Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Information Technology and Electrical Engineering
Department of Computer Science

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Classifying Combined MicroRNA Data Sets

Master's thesis in Informatics
Supervisor: Pål Sætrom
June 2019

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Kunnskap for en bedre verden

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Abstract

Since the discoveries of the first microRNAs in 1993, researcher have found several thousand different microRNAs in the human genome over the last decades. MicroRNAs as a topic has become especially hot when researching cancer due to its ability to regulate many of the protein-coding RNAs. Various machine learning methods have been employed in cancer diagnostic research. Machine learning methods can generate more accurate diagnoses or prognoses than traditional statistical methods can.

In cancer, cell division become abnormal and uncontrolled, which arises from the misregulation of several genes. MicroRNAs are major regulators of gene expression and thus it is not surprising that microRNAs are actively altered in different types of cancer.

In this paper several techniques are used to classify combined microRNA data sets for both colorectal- and hepatic cancer. Techniques includes several types of normalization, feature selection, algorithms and Gene Set Enrichment Analysis. The most important features for the different classification techniques is also extracted for both diseases.

The results indicate that microRNA data sets can be combined and classified with scores ranging from 0.89 to 1.00 in receiver operating characteristic area under curve score. This is done by utilizing two forms of normalization prior to training a classifier. In addition, this paper proposes a method for combining gene set enrichment analysis with support vector machines in classification for a robust unbiased classification of microRNA gene expressions. This method is favored for higher imbalanced and small data sets by being unaffected by both feature scaling and different feature subsets.

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Chapter 1

Introduction

This introduction to background and motivation states where this project is situated in the field and what the key driving forces motivating this research are. An outline for the projects goal and its main contributions are also listed.

1.1 Background and Motivation

The first microRNAs (miRNAs) were discovered in Lee et al. (1993). MiRNAs are small non-coding RNAs that regulate the translation process of messenger RNAs (mRNAs) into proteins. Since then, a lot of research has gone into discovering new miRNAs, finding miRNAs target mRNAs and linking miRNAs to several diseases including different types cancer. Great steps in bioinformatics, machine learning, new algorithms and increased processing power have facilitated this booming research. In addition several online sources such as mirbase.org are now available to make published miRNA sequences searchable.

Traditional statistical methodology called gene set enrichment analysis were invented to catch subtle changes in groups of genes. Additive relationships had to be considered as studying individual genes were not sensitive enough to detect subtle differences. This methodology has been mostly used on mRNAs, but can also be used for miRNAs. Gene set enrichment analysis can create scores that are bias independent of data sets.

Studying the relationships between miRNAs and different diseases can help us better understand the disease, and it can help produce better diagnosis, prognosis and in recent years therapies for patients. MiRNA data sets are prone to be small in samples compared to features because of a relative high cost of producing them. Therefore, making a classifier to separate a single data set will in many cases overfit the biases from that data set. To make a classifier for several data sets one would first have to eliminate an internal bias of each data set. This internal bias can come from several sources most of which are laboratory related and to varying degree unavoidable. The focus in this project is to examine different ways to eliminate internal biases and in doing so making a more universal classifier for the miRNA data sets.

1.2 Goals and Research Questions

The goal of this project is to identify robust methods for combining miRNA data sets. Specifically, the methods should robustly combine data sets with different biases such as those found in microarray and sequencing data. This involves using feature scaling, external calculations and feature selection in combination with different classification algorithms and combined data sets of different imbalance and bias. The methods are evaluated in terms of how well the combined data sets can be used in the binary classification problem separating normal from tumor samples. To help address these problems the following research questions were used.

RQ1 What are the existing machine learning solutions for classifying cancer gene expression data with different biases?

RQ2 How does standardization and scaling features affect classification performance for combined miRNA data sets?

RQ3 How beneficial is feature selection in combined miRNA data sets?

RQ4 How does the solutions to RQ2 and RQ3 translate to an imbalanced data set and how well does it perform compared to Gene Set Enrichment Analysis?

RQ5 How do the miRNAs with highest importance for classification compare to what is currently known about them and relations to diseases?

1.3 Research Method

As no previous work was found combining different miRNA data sets, the work was based of work in classification of single miRNA data sets. No similar prior work also indicated the need for experiments to evaluate the advise from classification of single miRNA data sets with regard to this thesis's goals. These advises gave a broad overview over how classification can be done and created a outline for what experiments should be done and how they should be done.

Eight data sets from patients with either colorectal cancer and hepatic cancer were provided for this work. Using these data sets a series of experiments were done testing different pre-processing combinations and algorithms. Experiments for imbalance in data sets were also done testing the previous found methods performance in data sets down to single samples. Finally, an experiment were done extracting the features who's importance were highest for different methods.

1.4 Contributions

The contributions in this project is presented in section 5.3. The main contributions of this project are:

- A literature review based on classification of miRNA data sets.
- Classification results of combined miRNA data sets using different feature scaling, feature selection and algorithms.
- A comparison of classification results between classification using miRNA expressions and GSEA enrichment scores on combined data sets.
- A method that utilizes GSEA enrichment scores with support vector machines classification to achieve unbiased classification of miRNA data sets.

1.5 Thesis Structure

Chapter two contains the background theory of both biological and algorithmic aspects, summary of related works and motivation. The related works is also where RQ1 will be addressed. Chapter three consists of the different feature scaling techniques, performance metrics, feature selection methods and cross-validation techniques used in this project. Chapter four contains the different experiments, their setups and the results of these. These experiments are highly related to the research questions. Section 4.2 address research questions RQ2 and RQ3. RQ4 is addressed in section 4.3 and tables with importance for RQ5 can be found in section 4.4. Chapter five contains a discussion of the results, conclusions found and what future work can be done. Bibliography and an appendix are the last chapters.

Chapter 2

Background Theory and Motivation

This chapter will present the current state-of-the-art regarding cancer classifications using microRNAs. First, the relevant knowledge of biology and bioinformatics is presented. Second, an in-depth summary of relevant background in machine learning is presented. Third, the research done on similar problems with similar constraints is summarized. A protocol for where this research can be found and why it is included can be found in the structured literature review protocol. The chapter ends with a motivation for possible applications and benefits for using machine learning on microRNAs.

2.1 Background Theory on Biology

This section presents current state-of-the-art in bioinformatics and biology with regards to microRNAs.

2.1.1 Transcriptome, Microarrays and Sequencing Technology

The transcriptome can roughly be described as a complete set of transcripts in a cell and their quantity, i.e. it is the set of all RNA molecules in one cell. Understanding the transcriptome is essential when interpreting the functional elements of the genome. Furthermore it represents the molecular constituents of cells and tissues, which allows for better understanding of development and disease. Transcriptomics seek to catalogue all species of transcripts to determine the transcriptional structure of genes and quantifying the changing expression levels for each transcript during development and under different conditions and diseases, such as cancer. The species of transcript include messenger RNA (mRNA) and small non-coding RNA called microRNA (miRNA). The transcriptional structure is expressed in terms of their start sites, 5' and 3' ends.

There exist several techniques to deduce and quantify the transcriptome, including microarray and RNA-sequencing. A microarray is typically a two-dimensional array with single-stranded DNA probes. RNA molecules from cells are transformed to complementary DNA, flourescently labelled, and allowed to hybridize to the microarrays probes. This

allows for measuring the expression levels of a large number genes simultaneously by scanning the microarray with a laser.

RNA-sequencing uses high-throughput sequencing to identify which genes are active, and how much they are transcribed. To do this the RNA-sequencing must have a library of cDNA fragments with adapters attached to one of both ends. The libraries are usually constructed from a population of RNA, however several manipulation stages are involved in constructing the cDNA libraries. Each molecule is sequenced in a high-throughput manner to obtain short sequences from one end or both ends. The result is a precise discrete value for all genes expression levels.

The different techniques each have their pros and cons, and therefore one is not strictly better than the other. Microarrays expression powers are limited because of its reliance upon existing knowledge about genome sequences. In addition comparing expression levels across different experiments can require complicated normalization methods due to both background and saturation of signals. RNA-sequencing does not have a problem with being limited to detecting transcripts that correspond to existing genomic sequence. Nor does it have any background signal because the DNA sequences can be unambiguously mapped to unique regions of the genome. Lastly the RNA-sequencing offers higher precision and requires less sample than microarrays. RNA-sequencing do face some challenges itself regarding its library construction. First of the larger RNA molecules must be fragmented into smaller pieces, approximately 200-500 base pairs (bp) to be compatible with most deep-sequencing technologies. These fragmentation techniques creates a different bias in the outcome. Furthermore some manipulations during library construction also complicate the analysis of RNA-sequencing results. Lastly RNA-sequencing faces some bioinformatics challenges with storing, retrieving and processing large amounts of data (Wang et al., 2009).

2.1.2 MicroRNAs

MicroRNAs are a class of short non-coding RNA sequences. The miRNAs regulate many genes by base pairing to sites in mRNAs. This makes them appealing targets for screening, diagnosis, prognosis, monitoring tumor progression, biomarker discovery and evaluation of correct treatment for patients. Bertoli et al. (2016) stated that they are specifically of interest as they regulate the expression of specific target genes, including tumor suppressors and oncogenes. That is the genes that protect a cell from one step on the path to cancer and the genes that has potential to cause cancer.

In the review Saito and Sætrom (2010), the targeting and target prediction of miRNAs are explained. Furthermore they also state that miRNAs regulate protein-coding genes post transcription. This is done by guiding a protein complex known as the RNA-induced silencing complex (RISC) to mRNAs with partial complementary to the miRNA. In general the miRNAs bind to the 3'-UTR (untranslated region) of their target mRNAs and repress protein production by destabilizing the mRNA and translational silencing. Although the full mechanism of this is not yet fully determined.

To summarize in simplistic terms: The miRNAs regulate mRNAs and represses protein production. This is of interest because it also involves tumor suppressors and oncogenes. A high regulation of a tumor suppressor could cause the gene not to protect the cell on

the way to cancer and a low regulation on an oncogene could cause the gene to develop cancer.

2.1.3 Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA) is a technique that considers expression profiles from samples belonging to two classes. By considering a predefined set of genes, the GSEA aims to determine whether the members of the set of genes are randomly distributed throughout the sample or is primarily found at the top or bottom.

In GSEA there are three main steps: Calculation of an Enrichment Score (ES), estimation of significance level of ES and adjustment for multiple hypothesis testing. Whereas the first is the most important for this thesis. For calculation a predefined set of genes must first be provided. Then for a given sample the genes are put into a ranked list. This ranked list is walked through and the degree to which the predefined set is present at top or bottom i calculated. The ES is increased when a gene from the predefined set is encountered and decreased when a gene not in the predefined set is encountered. This calculation corresponds to a weighted Kolmogorov-Smirnov-like statistic (Subramanian et al., 2005). The second step is essentially validation of the ES using phenotype labels and thus create a more biologically reasonable assessment of significance. The last step is normalization of the enrichment scores and adjusting scores for when running multiple predefined sets giving multiple hypotheses.

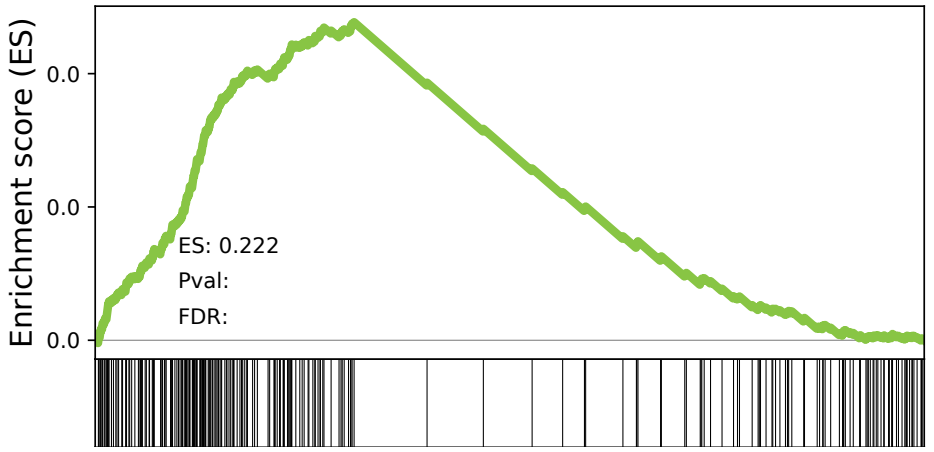

Figure 2.1: Example of the first step in GSEA, enrichment score calculation, on a sample. The green line indicates the enrichment score as the sample is walked through. The black bars indicate hits on the predefined set of genes. ES, P value (Pval) and false discovery rate (FDR) represents the three steps in GSEA.

2.2 Background Theory on Algorithms

In this section the relevant knowledge of the most common algorithms in classification of microRNAs are presented. Books and papers in machine learning were cherry picked with regards to material that I have previously had as syllabus in courses. In the cases where the topic had not been syllabus the paper with most citation for the specific term were chosen as a source.

2.2.1 Classification Problems

A classification problem is about grouping data by particular criteria. Classification is the process where a set of input for a new observation is mapped to an output based on earlier observations whose group is known. A popular example for classification is grouping email by spam or non-spam. Classification is considered an instance of supervised learning, i.e. learning where training set of correctly identified observations are available. The corresponding unsupervised procedure is called clustering and groups observations based on some measure of inherent similarity. Classification often requires analyzing data into a set of quantifiable properties, known variously as explanatory variables or features.

In formal definitions. Letting X represent the vector space of inputs and Y be the vector space of possible outputs. The classification problem consists of finding a function $f : X \to Y$ such that $f(\vec{x}) \sim y$ for all $\vec{x}$ and y pairs. The space of possible functions $f : X \to Y$ is the space classification algorithms will search through. The function that minimizes the combined difference between the predicted value $f(\vec{x})$ and the actual value y is the function chosen by the classification algorithm. This difference between predicted and actual value is usually called a loss function. This loss function is logically either zero, if predicted and actual label are the same, or one if they are not.

2.2.2 Decision Trees

Russell and Norvig (2016) stated decision trees are one of the simplest yet most successful forms of machine learning. Its rather simplistic metrics and natural flowchart like representation makes it easy to fathom and allows it to be an essential building block for more advanced algorithms.

In general a decision tree takes a vector of attributes, discrete or continuous, and returns its classification or the ”decision”. To reach a decision the decision tree perform a series of tests for the attributes. Each node in the tree represents such a test and each branch is the result of the test. The ”decision” returned lies in the tree’s leaf nodes. An example of this can be seen in Figure 2.2.

As with most classifiers, when constructing a decision tree classifier the set of examples is crucial. They should ideally convey a representative subset of the data for best performance. To build a decision tree, the decision tree learning algorithm selects the feature that best splits the data into correct classes. The selection of features are done through splitting criteria found in section 3.1.4. This process of selecting splitting features is then repeated for the leaf nodes until all samples are correctly classified through the decision tree.

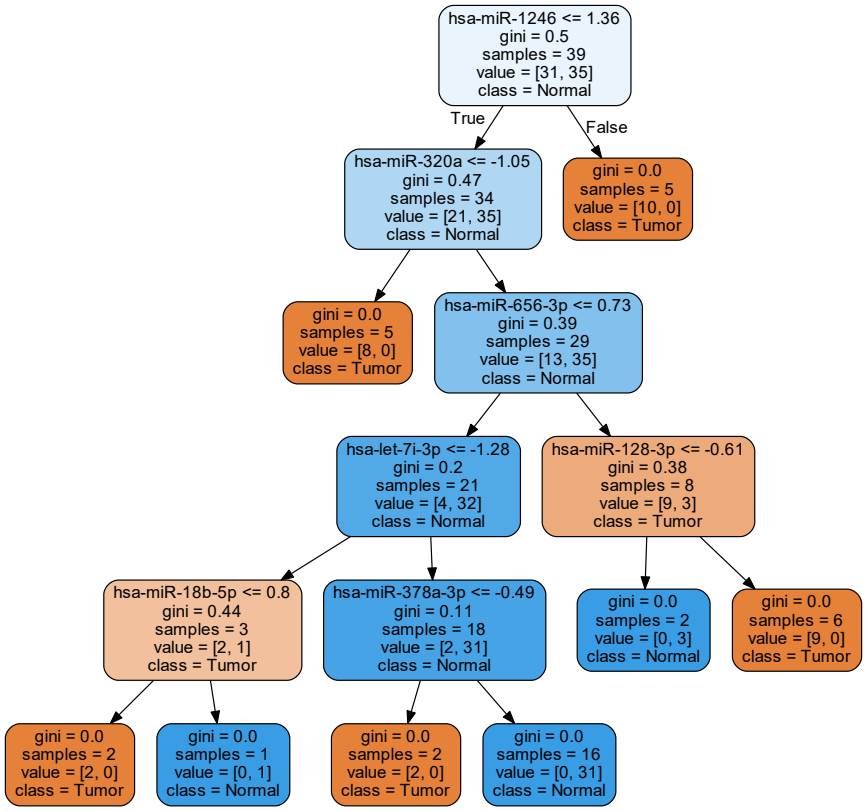

Figure 2.2: A decision tree estimator from the project. For each node the first row is the test. True is left and False is right for all tests. The second row is the gini score, further explained in section 3.1.4, which is a measure for how well this test splits the samples. The third row is the distinct number of samples. The fourth row is the number of tumor and normal samples in this node. The third and fourth row does not match in number of samples due to random forests bootstrapping. The fifth row is the most common class before a split is done. Note that the leaf nodes do not have a test.

