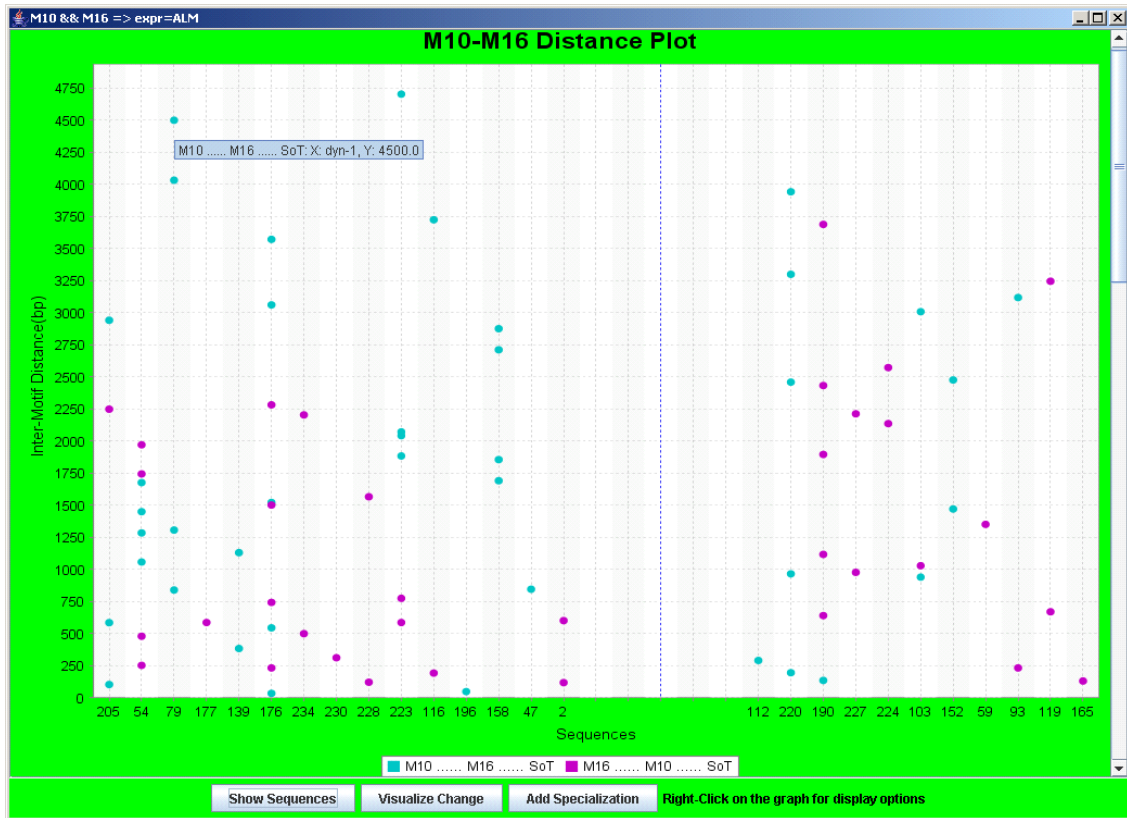


Figure 4.3: Sample Inter-Motif Distance Frame.

Each graph is displayed with the rule used to establish the context as the title of the frame. Each graph displays the pairwise inter-motif distance plots(M10 & M16 \Rightarrow expr=ALM, in this case). Along the x-axis of the plot are the id's of the genes in question and along the y-axis are the inter-motif distances between the pair of motifs. For each pair (a,b) of motifs, inter motif distances of all occurrences of motif a from all occurrences of motif b are plotted. The color of each point is indicative of the order of occurrence of motifs a and b relative to the SoT. In this graph, aqua (light) denotes points in which the occurrence of M16 appears in between the occurrence of M10 and SoT; and magenta (dark) denotes points in which the occurrence of M10 appears in between the occurrence of M16 and SoT. Each graph lists only those genes on the x-axis, that support the antecedent of the rule. That is, genes whose promoter regions contain at least one occurrence of each motif. Genes on the left of the dotted line also support the consequent of the rule.

dotted line. The genes on the left of this dividing line are the ones that support the antecedent and the consequent of the rule and hence support the rule. The ones on the right are the genes that support only the antecedents of the rule. This provides the user with an easy mechanism to discover inter-motif distance based patterns on the left-hand side of the plot that are not as frequent on the right hand side, as this would let us explore specializations with improved classification accuracy (and/or confidence). Once the user has utilized the dotted separation and inter-motif distance plots to define a range of interest, for instance a range of (0-500) between motifs M10 and M16, the user can invoke the “Visualize Change” command to visualize the data in the context of the specialization as depicted in Figure 4.4. Subsequently the specialized rule could be added to the Analysis Frame using the “Add Specialization” command on the inter-motif distance plot. This causes a new entry to be inserted in the Analysis Frame (Figure 4.5) with the following specialization

$$M10(0 - 500)M16 \Rightarrow expr = ALM \quad (4.1)$$

Note that the Id field is auto-generated in a fashion that always lets a user trace back the steps in case we want to later recall which rule was used to derive the specialization.

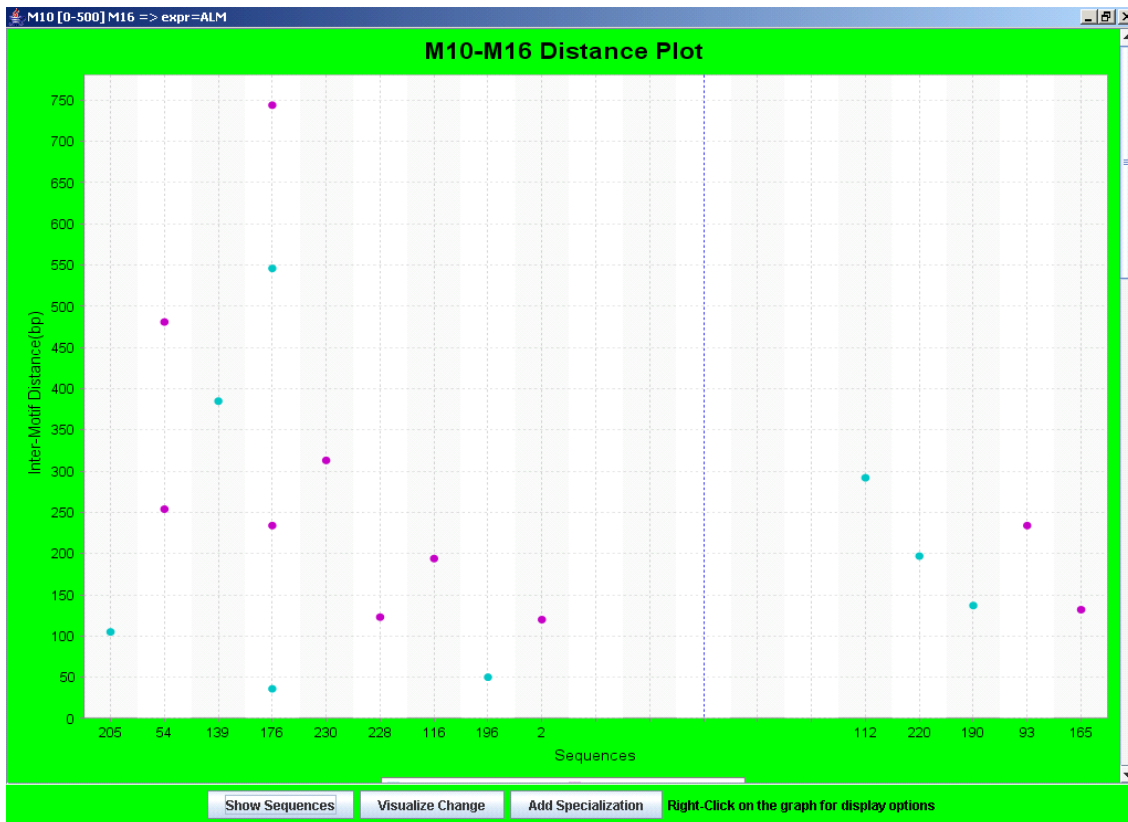


Figure 4.4: Visualize change command from the inter-motif distance plot. The Visualize Change command from the inter-motif distance plot enables the user to visualize the data in the context of the specialization. This plot depicts the specialization $M10 [0-500] M16 \Rightarrow expr=ALM$ of the original rule $M10 \&\& M16 \Rightarrow expr=ALM$ from Figure 4.3.

Analyze Rules							
Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
013.01	M10 [0-500] M16	expr=ALM	0.64285713	0.14754099	1.4005102	1.1581367E-1	0.32142857
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572

Inter-Motif Distance Plot Sequence Plot Add Rule Delete Rule Export Rules Import Rules Hide Current Column

Figure 4.5: A row representing the addition of a specialization to the Analysis Frame.

4.2.3 Sequence Plot

Selecting a Rule in the rules area and then clicking the sequence plot button displays a visualization all the qualifying gene sequences in the context of the rule. This action enables a user to perform exploratory analysis in the context of the hypothesis - “Distance of motifs from the SoT influences gene expression”. A qualifying gene sequence is one that has at least one occurrence of each motif that appears in the rule (selected in the Analysis Frame). Invoking this command causes a new frame with the sequence plot overlaid with the motif information to be displayed.

This sequence plot graph provides the user with an easy mechanism to discover “Distance from SoT” based patterns in the upper part of the plot that are not as frequent in the lower part as this would let us explore specializations with improved classification accuracy(and/or confidence). Once the user has utilized the dotted separation of the plot (into rule supporting and antecedent supporting) and the

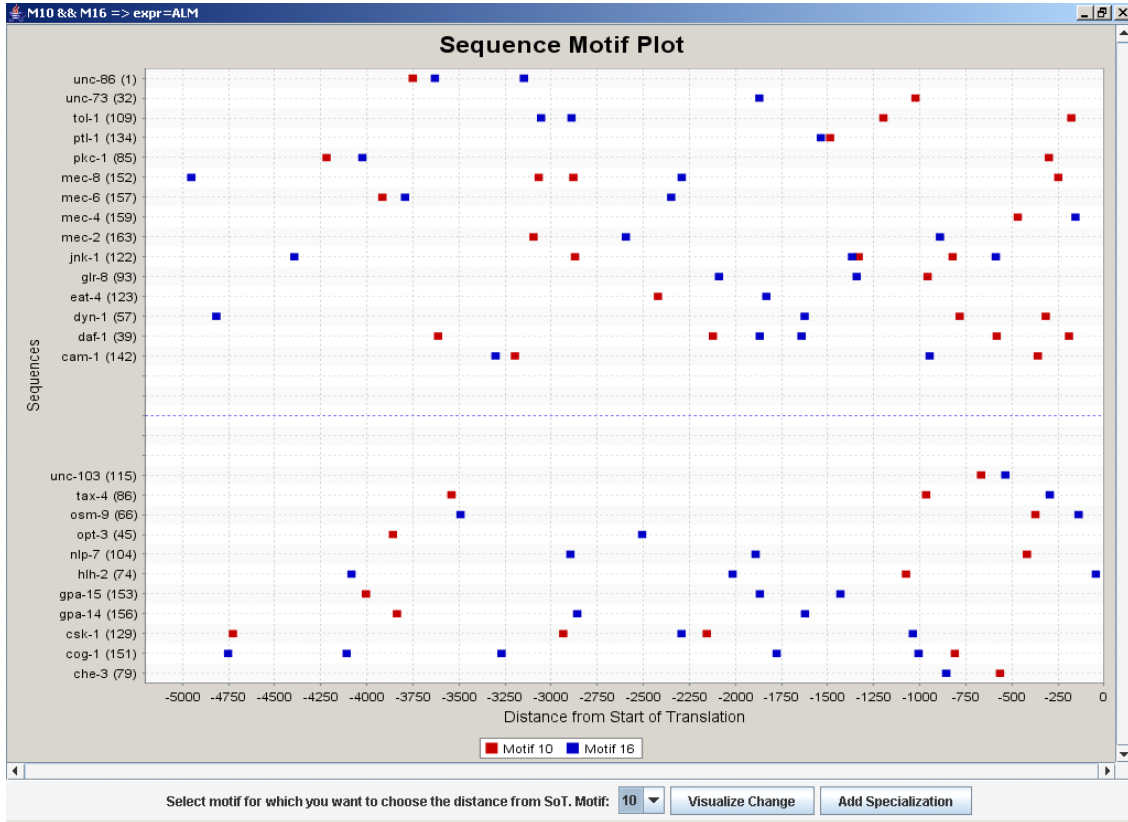


Figure 4.6: Sample Sequence Plot Frame.

Each graph is displayed with the rule used to establish the context as the title of the frame ($M10 \ \&\& \ M16 \Rightarrow \text{expr}=\text{ALM}$, in this case). This sequence plot displays all gene sequences that contain occurrences of the participating motifs (i.e., motifs M10 and M16). Along the y-axis is the list of gene promoters, that support the antecedent of the rule. That is, the gene promoters that contain at least one occurrence of each of the rule motifs. The x-coordinate of each point in the plot is the distance of the motif from the SoT, which is the far right end of the plot. The color of the point is used to identify the motif. The graph is sliced into two parts by a dotted line. The genes in the upper part of this dividing line are the ones that support the consequent of the rule and hence support the rule. The ones in the lower part are the genes that support only the antecedents of the rule.

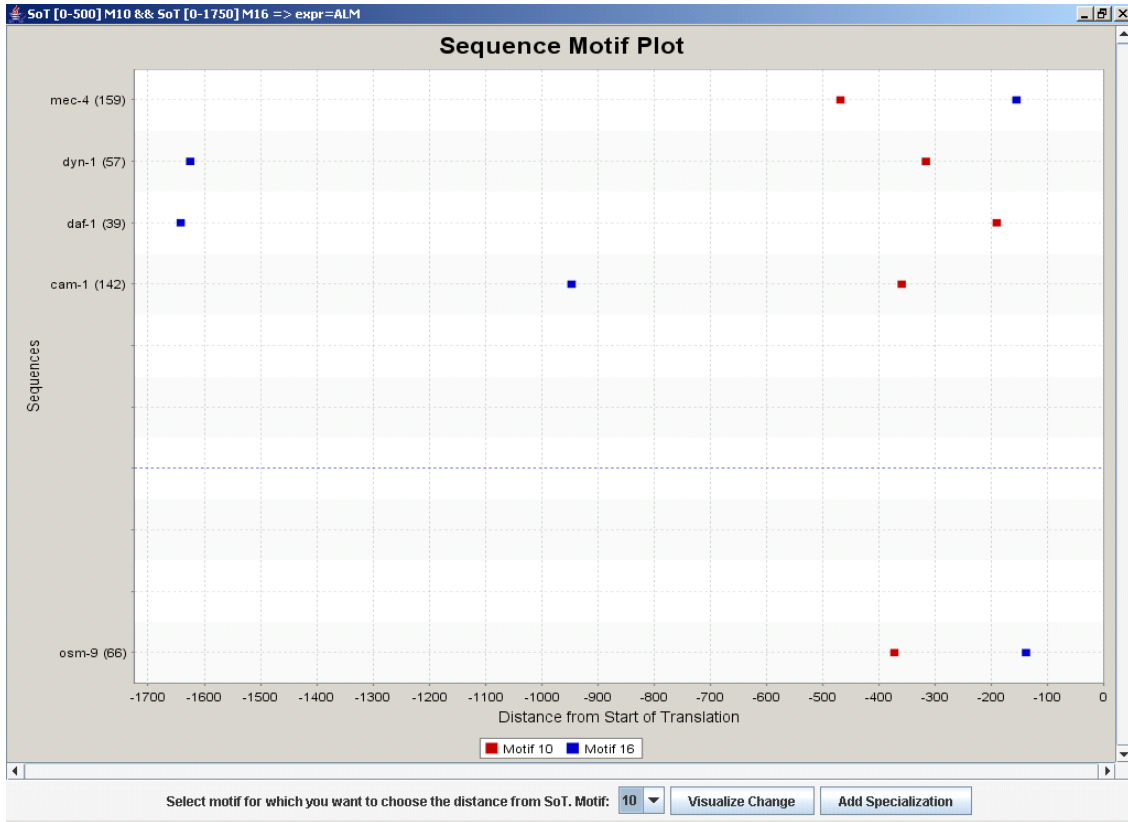


Figure 4.7: Visualize change command from the sequence plot.

The Visualize Change command from the sequence plot enables the user to visualize the data in the context of the specialization. This plot depicts the specialization $SoT [0-500] M10 \ \&\& \ SoT [0-1750] M16 \Rightarrow \ expr=ALM$ of the original rule $M10 \ \&\& \ M16 \Rightarrow \ expr=ALM$ from Figure 4.6.

rule specific sequence plots to identify a “distance from SoT” clause of interest, the “Visualize Change” command could be invoked to visualize the data in the context of the specialization rather than the original rule as depicted in Figure 4.7. Again the title of the new window is indicating the context setting rule/specialization. If the user finds the specialization of interest, it can be added to the Analysis Frame using the “Add Specialization” command on the new sequence plot. Again this causes the specialization to appear as a new entry in the Analysis Frame with an auto-generated Id that again lets a user trace back the steps in case the user wants to later recall which rule was used to derive a specialization (Figure 4.8). In case

the base rule consisted of more than 1 motif and multiple “distances from SoT” relationships are defined (one for each motif) each such relationship is represented as a term and a collection of independent terms constitutes the specialized rule. For instance see Figure 4.8 for the following specialization, which is interpreted as: “An occurrence of Motif 10 within 500 bp from the SoT and the presence of an occurrence of Motif 16 within 1750 bp from the SoT implies that the gene is expressed in cells of type ALM”.

$$SoT [0 - 500] M10 \ \&\& \ SoT [0 - 1750] M16 \Rightarrow expr = ALM \quad (4.2)$$

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
013.01.01	M16 && SoT [0-500] M10	expr=ALM	0.7777778	0.114754096	1.6944444	3.7665958E-2	0.25
013.01.03	SoT [0-500] M10 && SoT [0-1750] M16	expr=ALM	0.8	0.06557377	1.7428571	1.1028346E-1	0.14285715

Figure 4.8: Analysis Frame with two distance from SoT based specializations.

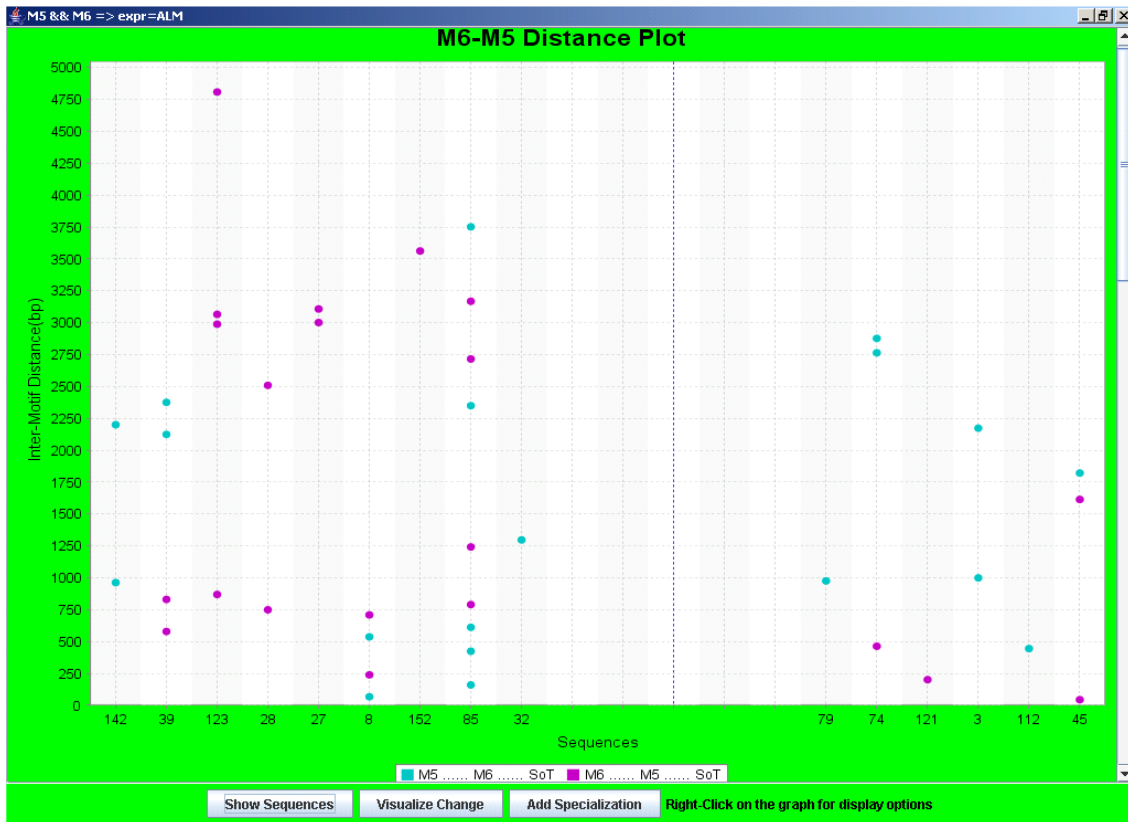


Figure 4.9: Inter-Motif Distance Plot for Motifs M5 and M6. Observe the lack of magenta (dark) dots in the right part of the frame.

4.2.4 Order of occurrence of motifs

We wanted the visual extensions to also accommodate exploratory analysis based on the hypothesis “The order of occurrence of motifs influences gene expression.” But during the system design and the system use by the team (including the domain expert) it was observed that we already had a few ways to visualize gene sequence data in the context of the “order of the occurrence” of motifs and hence a new plot was not created. If order of occurrence of motifs was important it could be easily identified by one of three ways: the color of the points in inter-motif distance plot, a repeating sequence of color in the sequence plot, or through the operation of the ASAS mining algorithm itself.

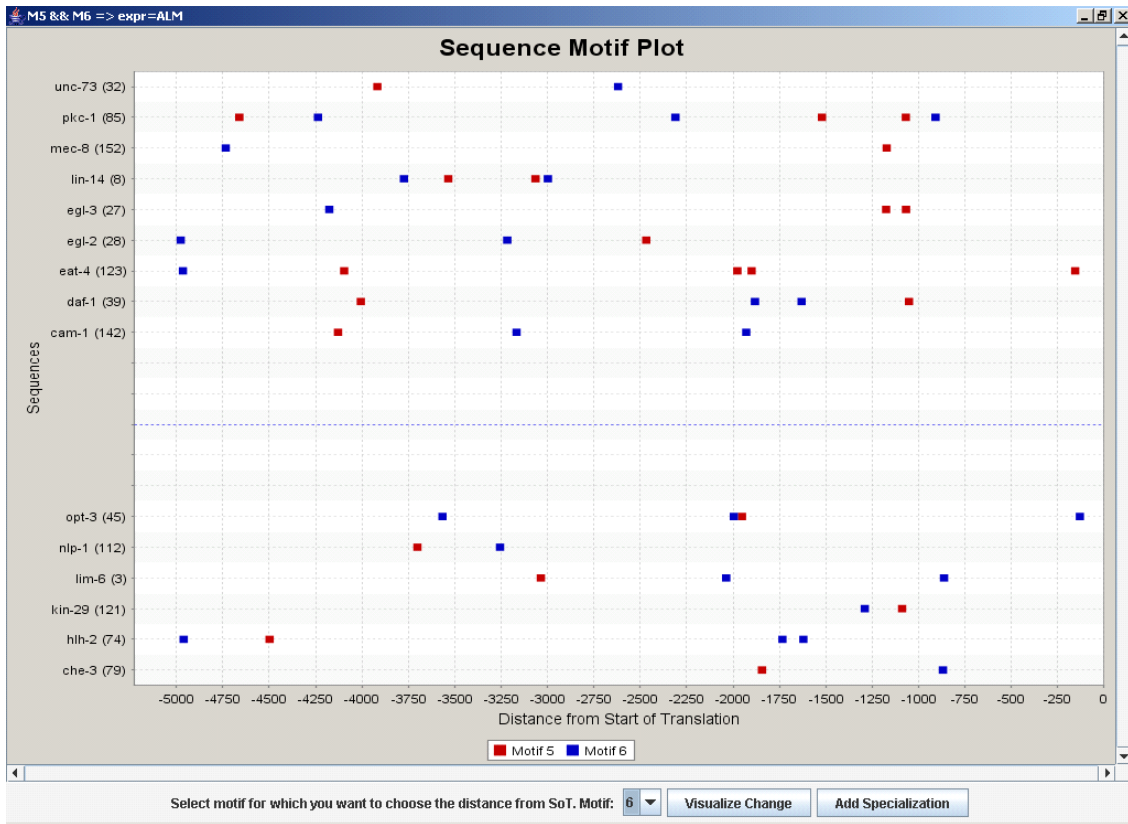


Figure 4.10: Sequence Plot for Motifs M5 and M6. Observe that in the rule supporting sequences (upper part) a red-dot is usually followed by a blue dot scanning the gene sequence from right end (SoT) to left.

Color of the points in an inter-motif distance plot. The order of the motifs in the inter-motif distance plot is represented by color. For instance in Figure 4.3, M10 to the right of M16 (i.e., M16..M10..SoT) is represented by a magenta (dark) dot, while M16 to the right of M10 is represented by a aqua (light) dot. Thus, color provides a quick visual clue whether the order of occurrence of motifs affects gene expression. If that is the case, the left part of the plot should have more points of one color than the other part.

Repeated sequence of color in the sequence plot. As mentioned in Section 4.2.3, the sequence plot displays all instances of participating motifs for qualifying

sequences as they occur on the gene relative to the SoT. Since each motif appears in its own color and the data is being visualized in the context of a single rule, one can often see a repetitive pattern of color in the upper part and a lack of the same in the lower part of the plot. Such a display could also indicate an influence of order of occurrence on gene expression.

ASAS mining algorithm. The association rule mining module from the WPI-Weka System [PR05] is capable of mining association rules with order/position based information and hence it is possible to have some of these rules with order of occurrence of motifs available already at the beginning of the exploratory analysis. Either of the two means mentioned above could be used to visually confirm/observe the order of occurrence relationship.

4.2.5 Adding Rules Manually

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3588956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
015	M5 [rp0-rp1] M6 [rp2-rp3]	expr=ALM	0.7	0.114754096	1.525	9.4429519E-2	0.25

Toolbar buttons: Inter-Motif Distance Plot, Sequence Plot, Add Rule, Delete Rule, Export Rules, Import Rules, Hide Current Column

Figure 4.11: Add Rule option in the Analysis Frame provides for free text option to add rules.

We wanted to allow users to type in specialized rules manually, particularly in the cases of rules involving order, as well as certain more complex 'hybrid' rules

discussed in detail below. To allow this option, we needed to write a grammar (Figure 4.12) to parse the rules. JavaCC [JCC], Java equivalent of LEX and YACC, was used to code the grammar and auto-generate the rule parser. The user can then simply type in the Antecedents and the Consequent of the rule to calculate the different statistics indicating the interestingness metrics of the rule as shown in Figure 4.11. Simply typing a complete rule computes the statistics indicating the interestingness of the rule. The user could also visualize the new rule using either the sequence plot or the inter-motif distance plot.

A rule keyed in by the user which does not adhere to this grammar results in an error as shown in Figure 4.13

4.2.6 Hybrid Rule

As described in the grammar governing rule definitions, each rule consists of an antecedent and a consequent. Antecedents in turn consist of terms. A rule could also include specialized term, extra hypothesis-based information(constraints) that the instances of the participating motifs must satisfy in order for a gene sequence to support the rule.

The system also supports hybrid rules, rules that consist of specialized terms based on different hypotheses and a gene sequence must satisfy all constraints in order to support the rule. In Figure 4.10 note that there exists an occurrence of M5 (the red dot) usually within the first 1600 bp from the SoT (far right end of the plot). Also note that there is an occurrence of Motif M5 between an occurrence of Motif M6 (blue dot) and the SoT. It is interesting to combine the two observations into a rule as follows and visualize it or calculate its interestingness. As we see in Figure 4.14 that this hybrid specialization:

Grammar

The grammar is defined as a 4-tuple: (SIGMA, N, P, S):
 SIGMA is an alphabet of terminal symbols
 N is an alphabet of non-terminal symbols
 P is a set of production rules
 S in N is the start symbol

```

Sigma := {SoT, Mn, Mn(rp i - rp i+1), \&\&, (x-y)}
N     := {S, L1, L2, C1, C2, CL1, CL2, CL3, CL4, S}
P := {
  S = Term | (Term C1 Term) | (T C1 Term)
  C1 = &&                                     # And
  Term = CL1 | CL2 | CL3 | CL4
  CL1 = L1 C1 L1 | CL1 C1 L1                 # Covers rules based on presence.
  CL2 = L1 C2 T                               # Covers literals of the form distance from SoT.
  CL3 = L1 C2 L1 | CL3 C2 L1                 # Covers literals of the form Mi at a distance of
                                              # x-y from Mj
  CL4 = L2 C1 L2 | CL4 C1 L2                 # Covers literals of the form Mi occurs before Mj
  L1 = Mn                                     # Motif n
  T = SoT                                     # Start of Translation
  C2 = (x-y)                                  # Where x and y are integers such that y > x
  L2 = Mn (rp i - rp i+1)                    # Motif n exists from Relative position i to i+1
                                              # on the gene sequence.
}

```

A sample derivation of an antecedent from the grammar is shown below:

```

S := Term
    CL1
    CL1 C1 L1
    CL1 && M3
    L1 C1 L1 && M3
    M1 && M2 && M3

```

This antecedent is interpreted as:

If Motif 1 is present and Motif 2 is present and Motif 3 is present.

Figure 4.12: Grammar to parse rules.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.3888889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
007	M10	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
008	M10 && M16	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
009	M25	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
010	M25	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
011	M26	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
012	M26	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
013	M10 && M16	expr=ALM	0.42102449	0.3125	1.0776987	4.9832669E-1	0.78571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
015	M5 && M6	expr=ALM	0.6	0.14754099	1.3071429	2.0701702E-1	0.32142857
017	M5 (rp0-rp1)	expr=ALM	0.7	0.114754096	1.525	9.4429519E-2	0.25

Figure 4.13: Grammar based parsing helps identify user errors in typing the rule.

$$M5 [rp0-rp1] M6 [rp2-rp3] \&\& \text{SoT} [0-1600] M5 \Rightarrow \text{expr=ALM}$$

$$[Confidence = 0.8333333, Support = 0.08196721] \quad (4.3)$$

has a higher confidence as compared to the following simpler “order of occurrence” specialization

$$M5 [rp0-rp1] M6 [rp2-rp3] \Rightarrow \text{expr=ALM}$$

$$[Confidence = 0.8333333, Support = 0.08196721] \quad (4.4)$$

As seen above hybrid specialization could have multiple specialized terms that relate to a single motif. A hybrid specialization could post multiple constraints on the same motif like Distance from SoT and Order of occurrence relative to another motif. It is important to note that although the rule may have multiple constraints for the same motif, it is not required that the same instance of the motif satisfies each of them. In the context of the 4.3 above, it is not required that the instance

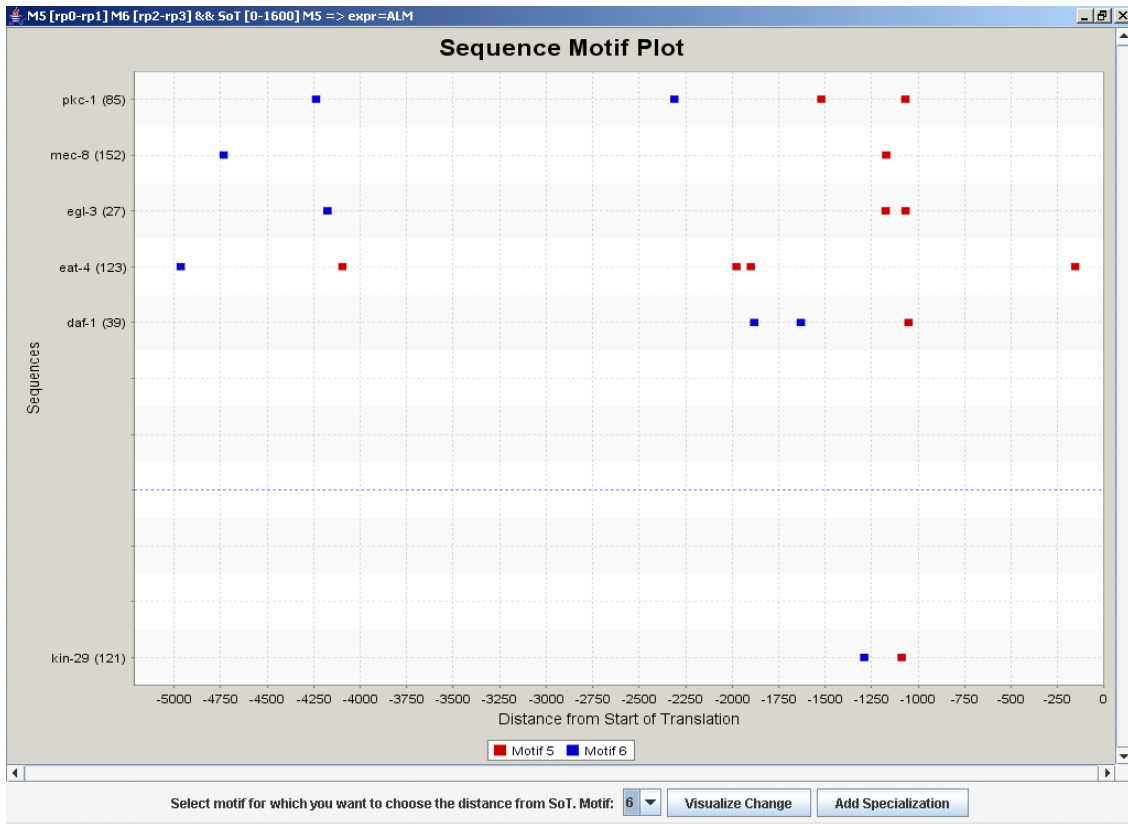


Figure 4.14: Hybrid rules help specify multiple constraints (based on different hypotheses) within a single specialization.

of Motif 5 that satisfies the order of occurrence condition is the same M5(instance) that lies within 1600 base pairs of the SoT. However, there might be a need for the user to actually specify constraints which are inter-related, aliases are supported by our rule grammar for exactly this reason.

4.2.7 Aliases

Aliases were included in the grammar to provide the user with an option of defining inter-related constraints or specialization terms. Consider the following Inter-Motif

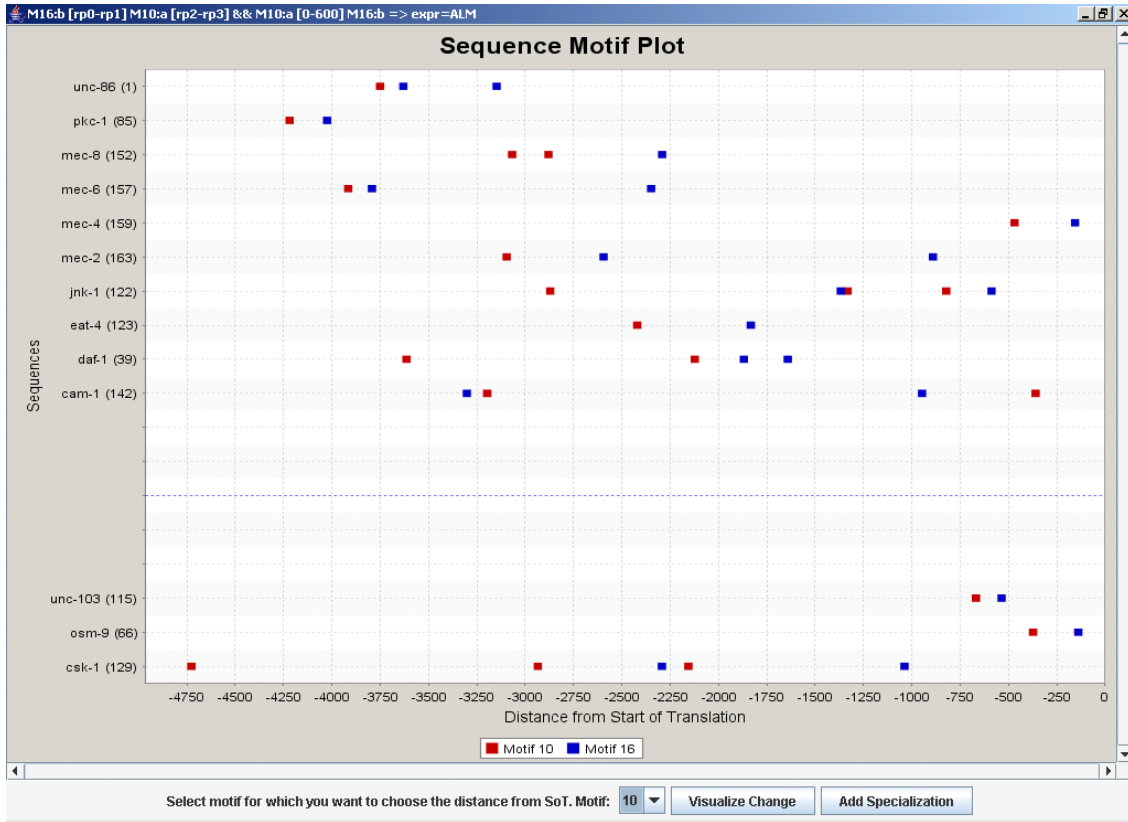


Figure 4.15: Aliases let user define specializations with inter-related constraints.

Distance based specialization from Figure 4.5:

$$M10[0 - 500]M16 \Rightarrow expr = ALM \quad (4.5)$$

Visualizing this specialization using a sequence plot (Figure 4.6), one can see distinctly that not only do motifs M10 (red dot) and M16(blue dot) occur close together but they also occur in a pattern such that the same instances of M10 and M16 that are involved in the distance-based relationship also occur in the same order relative to the SoT. Aliases enable the user to specify such complex relationships in the rule as follows (Figure 4.15):

$$M10:a [0-500] M16:b \&\& M16:b [rp0-rp1] M10:a [rp2-rp3] \Rightarrow expr=ALM \quad (4.6)$$

For details of system operation, see User Guide.

Chapter 5

System Architecture

This tool was conceptualized as an extension to the WPI-Weka system and hence is also referred to as the “*Visualization and Specialization Modules(VSM)*”. This chapter describes the interaction of this tool with the WPI-Weka system and a high level design overview.

5.1 Component Interaction

Figure 5.1 illustrates how VSM interacts with the WPI-Weka system, to let the user perform hypothesis-driven exploratory analysis of genetic data in order to create specialized association rules that predict gene expression. The enumerated arrows with italicized text denote the different steps that constitute the process of exploratory analysis to discover specializations predicting gene expression. Each of the steps along with the inputs and outputs to the process are listed below:

1. Mine association rules. One of the contributions of [Rudss] was to integrate into a single classifier (AssociateClassifier) within the WPI-Weka system([WPI]), contributions of previous work at WPI in the field of association rule mining.

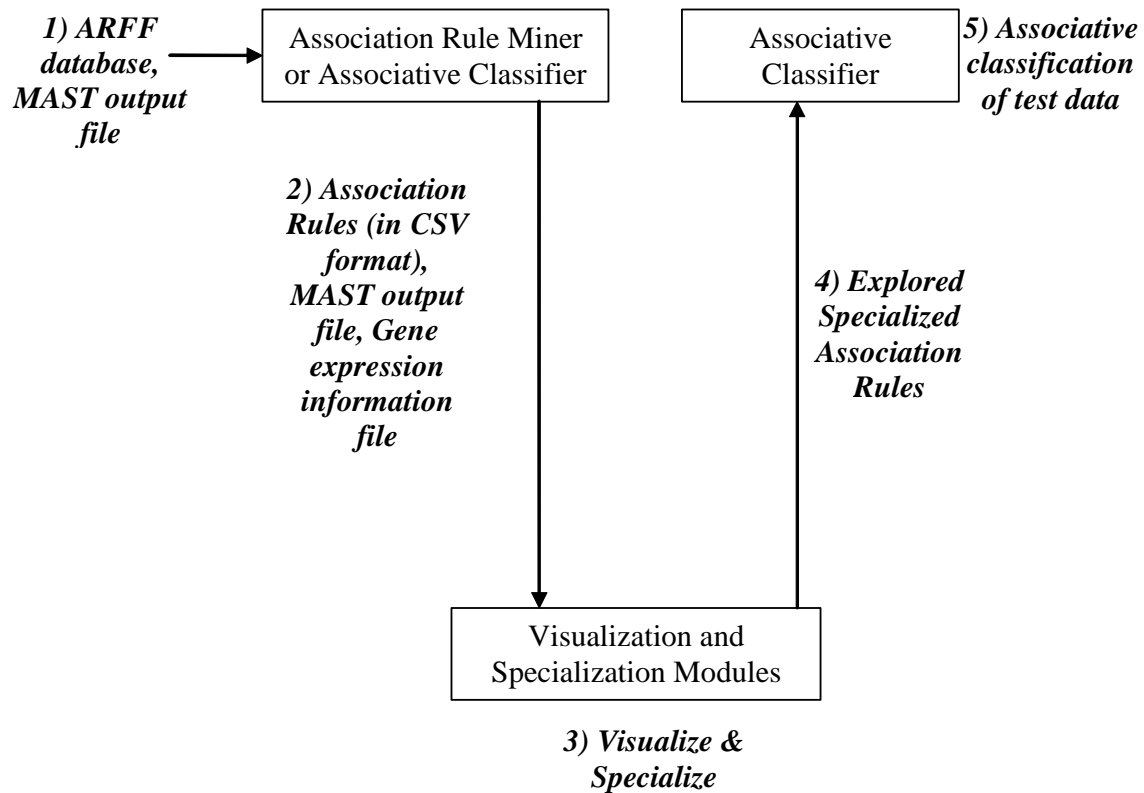


Figure 5.1: Process depicting the flow of data (interactions) between the WPI-Weka system and the VSM.

The data exchange between the WPI-Weka system and the VSM is in a comma separated value format which is explained in detail in Figure 5.2.

This classifier or the association rule miner (ARMiner) module of the WPI-Weka system, takes as input an ARFF file consisting of genetic data and a MAST output file (Section 3.3) and mines for association rules predicting gene expression.

2. Transfer mined association rules to VSM. As a part of the integration plan it was decided that both [Rudss] and this work would support import/export of rules in a predefined comma-separated values (CSV) format. So as a first step to analyzing gene expression association rules, [Rudss] could be used to invoke the VSM with the set of mined rules (Figure 5.2), the MAST output file and

CSV interface to transfer rule sets between WPI-Weka and VSM									
HEADER									
Id,	Antecedent,	Consequent,	Confidence,	Support,	Lift,	p-Value,	Event Wt. ,	Within Cell-Type(s)	support
DATA									
001,	M17,	expr=ALM,	0.48148146,	0.325,	1.2037036,	4.9873279E-1,	0,	0.42857143	
002,	M12,	expr=ALM,	0.52830184,	0.35,	1.3207545,	5.021099E-1,	0,	0.10714286	

Figure 5.2: CSV interface to transfer rule sets between WPI-Weka system and VSM. The header section defines a comma separated list of the headers describing the attributes contained in each data instance. The Data section consists of the actual set of rules being exported or imported.

the gene expression information.

3. Visualize and Specialize. The implementation of this work, that is the VSM, would let the user perform hypothesis-driven visualization of data that helps specialize a set of association rules. See Section 4.2.
4. Transfer the set of specialized rules to the AssociativeClassifier. Again the VSM as well as [Rudss] support the transfer of set of specialized rules explored using the VSM to the AssociativeClassifier using the predefined CSV format.
5. Use the classifier to predict gene expression for a novel set of genes. The classifier can be used to calculate the classification accuracy of the imported specializations over a test set consisting of novel genes (i.e., genes that have not been used during mining or specialization) in order to determine the predictive power of the relationships identified.

5.2 System Design

This section describes the high level design of the VSM tool from a functional perspective that is also illustrated in Figure 5.3. We describe the different modules within the VSM subsystem implementation:

1. **MAST Parser.** The VSM could be invoked from within the WPI-Weka System or as a standalone application. Irrespective of the invocation mechanism (i.e., as a standalone application or from within WPI-Weka) it requires as input the gene expression information (Figure 4.2) and a MAST output file (Figure 3.5). The information from the MAST output file and the gene expression information is parsed using this module to populate a multi-level internal data structure (Figure 5.4) and subsequently the analysis frame (Figure 4.1) is displayed.
2. **Rule Parser.** Each base rule, either belonging to the set of rules mined using the WPI-Weka system or by using the “Add Rule” (Section 4.2.5) command from the analysis frame is parsed by using this module, that is, an implementation of the grammar defined in Figure 4.12.
3. **Charting Extensions.** The parsed information from rule parser and the internal data structure are used as input to the charting infrastructure to generate hypothesis-specific plots for data visualization. The charting infrastructure is created by extending the open source charting library JFreeChart. JFreeChart separates its data from its presentation layers which means the system could be extended to add new features to plots with minimal changes. The user’s exploratory analysis using these plots (data visualization) subsequently produces specialized rules which in combination to the internal data structure can be

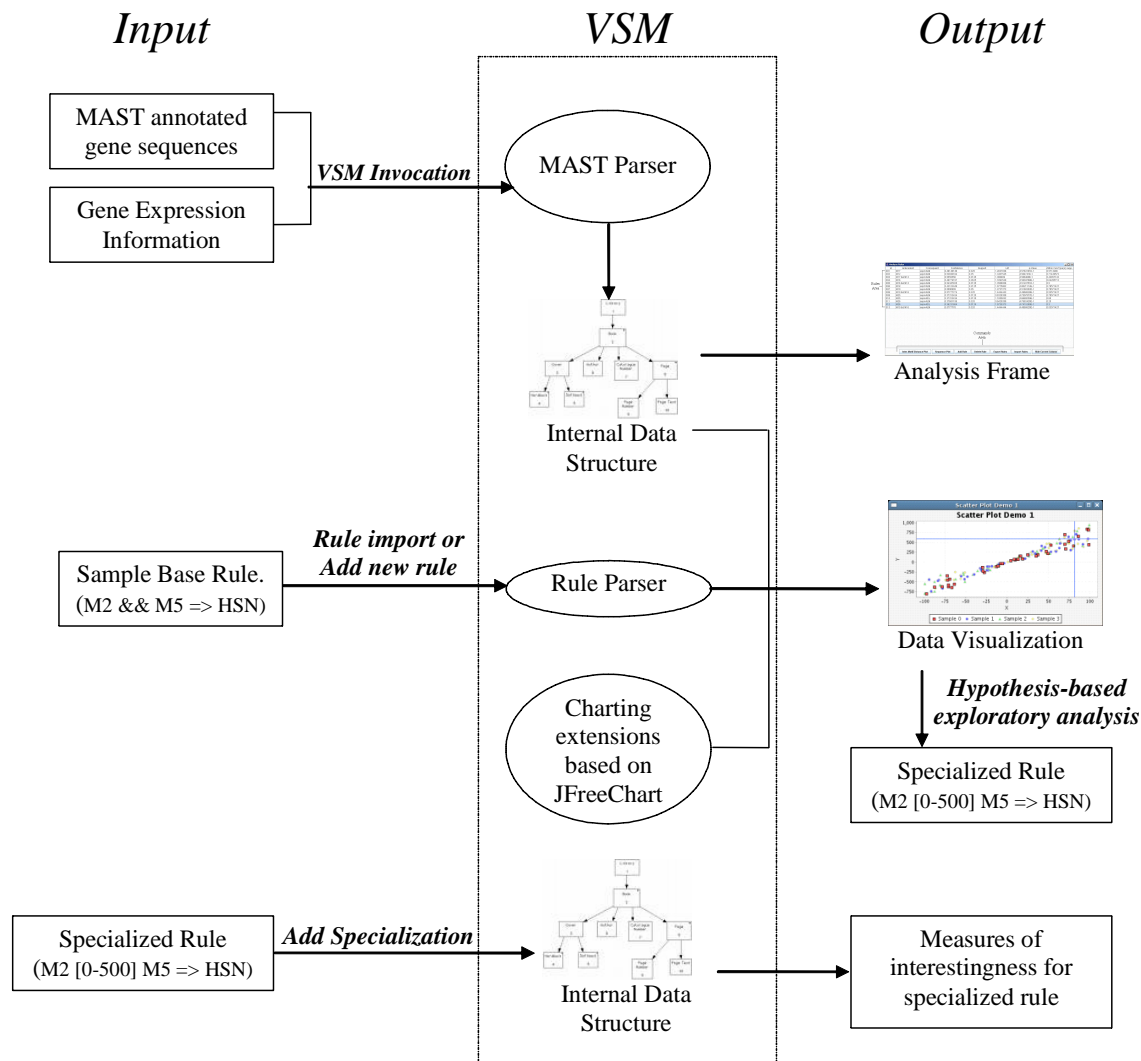


Figure 5.3: VSM System Design.

Functional design of the VSM. Each step in the exploratory analysis process is described in terms of the input(s) to the VSM subsystem, corresponding modules within the VSM invoked, and the output. The vertical rectangle represents the VSM subsystem with each oval representing a module within the VSM implementation. As delineated, the entities to the left of the VSM subsystem are the input(s) provided to each module and the entities to the right are the output(s) from the VSM at each step.