2.2.3 Random Forest

Random forest is an ensemble machine learning technique which uses several decision trees to do its classifications or predictions. This technique is more robust than a single decision tree because it is less prone to overfitting the training data. Breiman (2001) suggests that because of the Strong Law of Large Numbers the random forest always converge which implies overfitting is not a problem. Furthermore, the accuracy in random forest depends on the strength of each tree classifier.

To do a prediction the random forest runs the a sample trough all the decision trees in the forest and can return both probability for each class or the most common class

predicted in the forest.

The random in random forest comes from the fact that there needs to be employed some randomization to make each tree independent of each other. First random forest gives each tree a random sample with replacement from training set. Second each individual tree is given a random subset of features to be used when searching for splits or tests. The key in random forest is therefore injecting the right kind of randomness to make them accurate predictors and regressors.

Breiman (2001) concludes that random forests gives results competitive with boosting and adaptive bagging. The accuracy in random forests also indicate that they act to reduce bias, however the concrete mechanisms that reduce bias is not obvious. He also concludes the improvement in random forest are greater in larger data sets then smaller ones.

2.2.4 Ensemble Techniques

Ensemble techniques are meta-algorithms that combine several machine learning techniques into one in order to improve prediction and decrease variance or bias. These meta-algorithms are either sequential or parallel.

Sequential methods are typically called boosting. In boosting the overall performance can increase by weighing samples that are misclassified with higher weight. This is done by first training a classifier on the data then create a weighted version of the data based on what samples it predicts wrong. In sequential rounds data points that are misclassified receive higher weights and data points that are correctly classified receive gets their weight decreased.

Parallel method are called bagging. In bagging algorithms such as random forest several classifiers are ran in parallel with different subsets of features and samples. The predictions of these can be voting for classification or averaged for regression.

Both bagging and boosting are mostly used with one type base classifier. Combining multiple types of classification or regression models are also possible and are commonly known as stacking. In stacking the meta-classifier is trained on the output of the base classifiers. However, adding more models or layers to a classifier does not always lead to better prediction. In addition, to the increased difficultly of explaining the model and its predictions. Thus can a more complex model have less real world value depending on the problem it solves.

A good example of a complex model using all techniques is the winner of a Kaggle data science competition in 2015 called Otto Group Product Classification Challenge. This model uses 33 meta features from other models and 7 features from the original data set. These features are then put into two boosting algorithms and a neural network and their predictions again are averaged.

2.2.5 Support Vector Machines

Support vector machines or SVMs is based around the notion of a "margin", the distance of either side of a hyperplane that separates two data classes. Maximizing the margin, and thus the distance between the margin and either class, has been proven to reduce the upper bound of expected generalization error (Kotsiantis et al., 2007).

Assuming a weight vector w, an input vector x and bias b, then a hyperplane can be formulated as

$$H_0 : w^T x + b = 0 \tag{2.1}$$

in addition this hyperplane should separate either class such that

$$\begin{aligned} w^T x_i + b \geq 1 \quad & when \;\; y_i = 1 \\ w^T x_i + b \leq -1 \quad & when \;\; y_i = -1 \end{aligned} \tag{2.2}$$

creating two new hyperplanes $H_1 : w^T x + b = 1$ and $H_2 : w^T x + b = -1$ that defines each side of the margin of H_0. Equation 2.2 can be simplified to $y_i(w^T x + b) \geq 1$ giving us the restriction for the hyperplane.

The distance from H_0 to H_1 can then be formulated as

$$d = \frac{|w^T x + b|}{\|w\|} = \frac{1}{\|w\|} \tag{2.3}$$

To maximize the margin d, the $\|w\|$ must be minimized. This is rewritten as $\frac{1}{2}\|w\|^2$ because of problems to provide efficient, stable solutions to l^1-norm minimization, compared to l^2-norm minimization.

Thus, if two classes are linearly separable, the optimum separating hyperplane can be found by solving a convex quadratic programming (QP) problem:

$$\begin{aligned} \underset{w,b}{\text{minimize}} \quad & \Phi(w) = \frac{1}{2}\|w\|^2 \\ \text{subject to} \quad & y_i(w^T x_i + b) \geq 1, i = 1, ..., l. \end{aligned} \tag{2.4}$$

When an optimal separating hyperplane is found, it is represented as a linear combination of the points that lie on its margin, called support vectors. This helps the model complexity of a SVM to be unaffected by the number of features encountered in training. Thus making it suitable to deal with learning problems with a large number of features compared to samples.

In cases where no separating hyperplane can be found, a soft margin that accepts some misclassification in the training samples can be used. This soft margin is usually called C and is a hyperparameter in the SVM.

When the data is not separable, and thus no hyperplane exists that successfully separates the classes of the training set, the inseparability problem can be mapped onto a higher-dimensional space. This higher-dimensional space is called the transformed feature space. In the higher-dimensional space of sufficient dimensionality any consistent training set can be made separable. A separable transformed feature space corresponds to a non linear separation in the original input space.

To map data from the input space onto a transformed feature space is however not trivial. Luckily training does only depend on dot products in the transformed feature space. This allows for a kernel function that calculates inner products in input space without ever actually determining the mapping. Once the hyperplane has been created the kernel functions can again be used to map new points into the transformed feature space for classification. Kernel functions is the second hyperparameter used in SVMs.

There is not a given best option for a kernel function and common practice is to estimate a range of potential kernel functions with different hyperparameters in cross-validation to find the best ones. As long as the kernel function is legitimate, a SVM will operate correctly even if the creator does not know exactly what features are being used in the kernel-induced feature space.

The most common kernels are:

1. Linear : $< x, x' >$
2. Polynomial : $(\gamma < x, x' > +r)^d$
3. Radial Basis Function (RBF) : $\exp(-\gamma \|x - x'\|^2)$
4. Sigmoid : $\tanh(\gamma < x, x' > +r)$

However, custom kernels are also possible. The more complex kernel functions gives further hyperparameters such as γ which is a complexity parameter.

The pros of SVM is that the training always reaches a global minimum and are good at dealing with high dimensional data even on small data sets. However the methods of SVM are binary and for multi-class classification one must first reduce the problem to a set of multiple binary classification problems. In addition picking the right kernel and parameters can be computationally intensive (Kotsiantis et al., 2007).

2.3 Structured Literature Review

2.3.1 Protocol

This thesis is based on an extensive literature search for research and review papers evaluating machine learning on miRNAs in hepatic and colorectal cancer. Research was independently conducted on Scopus, ACM, IEEE Xplore, ScienceDirect and CiteSeer databases using the following key words (with both extended names and abbreviations): Machine Learning AND miRNA AND cancer AND classification. In addition, reference lists of identified papers were hand searched to obtained additional articles. The search was concluded on September 2018. Papers were considered for inclusion only if (IC1) they provided full text in the English language; (IC2) contained information on machine learning with miRNA; and (IC3) used data of miRNAs or gene expression. The papers were then ranked through on several quality criteria where some were dismissed because of a low score. The final resulting papers were considered eligible for the systematic review. Full list of search terms, inclusion criteria and quality criteria can be found in the protocol in Appendix A.1.

2.3.2 Related Work

In this subsection the related works found through the structured literature review is summarized; see Table 2.1 for an overview of the papers included in this review. The first subsection contains information found in reviews. The remaining research papers are presented in terms of how they solved four machine learning steps: choice of data, preprocessing of data, classification and validation. In each of these sections there is also tables for a better overview of similarities between the papers.

Reviews

Five papers were reviews and one (Bertoli et al., 2016) did experiments on their own. Banwait and Bastola (2015) aims to summarize various existing computational approaches and potential use of bioinformnatics in the field of cancer biology. In the role of miRNAs in human cancer they state that miRNA that are up-regulated in cancer can potentially act as oncogenes through negative regulation of tumor suppressor genes leading to uncontrolled cell proliferation, and down-regulated miRNA can act as tumor suppressors by inhibiting oncogenes or genes involved in cell proliferation and apoptosis preventing tumor development. In therapeutics they state that restoring the expression of miRNA as potential approach. They also summarize the state of the art in studies with aims to identify miRNAs. Lastly the review states how the detailed mechanism behind miRNAs as oncomiRs or tumor suppressors has not yet been achieved and points to the importance of integrating systems biology, cancer research and bioinformatics to gain a more complete and accurate picture of cancer.

Bertoli et al. (2016) reviews the use of miRNAs as biomarkers for diagnosis, prognosis and theranostics in prostate cancer. They point out the problems with circulating prostate-specific antigen (PSA) and Gleason score and propose using miRNAs as biomarkers for a more accurate prognosis. They also extract 29 miRNAs in a meta-analysis approach with

Table 2.1: Overview of related works articles. Scores indicate to what degree different quality criteria were fulfilled in relevance to this thesis. A full overview is available in Appendix A.1.

Article ID	Author	Score
0	Brown et al. (2000)	3
1	Guyon et al. (2002)	3
2	Furey et al. (2000)	3.5
3	Önskog et al. (2011)	3
4	Liaw et al. (2002)	2
5	Banwait and Bastola (2015)	2
6	Bertoli et al. (2016)	3.5
7	Erson and Petty (2009)	2
8	Li et al. (2010)	2.5
9	Razak et al. (2016)	2.5
10	Batuwita and Palade (2008)	2.5
11	Liao et al. (2018)	3
12	Chakraborty and Maulik (2014)	3
13	Kim and Cho (2010)	4
14	Kothandan and Biswas (2015)	3
15	Ibrahim et al. (2013)	4
16	Piao et al. (2017)	4
17	Saha et al. (2015)	3
18	Saha et al. (2016)	4
19	Tran et al. (2011)	3.5
20	Wang et al. (2017)	2.7
21	Yang et al. (2015)	3
22	Iorio and Croce (2012)	3

diagnostic properties which they suggest to be used as a non-invasive blood test in prostate cancer.

Erson and Petty (2009) reviews the relationship between miRNAs and cancer, miRNA detection techniques, miRNA target identification, miRNA as cancer biomarkers. In cancer, many miRNA genes within region of genomic instability and chromosomal fragile sites are shown to have abnormal DNA copy numbers in cancer cell. A global insight into deregulated miRNA expressions in different tumors and our understanding of individual miRNA functions are being developed, e.g. miR-21 being over-expressed in multiple cancer types. As cancer biomarkers for prognosis, miR-26 levels appear low in patients who had shorter overall survival but a better response to interferon therapy in hepatacellular carcinoma patients. However exiting in most studies a larger number of patients need to be screened before miRNAs may function as reliable cancer biomarkers to be used for detection of cancer in very early stages.

Iorio and Croce (2012) reviews the dysregulation of miRNAs in cancer. This review suggests that the over-regulation of tumor suppressor genes and under-regulation of oncogenes by alterations of miRNA expressions are not exceptional but rather the rule in human cancer. There has also been shown that different types of cancer can be discriminated with

high accuracy while mRNA profiles by contrast were highly inaccurate indicators of tissue or cancer type. This suggests that tumors more clearly maintain a unique tissue miRNA expression profile. In addition miRNAs are more stable due to their small size compared to long mRNAs.

Li et al. (2010) reviews three aspects of miRNA: miRNA gene finding, miRNA target prediction and regulation of miRNA genes. Although all of these aspects are interesting regulation of miRNA genes is of the highest relevance for this thesis. This review summarizes that there exists miRNA promoters that are experimentally verified and should help understand the regulation mechanism of miRNAs. These studies are done by looking at interactions between miRNA promoters and their predicted target proteins. Another study included in this review also found a case where one miRNA targets both a transcription factor and the regulating gene of that transcription factor thus having a regulated feedback loop.

Data sets

In the included related works all papers had data sets consisting of expression profiles, either mRNA or miRNA expressions, and all had samples which were either a cancer type or normal; see Table 2.2. Most papers also used paired samples from humans, i.e. both the tumor and normal sample were from the same patient. Several papers were based on data sets generated from microarrays. Some contained mRNA expressions, while others contained miRNA expressions. Others data sets were generated using RNA-sequencing technology. Some papers included several data sets, and some where focused on a single data set. A select few included both microarray data sets and RNA-sequencing data sets but used these in different experiments. Six papers had data from the cancer genome atlas (TCGA) , and five papers used data from Lu et al. work. The data sets that were used in more than one paper are listed in Table 2.3.

Kim and Cho; Tran et al. used a single microarray data set. Both originally published in Lu et al. (2005). The data set contains several types on cancer. Guyon et al.; Furey et al.; Önskog et al.; Razak et al.; Batuwita and Palade; Chakraborty and Maulik; Ibrahim et al.; Saha et al. used multiple microarray data sets. These were quite different where some focused on a single type of cancer while others had multiple cancer types. Chakraborty and Maulik; Ibrahim et al. also combined datasets with both mRNA gene expressions and miRNA. Liao et al. (2018) focused on multiple types of cancer using a single RNA-sequencing data set. Saha et al. (2015); Wang et al. (2017) focused on breast cancer using a single RNA-sequencing data set. Yang et al. (2015) used six RNA-sequencing data sets of different cancers where they extracted only paired samples. Piao et al. used both RNA-sequencing and microarray data sets. The RNA-sequencing data set were downloaded from TCGA while the microarray data set were from Lu et al.. These were though not combined but used in different experiment to show results can hold in different data sets. Brown et al. uses a single hybridization microarray data set of yeast with mRNA expression profiles. The hybridization experiment represents the ratio of expression levels of a particular gene under two different experimental conditions i.e. a measured condition divided by a reference condition. Kothandan and Biswas built their own data set from a list of genes involved in cancer using several online resources: catalog of somantic mutions

(COSMIC), tumor associated gene database (TAG), miRecords and miRTARBASE. This is to find miRNAs involved in cancer pathways and thus not comparable with the other data sets.

Table 2.2: Data set overview. Data set IDs are unique IDs for data sets used internally in this thesis. Samples indicate how many samples, and if available how many normal and tumor samples, indicated as (normal/tumor). Genes refers to how many genes are in the original data set. Set type is whether the data set are generated using microarrays or RNA-sequencing and also if the samples are mRNAs (m) or miRNAs (mi). Data type has abbreviations for the different diseases the data set are generated from. Author points to the original paper the data set was published with or to what organization has them online i.e. The Cancer Genome Atlas (TCGA) and The European Bioinformatics Institute (ebi). Data sets from three papers Batuwita and Palade (2008), Kothandan and Biswas (2015) and Ibrahim et al. (2013) were not included in this table as their paper lacks sufficient information about their data sets to be included. Abbreviations: DLBCL - Diffuse large B-cell lymphomas, iNFPAs - Invasive non-functioning pituitary adenomas, BRCA - Breast Invasive Carcinoma, LUAD - Lung Adenocarcinoma, LUSC - Lung Squamous Cell Carcinoma, STAD - Stomach Adenocarcinoma, THCA - Thyroid Carcinoma, UCEC - Uterine Corpus Endometrial Carcinoma, SRBCT - Small Round Blue Cell Tumors, PRAD - Prostate adenocarcinoma, HNSC - Head and Neck Squamous Cell Carcinoma, KICH - Kidney Chromophobe, Cancer - multiple cancers in the data set.

ID	Samples	Genes	Set type	Data type	Author
DS0	79	2467	Array(m)	Yeast	Eisen(1998)
DS1	72(47/25)	2000	Array(m)	Leukemia	Golub(1999)
DS2	72(22/50)	7129	Array(m)	Colon cancer	Alon(1999)
DS3	31(17/14)	97802	Array(m)	Ovarian cancer	Furey(2000)
DS4	133(65/68)	7806	Array(m)	DLBCL	Alizadeh(2000)
DS5	66(34/32)	33491	Array(m)	Epithelial	Finak(2006)
DS6	40(22/18)	40475	Array(m)	iNFPAs	Galland(2010)
DS7	104(58/46)	19718	Array(m)	High ER	Herschkowitz(2007)
DS8	91(72/19)	40233	Array(m)	Cancer	Jones(2004)
DS9	73(55/18)	8033	Array(m)	High ER	Srlie(2001)
DS10	87(65/22)	8911	Array(m)	Metastasis	Ye(2003)
DS11	353(169/184)	315	Array(mi)	Gastric cancer	E-TABM-341 / ebi
DS12	84	1569	Array(mi)	Ovarian cancer	E-TABM-343 / ebi
DS13	770(87/683)	1047	Seq(mi)	BRCA	TCGA
DS14	482(46/436)	895	Seq(mi)	LUAD	TCGA
DS15	376(45/331)	839	Seq(mi)	LUSC	TCGA
DS16	299(38/261)	857	Seq(mi)	STAD	TCGA
DS17	566(59/507)	904	Seq(mi)	THCA	TCGA
DS18	404(21/383)	765	Seq(mi)	UCEC	TCGA
DS19	83	2308	Array(m)	SRBCT	Khan(2001)
DS20	77(19/58)	7070	Array(m)	DLBCL	Shipp(2002)
DS21	334	217	Array(mi)	Cancer	Lu(2005)
DS22	215	1047	Seq(mi)	PAAD	TCGA
DS23	162(81/81)	906	Seq(mi)	HNSC	TCGA
DS24	82(41/41)	796	Seq(mi)	KICH	TCGA

Table 2.3: Data set and article relations. Contains the data set and in what papers they were used. Only the data sets that were used in multiple papers are listed.

Data set ID	Paper by ID
DS1	1,2,12
DS2	1, 2
DS13	11,17, 20, 21
DS14	11, 21
DS16	11, 21
DS17	11, 21
DS21	12,13,16,18,19

Pre-processing of data

Regarding pre-processing of gene data, there are three main ways this can be done. First, altering the values e.g. by scaling the raw data; see Table 2.4. Second, not use all the data e.g. using a selection either of samples and/or features; see Table 2.5. Third, adding additional data e.g. getting information from the data in an external analysis. More often than not, all of these methods are used in a way.

For feature scaling Batuwita and Palade (2008); Chakraborty and Maulik (2014); Kim and Cho (2010); Tran et al. (2011) all chose to scale features to a range between either 0 to 1 or -1 to 1. Guyon et al. (2002); Furey et al. (2000) chose to standardize their features such that the mean of each feature is 0 and the standard deviation is 1. All of these papers do though seem to think it advantageous to scale features following guidelines from e.g. Hsu et al. (2003). Brown et al. (2000) also has its raw data through a normalization algorithm such that each expression vector has euclidean length 1.

Önskog et al. looked specifically on synergistic effects between normalization, gene selection and machine learning. In their paper they implement five different feature scaling strategies and three different gene selection strategies. They conclude that there are significant positive effects from using normalized data on their best methods. In addition a larger number of genes selected imply better performance but that this effect decreases when there are many more genes than observations. In their experiment there were no significant improvement from including more than 200 genes.

Brown et al. (2000); Guyon et al. (2002); Furey et al. (2000); Razak et al. (2016); Batuwita and Palade (2008); Chakraborty and Maulik (2014); Kim and Cho (2010); Kothandan and Biswas (2015); Piao et al. (2017); Saha et al. (2015, 2016); Tran et al. (2011); Yang et al. (2015) all do some form of feature selection but the techniques greatly varies. The number of total features left also varies between as few as 8 up to several hundred, although the consensus on best performance seems to be at the higher end. This is due to that selection of fewer miRNAs does exclude the important interactions miRNA have on each other. Some of the selection techniques can be found in section 3.2.2.

Four papers did compare different feature selection methods in terms of performance. Chakraborty and Maulik (2014) used a kernelized fuzzy rough set (KFRS) for feature selection. In addition to study the performance of the proposed method they have also used fuzzy preference based rough set (FPRS) and consistency based feature selection (CBFS). In this experiment KFRS had the best performance. Kim and Cho (2010) tried

four similarity-based methods: inverse of Euclidean distance measure, Pearson correlation, Cosine coefficient and Spearman correlation. In addition information gain, mutual information and signal-to-noise ratio were used. These were compared and results shown that cosine coefficient proved to be best for feature selection in their experiment. Saha et al. (2015) compared Gravitational Search Algorithm (GSA), signal-to-noise ratio, Welch's t-test, Wilcoxon ranksum test, Joint Mutual Information (JMI), minimum Redudancy Maximum Relevance (mRMR) and Mutual Information Feature Selection (MIFS) in conjugation with a SVM as well as with the SVM itself alone. In this experiment Gravitational Search Algorithm outperformed the other six feature selection methods. Yang et al. (2015) removed all samples where the sum of expression levels for that sample were less than 10 in raw sequencing data expression. For selection of features seven feature selection algorithms were tested but failed to compare the algorithms in real data due to lack of a gold standard.

Three papers did not mention any feature scaling or feature selection. Liao et al. (2018) uses IsomiR expressions and no scaling or selection is explained. However using IsomiR expressions this is not directly comparable to the other papers. Wang et al. (2017); Ibrahim et al. (2013) does not specify that they use any specific scaling or feature selection though the last mentioned has feature selection methods in their related works.

Table 2.4: Feature scaling overview. This table shows what type of feature scaling were done by which papers. Techniques are explained in section 3.1.1.

Feature scaling technique	Papers by ID
Standardization	0, 1, 2
Min-Max	10, 12, 13, 19
Tested Several	3
None / Not mentioned	4, 5, 6, 11, 14, 15, 16, 17, 18, 20, 21

Table 2.5: Feature selection overview. This table shows which papers utilized feature selection and if they tested multiple techniques.

Feature Selection technique	Papers by ID
Single feature selection technique	0, 1, 2, 9, 10, 12, 13, 14, 16, 17, 18, 19, 21
Multiple techniques	3, 12, 13, 17, 21
None	11, 20, 15

Classification

MiRNA gene expressions being high dimensional in features and low in samples somewhat limits what type of classifiers that can effectively give accurate predictions. Suitable methods are those who use some form of regularization and the primarily methods are SVMs, KNNs and ensemble methods e.g. random forest. This is also reflected in the selected related literature; see Table 2.6. In addition to what kind of classifiers are used, it is interesting to see what parameters are chosen or optimized; see Table 2.7.

Razak et al. (2016) focused solely on a random forest classifier. This classifier is not sensitive to outliers or noise (Liaw et al., 2002). Gini index (section 3.1.4) is chosen as

splitting criteria. The number of estimators or trees in the forest is however not revealed in the paper.

Five papers focused on SVMs. Guyon et al. (2002) only focuses on a linear kernel and leaves the non-linear kernels as future work. In this paper the diagonal factor C is set to 100 because the problem is insensitive to the value of C as the training data set are linearly separable down to just a few features. They also concludes that the number of features selected matter more than the classifier used when compared to Golub et al. (1999) classifier and Fisher's linear discriminant. Furey et al. (2000) focus on a polynomial kernel with default parameters except for the C which is tuned in a hold-one-out cross-validation. Saha et al. (2015); Kothandan and Biswas (2015); Batuwita and Palade (2008) used a single SVM with the RBF kernel. Saha et al. preset parameters γ and C to 0.5 and 2.0 respectively. Kothandan and Biswas optimized the parameters through a exhaustive grid search. Batuwita and Palade used a more complex method to optimize the parameters. Initially they find the optimal C with a linear kernel called $\widetilde{C}$, using this $\widetilde{C}$ the remaining γ parameter can be found in a linear search and corresponding C using (2.5). The derivation of this relationship can be found in (Keerthi and Lin, 2003).

$$log_2 C = log_2 \widetilde{C} - (1 + log_2 \gamma) \tag{2.5}$$

Brown et al. (2000) solves a multi-class classification problem (dealing with different cancer illnesses) by using several classifiers. In this paper SVM with a higher dimensional kernel outperforms Parzen windows, Fisher's linear discriminant, two decision tree classifiers, and SVMs that use the simple dot product kernel. Önskog et al. (2011) tested decision trees with both gini index and information gain, neural networks with one hidden layer and no hidden layer, and SVM with linear, polynomial and RBF kernels. SVM with RBF kernel had the highest accuracy. Kim and Cho (2010) used KNNs with Euclidean distance, Pearson correlation, cosine coefficients and Spearman correlation, Multi-layer Perceptron and SVM with a linear kernel. Lowest error rate came from KNN with Euclidean distance. Tran et al. (2011) used a SVM with linear, polynomial with degree 3 and RBF kernel. RBF had best performance in terms of F1-score and AUC. This SVM also outperformed other classifiers which included decision trees, bayesian networks and backpropagation neural networks. Yang et al. (2015) used the classification algorithms of logistic regression, random forest and SVM with RBF kernel. They conclude that logistic regression is unsuitable for the high dimension and small sample data, and that random forest performed better than SVM.

Liao et al. (2018) used both a random forest and libD3C, an ensemble classifier, were the latter gave the best results. Piao et al. (2017) used C4.5 decision tree and SVM as base classifiers in their own ensemble with multiple independent feature subsets then uses averaging to produce a classification. This classifier is compared with random forest, bagging and boosting using the same base classifiers and finds their ensemble to outperform the other ensembles. Wang et al. (2017) uses random forest, eXtreme Gradient Boosting (XGBoost) and Light Gradient Boosting Machine(LightGBM). LightGBM outperformed the other classifiers in several aspects.

Chakraborty and Maulik (2014) proposes using a Transductive SVM (TSVM). This is a semisupervised SVM that utilizes unlabeled data. Traditional supervised learning or inductive learning is more general, and presumable harder, than transductive learning.

The TSVM outperformed inductive SVM, Naive bayes and a KNN. The TSVM had a RBF kernel and used a grid search with cross-validation to find the optimal parameters. Ibrahim et al. (2013) used random forest with 10 decision trees and SVMs as base classifiers. Then tries to improve these using two semisupervised machine learning approaches called self-learning and co-training. Saha et al. (2016) used a two step approach. The first step uses a multiobjective optimization technique in combination with multiple classifiers automatic determines classifier type. The second step has two different approaches. First a frequency based approach and the second approach is an simple ensemble approach. Both of which is developed to combine the outputs of the solutions obtained from the first stage. Classifiers in the first stage included random tree, random forest, Sequential Minimal Optimization (SMO) and Logistic regression.

Table 2.6: Classification technique overview. Overview over which papers used particular algorithms. Algorithms not specified were either labeled as an ensemble technique or a simple learner (a non ensemble technique).

Classification algorithm	Papers by ID
Random Forest	9, 11, 16, 20
SVM	1, 2, 3, 10, 12, 14, 17
Simple learners	0, 3, 13, 19, 21
Other ensemble techniques	11, 16, 20

Table 2.7: Parameter selection overview. This table gives a overview over which papers optimized their parameters through cross-validation and which used presets.

Parameter Optimization	Papers by ID
Cross-validation	2(onlyC), 3, 10, 12, 14, 19
Preset or multiple presets	0, 1, 2, 9, 13, 15, 17, 18, 21
None / Not stated	9, 11, 16, 20

Metrics and validation

There are several metrics that can be used to measure performance in a classification of miRNA expression levels. These depend on whether it is a binary or a multiclass classification, and on what type of balance between classes is in the data set. The most popular performance metrics, including all used in this thesis, can be found in section 3.1.2. Related literature's use of performance metrics is found in Table 2.8 and use of cross-validation is found in Table 2.9.

Furey et al. (2000) only used the metric of False Positives(FP), False Negatives (FN), True Positives (TP) and True Negatives (TN).

Önskog et al. (2011) used error rate, i.e. the percentage of misclassified observations in a test set, as a measure of performance. Error rate was adjusted by dividing by the theoretical error rate obtained by randomly assigning classes given the distribution of the two classes. Chakraborty and Maulik (2014); Kim and Cho (2010); Wang et al. (2017); Liao et al. (2018); Saha et al. (2015) used overall accuracy (ACC) as metric. The two

latter also calculated Matthew's Correlation Coefficient (MCC) to determine the trade-off of sensitivity and specificity. In addition Saha et al. (2015) also calculated F-measure and AUC, while Wang et al. (2017) used logistic loss.

Kothandan and Biswas (2015) chose to not use accuracy as class imbalance existed in the dataset. Hence, performance measures were chosen in compliance with the cross-validation rate and MCC.

Razak et al. (2016); Piao et al. (2017); Tran et al. (2011); Yang et al. (2015) used AUC obtained from a ROC curve as metrics. Piao et al. also calculated accuracy, sensitivity and specificity. Tran et al. also used precision, recall and F-measure. Yang et al. also calculated positive predictive value (PPV) and negative predictive value (NPV).

Ibrahim et al. (2013); Saha et al. (2016) used precision, recall and F-measure.

Three papers used slightly different metrics than the others. Brown et al. (2000) defines cost savings of using the learner procedure M as $S(M) = C(N) - C(M)$ where $C(N) = fp(N) + 2 \times fn(N)$ false negatives is higher weighted because the number of positive examples are low. Samples are tested against the null learning procedure which classifies all data as negative. Guyon et al. (2002) used error, reject, extremal margin and median margin. Error rate is the fraction of samples that are misclassified with its compliment the success rate. The rejection rate is samples that are rejected (low confidence) complemented by acceptance rate. Batuwita and Palade (2008) used $G-mean = \sqrt{SE * SP}$, where SE is the proportion of positive samples correctly classified and SE is the proportion of negative samples correctly classified.

Table 2.8: Overview over used performance metrics by papers.

Metric	Papers by ID
ROC (AUC)	9, 16, 17, 19, 21
F-score	15, 17, 18, 19
ACC/ Error rate	3, 11, 12, 13, 17, 20
MCC	11, 14, 17
Other	0, 1, 2, 10, 20

Table 2.9: Overview over how results were validated.

Validation technique	Papers by ID
K-fold	0, 3, 12, 13, 14, 16, 20, 21
LOOCV	1, 2, 9, 16, 18, 19
Stratified K-fold	10, 17
None / Not stated	11, 15

Depending on which specific problem an experiment tries to solve and how much data is available, several different ways to validate the results are also used. Gene data is prone to low amount of samples which again makes low confidence when going from e.g. 50-100 samples to an general solution. The most common techniques are using a form of

Cross-Validation (CV) and, depending on the experiment and goals, do a statistical test to show confidence in the results. The CV techniques are explained in section 3.3.

Furey et al. (2000); Razak et al. (2016); Tran et al. (2011); Saha et al. (2016); Guyon et al. (2002) applied Leave-one-out Cross-Validation (LOOCV) to asses feasibility and validity. The results was then averaged to produce an estimate of the accuracy of the system. Guyon et al. also computed metrics for each value in a separate test set.

Kothandan and Biswas (2015); Chakraborty and Maulik (2014); Yang et al. (2015); Brown et al. (2000) all used a K-fold cross-validation with respectively 10, 5, 5 and 3 folds. Chakraborty and Maulik only used this to optimize SVM parameters. Yang et al. replicated this K-fold 100 times to average out results. Brown et al. also repeated this procedure for best classifiers to show relatively low standard deviation in results.

Saha et al. (2015); Batuwita and Palade (2008) applied a Stratified K-fold CV. With 10 and 5 folds respectively.

Two papers combined cross-validation folds to validate their experiments. Önskog et al. (2011) used an inner 10-fold CV for optimization, and an outer 5-fold CV to estimate final classification performance. Piao et al. (2017) applied LOOCV and 10-fold CV to gain two separate results. In addition, the experiment was repeated 50 times where results of each method were recorded and finally averaged.

Two papers also applied additional statistical tests to show significance in their results. Chakraborty and Maulik used a Student's t-test and Wilcoxon signed rank test at 5% significance level. Saha et al. (2015) used the non-parametric test Friedman test with 5% significance level. This showed statistical significance of their results produced by their proposed method with respect to the results of other methods.

2.3.3 Reported Performance and Confidence

As the previous section and tables 2.1-2.9 has shown there are several factors which makes it hard to compare results and draw conclusions about how this type of work should be done and address RQ1. There are some key points most papers explicitly agree on. First, scaling raw gene expressions do increase the performance of a classifier. Second, several papers put forth the notion that feature selection is necessary to achieve somewhat good results. Third, a classifier that handles high dimensional data should be chosen. Fourth, cross-validation should be used both for optimizing parameters and to establish a correct performance estimate.

For DS1 three papers did classify this data set. Guyon et al. (2002); Furey et al. (2000) both achieved perfect classification using SVMs and Chakraborty and Maulik (2014) achieved 98.89 % accuracy. For DS2 two papers used this data set. Furey et al. (2000) had 6 miss-classifications of 72 samples and Guyon et al. (2002) held a 98% accuracy.

The next three data sets were from TCGA and all authors used different number of genes and subsets of samples of this data. Some split the data into paired samples while others combined data from patients from other sets to balance the imbalanced set of samples TCGA provides. Four papers used DS13. Liao et al. (2018); Saha et al. (2015) achieved approximately 95% accuracy using random forest and SVM respectively. Wang et al. (2017); Yang et al. (2015) achieved perfect classification using LightGBM and random forest respectively. Two papers used DS14. Liao et al. (2018) using IsomiR expressions and random forest achieved approximately 92% accuracy while Yang et al. (2015)

achieved 65% AUC score using a SVM. Two papers used DS16. Liao et al. (2018) using IsomiR expressions and random forest achieved approximately 94% accuracy while Yang et al. (2015) achieved 71% AUC score using a SVM and logistic regression.

DS21 were used by five papers. This is a data set that holds multiple types of cancer and most papers also used different subsets of the original data set. Kim and Cho (2010) had a 95% accuracy using a KNN and feature selection. Piao et al. (2017); Saha et al. (2016) had approximately 98% accuracy using self defined ensembles. Chakraborty and Maulik (2014); Tran et al. (2011) also had approximately 98% accuracy using SVMs.

2.4 Motivation

The review of related works on using the transcriptome to classify cancers identified two main points: (1) miRNA expression profiles are useful both for classifying cancers and for understanding cancer biology, but (2) there are technical challenges in classifying such data, especially when using multiple data sets at once with different errors and biases in their technology.

During the recent decade the number of diseases that are linked to misregulation of miRNA has dramatically increased. For cancer approximately 50% of miRNA genes are localized in genomic regions that are associated cancer. MiRNA expression profiling has been shown to be associated with tumor development, progression and response to therapy. This suggests that there are potential clinical use of miRNA in diagnostic, prognostic and possibly as a therapeutic tool. Several studies has already shown potential use of miRNA for diagnosis and prognosis. There are also potential use of miRNA as oncogenes and oncosupressor genes that can improve disease response and cure rates. MiRNA-based anticancer therapies have also recently been exploited, either alone or in combination with current targeted therapies.

In terms of the classification challenge there are several interesting aspects. Each sample has some individual differences associated with age, sex, ethnicity etc. However the larger difference is usually between data sets. This is differences connected to what lab has made the data sets, how each sample has been preserved and what technologies are used to deduce and quantify the transcriptome. Most samples also come in pairs meaning one sample has been harvested from the disease site and one sample has been harvested from neighbouring healthy tissue. For different types of cancer both of these samples may come from different sites; e.g. for colorectal cancer the colon is quite a large organ and tumors may form in different parts of the colon. The tumors may also have different degrees of compositions of cancerous and normal cells. Questioning how tumorous is the tumor sample and how normal is the normal sample. Even through all of these individual differences, some of which can be rectified by normalizing the data, the individual data sets can often be linearly separable and quite easy to classify.