Key (Gene Name)	Value		
ceh-43	Expression Pattern	Neural	
	Gene Name	ceh-43	
	Length	993	
	Motif-based location info	M1	250, 523
		M2	944
nlp-5	Expression Pattern	Muscle	
	Gene Name	nlp-5	
	Length	5000	
	Motif-based location info	M1	3555
		M2	45, 856

Figure 5.4: Hierarchical internal data structure - Hash of hashes.

The top level hash has the gene name as the key attribute. The value element corresponding to each gene is also a hash consisting of sequence information which, besides gene specific information like length and expression pattern, contains location information organized on a per motif basis. The location information for each motif (key) consists of all occurrences of the motif on the gene sequence relative to the SoT. The sample here illustrates the organization of information for two sample sequences, ceh-43 and nlp-5, each of which contains one or more occurrences of motif M1 and M2.

used to calculate the different measures of interestingness for each specialized rule.

5.3 Implementation Details

Since the WPI-Weka system, which is an extensive collaborative effort within the KDD Research Group at WPI, is Java based, Java was chosen to implement VSM.

It was during the course of this project and [Rudss] that the WPI-Weka system was hosted in the WPI sourceforge server. As a part of this project an ant-script (Java equivalent of a makefile) was also developed that enables users to build the system from the source with minimal instructions. Eclipse was our choice of the IDE used as it provides a easy to use interface for both Java based development as well as CVS based version control.

Elements of good design were extensively applied throughout the development process. For instance, the support for import/export of CSV file containing the rules is implemented as a Java interface called the Analyzable interface which makes it easy for a new implementation to support a certain functionality without restricting a specific type of implementation. For instance if a new mining algorithm that mines gene expression rules is added to the WPI-Weka System, it could invoke VSM as long as it implements the Analyzable interface.

Chapter 6

Experimental Evaluation

Chapter 3 (Figure 3.2) describes in detail the process to create a dataset in a mining compatible format (ARFF) starting with data collection. As mentioned in Section 3.2 and again in Section 3.4, to avoid mining and exploring relationships that over fit the data, the sequences were divided into a training set (90% of the sequences) and a test set (10% of the sequences). Figure 6.1 illustrates the experimental setup.

6.1 Experimental Protocol and Parameters to be measured

Mining the training dataset using the WPI-Weka Association Rule Miner produces gene expression association rules. Different measures of interestingness, including the traditional measures of support and confidence, are calculated to estimate the statistical significance of the rules. Once the domain user identifies an interesting base rule (e.g., based on the values of different measures of interestingness), VSM could be used to perform hypothesis-driven exploratory analysis of the selected rule

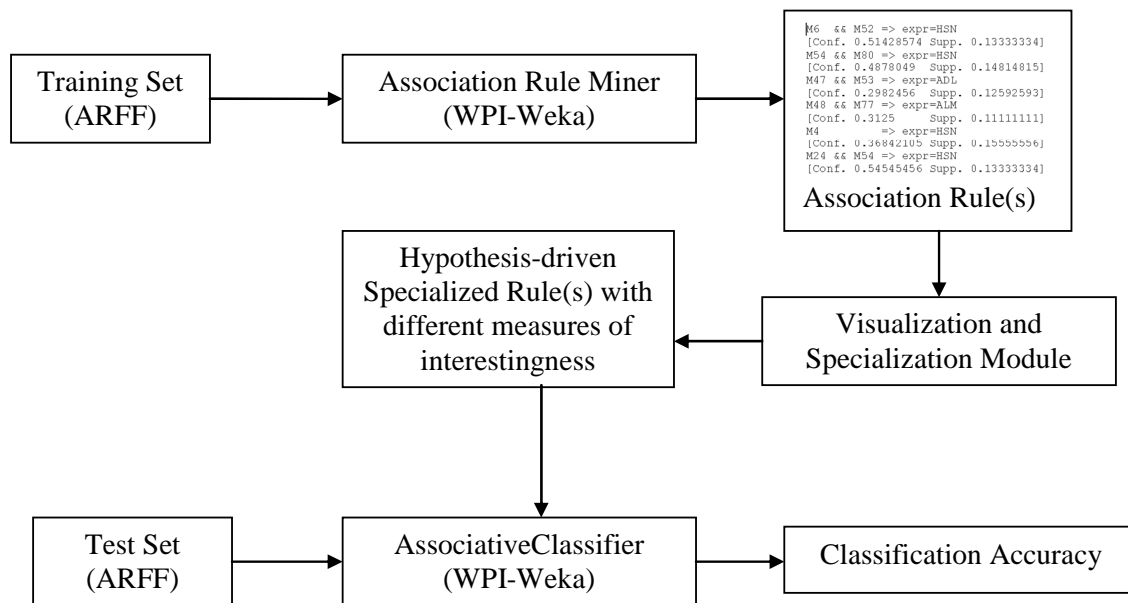


Figure 6.1: Experimental Setup.

Starting with mining of rules over the training data to the classification of the test dataset using the explored specialized rules.

to derive a specialized association rule such that the antecedent of the rule consists of additional constraints based on positional information of the motif(s). This process is repeated a few times with different base rules to derive a set of specialized rules that, at least statistically, seem to provide a more accurate representation of the underlying regulatory mechanism governing gene expression. This set of specialized rules is then tested for accuracy over a dataset consisting of novel genes (i.e., genes which were not used to elicit motifs and were also not involved in the mining or the specialization processes). The classification accuracy is a measure of predictive power. An improved classification accuracy as compared to the set of base rules translates to an increase in the confidence of the specialized rule with a reasonable decrease in the support of the rule and is used to estimate the potential biological validity of the relationship. Other measures of interestingness like the p-value and the lift also help to estimate the effectiveness of the rule. The system is extensible

from the measures of interestingness perspective; that is, it would require minimal changes for another measure of interestingness to be added to the system.

6.2 Experimental Results and Analysis

The target audience for the tool developed are domain experts trying to identify biologically interesting relationships. We present a walk through of a small scale experiment using real genetic data that helps establish the work flow for the process of performing hypothesis-driven specializations.

As discussed in Section 3.1, the training set consists of 151 gene sequences in ARFF format. In the experiment reported here, these sequences were mined for presence based association rule mining with a minimum support of 0.1 and a minimum confidence of 0.3. It is important to understand the reason for choosing the relatively low values for support and confidence. The choice of the low value for the minimum support is based on the way the dataset is put together. Since we tried to identify gene sequences for 9 different cell types, each cell type has approximately 11% representation in the dataset, then the motif elicitation and annotation process were performed with an intent to find cell-type specific motifs. So if a biologically valid cell-type specific regulatory mechanism was found during the mining process it is reasonable to expect that it should have low support(i.e., 11% or less). Since we intend to explore specializations that represent the biological relationship more effectively as compared to the base rules from which they are derived, we can select base rules with low confidence measure. The mining process resulted in a total of 269 rules, out of which the following 6 rules were chosen to illustrate effectively all forms of hypothesis-driven specializations. So we intend to visualize and specialize 2 rules per hypothesis type to illustrate the work flow.

Motif	Consensus sequence
M6	GGAAGAAGAG
M47	GAGAAGAG
M48	TGAGAAA
M52	GAAGAAGAAGAA
M53	GAAGAAGAAGGA
M54	GAGTGAGAGGGG
M69	GGGGGGGAGG
M77	GAGACGAAGA
M80	GAGAAGAAGAAG

Figure 6.2: List of motifs from the base rules under consideration along with their consensus sequences.

$$M6 \ \&\& \ M52 \Rightarrow \text{expr=HSN} \ [\text{Conf.} = 0.51428574, \text{Supp.} = 0.13333334] \quad (6.1)$$

$$M54 \ \&\& \ M80 \Rightarrow \text{expr=HSN} \ [\text{Conf.} = 0.4878049, \text{Supp.} = 0.14814815] \quad (6.2)$$

$$M47 \ \&\& \ M53 \Rightarrow \text{expr=ADL} \ [\text{Conf.} = 0.2982456, \text{Supp.} = 0.12592593] \quad (6.3)$$

$$M48 \ \&\& \ M77 \Rightarrow \text{expr=ALM} \ [\text{Conf.} = 0.3125, \text{Supp.} = 0.11111111] \quad (6.4)$$

$$M69 \Rightarrow \text{expr=HSN} \ [\text{Conf.} = 0.46341464, \text{Supp.} = 0.14074074] \quad (6.5)$$

$$M6 \Rightarrow \text{expr=HSN} \ [\text{Conf.} = 0.13636364, \text{Supp.} = 0.044444446] \quad (6.6)$$

6.2.1 Order of motif occurrence specialization of Rules (6.1) and (6.2)

In this section we specialize Rules (6.1) and (6.2) based on the hypothesis “The order of occurrence of motifs influences gene expression”. Figures 6.3 and 6.5 depict the process followed to specialize the base rules from an order of occurrence of motifs perspective.

An inter-motif distance plot, for instance Figure 6.3, is split into two parts: the left part containing the sequences that support the rule and the right part containing the sequences that support the antecedent only. This provides the user with an easy mechanism to discover order of occurrence based patterns in the left part that are not as frequent in the right part, as this would let us explore specializations with improved classification accuracy (and/or confidence). For instance, in Figure 6.3, many genes on the right-hand side lack magenta (dark) dots. This implies that the occurrence of Motif 6 in between Motif 52 and the SoT (i.e., M52-M6-SoT) may positively influence a gene to be expressed in cells of type HSN. Once a user has identified such an order based relationship that is of interest, the following corresponding specialized rule can be added to the analysis frame:

$$\text{M6 [rp0-rp1] M52 [rp2-rp3]} \Rightarrow \text{expr=HSN [Conf. = 0.6363636, Supp. = 0.1037037]} \quad (6.7)$$

The analysis frame enables the user to estimate the significance of the rule using the different measures of interestingness computed for the specialized rule. The user can also compare the interestingness of the specialized rule with that of the base rule (Figure 6.4).

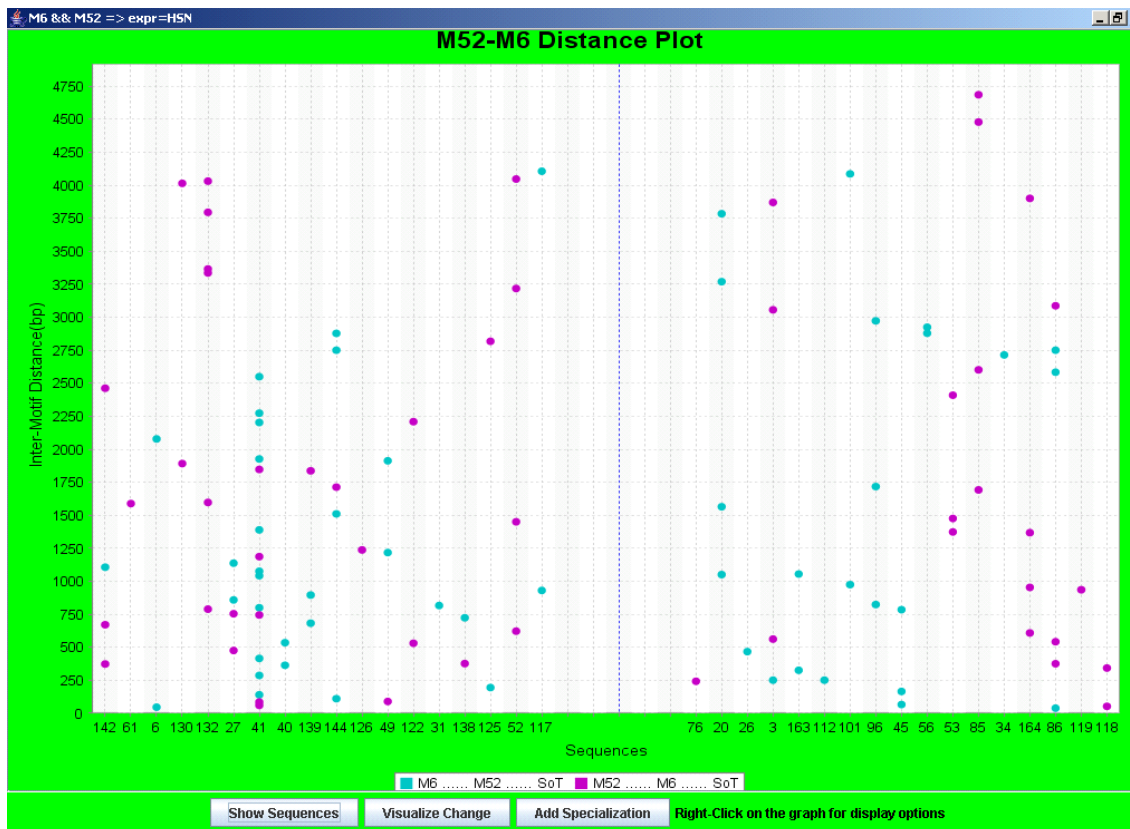


Figure 6.3: Inter-motif distance plot for the base rule $M6 \ \&\& \ M52 \Rightarrow \ expr=HSN$. Along the x-axis are the Id's corresponding to the genes. Along the y-axis is the inter-motif distance. Each point in the plot, irrespective of the color, represents the distance between an occurrence of Motif 6 from an occurrence of Motif 52. Also notice the division of the graph into two parts. Each colored dot is representative of the relative ordering of the occurrence of the motifs relative to the Start of Transcription. A magenta (dark) dot represents the inter-motif distance between an occurrence of Motif 6 and Motif 52 such that the occurrence of Motif 6 lies between the occurrence of Motif 52 and the SoT (i.e., M52-M6-SoT). A aqua (light) dot represents an inter-motif distance such that the order of occurrence of motifs is M6-M52-SoT. The left-side part consists of gene sequences which support the rule and the right-side part consists of the gene sequences that support the antecedent of the rule only.

Analyze Rules							
Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
I01	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
I01.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
I02	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	9.5348043E-1	0.47619048
I02.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	3.2186886E-1	0.0
I03	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	3.2552208E-1	0.7083333
I03.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.1845553E-1	0.0
I04	M48 && M77	expr=ALM	0.3125	0.11111111	1.5066965	3.4994598E-1	0.53571427
I04.01	M48:b [0-500] M77:a && M77:a [rp0-r...	expr=ALM	0.53846157	0.05185185	2.5961537	9.6472486E-1	0.0
I05	M69	expr=HSN	0.46341464	0.14074074	1.489547	9.1427948E-1	0.45238096
I05.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	8.960148E-1	0.0
I06	M6	expr=ADL	0.13636364	0.044444446	0.76704544	5.368088E-1	0.25
I06.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	8.1576099E-1	0.0

Inter-Motif Distance Plot
Sequence Plot
Add Rule
Delete Rule
Export Rules
Import Rules
Hide Current Column

Figure 6.4: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.7), with the base rule, Rule (6.1).

Similarly in Figure 6.5, observe that many genes in the right part of the plot lack magenta (dark) points. This again indicates that the occurrence of motif 54 between an occurrence of Motif 80 and the SoT (i.e., SoT-M54-M80) may positively influence gene expression. Again the user could add the following corresponding specialization to the analysis frame and compare its interestingness to that of the base rule.

$$M54[rp0-rp1] M80[rp2-rp3] \Rightarrow \text{expr=HSN} \text{ [Conf.} = 0.6, \text{ Supp.} = 0.11111] \quad (6.8)$$

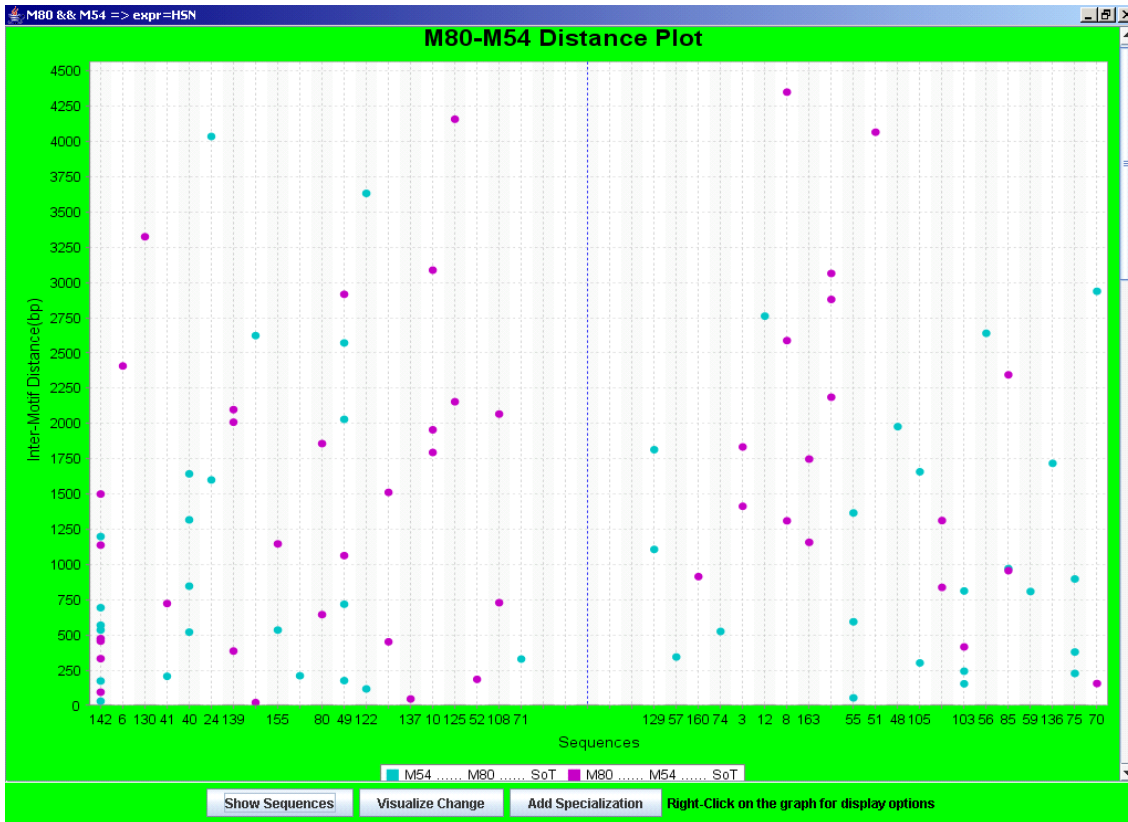


Figure 6.5: Inter-motif distance plot for the base rule $M54 \ \&\& \ M80 \Rightarrow \ expr=HSN$.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	9.5348043E-1	0.47619048
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	3.2186886E-1	0.0
003	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	3.2552208E-1	0.7083333
003.01	M47 [0-250] M53	expr=ADL	0.47828087	0.08148148	2.6902175	3.1845553E-1	0.0
004	M48 && M77	expr=ALM	0.3125	0.11111111	1.5066965	3.4994598E-1	0.53571427
004.01	M48.b [0-500] M77.a && M77.a [rp0-r...	expr=ALM	0.53846157	0.05185185	2.5961537	9.6472486E-1	0.0
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	9.1427948E-1	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	8.960148E-1	0.0
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	5.368088E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	8.1576099E-1	0.0

At the bottom of the table, there are six buttons: "Inter-Motif Distance Plot", "Sequence Plot", "Add Rule", "Delete Rule", "Export Rules", "Import Rules", and "Hide Current Column".

Figure 6.6: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.8), with the base rule, Rule (6.2).

6.2.2 Inter-motif distance specialization of Rules (6.3) and (6.4)

In this section we specialize Rules (6.3) and (6.4) based on the hypothesis “Inter-motif distance influences gene expression”. Figures 6.7 and 6.9 depict the process followed to specialize the base rules from an inter-motif distance perspective.

An inter-motif distance plot, for instance Figure 6.7, displays for qualifying gene the inter-motif distances between each occurrence of Motif 47 from each occurrence of Motif 53. A qualifying gene is one that contains at least one occurrence of each motif in the base rule. The splitting of the plot into two parts (as described in Section 6.2.1), provides the user with an easy mechanism to discover inter-motif distance pattern in the left part that are not as frequent in the right part, as this would let us explore specializations with improved classification accuracy (and/or confidence). For instance, in Figure 6.7, many genes on the right-side part lack dots in the shaded area of the plot, that is, an inter-motif distance range of 0-250 bp which implies that the occurrence of motifs 47 and 53 within 250 bp from each other may be positively related to the gene being expressed in cell type ADL. Once a user has defined an inter-motif distance relationship that is of interest the relevant region of the plot gets shaded as illustrated in Figure 6.7. Subsequently the following corresponding specialization can be added to the analysis frame.

$$\text{M47 [0-250] M53} \Rightarrow \text{expr=ADL [Conf. = 0.47826087, Supp. = 0.08148148]} \quad (6.9)$$

Again the user can estimate the significance of the rule by using the different measures of interestingness computed for the specialized rule. The user can also compare the interestingness of the specialized rule as compared with that of the base rule (Figure 6.8).

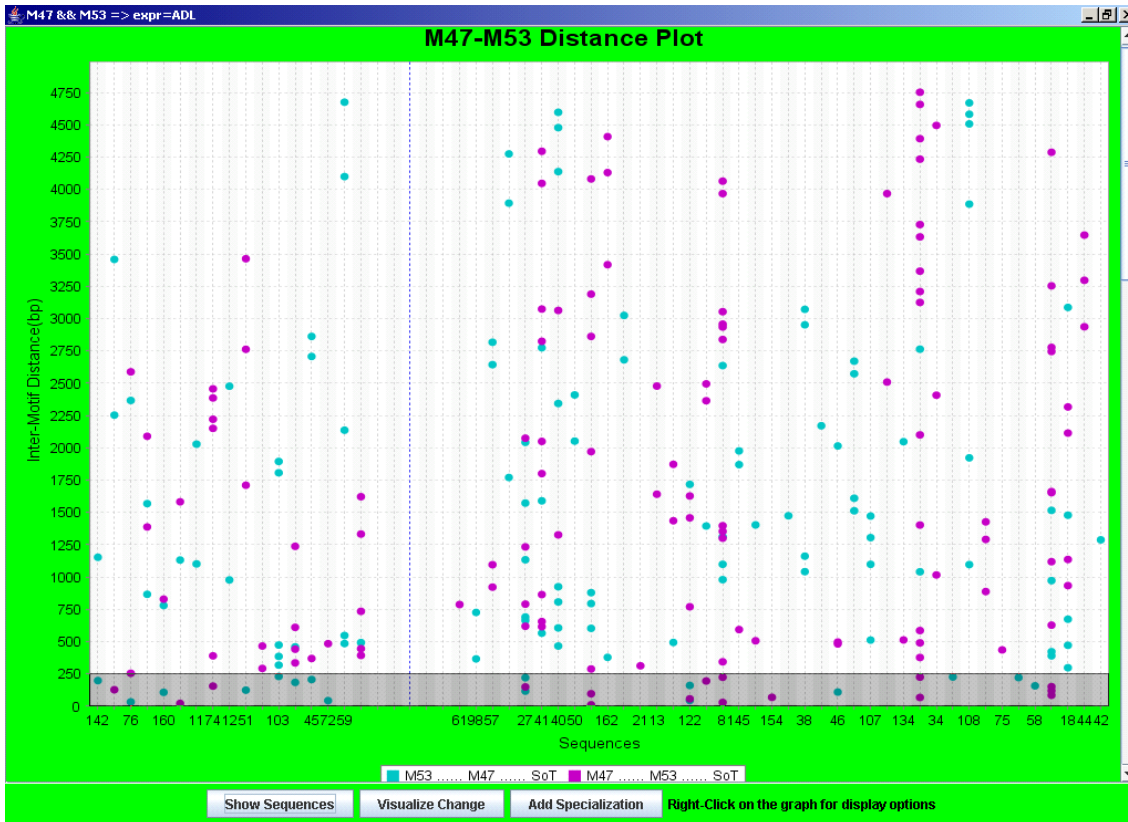


Figure 6.7: Inter-motif distance plot for the base rule $M47 \ \&\& \ M53 \Rightarrow \ expr=ADL$.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	9.5348043E-1	0.47619048
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	3.2186886E-1	0.0
003	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	1.7501895E-3	0.7083333
003.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.5008453E-5	0.45833334
004	M48 && M77	expr=ALM	0.3125	0.11111111	1.5066965	3.4994598E-1	0.53571427
004.01	M48:b [0-500] M77:a && M77:a [rp0-r...	expr=ALM	0.53846157	0.05185185	2.5961537	9.6472486E-1	0.0
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	9.1427948E-1	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	8.960148E-1	0.0
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	5.368088E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	8.1576099E-1	0.0

At the bottom of the window, there are six buttons: "Inter-Motif Distance Plot", "Sequence Plot", "Add Rule", "Delete Rule", "Export Rules", "Import Rules", and "Hide Current Column".

Figure 6.8: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.9), with the base rule, Rule (6.3).