The data sets are also quite large in terms of features (miRNAs) and small in samples which increases the importance of trying to combine data sets. These dimensions also suggest that the majority of features are not necessarily useful for classification. In addition the features can be validated by looking at the known relations between miRNA and cancer.

Chapter 3

Method

This chapter contains all necessary information and techniques to reproduce the experiments in chapter 4. The implementations of these were used through scikit-learns API (Pedregosa et al., 2011) and are easily found in their documentation. First, different techniques for scaling data and performance metrics are explained. Second, six feature selection techniques are presented. Third, the different techniques used for cross-validation are explained.

3.1 Feature Scaling and Performance Metrics

3.1.1 Feature Scaling and Normalization Techniques

In this project the term normalization is used for techniques scaling along the sample axis and feature scaling is used for techniques scaling along the feature axis.

Min-max scaling:

$$z_i = r \times \frac{x_i - \min(x)}{\max(x) - \min(x)} - c \tag{3.1}$$

where z_i is the scaled value, x_i is the original value, $min(x)$ and $max(x)$ is the minimum and maximum value of the feature, r and c are parameters to adjust range and center of the scaled data. e.g. by default r is set to 1 and c to 0 giving the range [0,1] to use the range [-1, 1] set r to 2 and c to 1. This scaling has some drawbacks in that it is sensitive to outliers.

Z-score scaling:

$$z_i = \frac{x_i - \mu}{\sigma} \tag{3.2}$$

where z_i is the scaled value, x_i is the original value, μ is the mean of the feature and σ is the standard deviation of the feature. This gives the scaled data a mean of 0 and 1 in standard deviation. Standardization is better than min-max scaling to outliers but do not guaranty a specific range. In general this scaling works better the more or less the features

look like standard normally distributed data.

Robust scaling:

$$z_i = \frac{x_i - \tilde{x}}{q_3 - q_1} \tag{3.3}$$

where z_i is the scaled value, x_i is the original value, $\tilde{x}$ is the median and q_3, q_1 is typically the 3rd and 1st quantile but any interquartile range can be specified. This gives the scaled data a 0 in median and values inside the interquantile range is between (-1, 1). This method is more robust to outliers than normal standardization.

Reads per million (RPM) normalization:

$$n_i = \log_2\left(\frac{(c_i + 0.5)}{\sum_j c_j} \times 10^6\right) \tag{3.4}$$

where n_i is the normalized sequencing gene expression, c_i is a unnormalized sequencing gene expression. RPM is used to normalize gene sequencing data to comparable values of microarray data. Equation (3.4) accounts for differences in library sizes and the relative value reads per million is then log-transformed to stabilize the variance as variance increases with mean.

Closest scaler:

$$s = \sqrt{\sum_i (\mu_i^{test} - \mu_i^{train})^2} + \sqrt{\sum_i (\sigma_i^{test} - \sigma_i^{train})^2} \tag{3.5}$$

This is a self defined scaling using the same scaling as the closest full training set. Where the closest full training set is defined by the lowest value to Equation 3.5, where μ_i and σ_i is the mean and standard deviation of feature i. These values are the means and standard deviations before any scaling were done.

3.1.2 Performance Metrics

When considering a binary classification problem a given sample can be classified to one of four cases: (1) a positive case classified as positive (True Positive - TP), (2) a negative case classified as positive (False Positive - FP), (3) a negative case classified as negative (True Negative - TN) or (4) a positive case classified as negative (False Negative - FN). These are the basic building blocks for most performance metrics in a binary classification problem.

Sensitivity / Recall / true positive rate

$$TPR = \frac{TP}{TP + FN} \tag{3.6}$$

Specificity / true negative rate

$$TNR = \frac{TN}{FP + TN} \tag{3.7}$$

Precision / Positive predictive value (PPV)

$$PPV = \frac{TP}{TP + FP} \tag{3.8}$$

Negative Predictive Value (NPV)

$$NPV = \frac{TN}{TN + FN} \tag{3.9}$$

Accuracy

$$ACC = \frac{TP + TN}{TP + TN + FP + FN} \tag{3.10}$$

Balanced accuracy

$$BACC = \frac{TPR + TNR}{2} \tag{3.11}$$

Matthew's correlation coefficient

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TN + FN)(TN + FP)(TP + FN)(TP + FP)}} \tag{3.12}$$

F1 Score

$$F_1 = 2 \times \frac{PPV \times TPR}{PPV + TPR} \tag{3.13}$$

G-mean

$$\overline{G} = \sqrt{TPR * TNR} \tag{3.14}$$

3.1.3 Receiver Operating Characteristic

A Receiver Operating Characteristic (ROC) curve is a graphical plot that shows the diagnostic ability of a binary classifier. The curve can also be used as a metric through its Area Under Curve (AUC) score. The ROC Curve is created by plotting the True Positive Rate (Sensitivity) in function of the False Positive Rate (1-Specificity) for different cutoff points. This implies that a perfect ROC AUC score is a curve that curves all the way to the top left corner of the plot (Schoonjans, 2018).

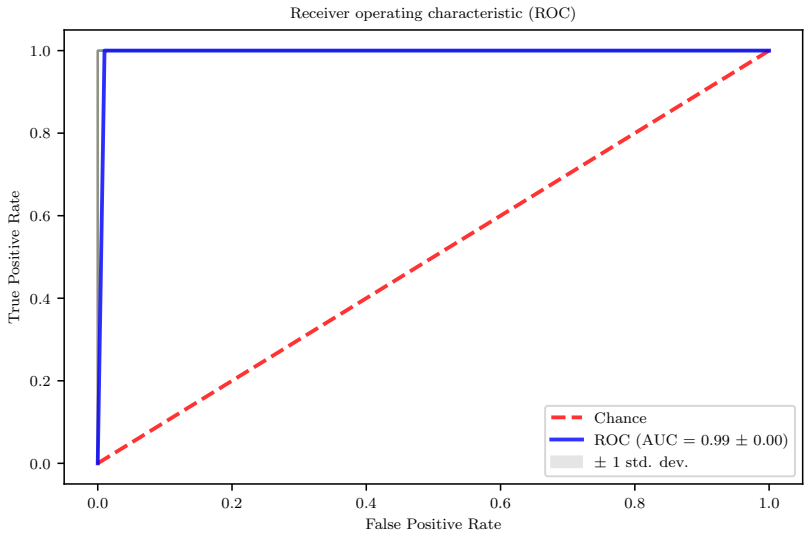

Figure 3.1: A ROC curve example. The vertical axis is the Sensitivity and the horizontal axis is the inverse Specificity. The further the ROC curve (blue line) is to the top left corner the better ROC AUC score.

3.1.4 Random Forest Splitting Criteria

Random forests decision trees has criteria in which it marks a feature as a good splitting condition. In general these are just calculations to minimize the amount of decision nodes by splitting on a feature that best separates the data. Here two different criteria is explained Entropy and Gini importance.

Entropy

Entropy for information gain contains two steps. First the entropy of the target feature S is calculated.

$$E(S) = -\sum_{j=1}^{J} p_j \log_2 p_j \tag{3.15}$$

where J is the set of classes and p_j is the probability of class j. Second the entropy of the child nodes are calculated, then added proportionally, to get the total entropy for the split.

$$E(S, X) = \sum_{c \in X} p_c \times E(c) \tag{3.16}$$

where X is the set of child nodes, c is a child node, p_c is the probability of node c and $E(c)$ is the entropy of the of child node c. This entropy is subtracted from the step one entropy. The result is the Information Gain, or decrease in entropy.

$$IG(S, X) = E(S) - E(S, X) \tag{3.17}$$

The higher the information gain the better it is as a splitting condition.

Gini Importance

Nembrini et al. (2018) explains the popularity of gini importance to the fact that it is relative fast to compute. In addition they give this definition of Gini impurity:

$$\hat{\Gamma}(t) = \sum_{j=1}^{J} \hat{\phi}_j(t)(1 - \hat{\phi}_j(t)), \tag{3.18}$$

where $\hat{\phi}_j(t)$ is the class frequency for class j in the node t. The decrease of impurity is the difference between a node's impurity and the weighted sum of the impurity measures of the two child nodes (the Gini index).

An example of gini importance can be calculated using the decision tree in Figure 2.2. Using the top most node with 31 tumor samples and 35 normal samples the calculations would be:

$$\hat{\Gamma}(t) = \frac{31}{66} \times (1 - \frac{31}{66}) + \frac{35}{66} \times (1 - \frac{35}{66}) = 0.498$$

The decrease of impurity would then be $0.498 - (0.47 + 0.0)/2 = 0.235$ where the parenthesis numbers are the gini impurity of the child nodes.

3.2 Principal Component Analysis and Feature Selection Methods

3.2.1 Principal Component Analysis

A Principal Component Analysis (PCA) is a statistical procedure that transform features into a set of principal components. In mathematics this procedure is an orthogonal linear transformation that transforms the data to a new coordinate system such that the greatest variance of the data is in the first principal component, the second greatest orthogonal variance of the data is in the second principal component, and so on. This is often used as a technique to view high dimensional data and get insight into how linearly separable it is.

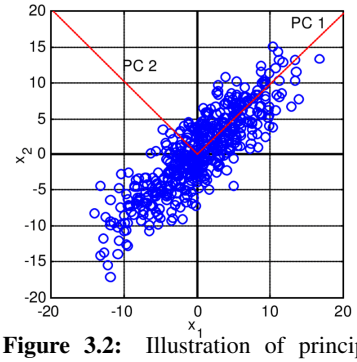

Figure 3.2: Illustration of principal components in two dimensional data.

3.2.2 Feature Selection

Score Based

Selects the features that on average differs least between classes. Then removes the selected feature until a desired subset with features that on average differs most between classes.

Recursive Feature Elimination

Recursive Feature Elimination (RFE) uses an external supervised learning classifier that can provide information about how important a feature is, then recursively prunes the least important feature until a predefined number of features are left.

Symmetrical Uncertainty

Symmetrical Uncertainty (SU) is calculated as follows (Singh et al., 2014):

$$SU(X,Y) = \frac{2 \times MI(X,Y}{E(X) + E(Y)} \tag{3.19}$$

where E is the Entropy and MI is the Mutual Information,

$$MI(X,Y) = E(X) - E(X,Y) = E(X,Y) - E(X|Y) - E(Y|X) \tag{3.20}$$

χ^2-Test

χ^2-test measures dependence between stochastic variables which requires non-negative features. Thus will this test find features that are most likely to be independent of classes

and therefore irrelevant for classification. The χ^2 is calculated as follows:

$$\chi^2 = \sum \frac{(f_0 - f_e)^2}{f_e} \tag{3.21}$$

where f_0 is the feature count and f_e is the expected count.

Signal-to-Noise Ratio

Signal-to-Noise Ratio (SNR) is defined as follows:

$$SNR = \frac{\mu_1 - \mu_2}{\sigma_1 + \sigma_2} \tag{3.22}$$

where μ is the mean values and σ is the standard deviation. In the case of feature selection the denoted 1 and 2 represents the classes and each feature thus has a SNR score.

Fisher Score

Fisher score selects each feature independently according to their scores under the Fisher criterion.

$$FS = \frac{\sigma^2_{between}}{\sigma^2_{within}} \tag{3.23}$$

i.e. this is the ratio of variance between the two classes divided by variance within the class.

3.3 Cross-Validation

Cross-validation is a model validation technique to asses how the results of a model performs in unseen data. In general, a model is first trained on one part of the data and tested on another. This helps asses how well the trained model generalizes to an independent data set. However simply doing a train-test split is often not random enough to give an accurate statistics on how well the model performs on unseen data. Thus slightly more advanced validation techniques are required. There are three main reasons for doing cross-validation. First, cross-validation is essential to help us evaluate the quality of a model. Second, it helps choosing the model which performs best on unseen data. Third, it helps avoiding overfitting and underfitting of the data. Overfitting is when a model is over trained to the training data such that it captures unnecessary noise and is fitted to patterns that does not generalize to the training set. Underfitting is when a model is under trained and does not capture essential patterns to predict in unseen data.

3.3.1 Leave-one-out Cross-Validation

Leave-one-out cross-validation is done by leaving a single sample out as the test set and the remaining data is used as the training set. Then this process is repeated for each sample until all samples has been a test sample. The predictions for each iteration can then be

averaged to estimate how the model would perform on unseen data. The drawbacks of this technique is that it is computationally heavy and should only be used if there is small amounts of samples and the model is fast to retrain in each iteration.

3.3.2 K-fold Cross-Validation

K-fold cross-validation is essentially the generalized version of leave-one-out cross-validation. Here the data are split into K-folds. Then one fold is selected as the validation set and the remaining fold are selected as a training set. This process is repeated for each fold such that all folds has been run once as the validation set. The accuracy for each fold can then be collected and averaged to estimate actual accuracy in unseen data.

3.3.3 Stratified K-fold Cross-Validation

The stratification of a K-fold implies that the number of classes within each fold is similar. For binary classification this means that the number of positive and negative cases in each fold is similar. This is highly useful for smaller data sets, imbalanced data sets and in the cases of multiclass classification.

3.3.4 Nested Cross-Validation

To do a nested cross-validation implies having an outer cross-validation loop and an inner cross-validation loop. This is often needed in cases were the outer loop does a performance estimate and the inner loop does a parameter optimization. If the parameter optimization were done outside the outer loop it would not be limited to optimize on the correct training data thus cheating by optimizing for testing data as well. In this project nested cross-validation is utilized when doing parameter optimization and feature selection.

3.3.5 Feature Scaling in Cross-Validation

In this project feature scaling is done at one of two places. The first case is when full data sets are used and scaled independent of each other outside any cross-validation loop. The second case is when subsets of data sets are used. In this case a classifier is usually trained on other data sets and their scaled data inside a cross-validation loop. The new data subset is unscaled and must either run a similar scaling for itself which may work if the subset is large enough or run a closest scaler approach and scale itself using the scales from another similar data set.

Chapter 4

Experiments and Results

In this chapter experiments and the results of these are presented. The first experiment is a overview of the data sets and how to combine these. The second experiment looks into how scaling and feature selection should be done for combined data sets. The third experiment looks into algorithm performances on the combined and individual data sets. The fourth experiment relates to the problem of imbalanced data sets. The fifth and last experiment looks into which features were of the highest importance for the different methods used in previous experiments.

4.1 Data sets

The classification experiments will use different data sets from colorectal and hepatic cancer. These data sets have few samples but gather several hundred to thousands of microRNAs. The samples are either labeled as 'normal' or 'tumor'. For colorectal cancer samples different types of tissue from different parts of the colon are used e.g. rectal, ascending and sigmoid. These were initially split into separate groups but PCA plots, seen in section 4.2, showed these were quite comparable.

The samples are generated using different technologies. One data set is made using microarray technology and the rest is generated using RNA-sequencing technology. These different technologies are not inherently comparable, therefore Equation (3.4) is used to normalize gene sequencing data to comparable values to microarray data. Log normalized values are preferred as sequencing values are absolutes which leaves us to wonder if the sample was twice as large or if it had twice us much miRNA. Furthermore as sequencing technology picks up a lot more miRNAs, only miRNAs with at least a mean of 1.0 in normalized n_i values is kept. A overview over each data set can be found in Table 4.1 where miRNAs are already filtered.

Density plots give us an idea of how well the equation works to make the different technologies comparable. Figure 4.1 gives us such a plot for Hepmark-Microarray, Hepmark-Tissue and Hepmark-Paired-Tissue. In general, the ideal plot is overlapping lines equally stretched in width and with equal peaks. Although this is not exactly the case they still

Table 4.1: Overview of data sets. ID is the internal ID for the data set. In each data set samples are the number of rows and number of miRNAs are number of columns. Technology refers to what technology were used to generate the data set. Type refers to what type of disease the data set has. HCC - Heptatocellular carcinoma and CRC - Colorectal cancer.

Name	ID	Samples	MiRNAs	Technology	Type
Hepmark-Microarray	D_1	146	396	Microarray	HCC
Hepmark-Tissue	D_2	150	472	RNA-seq	HCC
Hepmark-Paired-Tissue	D_3	37	381	RNA-seq	HCC
ColonCancer_GCF-2014-295	D_4	92	424	RNA-seq	CRC
GuihuaSun-PMID_26646696	D_5	66	425	RNA-seq	CRC
PublicCRC_GSE46622	D_6	15	441	RNA-seq	CRC
PublicCRC_PMID_23824282	D_7	57	485	RNA-seq	CRC
PublicCRC_PMID_26436952	D_8	51	433	RNA-seq	CRC

do pair up quite well. By close inspection the outline of two main bodies stretching from 0 to 15 and from 0 to 20 is seen. The first main body consists of D_1, the microarray set, while the other is D_2 and D_3, the RNA-sequencing sets. The peaks for each body is also quite close at around 5 for D_1 and 8 for D_2 and D_3 and the peaks having the density in range 0.12 to 0.14. The separation of samples in -1 is due to the microarray set having its missing transcribed miRNAs filled as -1 from the technology.

One important problem is that the set of features between these data sets do not match. Initially, the missing features were filled in as -1 because missing certain miRNAs can itself be a biomarker for tumor. It was discovered that this filling for missing values worsened the overall performance in classification for both of the combined data sets and thus all features that were missing for one or more of the data sets were dropped from the combined set. The baseline ROC curves of using the filling of features can be found on page 73 and for intersection of features can be found on page 71 in the Appendix.

This had a couple of important complications. When considering the individual data sets of Figure 4.2 the density at its peak slightly differs from Figure 4.1. For D_1 the peak has slightly higher density alone while for D_2 the density is the same but at a higher normalized expression. This is because a different feature subset is used for the combined case. The expectation is that the dropping of features impacts the lower expressed features more than the higher expressed ones. In addition, samples that deviates a lot from the rest such the orange centered at -1 in Figure 4.1 occurs because most of the features that had a value were dropped when combining the data sets thus leaving it with mostly -1 values from the microarray technology. Density plots proved quite effective to identify such samples. In most cases these are samples that had been contaminated during the process of making the data sets and were simply removed from the data sets when found. The excluded samples can be found in the source code Appendix A.2.

Ironically the data sets are more similar in terms of density before a combination is done. However, the alternative of filling missing features created similar situations were more samples contained mostly -1 values. The RPM normalization is done to make the

samples comparable and is not the only normalization that has to be done to make the data sets comparable. More density plots with additional feature scaling can be found in Appendix A.5.3.

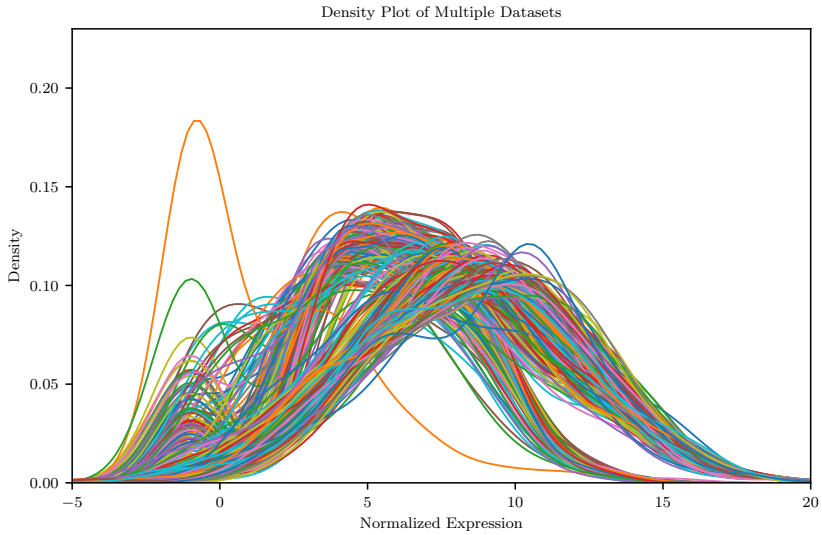

Figure 4.1: A density plot of hepmark data sets: D_1, D_2 and D_3. Each line represents a sample and its values, the higher the line is for some value the more common the value is in the sample.

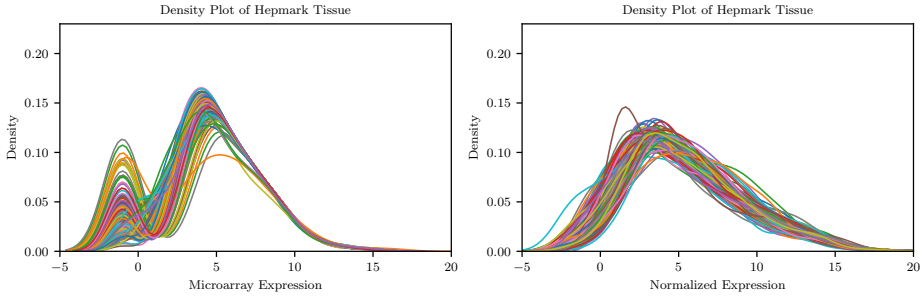

Figure 4.2: A density plot of hepmark data sets D_1 and D_2. Each line represents a sample and its distribution of values, the higher the line is at a value the more common the value is in the sample.

4.2 Scaling and Feature Selection of Data Sets

In this section the effects of scaling (RQ2) is first addressed before the benefits of feature selection (RQ3) is evaluated. As mentioned in section 3.1.1, there exists several ways to scale data points. Often such feature scaling of the data points are necessary to remove inherent bias in the data. All of the related work articles concerns themselves with scaling a single data set. However when several data sets should be combined and the classifier should work for even a single sample there is not clear cut for what is the best way to scale such data.

4.2.1 Problem Description

Generally for miRNA transcription the highest differences is often between data sets and not the differences between tumor and normal samples. Subsequently, there is no one scaling to handle all transcription data as the errors from both lab and samples differs. Thus each data set should be scaled individually. However, such feature scaling will only be applicable for full data sets. Therefore it is important to see what scaling gives best accuracy for different combinations of samples both in terms of set size and imbalance.

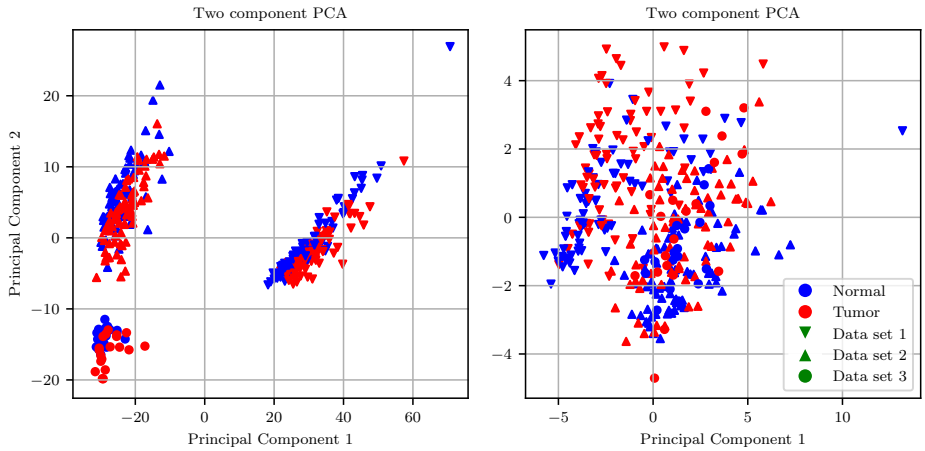

Figure 4.3: Principal component analysis for hepmark data sets D_1, D_2 and D_3. To the left the data sets are unscaled and to the right each data sets has been scaled using Z-score scaling.

In Figure 4.3 the greatest variance, or first principal component, is between the microarray and RNA-sequencing sets. The second greatest variance is between the two RNA-sequencing sets. After a feature scaling is done individually for each data set this difference is greatly reduced however somewhat still present in that a D_1 favors the left and D_2 and D_3 the right of the first principal component.

There are different tendencies for the colorectal data sets in Figure 4.4. In this figure there are two main bodies split by the first principal component consisting of D_4, D_7, D_8 and D_5, D_6 respectively. In the first body the difference of the second principal component

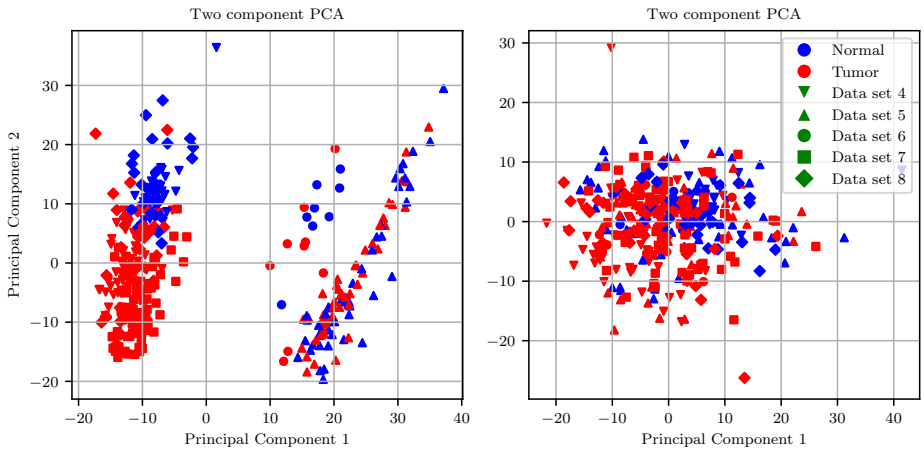

Figure 4.4: Principal component analysis for colorectal data sets D_4, D_5, D_6, D_7 and D_8. To the left the data sets are unscaled and to the right each data sets has been scaled using Z-score scaling.

seems to be the difference between tumor and normal samples, note that D_7 only consists of tumor samples. The second body is quite a bit more cluttered but D_5 and D_6 is quite separated in the first component. The amount of data points in the right-hand plot makes it hard to decipher. In general the differences between samples in the data sets are now greatly reduced. There are still distinct differences between the data sets, but these seem smaller than the differences between classes. The data set D_6 does seem to also be in this pattern once scaled but it is hard to determine as it has few samples. The data set D_5 does become more similar with the other data sets but is not split into classes by the first principal component like the others. Additional PCA plots were generated to visualize this for the colorectal data sets using subsets of the left body D_4 and D_8 with each set of the right body. These are in the Appendix A.5.4.

4.2.2 Scaler Performances

In this thesis the feature scaling techniques tested are mentioned in section 3.1.1. These are Min-max scaling, Z-score scaling and Robust scaling, referred to as minmax, standard (or standardization) and robust. The scalers were tested on full data sets scaling each set individually. Then they were ran in a stratified 10-fold cross-validation using AUC as performance metric. A SVM with RBF kernel and parameters optimized in a 5-fold cross-validation and Random forest with 200 estimators were used as test classifiers. Each of the combined data sets were tested. Additionally, a test without D_5 for the combined colorectal data set were done as this data set proved to be the hardest to classify.

The results, Table 4.2, showed that a minmax scaling adjusted to range [-1, 1] performed slightly better than both standardization and robust scaling for the hepmark data sets and colorectal data sets. The same tendency were also seen in the other permutations of these two groups.

Table 4.2: Feature scaling results for different feature scaling on combined data sets. Scores are the average ROC AUC score ± standard deviation estimated by a 10-fold cross-validation. The best score per row is highlighted in bold.

	Random Forest			
Data sets	MinMax	Standard	Robust	Unscaled
$D_1\ D_2\ D_3$	$\mathbf{0.94 \pm 0.04}$	0.93 ± 0.05	0.93 ± 0.05	0.93 ± 0.05
$D_4\ D_5\ D_6\ D_7\ D_8$	0.89 ± 0.15	0.79 ± 0.18	0.78 ± 0.18	$\mathbf{0.91 \pm 0.15}$
$D_4\ D_6\ D_7\ D_8$	0.98 ± 0.03	0.97 ± 0.05	0.97 ± 0.05	$\mathbf{1.00 \pm 0.00}$
	SVM			
Data sets	MinMax	Standard	Robust	Unscaled
$D_1\ D_2\ D_3$	$\mathbf{0.95 \pm 0.04}$	$\mathbf{0.95 \pm 0.04}$	$\mathbf{0.95 \pm 0.04}$	0.94 ± 0.05
$D_4\ D_5\ D_6\ D_7\ D_8$	0.86 ± 0.13	0.74 ± 0.24	0.73 ± 0.23	$\mathbf{0.91 \pm 0.11}$
$D_4\ D_6\ D_7\ D_8$	$\mathbf{0.98 \pm 0.04}$	0.97 ± 0.06	0.96 ± 0.06	$\mathbf{0.98 \pm 0.03}$

An interesting finding was that unscaled colorectal data sets performed better than the scaled ones. To further inspect the high performance of unscaled data a similar experiment were done replacing the stratified 10-fold with a leave one data set out setup with accuracy as metric leading to results summarized in Table 4.3. Accuracy was used as metric due to the fact that D_7 only consists of samples of a single class and thus has no AUC score by itself. These results indicate that three of the colorectal cancer data sets, D_4, D_7 and D_8 were a lot more similar than the hepatic cancer data sets, which was also seen in Figure 4.4. The effects of scaling these data sets thus improves the outlier data sets D_5 and D_6 but at a higher cost in loss of performance for the similar data sets.

Table 4.3: Feature scaling results for leave one data set out. A classifier were trained on data sets of the same disease then tested on a data set of the same disease that was unknown to the classifier. Scores are the accuracy of predictions on the unknown data set. Best accuracy per data set for each method is highlighted in bold.

	Random Forest		SVM	
Data set	MinMax	Unscaled	MinMax	Unscaled
D_1	**0.69**	0.47	**0.89**	0.50
D_2	0.87	**0.89**	0.71	**0.81**
D_3	**0.92**	0.51	**0.92**	0.67
D_4	0.51	**0.99**	0.50	**0.95**
D_5	**0.50**	0.40	0.40	**0.50**
D_6	**0.65**	0.47	**0.53**	**0.53**
D_7	0.91	**1.00**	0.80	**1.00**
D_8	0.80	**0.92**	0.62	**0.75**

4.2.3 Feature Selection Performances

For feature selection the same experimental setup was used with minmax scaling with range [-1, 1]. This experiment was also done using the filling for missing miRNA which left a larger number of features. This lowered the overall performance for the baseline, however having more features for the feature selection might give the feature selection methods an additional edge. To facilitate feature selection, RFE with cross-validation was used with an estimator of the same type of classifier. For random forest a random forest with equal amount of estimators were used. For SVM a linear kernel were used for feature selection because the RFE algorithms requires information about feature importances which is not available in a RBF kernel.

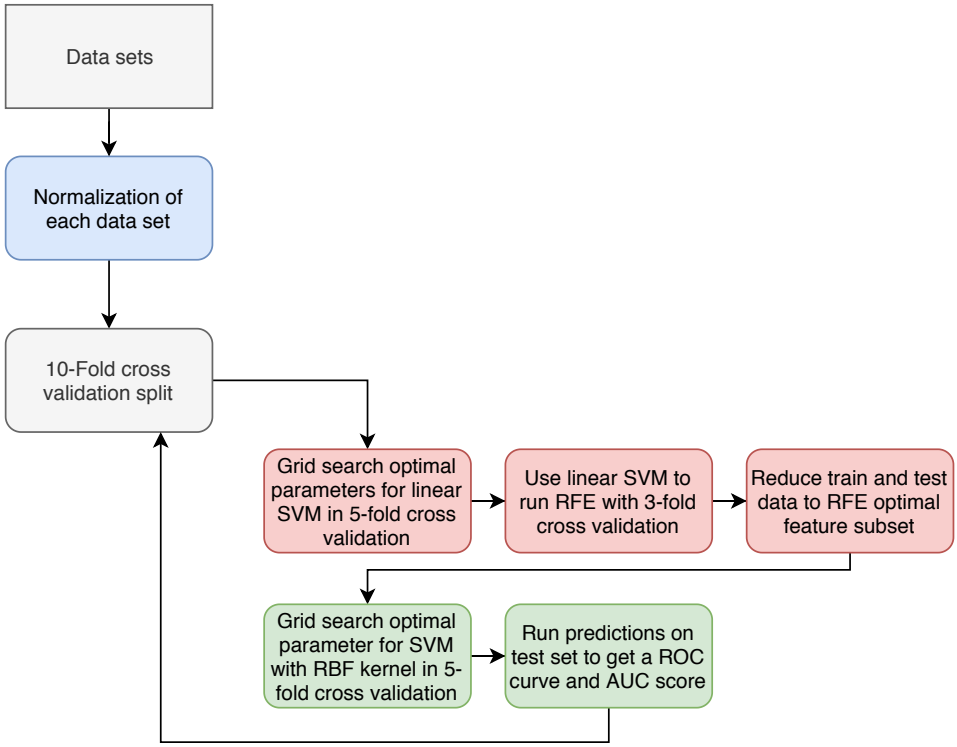

Figure 4.5: Overview over feature selection done with SVM. Blue boxes are normalization, red boxes are feature selection and green boxes are classification. Note that linear SVM were used for feature selection while RBF kernel were used in classification.

Figure 4.5 gives an overview over this process. First normalization and feature scaling of all data sets is done. Second, data is split in a stratified 10-fold cross-validation. Then ran through three steps for feature selection: (1) finding the best linear kernel parameters, (2) run RFE with this optimal linear kernel and (3) reducing the feature space of training and test data. Third, classification were done in two steps: (1) grid search for optimal parameters for the RBF kernel (this also trains the classifier) and (2) run predictions on the

test data.

To adapt this model to random forest none of the grid searches were used and the linear SVM kernel were swapped with a random forest classifier to be used as estimator for the RFE algorithm.

Table 4.4: Number of features selected in RFE for both SVM and random forest with the respective AUC score for each fold. Hepmark data sets refer to D_1 D_2 D_3 and Colon data sets refer to D_4 D_5 D_6 D_7 D_8. The initial set of features were 701 for Hepmark and 578 for Colon.

	Random Forest			
	Hepmark		Colon	
Iteration	Features	AUC score	Features	AUC score
1	41	0.90	338	1.00
2	66	0.87	83	1.00
3	41	0.93	78	0.71
4	161	1.00	448	0.63
5	146	0.98	568	0.57
6	66	0.89	243	0.98
7	146	0.93	413	0.99
8	541	0.89	318	0.94
9	111	0.97	508	1.00
10	286	1.00	463	1.00
	SVM			
	Hepmark		Colon	
Iteration	Features	AUC score	Features	AUC score
1	11	0.95	118	1.00
2	91	0.90	408	1.00
3	21	0.94	423	0.31
4	71	1.00	318	0.60
5	216	0.98	528	0.67
6	131	0.94	228	0.99
7	346	0.89	323	0.97
8	96	0.91	318	0.86
9	111	1.00	208	1.00
10	41	1.00	13	0.96

In Table 4.4 the number of features selected in RFE for each iteration of the outer stratified 10-fold cross-validation is presented along with the score of each fold. The high deviation between folds in the colon data set is a topic that is addressed in section 4.2.4. From this table there are no clear correlation between loss of performance in selecting fewer features but an indication that data exists in the training fold that are more similar and more easily separable.