Similarly in Figure 6.9, observe that many genes lack magenta (dark) points in the right-side part of the shaded area of the plot, that is, an inter-motif distance range of 0-500 bp. This implies that the occurrence of motif 77 and 48 within 250 bp from each other and in the order SoT-M77-M48 may positively influence gene expression in cell type ALM. Subsequent addition of the following corresponding specialization to the analysis frame enables the user to compare the interestingness of the specialized rule to that of the base rule (Figure 6.10).

$$\begin{aligned}
 & \text{M48:b [0-500] M77:a \&\& M77:a [rp0-rp1] M48:b[rp2-rp3]} \Rightarrow \text{expr=ALM} \\
 & \quad \quad \quad [\text{Conf.} = 0.53846157, \text{Supp.} = 0.05185185] \quad (6.10)
 \end{aligned}$$

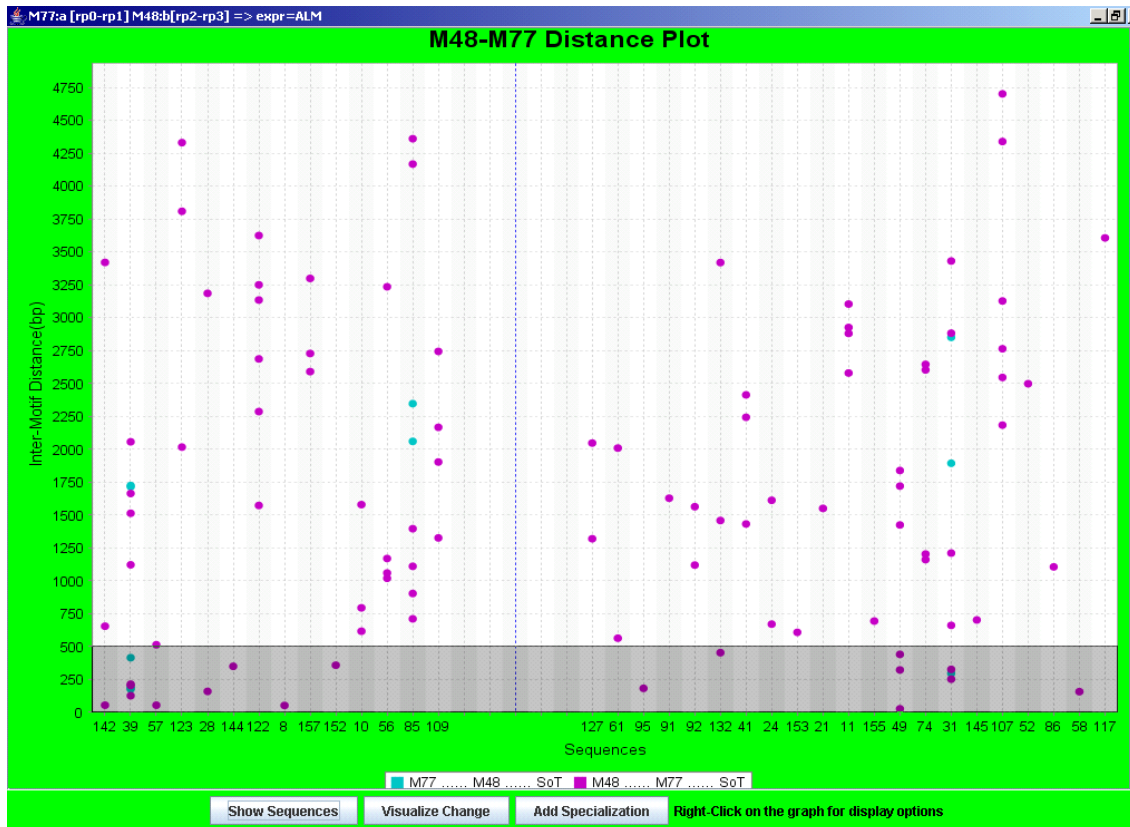


Figure 6.9: Inter-motif distance plot for the base rule $M77 [rp0-rp1] M48[rp2-rp3] \Rightarrow expr=ALM$.

Notice that the base rule selected is already an “*order of occurrence of motifs*” specialization. This was one of the rules generated by the ASAS algorithm as mentioned in Section 4.2.4.

id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	9.5348043E-1	0.47619048
002.01	M54 [rp0-rp1] M80 [rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	3.2186886E-1	0.0
003	M47 && M53	expr=HSN	0.2982456	0.12592593	1.6776316	1.7501895E-3	0.7083333
003.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.5008453E-5	0.45833334
004	M77 [rp0-rp1] M48 [rp2-rp3]	expr=ALM	0.4	0.1037037	1.9285715	1.0940551E-3	0.5
004.01	M48:b [0-500] M77:a && M77:a [rp0-rp1] M48:b [rp2-rp3]	expr=ALM	0.53846157	0.05185185	2.5961537	1.955872E-3	0.25
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	9.1427948E-1	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	8.960148E-1	0.0
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	5.368089E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	8.1576099E-1	0.0

Figure 6.10: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.10), with the base rule, Rule (6.4).

6.2.3 Distance from SoT specialization of Rules (6.5) and (6.6)

In this section we specialize Rules (6.5) and (6.6) based on the hypothesis “Distance of motif occurrence from SoT influences gene expression”. Figures 6.11 and 6.13 depict the process followed to specialize the base rules from a distance from SoT perspective.

A sequence plot, for instance Figure 6.11, displays qualifying genes with the motifs overlaid such that a point on the plot represents the distance of that occurrence of the motif from the SoT. A qualifying gene is one that contains at least one occurrence of each motif in the base rule. The color of the point in the plot is indicative of the motif in question. The splitting of the plot into two parts (as described in Section 6.2.1), provides the user with an easy mechanism to discover distance from SoT based pattern in the top part that are not as frequent in the bottom part, as this would let us explore specializations with improved classifica-

tion accuracy (and/or confidence). For instance, in Figure 6.11, many genes in the bottom part lack dots in the area delineated by the SoT (far right end of the plot) and the vertical line representing the 500 bp from SoT cutoff. This indicates that an occurrence of Motif 69 within a distance of 500 bp from the SoT may positively influence gene expression in cell type ADL. Once a user has defined such a “Distance from SoT” relationship it is indicated in the plot as the vertical line, in the same color as the one reserved for the motif, as illustrated in Figure 6.11 is added to the plot. Subsequently the following corresponding specialization can be added to the analysis frame.

$$\text{SoT [0-500] M69} \Rightarrow \text{expr=HSN [Conf. = 0.6666667, Supp. = 0.044444446]} \quad (6.11)$$

Again the analysis frame with the specialized rule enables the user to compare the interestingness of the specialized rule with that of the base rule (Figure 6.12).

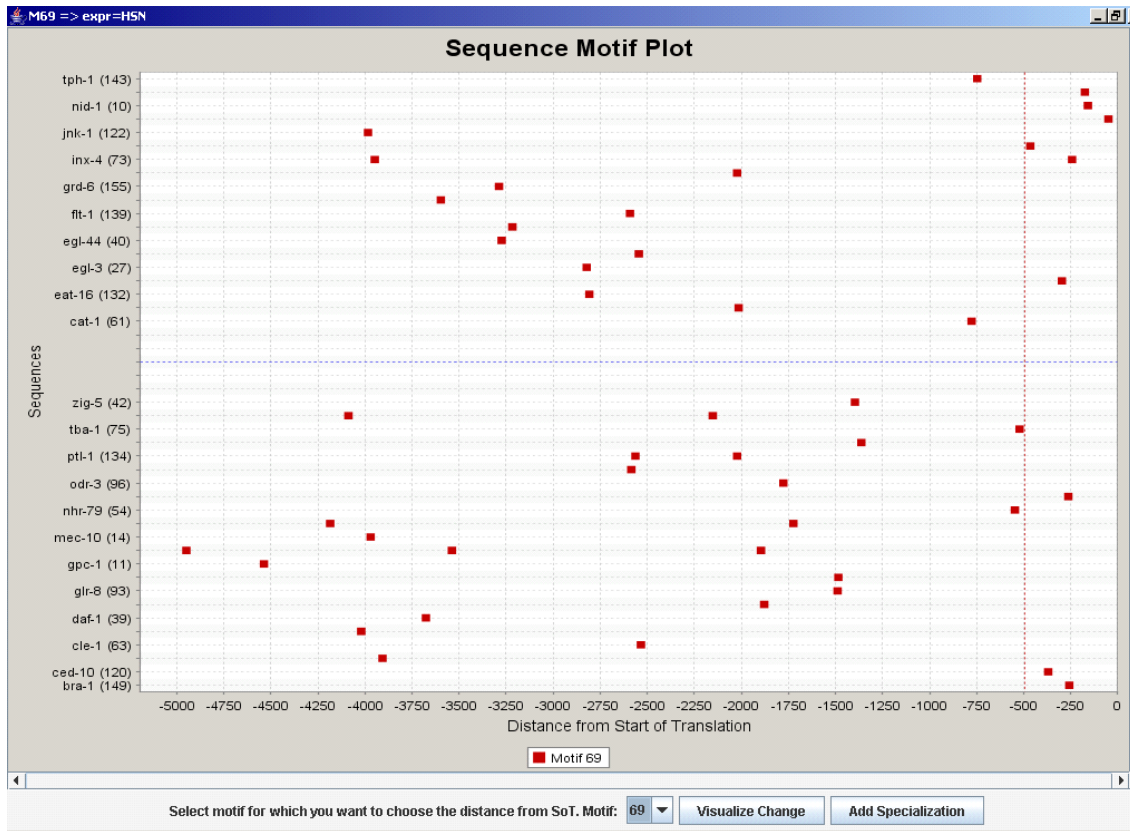


Figure 6.11: Sequence plot for the base rule $M69 \Rightarrow expr=HSN$. The sequence plot lists the genes along the y-axis and the distance from SoT along the x-axis. Each colored dot represents an occurrence of a specific motif. Notice the division of the graph into two parts using a horizontal line through the plot. The top part consists of gene sequences which support the rule and the bottom part consists of the gene sequences that support the antecedent of the rule. A user introduced vertical line marks the 500 bp distance from SoT.

id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	3.4031714E-3	0.47619048
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	5.4719937E-4	0.35714287
003	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	1.7501895E-3	0.7083333
003.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.5008453E-5	0.45833334
004	M77 [rp0-rp1] M48 [rp2-rp3]	expr=ALM	0.4	0.1037037	1.9285715	1.0940551E-3	0.5
004.01	M48:b [0-500] M77:a && M77:a [rp0-rp1] M48:b[rp2-rp3]	expr=ALM	0.53846157	0.05185185	2.5961537	1.955872E-3	0.25
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	1.1586854E-2	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	1.7081774E-2	0.14285715
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	3.8148643E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	5.4289247E-2	0.0952381

Figure 6.12: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.11), with the base rule, Rule (6.5).

Similarly in Figure 6.13, observe that many genes lack points in the top part in the 0-350 bp region of the plot, that is, a distance from SoT of 0-350 bp. This implies that the occurrence of motif 6 within 350 bp from the SoT may positively influence gene expression in cell HSN. Subsequent addition of the following corresponding specialization to the analysis frame enables the user to compare the interestingness of the rule to that of the base rule (Figure 6.14).

$$\text{SoT [0-350] M6} \Rightarrow \text{expr=HSN[Conf. = 0.6666667, Supp. = 0.02962963]} \quad (6.12)$$

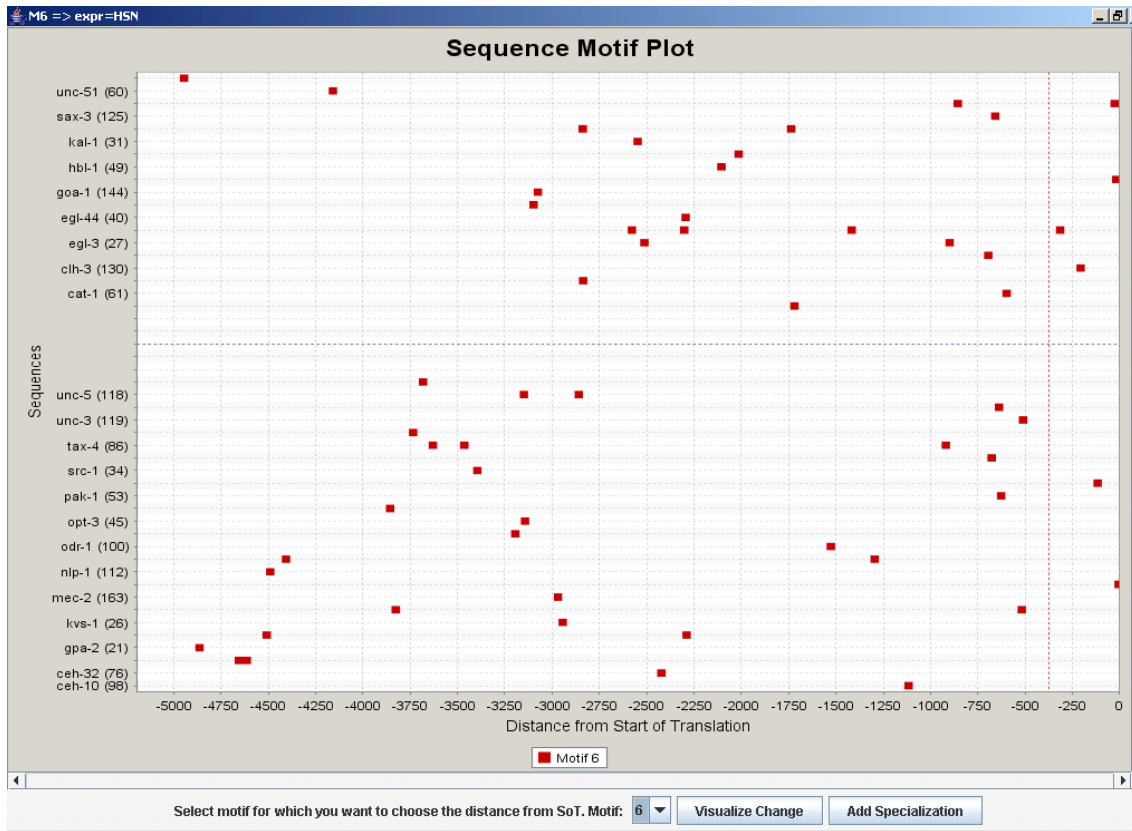


Figure 6.13: Sequence plot for the base rule $M6 \Rightarrow expr=HSN$.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	3.4031714E-3	0.47619048
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	5.4719937E-4	0.35714287
003	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	1.7501895E-3	0.70833333
003.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.5008453E-5	0.45833334
004	M77 [rp0-rp1] M48 [rp2-rp3]	expr=ALM	0.4	0.1037037	1.9285715	1.0940551E-3	0.5
004.01	M48:b [0-500] M77:a && M77:a [rp0-rp1] M48:b[rp2-rp3]	expr=ALM	0.53846157	0.05185185	2.5961537	1.955872E-3	0.25
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	1.1586854E-2	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	1.7081774E-2	0.14285715
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	3.8148643E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	5.4289247E-2	0.0952381

Inter-Motif Distance Plot Sequence Plot Add Rule Delete Rule Export Rules Import Rules Hide Current Column

Figure 6.14: Analysis frame providing a comparison of the interestingness of the specialized rule, Rule (6.12), with the base rule, Rule (6.6).

6.2.4 Experimental results

As mentioned all along the analysis phase, an important feature which aids the exploratory analysis is that as soon as a specialization is added to the analysis frame using any of the visualization plots the different measures of interestingness of the newly added specialization are calculated. Thus, the domain user can instantly observe whether the specialized rule is better or worse on a specific metric and by how much. For instance, Figure 6.15 lists all the base rules and their corresponding specializations as they would appear in the analysis frame with the different measures of interestingness computed over the **training data**. At this point the user could save the rule set using the ‘Export Rules’ option from the analysis frame. This saved rule set can later be imported into the ‘AssociativeClassifier’ within the WPI-Weka system to test the classification accuracy over a set of novel gene sequences (test dataset) to estimate the predictive power of the specializations discovered. It is worth noting that at this point we have established the process which can be used to identify relationships which are interesting at least over the training data. Subsequently the user might want to evaluate the quality of the relationships discovered by using a test set either via the associative classifier which would provide the classification accuracy of the specialized rules or evaluate the different measures of interestingness using the VSM. Since the functionality to support associative classification is currently work under progress as a part of another effort here at WPI [Rudss], we decided to use the VSM to evaluate the strength of the relationship. Figure 6.16 lists the different measures of interestingness over the **test dataset** by using the VSM. This helps to determine if the specializations are really interesting, or if they are a case of overfit to the training data. Observe specifically the various measures for rules 1.01, both of which have a positive lift and has all the measures of interestingness as good as the base rule. Given that no extensive process was

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.51428574	0.13333334	1.6530613	2.5550831E-3	0.42857143
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6363636	0.1037037	2.0454545	3.1594354E-4	0.33333334
002	M54 && M80	expr=HSN	0.4878049	0.14814815	1.5679443	3.4031714E-3	0.47619048
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.6	0.11111111	1.9285715	5.4719937E-4	0.35714287
003	M47 && M53	expr=ADL	0.2982456	0.12592593	1.6776316	1.7501895E-3	0.7083333
003.01	M47 [0-250] M53	expr=ADL	0.47826087	0.08148148	2.6902175	3.5008453E-5	0.45833334
004	M77 [rp0-rp1] M48 [rp2-rp3]	expr=ALM	0.4	0.1037037	1.9285715	1.0940551E-3	0.5
004.01	M48:b [0-500] M77:a && M77:a [rp0-rp1] M48:b[rp2-rp3]	expr=ALM	0.53846157	0.05185185	2.5961537	1.955872E-3	0.25
005	M69	expr=HSN	0.46341464	0.14074074	1.489547	1.1586854E-2	0.45238096
005.01	SoT [0-500] M69	expr=HSN	0.6666667	0.044444446	2.142857	1.7081774E-2	0.14285715
006	M6	expr=ADL	0.13636364	0.044444446	0.76704544	3.8148643E-1	0.25
006.01	SoT [0-350] M6	expr=HSN	0.6666667	0.02962963	2.142857	5.4289247E-2	0.0952381

Figure 6.15: Statistical measures over training data.

Base rules alongside their corresponding specializations and various measures of interestingness calculated over the training data, which help identify which specializations are interesting, and how much more interesting.

followed to divide the instances between the test set and the training set we believe this rule still might be an interesting discovery. Also we have hereby established the process that domain users might follow to verify the strength of a relationship which seems to be interesting based on the training data. It is important to understand at this point that this implementation equips the domain user with a tool and a work flow to identify potential relationships. The actual quality of the specializations is again contingent on many other things. For instance, the quality of the motifs elicited as well as the mining process both of which are outside the scope of this project. It is worth noting that both these areas are being researched in separate efforts here at WPI.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M6 && M52	expr=HSN	0.6666667	0.16666667	2.0	1.5729921E-1	0.5
001.01	M6 [rp0-rp1] M52 [rp2-rp3]	expr=HSN	0.6666667	0.16666667	2.0	1.5729921E-1	0.5
002	M54 && M80	expr=HSN	0.2	0.083333336	0.6	4.0762594E-1	0.25
002.01	M54[rp0-rp1] M80[rp2-rp3]	expr=HSN	0.33333334	0.083333336	1.0	1E0	0.25
003	M47 && M53	expr=ADL	0.5	0.25	1.2	5.5818464E-1	0.6
003.01	M47 [0-250] M53	expr=ADL	0.33333334	0.083333336	0.8	7.3531669E-1	0.2
004	M77 [rp0-rp1] M48 [rp2-rp3]	expr=ALM	0.6666667	0.16666667	2.0	1.5729921E-1	0.5
004.01	M48:b [0-500] M77:a && M77:a [rp0-rp1] M48:b[rp2-rp3]	expr=ALM	0.0	0.0	0.0	1E0	0.0
005	M69	expr=HSN	1.0	0.16666667	3.0	2.8459734E-2	0.5
005.01	SoT [0-500] M69	expr=HSN	1.0	0.083333336	3.0	1.3964939E-1	0.25
006	M6	expr=ADL	0.0	0.0	0.0	1E0	0.0
006.01	SoT [0-350] M6	expr=HSN	0.0	0.0	0.0	1E0	0.0

Figure 6.16: Specializations and various measures of interestingness evaluated over the test data.

Chapter 7

Conclusions and Future Work

The goal of this thesis was to computationally enable the discovery of gene expression association rules based on several biological hypotheses. We designed and implemented a tool that helps domain experts visualize genetic data in the context of various biological hypotheses and perform exploratory analysis of data to discover specialized gene expression association rules. This process of exploratory analysis allows for post mining specialization of association rules, which alleviates some of the shortcomings of incorporating hypothesis-driven information into the mining process.

This work sketched out a process work flow for exploratory analysis of genetic data to discover interesting association rules. This work facilitates the process of identifying interesting rules beyond the conventional support-confidence framework by adding other measures of interestingness to the analysis process. We established via the experimental evaluation (Section 6) that the data visualization capabilities provided by our tool helps human experts in identifying hypothesis-driven specialized rules that score better than their generic counterparts in terms of different measures of interestingness. In addition to the visual mining tool, this work pro-

vides an updated genetic dataset which is an important resource for future research.

Future work would involve verifying the scalability of the tool to ensure that the tool performs well with a substantially larger dataset (e.g., so as to support a genetic database from micro array experiments). Another potential area worth investigating is to provide the functionality wherein the tool suggests patterns to the user based on the data being visualized. For instance, the approach proposed in [Ice03] could be used in an inter-motif distance plot to suggest specialization tips (visual or textual) to the domain user based only on the section of the data relevant in the context of the rule being visualized.

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 Project@Repository: Bioinformatics@wekacode.