In Table 4.5 the overall performance of doing feature selection versus no feature selection is presented. From this data there seems to be a loss of performance from doing feature selection on SVM and random forest does not necessarily benefit either.

Table 4.5: Feature selection results comparing scores from doing feature selection to not doing feature selection. Scores are in the ROC AUC metric with standard deviation.

	Random Forest	
Data sets	No feature selection	Feature selection
D_1 D_2 D_3	0.932 ± 0.05	$\mathbf{0.936 \pm 0.05}$
D_4 D_5 D_6 D_7 D_8	$\mathbf{0.898 \pm 0.15}$	0.882 ± 0.16
	SVM	
Data sets	No feature selection	Feature selection
D_1 D_2 D_3	$\mathbf{0.954 \pm 0.04}$	0.951 ± 0.04
D_4 D_5 D_6 D_7 D_8	$\mathbf{0.844 \pm 0.21}$	0.836 ± 0.22

For random forest the impact of feature selection is quite clear. As the number of features decreases the amount of randomness in sub-feature space is decreased, and thus also the room for randomness between each estimator. This results in a model that, depending on the cross-validation split, is more overfitted to the training data and thus less robust to the test data. In cases were the split of training data is representative for the testing data this results in higher accuracy but in the cases were this is not the case it results in lower accuracy. This can also be seen in the full ROC curves in Appendix page 73.

For SVM the impact is not as easily explainable. An obvious source of error might the use of a linear kernel to select features. The linear kernel does generally perform worse than the RBF kernel. Thus one might conclude that the linear kernel have allowed for more error to fit the data pattern differently than the RBF kernel. There has been studies that show feature selection may be valuable analysis to include in preprocessing operations for classification by SVM (Pal and Foody, 2010). However, in our experiment the conclusion were that the extra effort of doing feature selection is not valuable towards overall performance.

Another problem with feature selection for these types of experiment is that the selected features will be the ones that separate the classes best and not necessarily the ones related to the overall problem. For example did Guyon et al. (2002) point out that a particular challenging problem with their colon cancer data set is that the tumor samples and normal samples differ in cell composition. Thus was the best split that tumor samples were generally rich in epithelial(skin) cells while normal samples held a variety of cells. This split of tumor and normal is not informative for tracking cancer related genes.

4.2.4 Algorithm Performances and Data Sets

As shown in previous sections the two main algorithms used for classification is random forest and SVM. The initial choice of these are connected to the use of similar classifications from related works. These have themselves several parameters that can be tuned. Random forest has number of estimators, choice of splitting criteria and number of max features. SVM has the choice of kernel, error term C, complexity γ, degree of polynomial and coefficient. Though not all of these are available for every kernel. In addition, there were some attempts to create bagging and boosting ensembles to achieve even better performance. Table 4.6 gives a short summary of this.

Table 4.6: Overview of algorithm performances in a 10-fold cross-validation using the two combined data sets. Scores are in the ROC AUC metric with standard deviation.

	Algorithm performance			
Data sets	Random Forest	SingleSVM	BaggingSVM	BoostingSVM
Hepmark	0.932 ± 0.05	$\mathbf{0.954 \pm 0.04}$	0.88 ± 0.05	0.92 ± 0.05
Colon	$\mathbf{0.898 \pm 0.15}$	0.844 ± 0.21	0.67 ± 0.16	0.76 ± 0.37

To select best parameters for random forest a simple experiment were done. A grid search through parameters in Table 4.7 on the Hepmark data sets and Colon data sets finding the optimal parameters in each of the 10 folds. The best parameters and the score for each iteration is listed in Table A.4 and Table A.5 located in Appendix page 68.

In this experiment deviation from standard parameters gave no improvement in score. The standard parameter of max features (square root) were best in half of the folds. In the criteria parameter gini and entropy were close in most folds each but gini had a slightly higher increase of performance compared to entropy in folds where it was best. For estimators between 200 and 500 were best in most folds.

Going forward a preset of parameters were used setting estimators to 200, criterion to gini and max features to square root of number of features. There are obviously faults to this procedure by not doing a full grid search through all parameters every time but the additional cost in processing time, approximately one hour on a single 4.4GHz core, seemed not to be worth it. For comparison, on the Hepmark data sets the preset is better, 0.942 AUC, than when doing a full grid search, 0.941 AUC, having the same standard deviation while being close to instantaneous. These scores suggest that the full grid search of parameters increases the chance of overfitting the classifier. The ROC curves from these runs can be found in Appendix page 75.

Table 4.7: Random forest grid search parameters. Square root and log_2 indicates that the square root and log_2 of number of features are used. Real numbers indicate the percentage of max features that can be used.

Parameter	Values
Estimators	10, 50, 100, 200, 500
Criterion	gini, entropy
Max features	$\sqrt{}$, $\log_2$, 0.5, 1.0

The grid search used on the SVM classifier used all parameters in Table 4.8 and the results for each fold is listed in Table A.6 and Table A.7. In most folds both the polynomial and RBF kernels performed best. RBF dominated the colorectal data sets and polynomial of degree 3 dominated the hepmark data sets. Neither linear kernel or sigmoid were best in any folds of the combined data sets. Head-to-head RBF kernel performed slightly more consistent than the polynomial kernel. Thus to speed up a reduced parameter list were created that only considered the RBF kernel. The full parameter search also performed worse than the reduced. Scoring 0.954 ± 0.04 on the reduced to 0.946 ± 0.05 on the full parameters list for the hepmark data sets and scoring 0.862 ± 0.13 on the reduced to 0.839 ± 0.21 on the full parameters list for the colon data sets. The ROC curves from these runs can be seen in Appendix page 76.

Table 4.8: SVM grid search parameters. C is used in all kernels. γ is used in RBF, polynomial and sigmoid kernels. Coef is used as an independent term in polynomial and sigmoid kernels. Degree is only used in the polynomial kernel.

Parameter	Values
Kernel	Linear, Polynomial, Sigmoid, Radial Bias Function
C	0.1, 1, 5, 10
γ	0.1, 0.01, 1e-3, 1e-4, 1e-5
Coef	0, 1
Degree	1, 2, 3

Table 4.9: Algorithm performances overview. D_7 is a tumor only data set and thus has no score by itself. Scores are in the ROC AUC metric with standard deviation. The best scores for each data set are in bold.

	Algorithm performance on individual data sets			
Data sets	Random Forest	SingleSVM	BaggingSVM	BoostingSVM
D_1	0.92 ± 0.10	$\mathbf{0.94 \pm 0.07}$	0.92 ± 0.08	0.92 ± 0.07
D_2	0.92 ± 0.09	$\mathbf{0.94 \pm 0.07}$	0.94 ± 0.08	0.84 ± 0.11
D_3	$\mathbf{1.00 \pm 0.00}$	0.23 ± 0.39	$\mathbf{1.00 \pm 0.00}$	$\mathbf{1.00 \pm 0.00}$
D_4	$\mathbf{1.00 \pm 0.00}$	0.95 ± 0.11	$\mathbf{1.00 \pm 0.00}$	$\mathbf{1.00 \pm 0.00}$
D_5	$\mathbf{0.66 \pm 0.16}$	0.51 ± 0.18	0.48 ± 0.17	0.40 ± 0.16
D_6	$\mathbf{0.75 \pm 0.38}$	0.58 ± 0.45	0.34 ± 0.47	0.17 ± 0.37
D_8	$\mathbf{1.00 \pm 0.00}$	$\mathbf{1.00 \pm 0.00}$	$\mathbf{1.00 \pm 0.00}$	$\mathbf{1.00 \pm 0.00}$

The data sets were of quite different difficulties to classify. Table 4.9 has a summary of how the different algorithms performed on the individual data sets. An important note here is that a small sample count of some of these data sets makes them harder to classify alone. As an example SingleSVM performed better for combined data sets, D_2 and D_3 gave 0.95 ± 0.06 and D_6 and D_8 gave 0.97 ± 0.08 the latter is also the case for BaggingSVM and BoostingSVM. While all but one data set could be classified with a fairly high AUC score, data set D_5 were consistently hard to classify. The best performance on this set were achieved by the random forest preset achieving 0.66 ± 0.16. Excluding this data set

from the overall colon data sets gives an ROC AUC score of 0.98 ± 0.03 and 0.98 ± 0.04 for random forest and SingleSVM respectively scaled using minmax scaling.

BaggingSVM and BoostingSVM were introduced as an attempt to improve the initial performances from random forest and SingleSVM. The bagging classifier has 3 parameters that can be tuned: base estimator, number of estimators and max features to be given to each estimator. For boosting, Adaptive boost classifier (Adaboost) were used. This also has 3 parameters that can be tuned: base estimator, number of estimators and learning rate. The base estimators for both of these could also be tuned in the same manner as the SingleSVM. However tuning the base estimator much is somewhat counter intuitive for the overall algorithm as the idea is to use SVM as a weak learner in the same way random forest uses decision trees. Thus were the base estimators kept to the linear and RBF kernel.

$$Binom(0, 9, 0.5) = P(X \leq 0) = 0.001953125. \tag{4.1}$$

The hypothesis, Equation 4.1, was there that there would be some improvement over random forest or SingleSVM in a single or combination of data sets. However in none of the nine combinations from Table 4.6 and Table 4.9 were bagging and boosting strictly better. Thus was the hypothesis rejected and further testing of bagging and boosting is left for future work.

4.3 Imbalance Problem

The next experiment is to see how well other data sets can be used to classify an unknown data set. This will be done by training on all other available data sets of the same disease and then sampling a subset of the testing set. In addition, the same procedure will be done using the GSEA signatures of the training data sets and testing how well these can be used to classify the testing subset. This procedure should demonstrate how previous findings translate to the imbalanced case and thus address RQ4.

To test this the random forest and SingleSVM classifiers from the previous section were used. Leaving one data set out as a test set, the remaining data sets were used to train the classifiers. The training sets were scaled individually using standard scaling, minmax scaling and unscaled. Then for each sample in the test set, take 0,1,2,4,8,16 and all positive samples and 0,1,2,4,8,16 and all negative samples from the test set. These combinations of samples were scaled using standard scaling, minmax scaling, closest scaler and unscaled. Then the results were saved in a csv file. The definition for closest scaler is explained in section 3.1.1.

These results were extracted onto heatmaps based on scaling of training set, scaling of test set and algorithm. On the heatmap the mean of each combination was generated for each data set and the combined sets for liver and colorectal cancer. The mean was the ROC(AUC) score where such is applicable (from at least two samples of both tumor and normal), and balanced accuracy where ROC score was not applicable.

To generate GSEA signatures a data set is first ran through a R function(page 83) that extracts the miRNAs that negatively and positively correlated with tumor samples for that data set. The negatively correlated genes are used as a normal gene set while the positively correlated genes are used as a tumor gene set and saved in a Gene Matrix Transposed

(GMT) file. This GMT file can be used in a GSEA package for python to run single or multiple samples though for an enrichment score per gene set in the GMT. Thus are two enrichment scores per training set generated for the test set.

These enrichment scores can be used in multiple ways. The first intuitive way was to evaluate the normal enrichment scores to the tumor enrichment scores. However in this process there were found some on average the normal enrichment scores had a higher mean than the tumor enrichment scores. Instead the enrichment scores from the training samples were used to train a SVM classifier, optimized with the same parameters used in Table 4.8, that could be used on the test samples enrichment scores. This process is illustrated in Figure 4.6.

One strictly advantageous feature of using the enrichment scores is that these are not scaled and thus are not reliant on the set scaling. Thus be unaffected by imbalance from the test data set. The only real variable in this scoring is the creation of the original gene sets to be used in GSEA. This allows for saving of the enrichment scores and new generation of heatmaps are fairly fast compared to the multiple types of scaling, algorithms and possibly feature selection used in the other heatmap generation. It is however true that SVM used to solve biases in enrichment scores also must be optimized but this training set only has two times the number of training sets as features, specifically four for hepmark and up to eight for colorectal, while the other has several hundred and possibly thousands for other miRNA data sets.

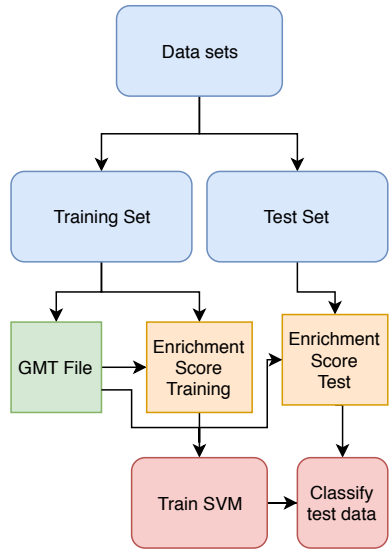

Figure 4.6: GSEA model with SVM classification.

The results from both hepmark and colorectal data sets can be found in Figure 4.7 and Figure 4.8. The best heatmap from the non-GSEA heatmap approach is shown alongside the GSEA heatmap for each disease. All other heatmaps can be found in the Appendix A.5.2. In these heatmaps the results gained inside the 25 squares with at least two positive and two negative samples differed a lot with the ones obtained outside. As a results the inner area refers to these squares and the outer area to the area outside these squares. One should also note that the performance metric of AUC is valid inside all of the inner area while the outer area used both balanced accuracy and accuracy. Balanced accuracy is used where it is valid i.e. when not at the leftmost tiles where TNR can not be calculated or at the topmost tiles where TPR can not be calculated. This change in performance metric also makes the methods which should not be affected by imbalance such as GSEA and unscaled has a slightly higher performance inside this area.

In the hepmark data sets SVM proved the best algorithm in all scalings, furthermore minmax scaling was best for the inner area. In the outer area minmax and closest scaling were imbalanced towards tumor and GSEA and unscaled performed pretty equal with a slight edge to GSEA which had the highest average performance of 0.787 in accuracy and

balanced accuracy with lower deviation that unscaled. The best for inner and outer can be seen in Figure 4.7.

In the colorectal data sets random forest were best in most cases. In the inner area minmax scaling was the worst and the remaining three were pretty equal but the best was the GSEA approach with an average of 0.77 AUC score. For the outer area minmax performed worst followed by GSEA and unscaled. The best performance in the outer area was actually a SVM with closest scaling scoring an average of 0.78 in balanced accuracy. In Figure 4.8 the two best performing heatmaps for the colon data set can be found.

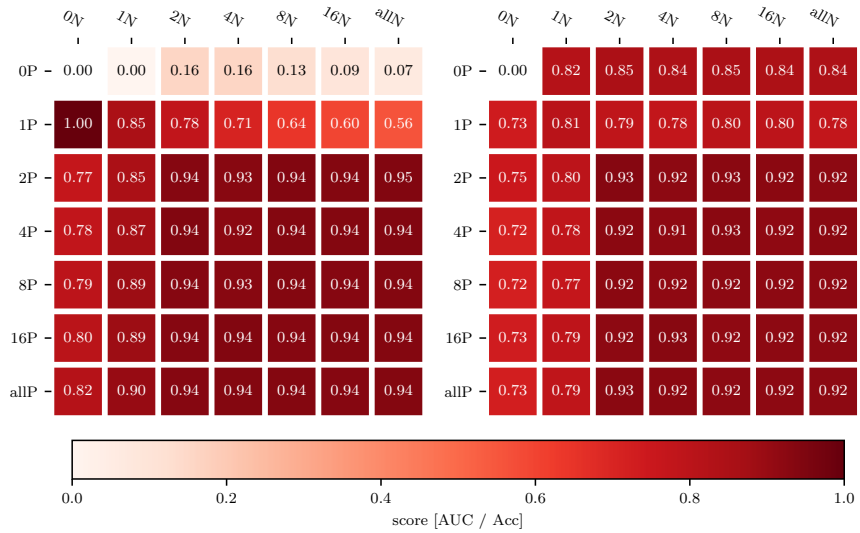

Figure 4.7: SVM and GSEA heatmaps for hepmark data sets. The left heatmap contains SVM scores from data scaled with MinMax to range [-1, 1], while the right heatmap contains scores on classification based of GSEA scores.

Assuming the methods are equally good the hypothesis can be formulated as a coin flip. Over the two data sets a total of 96 tiles can be compared. The methods that has no scaling, unscaled and GSEA is compared first. Choosing the best overall algorithm for unscaled for each of the data sets. In 72 out of the 96 tiles GSEA is equal or better than unscaled giving us Equation (4.2) which concludes that GSEA outperforms non-GSEA method with unscaled data.

$$Binom(72, 96, 0.5) = P(X \leq 72) = 0.999.. \tag{4.2}$$

$$Binom(23, 46, 0.5) = P(X \leq 23) = 0.56 \tag{4.3}$$

$$Binom(25, 50, 0.5) = P(X \leq 25) = 0.56 \tag{4.4}$$

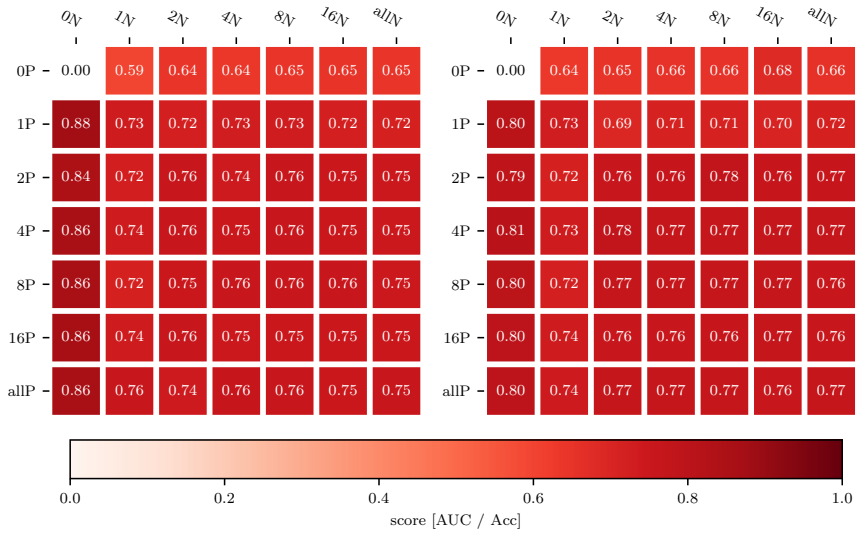

Figure 4.8: Random forest and GSEA heatmaps for colorectal data sets. The left heatmap contains random forest scores from data that are unscaled, while the right heatmap contains scores on classification based of GSEA scores.

For the methods with scaling, closest scaler is compared to GSEA for the outer area while minmax scaling is compared to GSEA for the inner area. Also this time using the best overall algorithm for closest and minmax scaling in each of the two combined data sets. This gives us Equation (4.3) and Equation (4.4) respectively. For the outer area the closest scaler outperformed the GSEA approach in 17 tiles for the colorectal data set and in 6 tiles for the hepmark data set. For the inner area minmax scaling beats the GSEA approach in all 25 tiles for the hepmark data set and is worse in all tiles for the colorectal data sets. Thus concluding that neither method is better than the other.

4.4 MiRNA Feature Importance

To address RQ5 this experiment looked into the most important features used for classification in the previous sections across different methods. This is done by looking into similarities in selected miRNA. One interesting part of this is whether the most important miRNAs used for classification has known connections to the disease. Another interesting part is if the selected miRNAs scored high by the different methods are the same of if they differ.

For the GSEA method the miRNAs found for the tumor gene sets and transported to the GMT file is used. In random forest an attribute called ”feature_importances” is used. This attribute calculates importance of each feature by first calculating the importance of each feature in each tree estimator then sum this and divide it by the number of tree estimators. The importance of each feature in a single tree is the normalized total reduction of the criterion brought by that feature. In this case this would be the features gini importance in that tree. One downside to this is that the computed importance of each feature becomes quite small in a large forest and that it does not indicate if the feature is more correlated with disease or normal.

For SVM there is no attribute available to indicate feature importance for most kernels. One possible solution to this is to remove features one by one and see how this affects the performance. The problems with this is the amount of computation time because of the relative high number of features and the base time required to train the model. Instead by using a linear kernel the coefficients for each feature can be used. Thus is the feature importance generated by this model not the same as the one used in the primary SVM model. The coefficients in the linear kernel is the weight assigned each feature. This means that a positive coefficient indicates that the feature is up-regulated in disease and a negative coefficient indicate that the feature is down-regulated in disease. This is illustrated in Figure 4.9.

To validate connections between miRNAs and diseases Human microRNA Disease Database (HMDD) is used. As this had to be done manually not all miRNAs were checked but the top 15 features for each method were used to validate. These results are summarized in Table 4.10 and Table 4.11.

To give GSEA a scoring in these tables rank GSEA is invented. Rank GSEA is calculated by giving each feature in the gene set sequence a number as it appears in the gene set. In addition to separate between normal and tumor gene sets the normal gene sets are given negative numbers while the tumor gene sets are positive numbers. For the cases where a feature is in several gene sets the smallest number is always chosen.

To select the top 15 features for each method the following were used. For random forest the highest values in the calculated feature importance. For SVM the highest positive coefficients were used. For GSEA the top features from each tumor gene set such that they total 15, e.g. if you have three tumor gene sets and no duplicates in the top five in each of these the top 15 features is then the top five features in each of those three sets.

In some cases the scoring between GSEA and the other methods does not make sense as either the GSEA does not have the feature in any of its gene sets or in the case were the gene set contains features that were excluded either in the reads per million normalization or a feature that is only present in some of the data sets. In these cases these values will be filled with N/A.

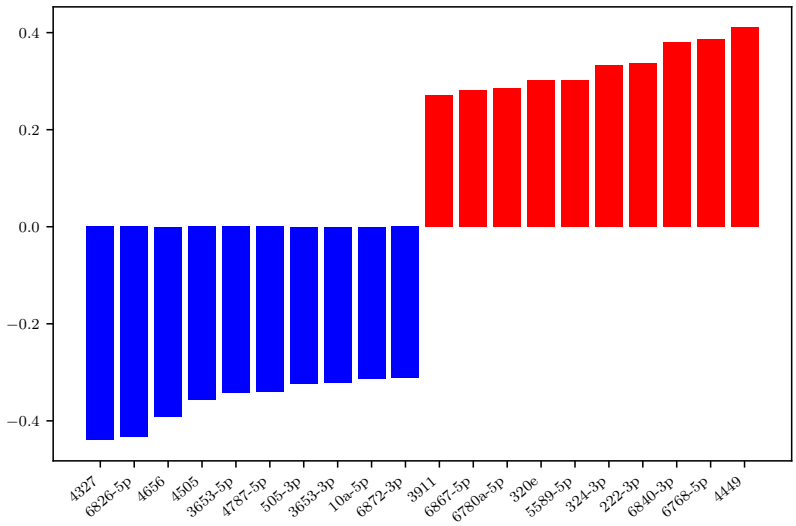

Figure 4.9: Feature importance in SVM for hepmark. Red bars are positively correlated with tumor i.e. up-regulated and blue bars are negatively correlated with tumor. Features has their prefix (hsa-miR-) removed to make space.

For the HMDD results the labels ”Yes”, ”No” and ”Other” were used. The first label, Yes, is if the searched miRNA is connected to the disease (HCC for hepmark sets and CRC for colon sets). Other is if the searched miRNA is connected to diseases but not the one in question. No is if the searched miRNA has no hits in the database.

In these two tables there are a couple of important observations. First, to what degree does the features extracted from each method overlap i.e. the feature is indicated as important by more than one method. Second, at what rate is the features marked as important actually known to be related to the disease. Third, is there a lot of mismatch between scoring of the same feature.

In Table 4.10 the answer to the first question is that there are only five features that overlaps between methods. To the second question the random forest approach has all its 15 top rated features as known targets while GSEA and SVM has 12 and 7 respectively. In terms of scoring one important observation is that most of the high ranking random forest features were in negative for GSEA and SVM which indicates that these miRNAs are down-regulated in tumor and may be a indication for why there is few overlapping features between methods. Scores from SVM and GSEA also do agree which features are up- and down-regulated in HCC in all but three cases and in these three cases there is the SVM score that is positive and and GSEA that is negative and the SVM score is lower than 0.05 in absolute value. To further check this the miRNAs hsa-miR-200b-3p, hsa-miR-200a-3p and hsa-miR-96-5p had their regulation confirmed by previous findings manually in HMDD.

In Table 4.11 the amount of overlap between methods are somewhat higher. Also here the random forest approach has the best prediction of miRNAs related to the disease with 14 of its 15 features being related, while GSEA and SVM had 13 and 12 respectively. The scoring of features from different methods also seem to be more correlated than the previous table. There are also more up-regulated or positive scored features. GSEA and SVM does not agree to the same degree which features are either up- or down-regulated as for HCC. The features that had a fairly high score (above 0.5) for SVM but were mismatched in regulation based on GSEA rank were checked manually for regulation in HMDD. These miRNA were hsa-miR-138-5p, hsa-miR-143-3p, hsa-miR-143-5p and hsa-miR-363-3p. The discovery was that the GSEA had the right regulation in all four cases.

Each of the three feature importance strategies had their uses. Random forest were the most accurate in terms of finding related targets. GSEA were the second best in accuracy and also had the correct notion which way the feature in question was regulated but does not have a rank for all features and does not enforce strict ordering of features. SVM was the most inaccurate but has a ordering of all features and its regulation.

Table 4.10: Feature importance for hepmark data sets. MiRNA is the feature. Rank GSEA refers to the what number the feature is in the gene set, positive values for tumor and negative values for normal gene signature. Rank RF is the features position in the sorted feature importance list from random forest. Score SVM is the linear kernels coefficient for the particular feature. Related to disease is whether the feature is linked to HCC in HMDD. The table is sorted by Rank RF.

MiRNA	Rank GSEA	Rank RF	Score SVM	Related to Disease
hsa-miR-200b-3p	-2	1	-0.17	Yes
hsa-miR-200a-3p	-18	2	-0.12	Yes
hsa-miR-96-5p	4	3	0.16	Yes
hsa-mir-130b-3p	7	4	-0.05	Yes
hsa-miR-30a-3p	-24	5	-0.12	Yes
hsa-miR-224-5p	23	6	0.12	Yes
hsa-miR-30a-5p	-17	7	-0.13	Yes
hsa-miR-483-5p	-21	8	-0.17	Yes
hsa-miR-199a-3p	-5	9	-0.07	Yes
hsa-miR-199a-5p	-6	10	-0.12	Yes
hsa-miR-221-3p	15	11	0.12	Yes
hsa-miR-452-5p	10	12	0.05	Yes
hsa-miR-30d-5p	33	13	0.09	Yes
hsa-mir-21-5p	3	14	0.22	Yes
hsa-miR-25-3p	17	15	0.08	Yes
hsa-mir-15b-5p	6	16	0.007	Yes
hsa-mir-1269a	1	17	0.15	Yes
hsa-mir-3651	4	20	-0.06	Yes
hsa-mir-93-5p	5	21	-0.004	Yes
hsa-miR-182-5p	3	32	0.02	Yes
hsa-miR-222-3p	21	36	0.34	Yes
hsa-miR-183-5p	2	38	0.09	Yes
hsa-miR-15a-5p	31	50	0.23	Yes
hsa-miR-320e	71	52	0.30	Yes
hsa-mir-6090	1	65	0.15	No
hsa-miR-1290	38	87	0.25	Yes
hsa-miR-147b	5	89	0.17	Other
hsa-miR-324-3p	128	92	0.33	Yes
hsa-miR-1180-3p	7	113	0.08	Yes
hsa-mir-3665	2	127	0.022	No
hsa-miR-5589-5p	N/A	140	0.30	No
hsa-miR-6768-5p	32	170	0.38	No
hsa-miR-1185-1-3p	N/A	219	0.26	Other
hsa-miR-4449	21	229	0.41	Other
hsa-miR-6840-3p	83	299	0.38	No
hsa-miR-939-5p	85	371	0.23	Yes
hsa-miR-6780a-5p	N/A	383	0.28	No
hsa-miR-6867-5p	N/A	387	0.28	Other
hsa-miR-10b-3p	1	546	0.77	Yes
hsa-miR-3911	N/A	575	0.27	No
hsa-miR-183-3p	2	N/A	N/A	Yes

Table 4.11: Feature importance for colon data sets. MiRNA is the feature. Rank GSEA refers to the what number the feature is in the gene set, positive values for tumor and negative values for normal gene signature. Rank RF is the features position in the sorted feature importance list from random forest. Score SVM is the linear kernels coefficient for the particular feature. Related to disease is whether the feature is linked to CRC in HMDD. The table is sorted by Rank RF.

MiRNA	Rank GSEA	Rank RF	Score SVM	Related to Disease
hsa-miR-181d-5p	15	1	0.02	Other
hsa-miR-93-5p	47	2	0.26	Yes
hsa-miR-92a-3p	7	3	0.27	Yes
hsa-miR-584-5p	4	4	0.31	Yes
hsa-miR-25-3p	23	5	0.88	Yes
hsa-miR-21-3p	3	6	0.34	Yes
hsa-miR-378a-3p	-3	7	-0.48	Yes
hsa-miR-31-5p	1	8	0.03	Yes
hsa-miR-9-5p	-6	9	-0.27	Yes
hsa-miR-1-3p	-4	10	-0.53	Yes
hsa-miR-20a-3p	47	11	0.20	Yes
hsa-miR-147b	-6	12	0.02	Yes
hsa-miR-30a-5p	-3	13	-0.07	Yes
hsa-miR-424-3p	7	14	0.64	Yes
hsa-miR-182-5p	6	15	0.26	Yes
hsa-miR-135b-5p	2	18	0.32	Yes
hsa-miR-183-5p	5	19	0.09	Yes
hsa-miR-224-5p	3	27	0.18	Yes
hsa-miR-125a-3p	N/A	42	0.69	Yes
hsa-miR-7641	26	54	0.80	Other
hsa-miR-138-5p	-15	66	0.63	Yes
hsa-miR-27b-5p	N/A	74	0.54	Yes
hsa-miR-21-5p	1	76	0.05	Yes
hsa-miR-10a-5p	N/A	83	0.60	Other
hsa-miR-181a-2-3p	N/A	86	0.74	Yes
hsa-miR-143-3p	-34	90	0.71	Yes
hsa-miR-143-5p	-16	181	0.49	Yes
hsa-miR-1271-5p	N/A	195	0.67	Yes
hsa-miR-323a-3p	N/A	227	0.63	Other
hsa-miR-210-3p	N/A	233	0.56	Yes
hsa-miR-363-3p	-31	235	0.54	Yes
hsa-miR-33a-3p	N/A	306	0.63	Yes
hsa-miR-549a	2	N/A	N/A	No
hsa-miR-135b-3p	1	N/A	N/A	Yes
hsa-miR-503-5p	4	N/A	N/A	Yes
hsa-miR-31-3p	3	N/A	N/A	Yes
hsa-miR-1273d	5	N/A	N/A	Other

Chapter 5

Discussion and Conclusion

In this chapter a discussion for findings in previous experiments is done. The second section contains conclusions and authors' suggestions to the problem of combining miRNA data sets. In addition, an overview of contributions and possible future work is included.

5.1 Discussion

Looking back at all experiments there are several important observations to be made. In the first experiment there were already several steps of pre-processing done. These preparation processes removes quite a bit of the miRNAs in the original data sets. Through the filtering following the reads per million, Equation 3.4, the miRNAs from the colorectal data sets were reduced from around 2438 unique miRNAs to 578 and the hepmark data sets were reduced from 2259 unique miRNAs to 701. In this step some information in low expressed miRNAs may have been lost that might further increase performance in classification.

Another problem was the handling of different feature subsets present in each data set. Two approaches were used, the first was a filling of missing features, i.e. using the union of features and the second was to use the intersection of features. It is expected that a filling would maintain a higher difference between data sets but having more features, that might be vital for specific data sets, to help classification. This is indeed also what is seen in Figure 5.1. The highest difference after feature scaling is still the individual data sets by a fair margin although the difference is greatly reduced by the shrinking of both axes. This effect differs from the effect seen in Figure 4.4, where the data sets overlap after feature scaling.

In the scaling experiments the most surprising result was how well the unscaled data did perform. Especially in the colorectal data sets. Recalling the PCA of the unscaled and standardized features shown in Figure 4.4. The first observation was the likeness of data sets D_4, D_7 and D_8 which actually overlaps quite well without any scaling. These sets already contribute 235 of the 348 samples in the combined colorectal data set. Close to perfect classification, 0.98 and above AUC score, were achieved from both unscaled and scaled when excluding D_5 in Table 4.2. Thus it seems that the actual performance mostly

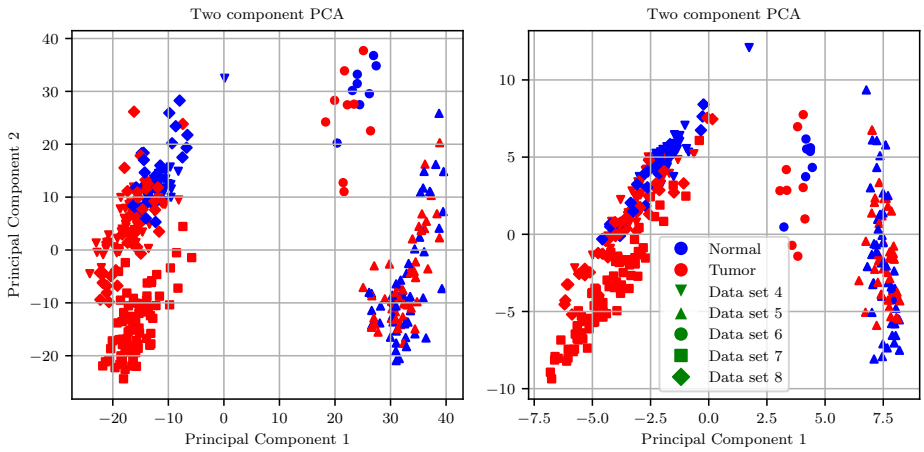

Figure 5.1: Principal component analysis for colorectal data sets D_4, D_5, D_6, D_7 and D_8. To the left the data sets are unscaled and to the right each data sets has been scaled using MinMax scaling.

relied on the classification of D_5. As D_5 held the majority of remaining samples, 95 out of 113, the best technique was to distinguish the samples from this data set rather than making them more relatable to the others. Unfortunately, by trying to make D_5 comparable the performance in the previously perfectly classified data sets dropped quite substantially as seen in Table 4.3.

The expectation would still be that an unscaled approach would not generalize as the unscaled values are not comparable from Figure 4.3 and Figure 4.4. This effect may further be demonstrated if a microarray data set had been included in the combined colorectal data set. In addition, the unscaled data is not entirely unscaled as it has been through the reads per million normalization to make samples comparable. This normalization has not eliminated any of the biases within the features inside each data sets that the other feature scaling methods do, but is a substantial part of making the data sets comparable.