Appendix A

Sample ARFF File

```
@relation test_ASAS
@attribute gene string
@attribute M1 string
%Width = 8
%Consensus = CCGGCAAT
%Log-Odds Matrix:
%-689 217 -684 -91
%-689 246 -684 -690
%-689 -685 264 -690
%-689 -685 264 -690
%-21 200 -684 -690
%165 -685 -684 -690
%165 -685 -684 -690
%-689 -685 -684 153

@attribute M2 string
%Width = 8
%Consensus = GGAAAACG
%Log-Odds Matrix:
%-736 -731 264 -737
%-736 -731 264 -737
%151 -89 -729 -737
%166 -731 -729 -737
%166 -731 -729 -737
%166 -731 -729 -737
```

```
%-736 232 -729 -181
%-736 -731 221 -41
```

```
@attribute M3 string
```

```
%Width = 8
```

```
%Consensus = GAGAGAGA
```

```
%Log-Odds Matrix:
```

```
%-689 -685 264 -690
%128 -685 50 -690
%-21 -685 218 -690
%165 -685 -684 -690
%-689 -685 264 -690
%128 -685 50 -690
%-689 -685 264 -690
%165 -685 -684 -690
```

```
@attribute M4 string
```

```
%Width = 10
```

```
%Consensus = GAGArAGAGA
```

```
%Log-Odds Matrix:
```

```
%-683 -679 263 -683
%143 -679 -16 -683
%-683 -679 263 -683
%158 -679 -172 -683
%44 -91 157 -683
%135 -679 -678 -84
%-683 -679 263 -683
%107 -679 -678 -4
%-683 -679 263 -683
%126 -679 57 -683
```

```
@attribute M5 string
```

```
%Width = 10
```

```
%Consensus = GAGAGrsAGn
```

```
%Log-Odds Matrix:
```

```
%-727 -722 264 -728
%150 -722 -62 -728
%-727 -235 259 -728
%166 -722 -720 -728
```

%-216	42	210	-728
%89	-722	137	-728
%-727	131	178	-728
%112	-722	59	-228
%-727	-722	264	-728
%80	-39	95	-728

@attribute M6 string

%Width = 10

%Consensus = rGAAGAAGAn

%Log-Odds Matrix:

%85	-679	125	-278
%-683	-679	263	-683
%135	-679	-16	-278
%165	-679	-678	-683
%-683	-679	263	-683
%165	-679	-678	-683
%143	-679	-678	-125
%-683	-679	263	-683
%151	-91	-678	-683
%85	-679	105	-182

@attribute M7 string

%Width = 12

%Consensus = AAwTTGCCGGAA

%Log-Odds Matrix:

%152	-685	-80	-690
%152	-685	-80	-690
%52	-685	-684	66
%-689	-685	-684	153
%-689	-685	-23	132
%-120	1	208	-690
%-689	246	-684	-690
%-120	200	-23	-690
%-689	-685	257	-284
%-689	-685	264	-690
%165	-685	-684	-690
%152	-98	-684	-690

```

@attribute M8 string
%Width = 12
%Consensus = rAGAAGArGAAr
%Log-Odds Matrix:
%46 -691 181 -696
%130 -691 44 -696
%-695 -691 264 -696
%166 -691 -690 -696
%159 -691 -185 -696
%-126 -691 243 -696
%166 -691 -690 -696
%83 -691 129 -291
%-5 -691 211 -696
%122 -691 70 -696
%166 -691 -690 -696
%60 -691 170 -696

```

```

@attribute M9 string
%Width = 12
%Consensus = GrGAGAGwGAGm
%Log-Odds Matrix:
%-91 -658 237 -662
%49 -658 179 -662
%-91 -658 237 -662
%139 -658 6 -662
%-18 -658 216 -662
%148 -658 -657 -160
%-50 -658 227 -662
%81 -658 -150 18
%-662 -167 255 -662
%139 -658 -657 -103
%-662 -658 263 -662
%49 161 -657 -662

```

```

@attribute M10 string
%Width = 8
%Consensus = GGGnGGnG
%Log-Odds Matrix:
%-748 -742 264 -749

```

```

%-313  -742  259  -749
%-748  -742  264  -749
%-19   151   37   -749
%-748  -742  264  -749
%-748  -742  264  -749
%105   -138  78   -749
%-748  -742  264  -749

```

```
@attribute M11 string
```

```
%Width = 8
```

```
%Consensus = GGGAGrAG
```

```
%Log-Odds Matrix:
```

```

%-716  -711  264  -717
%-716  -711  264  -717
%-716  -711  264  -717
%122   -711  70   -717
%-716  -711  264  -717
%31    -711  192  -717
%113   -711  92   -717
%-716  -711  264  -717

```

```
@attribute M12 string
```

```
%Width = 8
```

```
%Consensus = GGGnGGAG
```

```
%Log-Odds Matrix:
```

```

%0      -777  209  -785
%-784   -777  264  -785
%-784   -777  264  -785
%0      147   -774  -90
%-784   -777  264  -785
%-784   -777  264  -785
%141    -19   -774  -785
%-784   -777  264  -785

```

```
@attribute M13 string
```

```
%Width = 10
```

```
%Consensus = rnGGGnGGAG
```

```
%Log-Odds Matrix:
```

```
%94    -711  129  -717
```

```

%-27   -711   157   -40
%-27   -711   220  -717
%-716  -711   264  -717
%-716  -711   264  -717
%-27    140  -710  -40
%-716  -711   264  -717
%-716  -711   264  -717
%138   -5    -710  -717
%-716  -711   264  -717

```

```
@attribute M14 string
```

```
%Width = 10
```

```
%Consensus = GGGnGGnGnn
```

```
%Log-Odds Matrix:
```

```

%-781  -773   264  -781
%-348  -773   260  -781
%-781  -773   264  -781
%-22    134   -24  -134
%-781  -773   264  -781
%-781  -773   264  -781
%104   -42    43  -781
%-781  -773   260  -360
%-781   72    76   33
%-22   43    60  -50

```

```
@attribute M15 string
```

```
%Width = 10
```

```
%Consensus = AnnGGmGGAG
```

```
%Log-Odds Matrix:
```

```

%115   -773    89  -781
%-37   -773   152  -21
%4     -773   202  -360
%-73   -773   234  -781
%-781  -773   264  -781
%15    143     2   -781
%-781  -773   264  -781
%-781  -773   264  -781
%121   -773    76  -781
%-781  -773   264  -781

```



```

@attribute M16 string
%Width = 12
%Consensus = GGnGGrnGrGGr
%Log-Odds Matrix:
%-614 -612 263 -615
%-614 -612 263 -615
%-78 -612 198 -90
%-175 -612 217 -90
%-614 -612 234 -90
%20 -612 198 -615
%-21 -612 198 -186
%-614 59 217 -615
%78 -612 149 -615
%-614 -612 217 -33
%-614 -612 249 -186
%20 -612 198 -615

```

```

@attribute M17 string
%Width = 12
%Consensus = AwrkGGGmGGAG
%Log-Odds Matrix:
%112 -742 -62 -73
%26 -742 11 38
%97 -235 111 -749
%-217 -39 148 0
%-748 -742 264 -749
%-748 -742 264 -749
%-748 -742 264 -749
%89 119 -740 -749
%-217 -742 254 -749
%-61 -742 230 -749
%144 -742 -21 -749
%-748 -742 264 -749

```

```

@attribute M18 string
%Width = 12
%Consensus = kGrGkrrGkGkG
%Log-Odds Matrix:

```

%-657	-141	190	-4
%-657	-654	219	-36
%75	-654	154	-658
%-657	-654	263	-658
%-657	-654	190	22
%56	-654	131	-134
%56	-654	173	-658
%-657	-43	243	-658
%-657	-654	173	44
%-657	-654	263	-658
%-657	-654	131	80
%-657	-654	263	-658

@attribute M19 string

%Width = 8

%Consensus = GGGsGGrG

%Log-Odds Matrix:

%-836	-825	264	-837
%-836	-825	264	-837
%-63	-825	228	-414
%-836	110	193	-837
%-836	-825	264	-837
%-92	-825	238	-837
%43	-825	184	-837
%-836	-825	264	-837

@attribute M20 string

%Width = 8

%Consensus = GGGGGnGG

%Log-Odds Matrix:

%-834	-823	264	-835
%-36	-823	224	-835
%-834	-823	264	-835
%-74	-823	234	-835
%-106	-823	241	-835
%-60	74	162	-835
%-834	-823	264	-835
%-834	-823	264	-835

```

@attribute M21 string
%Width = 8
%Consensus = GGGnGGrG
%Log-Odds Matrix:
%-52 -836 228 -849
%-848 -836 264 -849
%-848 -836 264 -849
%-22 111 101 -427
%-848 -836 264 -849
%-848 -836 264 -849
%64 -836 167 -849
%-848 -836 264 -849

```

```

@attribute M22 string
%Width = 10
%Consensus = GAArAAGAAG
%Log-Odds Matrix:
%-763 -756 264 -764
%129 -756 50 -764
%166 -756 -754 -764
%41 -756 186 -764
%116 -756 86 -764
%166 -756 -754 -764
%-763 -756 264 -764
%166 -756 -754 -764
%161 -756 -754 -335
%-763 -756 264 -764

```

```

@attribute M23 string
%Width = 10
%Consensus = GAAGAAGAAn
%Log-Odds Matrix:
%-797 -789 264 -798
%166 -789 -786 -798
%166 -789 -786 -798
%-797 -789 264 -798
%166 -789 -786 -798
%151 -85 -786 -798
%-797 -789 264 -798

```

```
%134 -789 32 -798
%129 -789 49 -798
%-49 -53 202 -798
```

```
@attribute M24 string
```

```
%Width = 10
```

```
%Consensus = GnGrGsGrGG
```

```
%Log-Odds Matrix:
```

```
%-854 -842 264 -855
%-20 -842 190 -141
%-38 -842 224 -855
%23 -842 197 -855
%-4 -842 211 -855
%-854 104 197 -855
%-422 -842 262 -855
%29 -842 194 -855
%3 -842 208 -855
%-854 -842 264 -855
```

```
@attribute M25 string
```

```
%Width = 12
```

```
%Consensus = TGTGTrTGTGTG
```

```
%Log-Odds Matrix:
```

```
%-245 -685 -684 145
%-689 -685 264 -689
%-50 -685 -684 117
%-689 -685 264 -689
%-689 30 -684 117
%30 -685 193 -689
%-50 -685 -684 117
%-689 -685 264 -689
%8 -685 -684 95
%-245 -685 255 -689
%-148 -685 -684 136
%-689 -685 264 -689
```

```
@attribute M26 string
```

```
%Width = 12
```

```
%Consensus = AAGAAGAAGAAG
```

```

%Log-Odds Matrix:
%138   -768   14   -776
%153   -103  -766  -776
%-775  -768  264  -776
%166   -768  -766  -776
%148   -768  -44   -776
%4     -768  208   -776
%116   -61   14    -776
%133   -103  -44   -776
%-775  -768  264  -776
%166   -768  -766  -776
%138   -768  14    -776
%-775  -768  264  -776

```

```
@attribute M27 string
```

```
%Width = 12
```

```
%Consensus = TGTGTGTGTGTG
```

```
%Log-Odds Matrix:
```

```

%-50   -685   -684   117
%-689  -685   264   -689
%-148  -11    -684   106
%-18   -685   217   -689
%-689  -685   -684   153
%-689  -685   264   -689
%8     -685   -684   95
%-50   -685   227   -689
%-91   -685   -684   127
%-689  -685   264   -689
%-148  -685   -684   136
%-689  -685   264   -689

```

```
@attribute M28 string
```

```
%Width = 8
```

```
%Consensus = nGGmGGAG
```

```
%Log-Odds Matrix:
```

```

%-2    20    159   -738
%-737  -732  264   -738
%-737  -732  264   -738
%50    107   -730  -99

```

```

%-737  -732  264  -738
%-737  -732  264  -738
%166   -732  -730  -738
%-737  -732  264  -738

```

```
@attribute M29  string
```

```
%Width = 8
```

```
%Consensus = GAAGAAGA
```

```
%Log-Odds Matrix:
```

```

%-664  -661  263  -664
%165   -661  -660  -664
%165   -661  -660  -664
%-664  -661  263  -664
%165   -661  -660  -664
%165   -661  -660  -664
%-664  -661  263  -664
%165   -661  -660  -664

```

```
@attribute M30  string
```

```
%Width = 8
```

```
%Consensus = GAGAGAGA
```

```
%Log-Odds Matrix:
```

```

%-679  -676  263  -680
%149   -676  -59  -680
%-679  -676  263  -680
%111   -676  -675  -12
%-679  -676  263  -680
%165   -676  -675  -680
%-679  -676  263  -680
%165   -676  -675  -680

```

```
@attribute M31  string
```

```
%Width = 10
```

```
%Consensus = GATTACGrG
```

```
%Log-Odds Matrix:
```

```

%-723  -717  258  -309
%166   -717  -716  -723
%-723  -219  -716  148
%-297  -717  -716  148

```

```

%-723  -717  -716  153
%166   -717  -716  -723
%-723  240   -716  -309
%-201  -717  252   -723
%54    -717  175   -723
%-297  -717  258   -723

```

```
@attribute M32 string
```

```
%width = 10
```

```
%consensus = AGAAGAAGAW
```

```
%Log-Odds Matrix:
```

```

%166   -741  -739  -747
%-746  -741  264   -747
%166   -741  -739  -747
%166   -741  -739  -747
%-746  -741  264   -747
%166   -741  -739  -747
%110   -741  101   -747
%-746  -741  264   -747
%135   10    -739  -747
%29    -741  69    4

```

```
@attribute M33 string
```

```
%width = 10
```

```
%consensus = nAAGAAGAAG
```

```
%Log-Odds Matrix:
```

```

%-11   -707  186   -144
%116   -707  86    -712
%166   -707  -706  -712
%-712  -707  264   -712
%166   -707  -706  -712
%166   -707  -706  -712
%-712  -707  264   -712
%166   -707  -706  -712
%116   -707  -35   -103
%-712  -707  264   -712

```

```
@attribute M34 string
```

```
%width = 12
```

%Consensus = AAGAnGAAGAAG

%Log-Odds Matrix:

%146	-653	-652	-143
%146	-150	-133	-656
%-228	-653	254	-656
%165	-653	-652	-656
%98	-653	64	-143
%-131	-653	233	-239
%165	-653	-652	-656
%165	-653	-652	-656
%-656	-653	263	-656
%135	-52	-133	-656
%146	-52	-652	-656
%-656	-653	263	-656

@attribute M35 string

%Width = 12

%Consensus = ATGATGATGATG

%Log-Odds Matrix:

%117	-131	42	-637
%-637	-634	-634	153
%-637	-634	263	-637
%165	-634	-634	-637
%-112	-634	-634	131
%-637	24	228	-637
%154	-634	-634	-220
%-637	-634	-114	142
%-209	-634	252	-637
%165	-634	-634	-637
%-637	-634	-114	142
%-637	-33	241	-637

@attribute M36 string

%Width = 12

%Consensus = GAARAGAAGAr

%Log-Odds Matrix:

%-656	-653	263	-656
%165	-653	-652	-656
%124	-653	64	-656

%47	-653	180	-656
%165	-653	-652	-656
%146	-653	-652	-143
%-656	-653	233	-86
%146	-653	-35	-656
%165	-653	-652	-656
%-656	-653	263	-656
%111	-52	23	-656
%98	-653	122	-656

@attribute M37 string

%Width = 8

%Consensus = GGGmGGnG

%Log-Odds Matrix:

%-570	-569	262	-571
%-570	-569	262	-571
%-570	-569	262	-571
%65	145	-568	-571
%-570	-83	247	-571
%-570	-569	262	-571
%91	14	32	-571
%-570	-569	262	-571

@attribute M38 string

%Width = 8

%Consensus = AGGTAGGC

%Log-Odds Matrix:

%124	-633	64	-636
%-636	-633	263	-636
%-636	-633	263	-636
%-636	-633	-632	153
%124	-633	-632	-45
%-636	-633	263	-636
%-636	-633	263	-636
%-636	245	-632	-636

@attribute M39 string

%Width = 8

%Consensus = GAAGAAGA

```

%Log-Odds Matrix:
%-607  -605  263  -607
%165   -605  -604  -607
%165   -605  -604  -607
%-607  -605  263  -607
%165   -605  -604  -607
%165   -605  -604  -607
%-607  -605  263  -607
%165   -605  -604  -607

```

```

@attribute M40  string
%Width = 10
%Consensus = GAAGAAGAAG

```

```

%Log-Odds Matrix:
%-570  -569  262  -571
%132   -569  32   -571
%113   72    -568  -571
%-7    -569  211  -571
%164   -569  -568  -571
%164   -569  -568  -571
%-570  -569  262  -571
%164   -569  -568  -571
%164   -569  -568  -571
%-570  -569  262  -571

```

```

@attribute M41  string
%Width = 10
%Consensus = GrAGmAGAAG

```

```

%Log-Odds Matrix:
%-596  -594  263  -596
%8     -594  204  -596
%165   -594  -593  -596
%-596  -594  250  -197
%65    119   -92  -596
%165   -594  -593  -596
%-596  -594  250  -197
%139   -11   -593  -596
%165   -594  -593  -596
%-596  -594  263  -596

```

```

@attribute M42 string
%Width = 10
%Consensus = GnAGGCAGGC
%Log-Odds Matrix:
%-644 -641 263 -644
%-235 -158 55 90
%165 -641 -640 -644
%-644 -641 263 -644
%-644 -641 263 -644
%-644 195 -640 -22
%127 -641 -141 -95
%-644 -641 245 -152
%-235 -3 225 -644
%-644 218 -640 -95

```

```

@attribute M43 string
%Width = 12
%Consensus = AAGAArrAGAAG
%Log-Odds Matrix:
%145 -538 -34 -539
%164 -538 -537 -539
%-539 -538 262 -539
%145 -538 -34 -539
%164 -538 -537 -539
%97 -538 121 -539
%97 -538 121 -539
%164 -538 -537 -539
%-539 -538 262 -539
%164 -538 -537 -539
%164 -538 -537 -539
%-539 -538 262 -539

```

```

@attribute M44 string
%Width = 12
%Consensus = AGAAGAAGrAGA
%Log-Odds Matrix:
%164 -519 -519 -521
%-521 -519 261 -521

```

```

%164 -519 -519 -521
%164 -519 -519 -521
%-521 -519 261 -521
%164 -519 -519 -521
%116 -33 -16 -521
%-521 -519 261 -521
%84 -519 140 -521
%116 65 -519 -521
%-521 -33 239 -521
%116 65 -519 -521

```

```
@attribute M45 string
```

```
%Width = 12
```

```
%Consensus = GACGACGACGnC
```

```
%Log-Odds Matrix:
```

```

%-176 -195 242 -680
%137 -676 18 -680
%-21 191 -675 -284
%-680 -195 257 -680
%152 -676 -80 -680
%-680 217 -23 -284
%-79 -676 235 -680
%137 -195 -23 -680
%-680 246 -675 -680
%-21 -676 208 -284
%52 -676 99 -59
%-680 246 -675 -680

```

```
@attribute M46 string
```

```
%Width = 8
```

```
%Consensus = GnrGGnGG
```

```
%Log-Odds Matrix:
```

```

%-722 -717 264 -723
%-43 -717 74 54
%21 -717 198 -723
%-164 -717 249 -723
%-722 -717 264 -723
%-43 146 -716 -36
%-722 -717 264 -723

```

%-722 -717 264 -723

@attribute M47 string

%Width = 8

%Consensus = GAGAArAG

%Log-Odds Matrix:

%-718 -713 264 -718

%119 -713 -711 -32

%-718 -713 264 -718

%166 -713 -711 -718

%166 -713 -711 -718

%89 -713 137 -718

%166 -713 -711 -718

%-718 -713 264 -718

@attribute M48 string

%Width = 8

%Consensus = TGAGAAAA

%Log-Odds Matrix:

%-743 -738 -736 153

%-743 -738 264 -744

%148 -738 -48 -744

%-743 -738 264 -744

%153 -107 -736 -744

%166 -738 -736 -744

%166 -738 -736 -744

%166 -738 -736 -744

@attribute M49 string

%Width = 10

%Consensus = ArAGrAnGAG

%Log-Odds Matrix:

%120 -676 77 -680

%20 -676 198 -680

%165 -676 -675 -680

%-680 -676 263 -680

%78 -676 150 -680

%128 -676 50 -680

%78 -676 77 -91

```
%-680  -676  263  -680
%165   -676  -675  -680
%-272  -676  257  -680
```

```
@attribute M50  string
```

```
%Width = 10
```

```
%Consensus = rGAArAAAGA
```

```
%Log-Odds Matrix:
```

```
%28    -698   174   -212
%-703   -698   264   -703
%113    -698    94   -703
%142    -698    -5   -703
%54     -698   174   -703
%166    -698  -697   -703
%166    -698  -697   -703
%135    -698    27   -703
%-703   -698   264   -703
%166    -698  -697   -703
```

```
@attribute M51  string
```

```
%Width = 10
```

```
%Consensus = GAGArnAAGA
```

```
%Log-Odds Matrix:
```

```
%-123  -717   243   -723
%107    -717   106   -723
%-722   -717   264   -723
%166    -717  -716   -723
%21     -717   198   -723
%100    -717    32   -103
%114    -717    90   -723
%134    -717    32   -723
%-722   -717   264   -723
%166    -717  -716   -723
```

```
@attribute M52  string
```

```
%Width = 12
```

```
%Consensus = GAAnAnGAAGAA
```

```
%Log-Odds Matrix:
```

```
%-33    -663   212   -270
```

```

%165 -663 -662 -667
%142 -663 -9 -667
%103 -663 112 -667
%165 -663 -662 -667
%92 -663 90 -175
%-667 -26 240 -667
%142 -663 -662 -118
%165 -663 -662 -667
%-667 -663 263 -667
%158 -663 -164 -667
%165 -663 -662 -667

```

```
@attribute M53 string
```

```
%Width = 12
```

```
%Consensus = GAARAGAAnrA
```

```
%Log-Odds Matrix:
```

```

%8 -688 205 -692
%166 -688 -687 -692
%166 -688 -687 -692
%25 -688 196 -692
%124 -688 64 -692
%159 -688 -687 -296
%-11 -688 205 -296
%166 -688 -687 -692
%166 -688 -687 -692
%-692 -208 196 -4
%98 -688 122 -692
%166 -688 -687 -692

```

```
@attribute M54 string
```

```
%Width = 12
```

```
%Consensus = GARwGAGArrnG
```

```
%Log-Odds Matrix:
```

```

%-713 -708 264 -714
%137 -708 16 -714
%44 -708 174 -318
%31 -708 -16 43
%-713 -708 264 -714
%117 -708 83 -714

```

```

%-154  -708  247  -714
%110   -34   16   -714
%102   -708  115  -714
%66    -34   115  -714
%76    -2    83   -714
%-713  -708  264  -714

```

```
@attribute M55  string
```

```
%Width = 8
```

```
%Consensus = rGGCGGnG
```

```
%Log-Odds Matrix:
```

```

%83    -687  144  -691
%-691  -687  264  -691
%-126  -687  243  -691
%-53   194   -87  -691
%-691  -687  264  -691
%-691  -687  264  -691
%104   94    -686 -691
%-691  -687  264  -691

```

```
@attribute M56  string
```

```
%Width = 8
```

```
%Consensus = GGGmGGAG
```

```
%Log-Odds Matrix:
```

```

%-708  -703  258  -308
%-103  -703  240  -708
%-708  -703  264  -708
%77    135   -702 -708
%-200  -703  252  -708
%-4    -703  211  -708
%113   -23   -5   -708
%-708  -703  264  -708

```

```
@attribute M57  string
```

```
%Width = 8
```

```
%Consensus = GGnGnsGG
```

```
%Log-Odds Matrix:
```

```

%-678  -675  263  -679
%-678  -675  241  -125

```



```

%-113  88    170   -679
%-40   -675  224   -679
%8      8     157   -679
%-678  177   125   -679
%-678  -675  263   -679
%-678  -675  263   -679

```

```
@attribute M58 string
```

```
%width = 10
```

```
%consensus = nnnGGnGGnG
```

```
%Log-Odds Matrix:
```

```

%14    -202   192   -691
%-85   -104   181   -97
%83     -5    70   -691
%-691  -687   264   -691
%-691  -104   250   -691
%-691   174   -87   -17
%-691  -687   264   -691
%-691  -687   257   -290
%60     53    70   -691
%-691  -687   264   -691

```

```
@attribute M59 string
```

```
%width = 10
```

```
%consensus = GrnnnGGCGG
```

```
%Log-Odds Matrix:
```

```

%-159  -718   203   -51
%39    -718   187  -723
%-60   -718   187  -73
%-723   42    195  -130
%-60   -39    78    14
%-159  -718   248  -723
%-723  -718   264  -723
%-723  185    110  -723
%-723  -718   264  -723
%-723  -718   264  -723

```

```
@attribute M60 string
```

```
%width = 10
```

%Consensus = rGnGGGGGkG

%Log-Odds Matrix:

%25	-693	196	-697
%-33	-693	222	-697
%-285	88	-35	54
%-697	-693	264	-697
%-132	-693	222	-144
%-697	88	205	-697
%-697	-208	205	-24
%-697	-693	264	-697
%-59	-693	122	27
%-697	-693	264	-697

@attribute M61 string

%Width = 12

%Consensus = nGTGTGTGTGTG

%Log-Odds Matrix:

%88	-599	6	-45
%-601	-599	263	-601
%-90	-599	-598	126
%-90	-599	236	-601
%-601	-599	-598	153
%-601	-599	236	-102
%-186	-599	64	94
%-33	-599	221	-601
%-601	-599	-92	140
%-601	-599	263	-601
%-601	-599	-92	140
%-601	-599	263	-601

@attribute M62 string

%Width = 12

%Consensus = wGTGnGknkGTG

%Log-Odds Matrix:

%46	-687	-184	60
%-691	-5	236	-691
%-279	-687	-686	147
%-691	-202	257	-691
%14	-687	70	18

%-691	-687	243	-138
%-691	-104	128	60
%14	-687	202	-691
%-691	-687	144	71
%-126	-687	243	-691
%-691	-687	44	118
%-691	-687	264	-691

@attribute M63 string

%Width = 12

%Consensus = GARAAAGArrnnG

%Log-Odds Matrix:

%-45	-64	202	-708
%154	-122	-702	-708
%86	-703	140	-708
%154	-703	-104	-708
%166	-703	-702	-708
%-708	-703	264	-708
%166	-703	-702	-708
%96	-703	126	-708
%86	-703	140	-708
%54	-703	111	-83
%86	-703	94	-155
%-708	-703	264	-708

@attribute M64 string

%Width = 8

%Consensus = GGGmGGAG

%Log-Odds Matrix:

%-644	-641	263	-644
%-644	-641	263	-644
%-644	-641	263	-644
%16	118	55	-644
%-644	-158	254	-644
%-644	-641	263	-644
%165	-641	-640	-644
%-644	-641	263	-644

@attribute M65 string

```

%Width = 8
%Consensus = GGGrGGrG
%Log-Odds Matrix:
%-667  -663  263  -667
%-667  -663  256  -270
%-667  -663  263  -667
%15    -663  148  -77
%-667  -663  263  -667
%-259  -663  256  -667
%103   -663  112  -667
%-667  -663  263  -667

```

```
@attribute M66 string
```

```

%Width = 8
%Consensus = GkGkGrGG
%Log-Odds Matrix:
%-739  -734  264  -740
%-739  -734  201   4
%-25   -734  220  -740
%-739  -734  187   26
%-739  -734  264  -740
%82    -734  146  -740
%-739  -734  264  -740
%-739  -734  264  -740

```

```
@attribute M67 string
```

```

%Width = 10
%Consensus = GAAGnAGAnG
%Log-Odds Matrix:
%-680  -676  263  -680
%159   -676  -178  -680
%165   -676  -675  -680
%-680  -676  235  -91
%100   59    -80  -680
%144   -676  -23  -680
%-680  -676  263  -680
%165   -676  -675  -680
%100   -195  -80  -59
%-680  -676  263  -680

```

```

@attribute M68 string
%Width = 10
%Consensus = GAAGAAGAnG
%Log-Odds Matrix:
%-718 -713 264 -718
%166 -713 -711 -718
%166 -713 -711 -718
%-718 -713 264 -718
%161 -713 -217 -718
%119 -713 59 -323
%-159 -713 242 -323
%105 -713 110 -718
%61 -137 59 -73
%-718 -235 259 -718

```

```

@attribute M69 string
%Width = 10
%Consensus = GrGnGrGAGr
%Log-Odds Matrix:
%-735 -730 264 -736
%70 -730 160 -736
%-735 -730 264 -736
%32 -730 77 -4
%-137 -730 245 -736
%79 -730 130 -246
%-57 -730 229 -736
%142 -730 -8 -736
%-735 -730 264 -736
%52 -730 176 -736

```

```

@attribute M70 string
%Width = 12
%Consensus = CGATGCACCATG
%Log-Odds Matrix:
%-686 226 -681 -138
%-126 -682 243 -686
%159 -682 -681 -290
%-686 -201 -681 147

```

```

%-686  -682  264  -686
%-686  239  -681  -290
%165   -682  -681  -686
%-686  226  -681  -138
%-686  239  -681  -290
%159   -682  -184  -686
%-686  -682  -681  153
%-53   -682  228  -686

```

```
@attribute M71 string
```

```
%Width = 12
```

```
%Consensus = AAGAAGAAGAnG
```

```
%Log-Odds Matrix:
```

```

%165  -649  -648  -652
%129   30  -648  -652
%-91  -649  227  -255
%165  -649  -648  -652
%139  -649  -52  -255
%-652 -649  263  -652
%148  -166 -149  -652
%139  -166  -52  -652
%-652 -649  263  -652
%165  -649  -648  -652
%30   62   47  -160
%-652 -649  263  -652

```

```
@attribute M72 string
```

```
%Width = 12
```

```
%Consensus = GAAGAAGAnGAA
```

```
%Log-Odds Matrix:
```

```

%-652 -649  263  -652
%165  -649  -648  -652
%129  -649   47  -652
%-50  -649  227  -652
%165  -649  -648  -652
%165  -649  -648  -652
%-50  -649  227  -652
%148  -166 -149  -652
%95   -69  -648  -30

```

```
%-652  -649  263  -652
%129   -649  47   -652
%139   -166  -52  -652
```

```
@attribute M73  string
```

```
%Width = 8
```

```
%Consensus = GrkGGGGG
```

```
%Log-Odds Matrix:
```

```
%-718  -713  264  -718
%102   -713  115  -718
%-718  -713  164  54
%-718  -713  264  -718
%-718  -713  235  -94
%-718  -34   208  -94
%-718  -713  264  -718
%-718  -713  264  -718
```

```
@attribute M74  string
```

```
%Width = 8
```

```
%Consensus = GrAGAGAG
```

```
%Log-Odds Matrix:
```

```
%-697  -693  264  -697
%77    -693  151  -697
%146   -53   -691  -697
%-697  -693  264  -697
%139   -693  -691  -103
%-697  -693  264  -697
%159   -693  -191  -697
%-697  -693  264  -697
```

```
@attribute M75  string
```

```
%Width = 8
```

```
%Consensus = kGnkGGGG
```

```
%Log-Odds Matrix:
```

```
%-198  -753  164  29
%-759  -753  264  -760
%94    -753  20   -70
%-759  -753  185  29
%-125  -753  244  -760
```

```
%-759 -176 256 -760
%-759 -753 264 -760
%-759 -753 264 -760
```

```
@attribute M76 string
```

```
%Width = 10
```

```
%Consensus = GGrGrnGGAG
```

```
%Log-Odds Matrix:
```

```
%13 -718 203 -723
%-216 -718 253 -723
%71 -718 159 -723
%-312 -718 259 -723
%39 -718 187 -723
%13 61 124 -723
%13 -718 203 -723
%-723 -718 264 -723
%155 -718 -120 -723
%-723 -718 264 -723
```

```
@attribute M77 string
```

```
%Width = 10
```

```
%Consensus = nAGAnGAAGA
```

```
%Log-Odds Matrix:
```

```
%8 -693 196 -296
%166 -693 -691 -697
%-697 -693 264 -697
%159 -208 -691 -697
%66 88 6 -697
%-697 -693 264 -697
%116 69 -691 -697
%166 -693 -691 -697
%-697 -693 264 -697
%139 -693 6 -697
```

```
@attribute M78 string
```

```
%Width = 10
```

```
%Consensus = GGnGGnGGAG
```

```
%Log-Odds Matrix:
```

```
%-38 -735 224 -741
```



```

%-740  -735  264  -741
%-57   -735  150  -4
%-740  42    224  -741
%-79   -25   205  -741
%-38   74    150  -741
%-740  -25   240  -741
%-330  -735  259  -741
%107   -735  19   -117
%-740  -735  264  -741

```

```
@attribute M79  string
```

```
%Width = 12
```

```
%Consensus = TGnsTGTGyGnG
```

```
%Log-Odds Matrix:
```

```

%-702  -698  100  98
%-291  -698  258  -703
%-702  -58   100  70
%-65   114   132  -703
%-702  -698  -196  147
%-702  -698  264  -703
%-291  -698  -697  147
%-97   -213  231  -703
%-702  99    -697  89
%-702  -698  264  -703
%-291  -698  100  89
%-702  82    208  -703

```

```
@attribute M80  string
```

```
%Width = 12
```

```
%Consensus = rAGAAGAAGAAG
```

```
%Log-Odds Matrix:
```

```

%85    -675  142  -679
%165   -675  -674  -679
%-678  -675  241  -125
%165   -675  -674  -679
%117   -33   -674  -125
%-678  -189  256  -679
%126   -675  25   -277
%143   -675  -16  -679

```

```
%-72    -675    224    -277
%158    -189    -674    -679
%135    -33     -674    -277
%-678   -675    263    -679
```

```
@attribute M81 string
```

```
%Width = 12
```

```
%Consensus = GGGnGnrGAnnG
```

```
%Log-Odds Matrix:
```

```
%-718   -713   264   -718
%-14     -713   215   -718
%-33     -713   222   -718
%-33     -75    200   -718
%-718   -713   264   -718
%3       98    100   -718
%44     -713   183   -718
%-718   -2    235   -718
%131    -713   42    -718
%3      -75    174   -318
%-81    83    129   -166
%-718   -2    235   -718
```

```
@attribute expr string
```

```
@data
```

```
osm-6, '{}', '{}', '{28:35}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{
}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{
}', '{}', '{}', '{}', '{}', '{67:74}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '
{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{129:138
}', '{}', '{}', '{}', '{}', '{48:59}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '
{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '
{}', '{}', '{}', '{}', '{}', '{}', '{}', 'ASH^ASI^PHA^ADL^ASE^ASK'
```

```
unc-97, '{}', '{2158:2165~1927:1934}', '{}', '{474:483}', '{}', '{2124:2133}', '
{}', '{2087:2098~349:360~331:342~90:101}', '{1149:1160}', '{}', '{}', '{}', '{}',
', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{2560:2569}', '{}', '{}', '{5
05:516}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{2524:2535~589:
600}', '{}', '{}', '{1090:1097}', '{}', '{}', '{1300:1309}', '{}', '{}', '{}', '
{}', '{}', '{}', '{}', '{1886:1893~665:672}', '{}', '{617:626}', '{1288:1297}', '{}',
```

'{}', '{}', '{}', '{571:578}', '{}', '{1544:1553^416:425^141:150}', '{1106:1115}',
'{313:322}', '{}', '{160:171}', '{1022:1033}', '{}', '{}', '{991:998}', '{}',
'{}', '{}', '{}', '{}', '{2540:2551^1060:1071}', '{74:81}', '{}', '{}', '{}', '
'{}', '{}', '{}', '{}', '{2505:2516}', 'ALM'

flp-6, '{}', '{}', '{}', '{4198:4207}', '{513:522}', '{}', '{3836:3847^2013:2024}',
'{1779:1790}', '{3555:3566}', '{3947:3958}', '{}', '{}', '{}', '{}', '{}', '{447:
456}', '{4533:4544^301:312^274:285}', '{3975:3986^469:480}', '{}', '{3231:3238^4
61:468}', '{}', '{}', '{}', '{730:739}', '{}', '{}', '{}', '{2568:2579}', '{}',
'{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{4167:4176^30
82:3091}', '{}', '{}', '{1889:1900}', '{2480:2491}', '{}', '{}', '{4724:4731^414
9:4156^713:720^320:327}', '{3881:3888^2121:2128^932:939}', '{3166:3175}', '{2377
:2386^499:508}', '{3120:3129^864:873}', '{4415:4426^2986:2997}', '{4284:4295^245
1:2462^1854:1865}', '{573:584}', '{}', '{}', '{1833:1840}', '{}', '{428:437}', '
'{}', '{1166:1177}', '{2197:2208^1791:1802}', '{3694:3705}', '{}', '{3293:3300}',
'{}', '{2100:2109}', '{}', '{}', '{1199:1210}', '{3935:3946^384:395}', '{}', '{
550:557}', '{}', '{2862:2869}', '{}', '{3901:3910}', '{}', '{}', '{526:537}', '{
'{}', 'ASE'

unc-32, '{}', '{}', '{155:162}', '{}', '{1255:1264}', '{}', '{18:29}', '{1061:10
72}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{1315:1326}', '{}',
'{}', '{}', '{}', '{872:881}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}',
'{}', '{}', '{46:57}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}',
'{}', '{}', '{}', '{}', '{1243:1252^419:428^232:241}', '{1388:1397^853:862}',
'{}', '{}', '{1483:1494^1014:1025}', '{498:509}', '{}', '{1275:1282^118:125}',
'{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{
'{}', '{}', '{}', '{}', '{187:194}', '{}', '{}', '{338:347}', '{598:607}', '{}', '
'{386:397^165:176}', '{1423:1434}', 'ALM'

unc-86, '{4936:4943^4428:4435}', '{68:75}', '{}', '{1543:1552^1020:1029}', '{}',
'{1824:1833^1172:1181}', '{}', '{754:765}', '{1838:1849}', '{}', '{}', '{}', '{
'{}', '{4694:4703}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{2663:
2672}', '{}', '{1905:1916^188:199}', '{1622:1633}', '{480:487}', '{}', '{1579:15
86^1504:1511}', '{}', '{1555:1564^1292:1301}', '{}', '{4656:4667}', '{286:297}',
'{}', '{}', '{}', '{}', '{}', '{}', '{542:551^527:536}', '{}', '{}', '{842:853}',
'{}', '{787:794}', '{821:828}', '{2095:2102^1405:1412}', '{1969:1978}', '{4683:4692
^4512:4521^2802:2811^1871:1880^1476:1485^714:723}', '{}', '{2643:2654^901:912}',
'{957:968^804:815^48:59}', '{4618:4629}', '{}', '{}', '{}', '{3529:3538}', '{}',
'{}', '{2601:2612}', '{3704:3715}', '{}', '{}', '{3843:3850}', '{}', '{}', '{}'

, '}', '}', '}', '}', '{4461:4472^1733:1744^730:741}', '{3670:3677^695:702}', '{
, '{4439:4446}', '}', '{3750:3759^866:875}', '}', '}', '}', '{4597:4608}',
'ALM^HSN'

unc-103, '{2159:2166}', '}', '{3055:3062}', '{1165:1174}', '{4626:4635^669:678}'
, '}', '{3910:3921^3694:3705^1213:1224}', '{575:586}', '{3637:3648^3093:3104}'
, '}', '}', '}', '}', '}', '{3393:3402^1451:1460^369:378^75:84}', '{2045:20
56^1856:1867^904:915}', '}', '}', '}', '{3489:3496}', '{2721:2728}', '{2969:2
978}', '{2461:2470^265:274}', '}', '{761:772^11:22}', '{2772:2783^2638:2649^91:
102}', '}', '}', '}', '{3252:3259^3220:3227^857:864}', '{2194:2203}', '{2979:
2988^1364:1373}', '}', '}', '{2064:2075^938:949^282:293}', '{2104:2115}', '}'
, '}', '}', '}', '{4706:4715^2789:2798}', '{4953:4962^2218:2227^1135:1144^104
5:1054}', '{2923:2934^125:136}', '{3679:3690^3518:3529^2748:2759^2558:2569}', '{
4531:4542^4507:4518^4393:4404^3110:3121^2606:2617}', '}', '{3042:3049}', '{3983
:3990^3624:3631^3369:3376^3327:3334^1929:1936}', '{3752:3761^1870:1879^1375:1384
^700:709}', '{2007:2016^1277:1286}', '}', '{3063:3074^2026:2037^1073:1084^839:8
50}', '{3781:3792^3354:3365^3145:3156^3010:3021}', '{4231:4242^3764:3775^387:398
}', '{4378:4385}', '{235:242}', '{4315:4322^3806:3813^2096:2103}', '{4466:4475^4
454:4463^187:196}', '{4117:4126}', '{684:693}', '{145:156}', '{503:514}', '{3960
:3971^2544:2555^2495:2506^1352:1363}', '}', '}', '}', '{2626:2635^829:838}',
'{2711:2720}', '{419:428}', '}', '}', '{2863:2874}', '{4486:4493^1945:1952}',
'}', '}', '{199:208}', '}', '}', '{488:499}', '}', '}', 'HSN^PHA^ADL^ASK'

unc-129, '{1022:1029^523:530^277:284}', '{3597:3604^1177:1184}', '}', '{2711:27
20^1954:1963}', '{2758:2767}', '{654:663}', '{3216:3227^18:29}', '{1707:1718^565
:576}', '{3525:3536}', '}', '}', '}', '{99:108}', '}', '}', '}', '}'
, '}', '}', '}', '}', '}', '{4219:4228}', '}', '{3370:3381}', '}', '{178
7:1794}', '}', '{3537:3544}', '{3193:3202^1214:1223}', '}', '{880:889}', '}',
'{4844:4855^2931:2942^1971:1982}', '{417:428}', '}', '}', '}', '{4455:
4464^3734:3743}', '}', '{4192:4203}', '{2291:2302}', '}', '{3098:3105}', '}',
'{300:307}', '}', '{1765:1774}', '}', '{4622:4633^3000:3011^690:701}', '{4670
:4681^853:864^824:835^197:208}', '{3553:3564}', '}', '{792:799}', '{3481:3488}'
, '}', '}', '}', '}', '}', '{4010:4021^2786:2797^134:145}', '}', '}', '}'
, '}', '}', '}', '}', '{1828:1839^1668:1679}', '}', '}', '{3859:3866}', '
'}', '}', '{1857:1866}', '}', '{2851:2862}', '{3884:3895^3625:3636}', '{6:17}'
, 'CAN'

tax-6, '}', '{749:756^337:344}', '}', '{262:271}', '{419:428}', '}', '{658:66
9}', '{703:714}', '}', '}', '}', '{120:127}', '}', '}', '}', '}', '}', '}'

{}, {}, {}, {786:793}', {}, {}, {}, {}, {83:94}', {372:383}', {
}', {1283:1290}', {200:207}', {}, {}, {}, {}, {1041:1052}', {630:641
^407:418^287:298}', {}, {}, {}, {}, {}, {}, {167:178}', {}, {771
:782}', {470:477}', {497:504}', {}, {214:223}', {}, {}, {}, {1006:10
17}', {}, {834:841}', {}, {}, {}, {}, {}, {}, {431:442}', {},
{}, {110:117}', {}, {}, {}, {}, {}, {}, {}, {}, {}, {}, {},
{}, {805:814}', {}, {}, {}, {}, {}, ASH^ASI^PHA^ADL^ASE^ASK'

unc-76, {1201:1208}', {910:917}', {}, {439:448^159:168}', {}, {}, {167
3:1684^1159:1170^953:964^921:932}', {2049:2060^1837:1848^510:521}', {693:704^5
98:609^423:434}', {}, {}, {}, {}, {724:733}', {}, {}, {}, {},
{}, {}, {}, {}, {}, {1055:1064}', {681:692^410:421}', {}, {}, {}
, {}, {}, {}, {}, {}, {}, {}, {}, {1875:1886}', {}, {}, {}, {
}', {}, {}, {1719:1730}', {2134:2145^586:597}', {934:945^225:236^73:84}',
{}, {1646:1653}', {}, {}, {102:111}', {}, {}, {1983:1994}', {1783
:1794^52:63^22:33}', {}, {}, {1932:1939}', {179:188}', {876:885}', {},
{}, {}, {}, {}, {}, {}, {}, {1295:1304}', {}, {}, {}, {114
:125}', {}, {818:825^272:279}', {493:500}', {547:556}', {333:342^143:152}'
, {480:489}', {641:652}', {312:323^240:251}', {}, HSN^CAN'

ttx-3, {}, {1646:1653}', {}, {}, {1803:1812^51:60}', {}, {1313:1324}'
, {2352:2363^1450:1461}', {}, {}, {}, {4419:4426}', {}, {3890:3899}'
, {3977:3986^3128:3137^1996:2005}', {}, {}, {}, {}, {}, {}, {}, {
}', {4394:4403}', {}, {2322:2333}', {}, {}, {}, {}, {}, {}, {},
, {}, {}, {1528:1539}', {}, {}, {}, {}, {}, {}, {}, {3643:3654}'
, {}, {}, {1517:1524}', {4525:4532}', {3287:3294}', {1205:1214}', {452:46
1}', {}, {1486:1497}', {3556:3567}', {1172:1183}', {}, {}, {}, {1499
:1508}', {}, {2525:2534^701:710}', {}, {}, {}, {}, {}, {}, {},
{}, {}, {}, {}, {1558:1569}', {143:150}', {3268:3275}', {4483:4490}'
, {}, {1573:1582^1112:1121}', {}, {}, {}, {4428:4439}', ASI^ADL'

unc-73, {3306:3313^922:929^459:466}', {}, {}, {}, {1003:1012}', {1250:1
259}', {1548:1559^1491:1502}', {4088:4099}', {4164:4175^3751:3762^3504:3515}'
, {}, {}, {3819:3826}', {}, {}, {}, {}, {}, {3958:3969}', {},
{}, {}, {}, {}, {}, {4137:4148^3939:3950^3221:3232}', {}, {3909:39
20}', {}, {}, {3861:3868^2236:2243}', {243:252}', {3871:3880}', {}, {}
, {1072:1083}', {}, {}, {}, {}, {}, {}, {}, {2538:2547^1165:1174}',
{}, {}, {2388:2399^1137:1148}', {3790:3797}', {4045:4052}', {3019:3026^16
6:173}', {}, {2674:2683}', {}, {2272:2283^842:853}', {}, {3802:3813^644
:655}', {}, {}, {}, {3833:3842^1094:1103^938:947}', {}, {1742:1751}'

'{}', '{}', '{854:865^192:203}', '{}', '{}', '{}', '{}', '{}', '{4209:4218}', '{2074:2085}', '{}', '{442:453}', '{4111:4118^1153:1160^907:914}', '{}', '{}', '{}', '{1025:1034}', '{}', '{69:80}', '{}', '{4008:4019}', 'ALM^HSN^CAN'

osm-9, '{3944:3951^2699:2706^1460:1467}', '{3545:3552^2368:2375}', '{}', '{203:212}', '{4121:4130}', '{}', '{4836:4847^2925:2936}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{2452:2463^573:584}', '{}', '{}', '{}', '{}', '{682:691}', '{}', '{2207:2218}', '{}', '{3211:3222^2227:2238}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{}', '{855:866}', '{3952:3963}', '{}', '{1379:1386}', '{}', '{}', '{2270:2279^109:118}', '{}', '{2319:2330}', '{2153:2164}', '{2800:2811}', '{1239:1246}', '{}', '{4616:4623^3526:3533^1538:1545^552:559}', '{}', '{3597:3606}', '{}', '{3357:3368^1224:1235}', '{3831:3842}', '{693:704^406:417}', '{387:394}', '{}', '{}', '{2335:2344^71:80}', '{2349:2358^288:297}', '{4053:4062^3573:3582}', '{299:310}', '{3260:3271^2569:2580^366:377}', '{}', '{}', '{2428:2435}', '{}', '{}', '{}', '{}', '{}', '{}', '{4767:4778}', '{2625:2632}', '{2300:2307}', '{}', '{2387:2396}', '{}', '{3620:3629^1714:1723}', '{}', '{2597:2608}', '{}', 'ASH^ASI^PHA^ADL^ASE^ASK'

Appendix B

Perl script to convert probability matrix to log odds matrix.

```
#!/usr/local/bin/perl -w
use strict;
use Math::Complex;

#
#

my @args = @ARGV;

if(scalar(@args) != 3){
    print "\n The # of command line arguments supplied not correct";
    print "\nUsage: convert <Prob Matrix motif file> <background Probability File> <output file name>\n";
    exit(0);
}

my $probMatrix = shift @args;
my $bgFrequencyFile= shift @args;
my $outputFile= shift @args;

sub slurp {
    local $/ = undef;
```

```

    open my $fh, $_[0] or die "Can't open $_[0]: $!";
    my $slurp = <$fh>;
    return \$slurp;
}

# Open Gene Names file
open(PROB, $probMatrix) || &return_error("File Error","Unable to open " . $probMatrix . ". Reason $!");

# Open Sequence File
#open(BG, $bgFrequencyFile) || &return_error("File Error","Unable to open " . $bgFrequencyFile . ". Reason $!");

# Open Gene Names file
open(FD, "> $outputFile") || &return_error("File Error","Unable to open " . $outputFile . ". Reason $!");

my @bg;
my $bgline = slurp($bgFrequencyFile);
foreach $bgline (<BG>){
    @bg = trim($bgline) =~ /([\d]+\.[\d]+)\s+([\d]+\.[\d]+)\s+([\d]+\.[\d]+)\s+([\d]+\.[\d]+)/;
    #print $bgline;
    #last;
    #}

print join(@bg);

my @parts;

foreach my $line(<PROB>){
    #if($line =~ /^#/){
    #    print FD $line;
    #    next;
    #}

    if(@parts = $line =~ /([\d]+\.[\d]+)\s+([\d]+\.[\d]+)\s+([\d]+\.[\d]+)\s+([\d]+\.[\d]+)/){
        for(my $i=0; $i<4; $i++){
            print FD round(logn( $parts[$i]/$bg[$i], 2) * 100);
            print FD "\t";
        }
        print FD "\n";
    }else{

```



```

        print FD $line;
        next;
    }

}

sub trim {
    my @out = @_;
    for (@out) {
        s/^\s+//;
        s/\s+$//;
    }
    return wantarray ? @out : $out[0];
}

sub round {
    my($number) = shift;
    return int($number + .5 * ($number <=> 0));
}

```

Appendix C

User Guide

C.1 Analysis Frame

The visualization and specialization modules (VSM) could either be invoked from the mining interface of WPI-Weka system or could be invoked as a standalone application using exported set of mined association rules, the associated MAST results (HTML format) and a list of gene names alongside the known expression patterns. The primary interface of the VSM is the Analysis frame, that is the first screen to be displayed when VSM is invoked. See Figure C.1. We explain the Analysis frame below.

The analysis frame loads with two sections, the **Rules** area and the **Commands** area as shown in Figure C.1. We explain the Rules area below and we explain each of the options in the Commands area in subsequent subsections. The Rules area is used to display base association rules along with the corresponding values for certain measures of interestingness. The design is extensible, that is, new measures of interestingness could be added in the future with minimal code changes. In the current state, a rule tuple consists of the following items:

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.42857143
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.10714286
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.071428575
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.4642857
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.10714286
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832668E-1	0.32142857
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.10714286
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.035714287
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.64285713
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.41666666
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.39285713
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.20833333
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.035714287
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.32142857

Commands Area

Inter-Motif Distance Plot Sequence Plot Add Rule Delete Rule Export Rules Import Rules Hide Current Column

Figure C.1: Sample Analysis Frame.

- **Id** - this is a unique id assigned to the rule. The usability of this field increases once the user starts to generate specializations from the rule, as the Id column helps us trace the history or the specialization path of new rules.
- **Antecedent** - The left-hand side of the rule. It contains the motifs present in the rule.
- **Consequent** - The right-hand side of the rule. It contains the cell-types predicted by the rule.
- **Support** - As discussed in Section 1.2, Rule 1.1; the support of the rule is one of the popular scales to measure the interestingness of the rule
- **Confidence** - Confidence is the other popular metric to measure the interestingness of the rule.
- **Lift** - The lift value of an association rule is another measure to try to quantify the interestingness of the rule. It is defined as the ratio of the confidence of the

rule and the support of the consequent of the rule [BMS97]. In other words

$$lift(rule) = p(consequent|antecedent) / p(consequent)$$

- **p-Value** - The p-value of the rule is the probability that the correlation between the antecedent and the consequent is due to chance by using the chi square test.
- **Within Cell-Type Support** - Provides the support of the rule among only those instances of the data that contain the consequent of the rule.

The Commands area of the Analysis frame provides buttons to perform a range of functions. Each of the following subsections describe each of these functions provided by the visualization extensions via the analysis frame. It is important to note that most of these functions are invoked in the context of a specific rule and so it is necessary to select a rule in the rules area of the analysis frame before we invoke a command.

C.2 Inter-Motif Distance Plot

Selecting a Rule in the rules area and then invoking the inter-motif distance plot via the button with the same label, lets a user visualize the data in the context of the rule from a inter-motif distance perspective. This action enables a user to perform exploratory analysis in the context of the hypothesis - “Inter-motif distance influences gene expression”.

On invoking this command a new frame with the pairwise inter-motif distance plot(s) is displayed. It displays one graph for each pair of motifs in the rule (selected

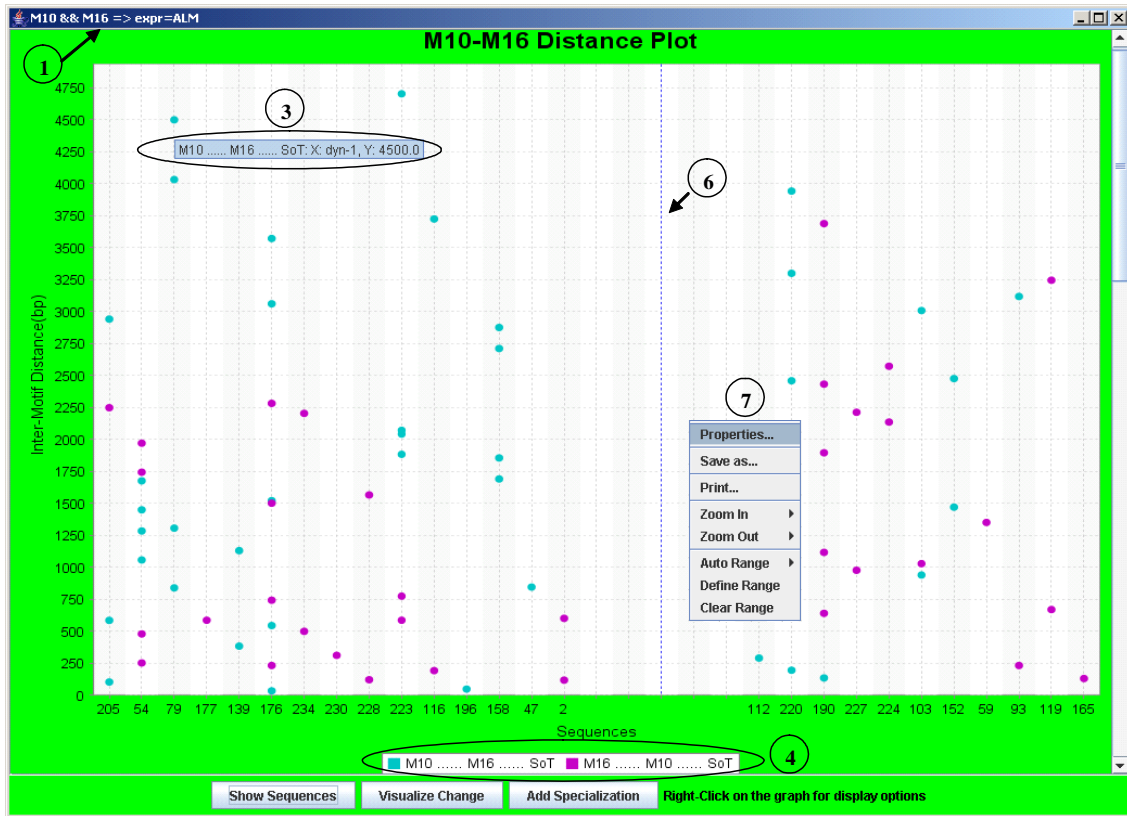


Figure C.2: Sample Inter-Motif Distance Frame - The numeric pointers refer to the enumerated text explaining Inter-Motif Distance Plot.

in the Analysis Frame). For sake of simplicity we start with a rule with only two motifs and we revisit plots originating from rules consisting of more than two motifs in item later in the section. We enlist below the highlights of the information displayed in an inter-motif distance plot and the numeric annotations in Figure C.2 are references to the information in the following enumeration.

1. Each graph is displayed with the rule used to establish the context as the title of the frame.
2. Each graph displays the pairwise inter-motif distance plots. For instance, in Figure C.2 the rule consists of motifs M10 and M16. So for each instance of M10 on a gene, a distance value from every instance of M16 is computed. Each

such value corresponds to a point on the graph with distance being plotted on the y-axis and the x-axis is an id for the gene in question.

3. Rolling your mouse over any such data point displays the relevant gene name and the distance computed.
4. Each graph could potentially contain points in two colours. The legend explains the difference. Each point is a distance of an instance of M10 from an instance of M16 but the color helps identify the order in which these motif instances occur in the gene sequence relative to the Start of Transcription(SoT).
5. Each graph lists only those genes on the X-axis, that support the antecedent of the rule. That is, genes whose promoter regions contain at least one instance of both motifs.
6. Another mechanism to aid visual exploration is that each graph is sliced into two parts by a dotted line. The genes in the left part are the ones that support the consequent of the rule and hence support the rule. The ones on the right are the genes that only support the antecedent of the rule. This provides the user with an easy mechanism to discover inter-motif distance based patterns on the left part of the plot that are not as frequent on the right part as this would let us explore specializations with improved classification accuracy(and/or confidence).
7. We used the charting library, JFreeChart [JFr], as the charting infrastructure for the visualization extensions. This was an obvious choice because it was an open source, well-documented API, supporting a wide range of chart types with a flexible design that is easy to extend. A right-click on the graph area displays a popup menu with the following options:

- **Zoom** - Lets you zoom in or out on the graph.
 - **Range** - Lets the user change the scale of either axis.
 - **Save** - Lets the user save an interesting graph as an image.
 - **Define Range** - This is an important extension we made to the charting infrastructure that lets the user define, in a graphical fashion, the inter-motif distance to be used in the specialization. An inter-motif distance based specialization places conditions on the distance between instances of the motifs involved. For instance, it could be worth noting that there are significantly more data points with an inter-motif distance value between 0 and 500 in the left part of the Figure C.2 as compared to the same distance range in the right-hand side. The definition of this distance condition is in the form of a range, for instance, $(0-500)$. Selecting this option from the menu changes the graph to a range define mode and a subsequent click and drag can be used to define the range.
 - **Clear Range** - Another extension to the charting infrastructure which lets a user clear a currently defined range providing an option to redefine a range.
8. Once the user has utilized the dotted separation and inter-motif distance plots to identify a range of interest, for instance a range of $(0-500)$ between motifs M10 and M16, the “Define Range” option from the pop-up menu can be used to graphically define this range. Once a range has been defined for a plot, it is highlighted on the graph in a shade of gray. For instance, refer to Figure C.3 numerical annotation 8.
 9. Even after a range has been defined the data being visualized is in the context of the original rule. At this point the user can invoke the “Visualize Change”

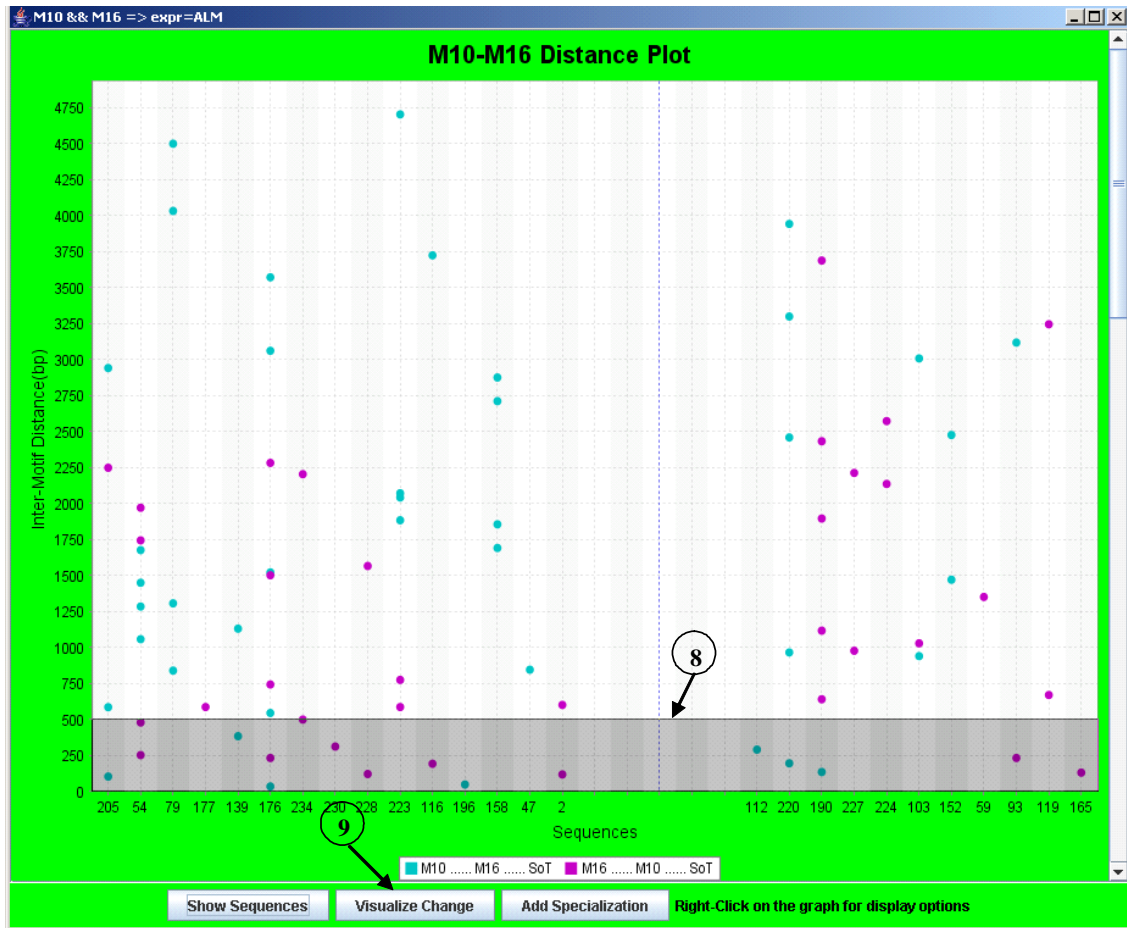


Figure C.3: Inter-Motif Distance Frame depicting an inter-motif range of 0-500 bp for Motif pair M10 and M16.

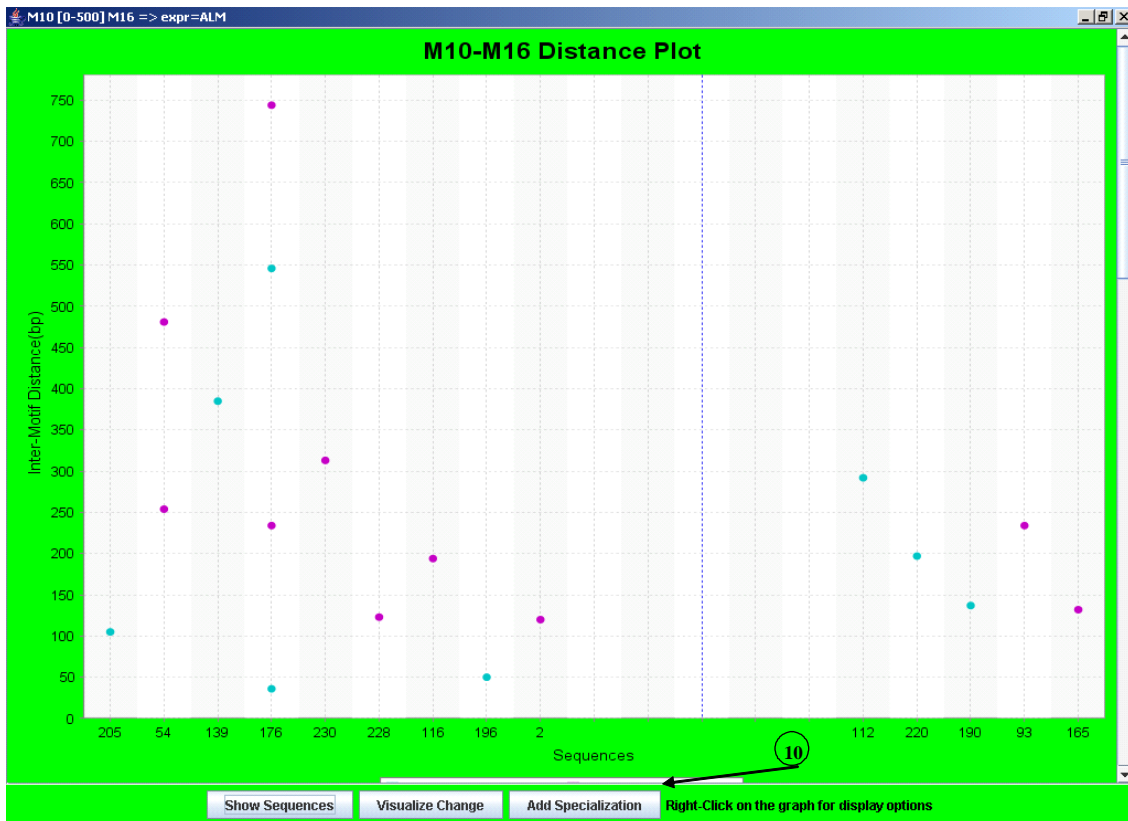


Figure C.4: Visualize command invoked inter-Motif distance plot displaying data in the context of the specialised rule $M10 (0-500) M16 \Rightarrow \text{expr} = \text{ALM}$.

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
013.01	M10 [0-500] M16	expr=ALM	0.64285713	0.14754099	1.4005102	1.1581367E-1	0.32142857
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572

Figure C.5: A row representing the addition of a specialization to the Analysis Frame.

(Figure C.3 numerical annotation 9) command to visualize the data in the context of the specialization rather than in the context of the original rule. For instance Figure C.4. It is worth noting that the title of the new window is indicating the new context.

- If the user finds this specialization (Figure C.4) of interest, the specialized rule can be added to the Analysis Frame using the “Add Specialization” command on the Inter-Motif distance plot. This causes a new entry to be inserted in the Analysis Frame with the following specialization

$$M10(0 - 500)M16 \Rightarrow expr = ALM \tag{C.1}$$

as shown in Figure C.5. Note that the Id field is auto-generated in a fashion that always lets a user trace back the steps in case we want to later recall which rule was used to derive the specialization. Also note the different measures of

interestingness are computed for the specialized rule.

11. If the base rule consisted of more than two motifs, an inter-motif distance plot for each pair of motifs is displayed in the same frame (Figure C.6). Each chart or plot individually provides for defining relationships between a pair of motifs. In case multiple relationships are defined for more than one pair of motifs each motif is represented as a term and a collection of independent terms constitutes the specialized rule. Mechanism to define advanced relationship's between each term is also provided and we would revisit the topic later.

C.2.1 Sequence Plot

Select a Rule in the rules area and then click the sequence plot button to visualize all the qualifying gene sequences in the context of the rule. This action enables a user to perform exploratory analysis in the context of the hypothesis - "Distance of motifs from the SoT influence gene expression". A qualifying gene sequence is one that has that has at least one instance of each motif that appears in the rule (selected in the Analysis Frame). Invoking this command causes a new frame with the sequence plot overlaid with the motif information to be displayed. We enlist below the highlights of the information displayed in an inter-motif distance plot and the numeric annotations in Figure C.7 are references to the information in the following enumeration.

1. Each graph is displayed with the rule used to establish the context as the title of the frame.
2. Displays the gene sequence plots with motif instances in the context of the rule. For example, in Figure C.7 the sequence plot displays all relevant gene

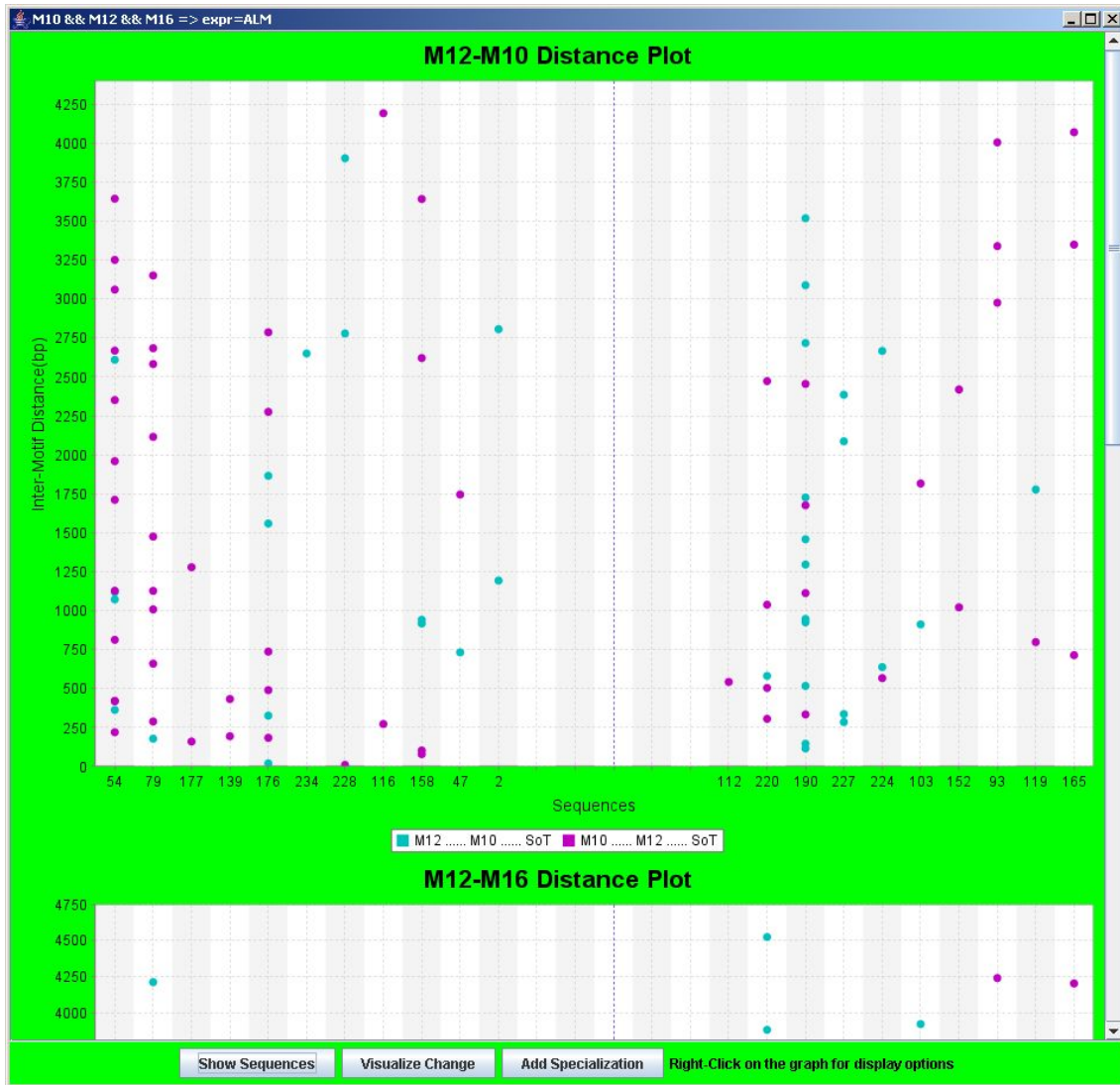


Figure C.6: Multiple pairwise inter-motif distance plots for a base rule with more than two motifs. For instance the base rule for this plot is $M10 \ \&\& \ M12 \ \&\& \ M16 \Rightarrow \text{expr} = \text{ALM}$.

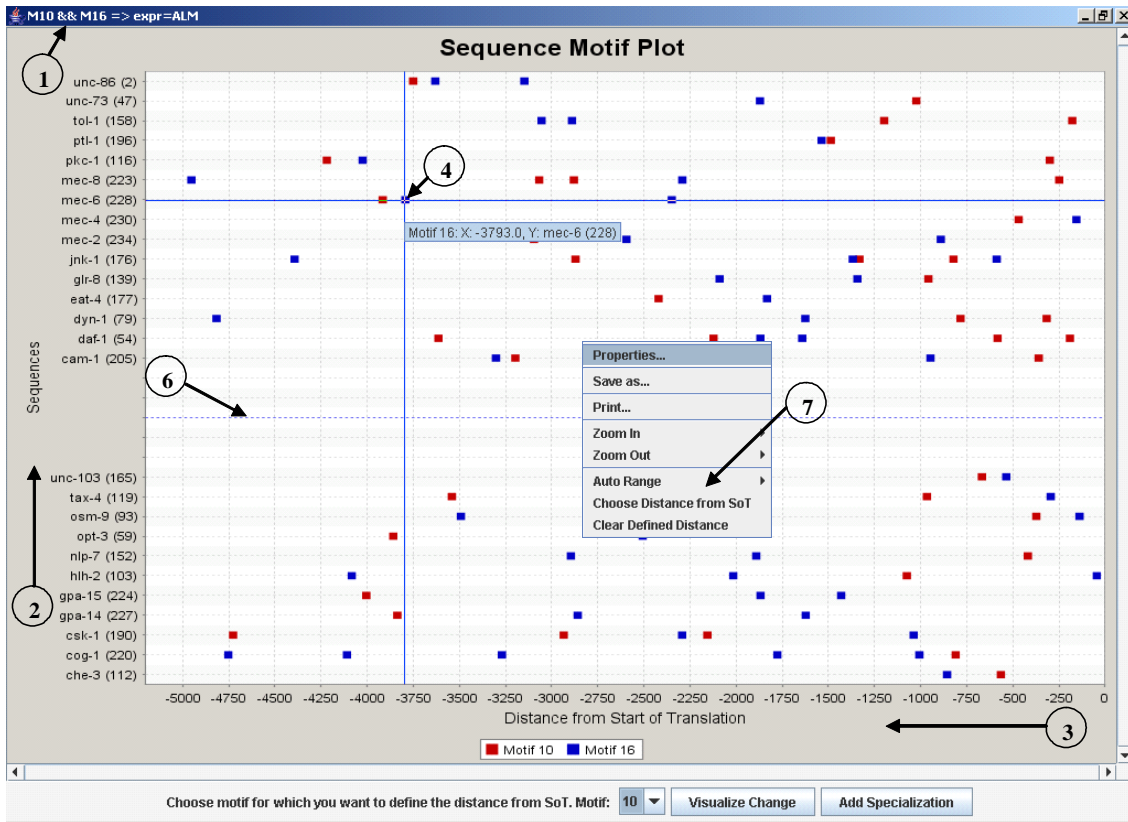


Figure C.7: Sample Sequence Plot Frame - The numeric pointers refer to the enumerated text explaining the Sequence Plot.

sequences with the instances of participating motifs (i.e., motifs M10 and M16). So along the y-axis is the list of qualifying gene promoters.

3. For each such gene sequence we plot all the instances of the participating motifs as they exist on the gene sequence relative to the Start of Transcription(SoT), which is the far right end of the plot. This makes the x-coordinate of each point in the plot the distance of the motif from the SoT and the color of the point is used to identify the motif.
4. Rolling your mouse over any such point displays the relevant gene name and the distance of the instance of the motif from the SoT.
5. Lists only qualifying genes on the Y-axis, that support the antecedent of the

rule. That is, the gene sequence has at least one instance of both motifs.

6. Another mechanism to aid visual exploration is that each graph is sliced into two parts by a horizontal dotted line. The genes in the upper part are the ones that support the consequent of the rule and hence support the rule. The ones in the lower part are the genes that support only the antecedents of the rule. This provides the user with an easy mechanism to discover “Distance from SoT” based patterns in the upper part of the plot that are not as frequent in the lower part as this would let us explore specializations with improved classification accuracy (and/or confidence).
7. The following context-specific options were added to the graph right-click popup menu in the charting infrastructure:
 - **Choose Distance from SoT** - This extension lets the user choose the “distance of a motif from the SoT” clause-based specialization in a graphical fashion. Selecting this option from the menu changes the graph to a distance selection mode. A subsequent click can be used to define the chosen value for the “distance from the SoT” and a visual confirmation of the defined distance clause is provided in the form of a vertical dotted line in the same color as the one reserved for the motif. One such distance can be defined for each participating motif. For example, in Figure C.8 a distance term of *SoT [0-500] M10* is chosen.
 - **Clear Distance from SoT** - This option lets a user clear all currently defined distances from SoT.
8. Once the user has utilized the dotted separation of the plot (into rule supporting and antecedent supporting) and the rule specific sequence plots to identify

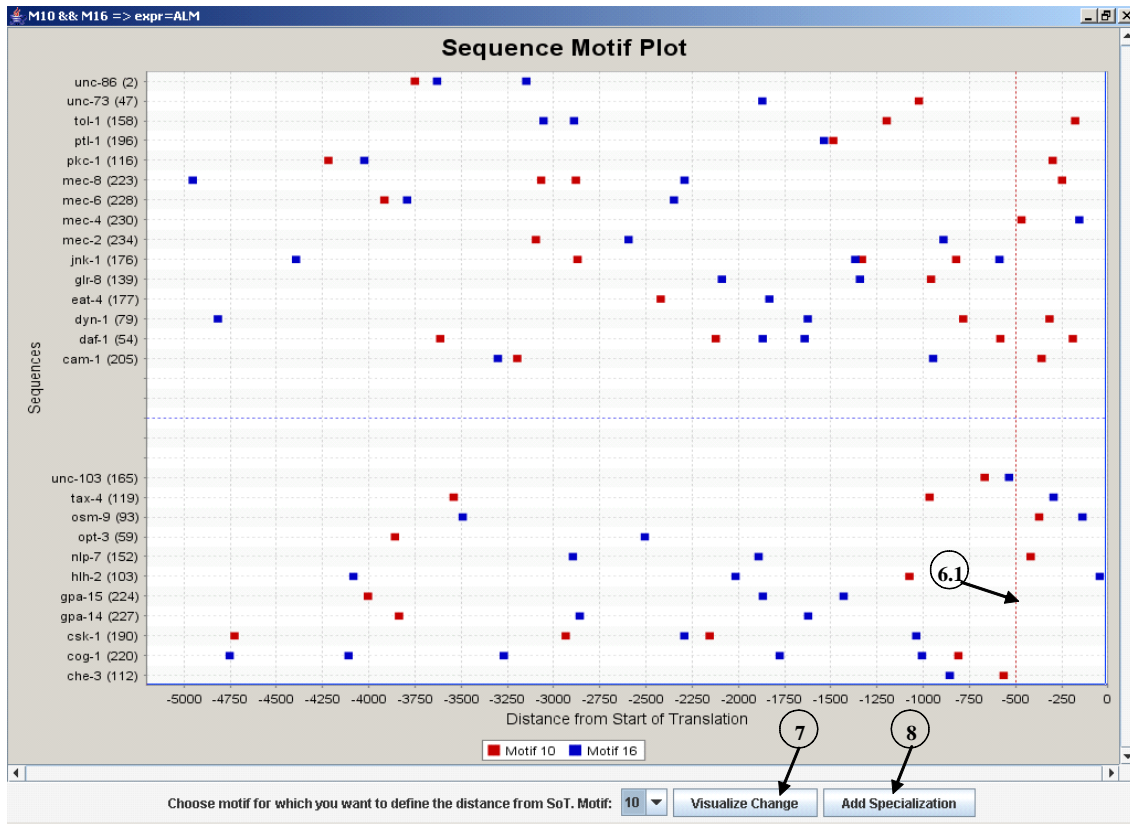


Figure C.8: Sequence Plot Frame depicting a distance of 500 bp from SoT for M10.

a “distance from SoT” clause of interest, the “Visualize Change” command could be invoked to visualize the data in the context of the specialization rather than the original rule. Again the title of the new window is indicating the context setting rule/specialization.

9. If the user finds the specialization of interest, it can be added to the Analysis Frame using the “Add Specialization” command on the new sequence plot. Again this causes the specialization to appear as a new entry in the Analysis Frame with an auto-generated Id that again lets a user trace back the steps in case the user wants to later recall which rule was used to derive a specialization (Figure C.9).

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775962	4.9832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
013.01.01	M16 && SoT [0-500] M10	expr=ALM	0.7777778	0.114754096	1.6944444	3.7665958E-2	0.25
013.01.03	SoT [0-500] M10 && SoT [0-1750] M16	expr=ALM	0.8	0.06557377	1.7428571	1.1028346E-1	0.14285715

Figure C.9: Analysis Frame depicting a couple of newly added distance from SoT based specializations.

- In case the base rule consisted of more than one motif and multiple “distances from SoT” relationships are defined (one for each motif) each such relationship is represented as a term and a collection of independent terms constitutes the specialized rule. For instance see Figure C.9 for the following specialization.

$$SoT [0 - 500] M10 \ \&\& \ SoT [0 - 1750] M16 \Rightarrow expr = ALM \quad (C.2)$$

C.2.2 Order of occurrence of motifs

We wanted the VSM to facilitate exploratory analysis based on the hypothesis “Order of occurrence of motifs influences gene expression”. But during the system design and the system use by the team (including the domain expert) it was observed that we already had a few ways to visualize gene sequence data in the context of the “order of the occurrence” of motifs. If order of occurrence of motifs was important

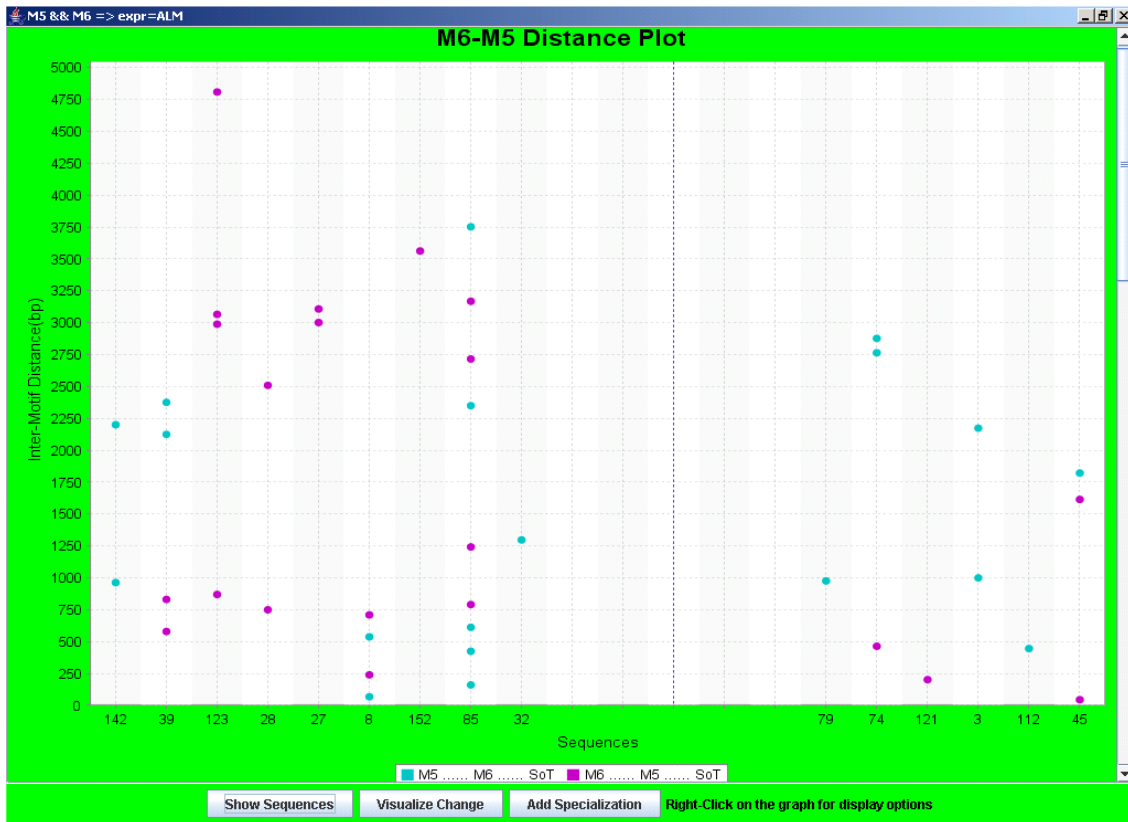


Figure C.10: Inter-Motif Distance Plot for Motifs M5 and M6. Observe the lack of magenta (dark) dots in the right half of the frame.

it could be easily identified by one of the following ways:

1. **Color of the points in inter-motif distance plot.** The order of the motifs in the inter-motif distance plot is represented by color. For instance in Figure C.2, M10 to the right of M16 (i.e., M16..M10..SoT) is represented by a magenta (dark) dot, while M16 to the right of M10 is represented by a aqua (light) dot. Thus, color provides a quick visual clue whether the order of occurrence of motifs affects gene expression; the left half of the plot should have more point of one color than the other in this case.
2. **Repeating sequence of color in the sequence plot** - As mentioned in

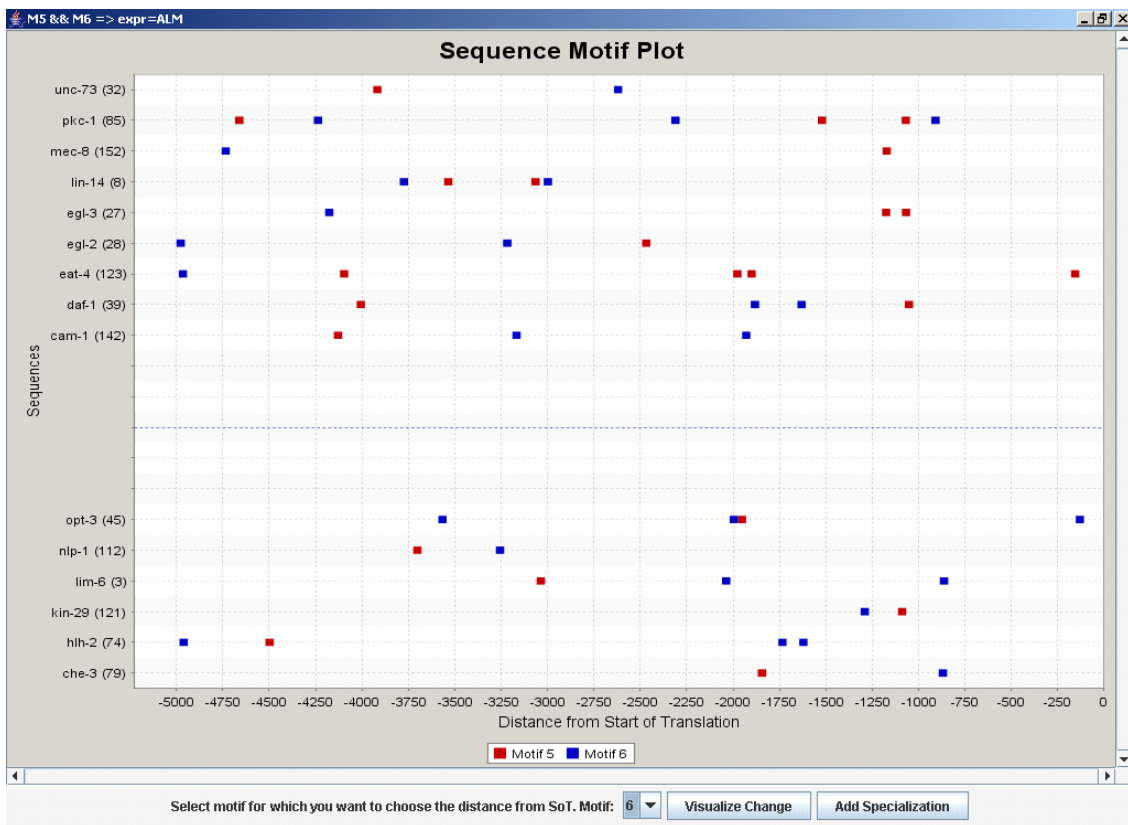


Figure C.11: Sequence Plot for Motifs M5 and M6. Observe that in the rule supporting sequences (upper part) a red dot is usually followed by a blue dot scanning the gene sequence from right end (SoT) to left.

Section C.2.1, enumeration item 2, the sequence plot displays all instances of participating motifs for qualifying sequences as they occur on the gene relative to the SoT. Since each motif appears in its own color and the data is being visualized in the context of a single rule, one can often see a repetitive pattern of color in the upper half and a lack of the same in the lower half of the plot. Such a display could also indicate an influence of order of occurrence on gene expression. In Figure C.11 one can observe that most sequences in the upper half have an occurrence of M5 (light point in the graph) closer to the SoT (the far right end of the plot) that is followed by a dark dot somewhere on the gene sequence. Also that this pattern is not so frequent in the lower part. Such pattern observation could indicate order of occurrence type relationship.

3. **ASAS mining algorithm** - The WPI implementation of association rule mining [PR05] used by us is capable of mining association rules with order based information and hence it is possible to have some of these rules being available already at the beginning of the exploratory analysis. Any of the two means mentioned above could be used to visually confirm/observe the order of occurrence relationship.

C.2.3 Add Rule

Irrespective of the method used to identify a potential order of occurrence relationship between participating motifs the following option from the Analysis Frame could be used to add order-based rules. Once a user has identified an order-based (or any other) relationship between motifs, it can use the “Add Rule” option in the Analysis Frame to add a blank row for the new rule. The user can then simply type in the Antecedents and the Consequent of the rule to calculate the different

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
015	M5 (rp0-rp1) M6 (rp2-rp3)	expr=ALM	0.7	0.114754096	1.525	9.4429519E-2	0.25

Figure C.12: Add Rule option in the Analysis Frame provides for free text option to add rules.

statistics indicating the interestingness metrics of the rule as shown in Figure C.12. Simply typing a complete rule computes the statistics indicating the interestingness of the rule. The user could also visualize the new rule using either the sequence plot or the inter-motif distance plot.

A rule keyed in by the user which does not have a valid syntax results in an error as shown in Figure C.13

Id	Antecedent	Consequent	Confidence	Support	Lift	p-Value	Within Cell-Type(s) support
001	M17	expr=ALM	0.48148146	0.325	1.2037036	4.9873279E-1	0.5714286
002	M12	expr=ALM	0.52830184	0.35	1.3207545	5.021099E-1	0.71428573
003	M17 && M12	expr=ALM	0.5555556	0.3125	1.388889	5.0202791E-1	0.42857143
004	M16	expr=ALM	0.46774197	0.3625	1.1693549	4.9947691E-1	0.64285713
005	M16 && M12	expr=ALM	0.54347825	0.3125	1.3586956	5.0175261E-1	0.5
006	M18	expr=ALM	0.43103448	0.3125	1.0775862	4.8832669E-1	0.78571427
007	M10	expr=ALM	0.5090909	0.35	1.2727273	5.0157473E-1	0.78571427
008	M10 && M16	expr=ALM	0.57777774	0.325	1.4444443	5.0248397E-1	0.53571427
009	M25	expr=ALM	0.37313434	0.3125	0.9328359	4.9043181E-1	0.78571427
010	M25	expr=ADL	0.37313434	0.3125	1.1055832	4.9446274E-1	0.85
011	M26	expr=ALM	0.37681156	0.325	0.9420289	4.8986432E-1	0.25
012	M26	expr=ADL	0.36231884	0.3125	1.0735373	4.9108282E-1	0.3
013	M10 && M16	expr=ALM	0.5777778	0.325	1.4444444	5.0248397E-1	0.53571427
014	M25 && M26	expr=ALM	0.42857143	0.09836066	0.93367344	3.7273299E-1	0.21428572
015	M5 && M6	expr=ALM	0.6	0.14754099	1.3071429	2.0701702E-1	0.32142857
017	M5 (rp0-rp1)	expr=ALM	0.7	0.114754096	1.525	9.4429519E-2	0.25

Figure C.13: Grammar based parsing helps identify user-errors in typing the rule.

C.2.4 Hybrid Rule

As described in the grammar governing rule definitions, each rule consists of an antecedent and a consequent. Antecedents in turn consists of terms. A rule could also include specialized term, extra hypothesis-based information(constraints) that the instances of the participating motifs must satisfy in order for a gene sequence to support the rule.

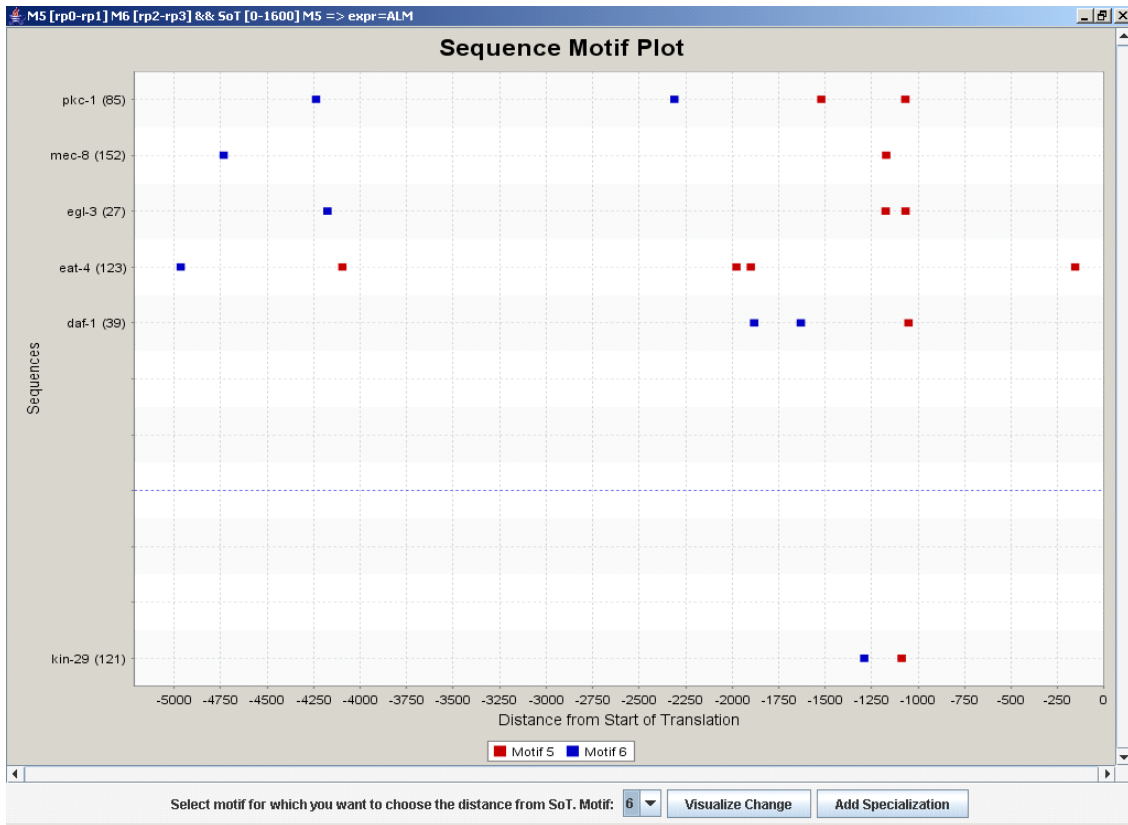


Figure C.14: Hybrid rules help specify multiple constraints (based on different hypothesis) within a single specialization.

The system also supports hybrid rules, rules that consists of specialized terms based on different hypothesis and a gene sequence must satisfy all constraints in order to support the rule. E.g. With reference to Figure C.11 note that there exists an instance of M5 (the red dot) usually within the first 1600 bp from the SoT (Far

right end of the plot). It is interesting to combine the two observations into a rule as follows and visualize it or calculate its interestingness. As we see in the figure C.14 that this hybrid specialization:

$$M5[rp0 - rp1]M6[rp2 - rp3]\&\&SoT[0 - 1600]M5 \Rightarrow expr = ALM \quad (C.3)$$

has a higher confidence as compared to the following simpler “order of occurrence” specialization

$$M5[rp0 - rp1]M6[rp2 - rp3] \Rightarrow expr = ALM \quad (C.4)$$

As seen above hybrid specialization could have multiple specialized terms that relate to a single motif. A hybrid specialization could post multiple constraints on the same motif like Distance from SoT and Order of occurrence relative to another motif. It is important to note that although the rule may have multiple constraints for the same motif, it is not required that the same instance of the motif satisfies each of them. In the context of the C.3 above, it is not required that the instance of Motif 5 that satisfies the order of occurrence condition is the same M5(instance) that lies within 1600 base pairs of the SoT. Although there might be a need for the user to actually specify constraints which are inter-related and aliases are supported by the rule grammar for exactly this reason.

C.2.5 Aliases

Aliases were included in the grammar to provide the user with an option of defining inter-related constraints or specialization terms. Consider the following Inter-Motif Distance based specialization from Figure C.5:

$$M10[0 - 500]M16 \Rightarrow expr = ALM \quad (C.5)$$

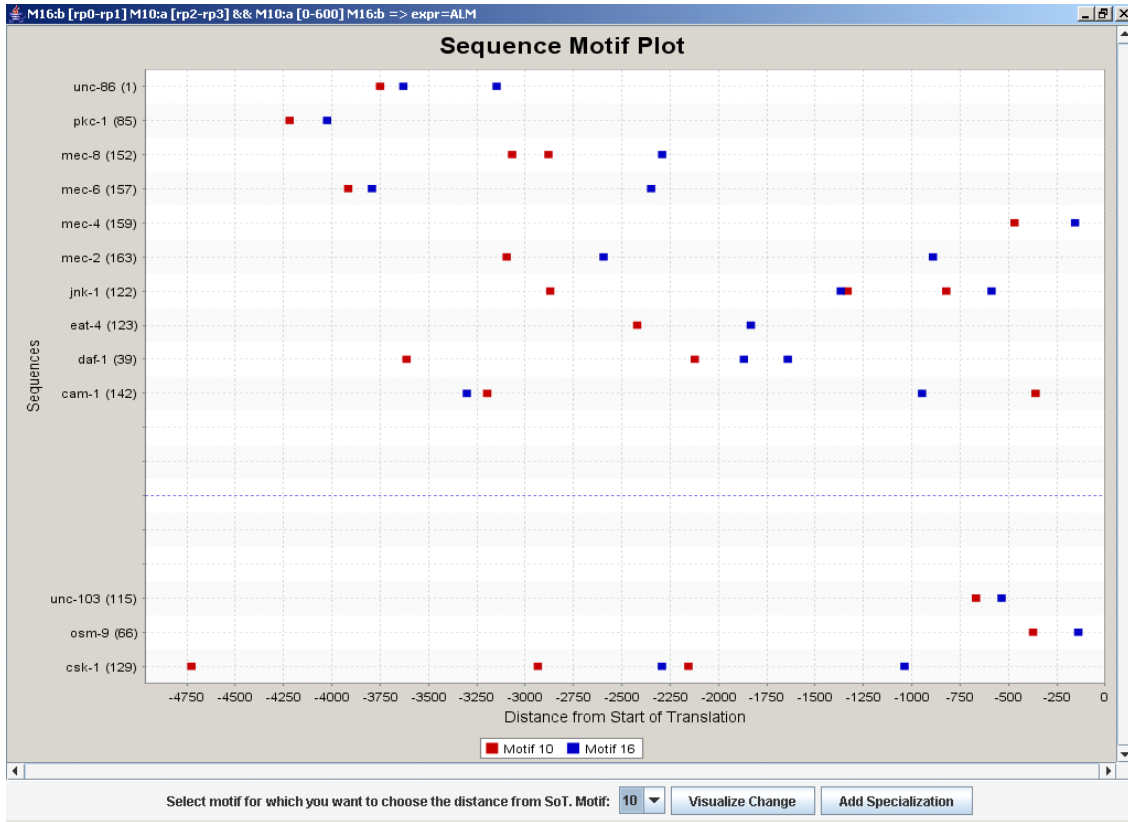


Figure C.15: Aliases let user define specializations with inter-related constraints.

Visualizing this specialization using a sequence plot (Figure C.7, one can see distinctly that not only do motifs M10 (red dot) and M16(blue dot) occur close together but they also occur in pattern such that the same instances of M10 and M16 that are involved in the distance-based relationship also occur in the same order relative to the SoT. Aliases enable the user to specify such complex relationships in the rule as follows (Figure C.15:

$$M10 : a[0 - 600]M16 : b\&\&M16 : b[rp0 - rp1]M10 : a[rp2 - rp3] \Rightarrow expr = ALM \quad (C.6)$$

C.2.6 Delete Rule

A user (usually a domain expert) can often identify rules which are of little biological significance and may want to delete such rules from the rule set. Simply selecting a rule from the Analysis frame and then clicking on the “Delete Rule” button could be used to accomplish exactly this.

C.2.7 Export Rules

This option enables a user to save a copy of the rule set currently in the Analysis Frame to a text file. This provides the user the facility to resume working on the rule set at a later point in time or maintain motif based rule sets. The extensions to WPI-Weka rule-miner [Rudss] ensured that the rule model can also be imported into the rule mining interface.

C.2.8 Import Rules

This option enables to import a rule model to be imported from a text-file. It could be either from a previous session of the analysis-frame or could be a rule set exported from the rule-mining interface. (Thanks Jon)

C.2.9 Hide Current Column

If a user thinks, that one of the columns in the analysis frame is not of use in the current context the user has the option of removing a column from the display. Simply select a cell in the column that the user wants to delete and click the “Hide Current Column” button.

C.2.10 Sorting in Analysis Frame

The Analysis Frame also provides the option of sorting the rule model in the analysis frame by simply clicking on the header of the column by which the rule model needs to be sorted. The order of sorting could also be reversed by simply clicking on the header column once more.