The effects of feature scaling also varies with what classification algorithm is used. For SVM the effect is quite high as there should be an assumption the all features may separate the classes with equal probability. As the features has different means and variance the higher expressed features would be favored to maximize the margin of the hyperplane. Feature scaling eliminates this favoring towards higher expressed values. For random forest and decision trees the effect of feature scaling is not as important. This is because random forest singles out a single threshold for a feature to make a split and thus is not affected by the value or margin of this threshold.

The feature selection experiments found few advantages for doing feature selection. Neither did it improve performance nor provide a stable subset of features that correctly separated the classes. Highly related to the number of initial features this may have been different if a lower threshold had been set in the initial pre-processing. In addition, for the case of SVM, the use of a linear kernel to reduce the feature space of a RBF kernel should be questioned. In the same manner as mentioned in the miRNA feature importance section

it is possible to use the RBF kernel to do feature selection. However this would require the implementation of a ranking of features once the classifier is trained and then run this ranking at each iteration a feature is removed. An example of this RFE-SVM for feature selection can be found in Guyon et al. (2002).

In the algorithm experiments few parameters were tested for both baggingSVM and boostingSVM. SVMs are not necessarily the best base estimators for bagging and boosting and other combinations should also have been tested. Due to some of the discoveries in related works section 2.3.3 the more complex models were not necessarily favored. Thus were the experiments to some degree favored to keep models as simple as possible.

The imbalance experiments were the most time consuming work as this required setup of GSEA for enrichment scores. This again required some analysis of single data sets to extract gene sets that made up the GMT files which was easiest achieved using R. Enrichment scores did though prove to be quite good at classifying and had the best overall performance in this experiment. As for the non-GSEA approach, random forest had better scores in the more ambiguous data while SVM had the highest measured performance in balanced full data sets.

The score sheets generated in the imbalance experiments are themselves good objects of investigation. These allow us to select specific data sets, algorithms and feature scaling strategies. In general, the performance greatly improves when there is at least two positive and two negative samples if normalized data is used. The use of a closest scaling proved only to be effective when data sets were quite similar and did not outperform the unscaled tests. For data set D_5 no algorithm, scaling or method gave good performance. The lack of general difference between the tumor and normal class for this data set was also discovered when creating gene signatures for GSEA. Here the information about pairing of samples had to be utilized to find genes that differed between the classes. At the very least this data set was much more difficult to classify than the others.

For the miRNA importance for classification three different methods were implemented. All three selected features that were mostly related to their respective disease based on information from HMDD. However all methods also did differ more than expected in what features were selected of a given importance. For instance SVM and GSEA found a feature, hsa-miR-10b-3p, in HCC that random forest had close to its bottom. Although random forest had the highest accuracy for hits in HMDD it would by itself still miss out on certain features and lack the information of which way the feature is regulated in tumor.

5.2 Conclusions

In this project the goal were to identify robust methods for combining miRNA data sets that handles bias and can be used in practice. Here are the general findings and authors' suggestions.

Related works gave several pointers for this problem. Random forest and Support Vector Machines were the algorithms that are favored for classification of data that are high dimensional in features and low dimensional in samples. Though for each data set that is combined the number of features either decreases or stays the same while samples increases thus making the typical ratio of features to samples not as applicable for the

combined case. Feature scaling and feature selection were suggested when working with gene expressions and should help improve performance. For RQ1 the general approach to remove some bias in the data sets would be normalization and feature scaling of the data. However this does not remove all bias for miRNA data sets. Working with multiple transcriptome technologies a reads per million formula, Equation (3.4), were used to make gene expressions between technologies comparable and remove the absolutes that were present in raw RNA-seq data and turn them into relatives. Combining the data sets the missing miRNAs between data sets are excluded from the combined data set.

RQ2 were concerned with the effects of feature scaling. Although unscaled data has performed really well in these experiments the general approach should be to normalize and scale the data as this is what makes the data sets properly comparable as seen in the PCA plots, Figure 4.3 and Figure 4.4. The process of doing reads per million is not enough by itself as the value ranges for each miRNA would still slightly differ between sets. Among feature scaling the minmax method with a range [-1, 1] had the best performance in these experiments.

There were no benefits in doing feature selection in these experiments. In general the authors suggestion should be to keep feature selection to an informed level when dealing with miRNA data. Not selecting features because they differ between classes alone, but because they are actually linked to the problem. This would avoids problems such as classifying tumor based of skin tissue instead of miRNAs that are linked to cancer.

The procedure from RQ2 and RQ3 translated quite well for cases where there was at least two tumor and two normal samples. However, gene set enrichment analysis approach performed equally good and more consistent with less drawbacks. This procedure avoided the problems of normalization and feature scaling, and thus were unaffected by smaller and unbalanced data sets. Overall GSEA were a great tool for classification of miRNA data sets.

In RQ5 the feature importance and currently known links between disease and miRNA were questioned for the different approaches. Here it was found that all methods had different benefits and some correlation in what features were deemed as important. These findings suggests that utilizing multiple techniques greatly reduce the chance of missing out on important features while giving increased precision by having multiple scores for each feature. The overall precision of disease relation for important features were also quite high. The additional information about expression impact in disease can also be used for studying specific miRNA.

5.3 Contributions

In this thesis I have provided several useful insights into using multiple miRNA data sets to classify miRNA samples. This work has lead into the more general problem of handling biases in different data sets. This problem can in many cases be solved by different feature scaling techniques, however when these fail there are to the best of my knowledge no solutions. This has in some ways identified an unknown area. One of which has possibly several reasons for when feature scaling alone fails and possibly unknown solutions to the problem.

In terms of literature review there was some surprise when no papers were found that used a combined miRNA data set. Furthermore there were only a few studies that actually had done any comparison on how to classifying a single miRNA data set. In this work the selected related literature has been structured to reflect how most other studies have done their work on single miRNA data sets.

By combining miRNA data sets results several classification runs were ran to compare effects of feature scaling, feature selection and algorithms. For feature scaling unscaled, minmax scaling, robust scaling and standardization were compared. For feature selection the effects of running RFE versus not doing any feature selection were compared. For algorithms random forest and SVM were both used extensively in all experiments of this project. Some attempts were also done to implement bagging and boosting ensembles with SVM as a base estimator.

The combined data sets were tested in different combinations of imbalance to further investigate the performance of both feature scaling and algorithms. GSEA were also done on the data sets and used to compare possible enrichment score classification to the other combinations. The GSEA enrichment score were by themselves biased towards the normal classification. To bypass this bias a SVM classifier were implemented to classify based of enrichment scores. This approach outperformed other unscaled approach tested for imabalance of combined data sets, and were equally good when ideal scaling were given the other approach. The GSEA-SVM approach were unaffected by both different scaling and feature subset within each data set. In addition, the method were fairly fast as it allows for saving of intermediary results such as enrichment scores.

Most parts of the generated material is provided as supplementary material to this delivery and is listed at page 66.

5.4 Future Work

Multiple adjustments can be made to further enhance performance including trying other ensemble classifiers, use new feature selection estimators, finding alternatives to read per million normalization and testing the methods introduced in this thesis in new data sets of same and different diseases. Specifically, the effects of scaling samples to unit norms is an interesting aspect that might have potential as an additional step to the reads per million procedure. For this reads per million procedure lower the mean requirement allowing more features in the data set to begin with is important and untested in this work. This might also benefit feature selection at later stages. Finally, testing the proposed techniques for additional data sets for both hepatic and colorectal cancer can help a long way to further substantiate the findings in this thesis.

The generalized case of combining data sets of different bias has also gained some insight and partial answers. First, the loss of features per additional combined data set is an important concern that were found working with this topic. Second, in making data sets comparable, tools such as principal component analysis and density plots are vital to visualize effects of feature and sample scaling. Third, the comparability between data sets will differ. These findings must also be further substantiated through tests in different domains.

GSEA has potential improvement both in its creation of enrichment scores, i.e. the GMT file, and classification using the enrichment scores. In this project only one set of GMT files were created using the statistical significance difference in expression levels from one data set at a time. Additionally, gene sets can be made to score a single samples correlation with multiple gene sets at once. This has potential application for looking into gene set likeness in several different diseases. Essentially with the data from this project, it is possible to combine the GMT files and score any sample in both hepatic and colorectal cancer enrichment score. Making larger GMT's that scores multiple diseases may help in finding connections between diseases and help understand functions of individual miRNAs.

There are additional information in most data sets that are not utilized here. The data for most data sets include paired samples. This information could be used to create a pair-based method for an extreme value of normalization, which should remove most technology and individual differences. This has somewhat more limited in practical use as it requires both the normal and tumor sample from the same individual. One potential use could be a prognosis using regression, estimating a survival time based on the given sample. The use of cox regression could also be utilized estimating the effects of single miRNAs and their impact for survival. This information would be helpful in both understanding the disease and possible therapies.

A data set that gave particular challenge was D_5. For this data set, no good classification score were obtained. This is of interest as all other data sets were quite easy to obtain a decent score for, and further clarification should be made regarding whether this data set is unique in this aspect or if this is fairly common for miRNA data sets. Possible solutions for achieving higher performance on this set might be to make subsets of disease states or even pairings.

Feature importance gave several high rated features that had no known relations in HMDD. These should be investigated further establishing why these are particularly good for splitting the classes of the combined miRNA data sets. This is because feature scaling might have helped classifiers picking up patterns in low expressed miRNA that traditional statistics might have missed when studying these types of sets due to their subtle nature.

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Appendix A

Appendix

A.1 Structued Litterature Review Protocol

Table A.1: Search terms table. One term from each group were used in every search to accumulate more candidate papers for the literature review.

Group 1	Group 2	Group 3	Group 4
Machine Learning	miRNA	Classification	Bias
Knowledge engineering	MicroRNA	Analysis	Weighted
Artificial intelligence	Gene expression	Clustering	Cancer

Search engines used to locate related papers. Search terms that were used are found in Table A.1.

1. Scopus
2. ACM
3. IEEE Xplore
4. ScienceDirect
5. CiteSeer
6. Google Scholar

Inclusion criteria (IC) and quality criteria (QC):

IC1 Provided full text in the English language.

IC2 Contained information on machine learning on miRNAs.

IC3 Article used data of miRNAs or gene expressions.

QC1 Article is cited.

QC2 Article is a primary study.

QC3 Article uses similar and more than one data set.

QC4 Article explains procedure and is reproducible.

QC5 Article tries more than one algorithm or approach to solve the problem.

Table A.2: Article review table. Each quality criteria is listed in the quality criteria list above. Article ID to paper relations can be found in Table 2.1. Scores to each quality criteria is in the range from zero to one.

Article ID	QC1	QC2	QC3	QC4	QC5	SUM
0	1	1	0	0.5	0.5	3
1	1	1	0	0.5	0.5	3
2	1	1	0	0.5	1	3.5
3	0	1	0	1	1	3
4	1	0	0	1	0	2
5	0	0	1	1	0	2
6	0	1	1	1	0.5	3.5
7	0	0	1	1	0	2
8	0.5	0	1	1	0	2.5
9	0	1	1	0	0.5	2.5
10	0	1	1	0	0.5	2.5
11	0	1	1	0.5	0.5	3
12	0	1	1	0.5	0.5	3
13	0	1	1	1	1	4
14	0	1	1	1	0	3
15	0	1	1	1	1	4
16	0	1	1	1	1	4
17	0	1	1	0.5	0.5	3
18	0	1	1	1	1	4
19	0	1	1	1	0.5	3.5
20	0	1	1	0	0.7	2.7
21	0	1	1	0.5	0.5	3
22	1	0	1	1	0	3

A.2 Code and User Guide

All source code for this project is publicly available at Github - vegabj/Mastersproject

Requirements

- Python 3.6 or above.
- R 3.5.2 or above.

Requirements Python

NOTE: Anaconda is recommended for Windows users.

- Numpy
- Pandas
- Scikit-learn
- Seaborn
- Graphviz
- gseapy
- tqdm
- rpy2

Requirements R

- limma
- edgeR
- statmod

Usage

Create enrichment scores

- generate_enrichment_score - Creates enrichment scores for a given dataset - This requires a GMT file.
- create_gmt - Creates a gmt file for a given dataset, see source code for instructions for non RNA-sequencing sets. The gmt files should be combined.

Create score spreadsheets

- generate_score_sheet - Creates a score sheet for selected data sets.
- generate_score_sheet_es - Creates a score sheet for selected data sets based on enrichment score.

Plots

- pca - Creates a PCA plots over selected data sets.
- visualize_decision_tree - visualizes a decision trees as pdf files.
- dual_heatmap - Creates heatmaps from two selected score sheets that are latex friendly.
- analyze_score_sheet - Creates a heatmap from a selected score sheet.
- print_feature_importance - Prints the feature importance in both SVM and Random Forest, makes a plot for the top 20 features in SVM, creates a scatter plot over SVM and Random Forest feature importance.
- roc_rf - Creates a ROC curve for selected data sets and feature scaling with random forest.
- roc_svm - Creates a ROC curve for selected data sets and feature scaling with a SVM.
- box_plot - Creates a box plot of miRNAs.
- density_plot - Creates a density plot of selected data sets.

A.3 Supplementary Material

with the delivery:

- Source code.
- Generated enrichment scores for data sets.
- Generated GMT files.
- Generated score sheets.
- Data sets for colorectal cancer. (Hepatic cancer data sets can be provided upon request.)

A.4 Additional Tables

Data set ID	Paper by ID
DS0	0
DS1	1,2,12
DS2	1, 2
DS3	2
DS4	3
DS5	3
DS6	3
DS7	3
DS8	3
DS9	3
DS10	3
DS11	9
DS12	9
DS13	11,17, 20, 21
DS14	11, 21
DS15	11
DS16	11, 21
DS17	11, 21
DS18	11
DS19	12
DS20	12
DS21	12,13,16,18,19
DS22	16
DS23	21
DS24	21

Table A.3: Data set ID and article ID relations.

Full parameter search random forest

Table A.4: Hepmark data sets random forest full parameter search. The best parameters for each iteration and its corresponding score is listed in each row.

Iteration	Estimators	Criterion	Max features	Score(AUC)
1	500	gini	log_2	0.89
2	200	entropy	log_2	0.86
3	100	gini	log_2	0.93
4	200	entropy	$\sqrt{}$	1.00
5	100	gini	$\sqrt{}$	0.97
6	200	entropy	log_2	0.87
7	50	gini	$\sqrt{}$	0.91
8	200	gini	$\sqrt{}$	0.94
9	50	gini	log_2	0.98
10	500	entropy	$\sqrt{}$	1.00

Table A.5: Colorectal data sets random forest full parameter search. The best parameters for each iteration and its corresponding score is listed in each row.

Iteration	Estimators	Criterion	Max features	Score(AUC)
1	200	entropy	log_2	1.00
2	200	entropy	$\sqrt{}$	1.00
3	500	entropy	$\sqrt{}$	0.85
4	500	entropy	$\sqrt{}$	0.64
5	100	entropy	log_2	0.54
6	200	entropy	log_2	0.95
7	500	gini	log_2	0.93
8	500	entropy	$\sqrt{}$	0.90
9	500	entropy	log_2	1.00
10	500	entropy	$\sqrt{}$	1.00

Full parameter search SVM

Table A.6: Hepmark data sets SVM full parameter search. The best parameters for each iteration and its corresponding score is listed in each row.

Iteration	Kernel	C	γ	Coef	Degree	Score(AUC)
1	poly	10	0.001	1.0	3	0.96
2	poly	10	0.001	1.0	3	0.88
3	poly	10	0.001	1.0	3	0.95
4	poly	10	0.001	1.0	3	1.00
5	poly	10	0.001	1.0	2	0.99
6	poly	5	0.001	1.0	2	0.89
7	poly	10	0.001	1.0	3	0.86
8	poly	10	0.001	1.0	2	0.94
9	poly	10	0.001	1.0	2	1.00
10	poly	5	0.001	1.0	3	0.99

Table A.7: Colorectal data sets SVM full parameter search. The best parameters for each iteration and its corresponding score is listed in each row.

Iteration	Kernel	C	γ	Coef	Degree	Score(AUC)
1	rbf	10	0.01			1.00
2	rbf	10	0.01			1.00
3	poly	0.1	0.001	0.0	2	0.35
4	rbf	5	0.01			0.63
5	rbf	10	0.01			0.69
6	rbf	10	0.01			0.96
7	rbf	5	0.01			0.91
8	rbf	5	0.01			0.85
9	rbf	5	0.01			1.00
10	rbf	5	0.01			1.00

A.5 Additional Plots

A.5.1 ROC Curves

Overview

- ROC Curves baselines

  A.1 Baseline ROC Curve for Random Forest on Hepmark data sets.

  A.2 Baseline ROC Curve for Random Forest on Colon data sets.

  A.3 Baseline ROC Curve for SVM on Hepmark data sets.

  A.4 Baseline ROC Curve for SVM on Colon data sets.

- ROC Curves from feature selection

  A.5 Baseline ROC Curve for Random Forest on Hepmark data sets with filling of missing features.

  A.6 Feature Selection ROC Curve for Random Forest on Hepmark data sets.

  A.7 Baseline ROC Curve for Random Forest on Colon data sets with filling of missing features.

  A.8 Feature Selection ROC Curve for Random Forest on Colon data sets data sets.

- ROC Curves from algorithms

  A.9 Full parameter search ROC Curve for Random Forest on Hepmark data sets.

  A.10 Full parameter search ROC Curve for Random Forest on Colon data sets.

  A.11 Full parameter search ROC Curve for SVM on Hepmark data sets.

  A.12 Full parameter search ROC Curve for SVM on Colon data sets.

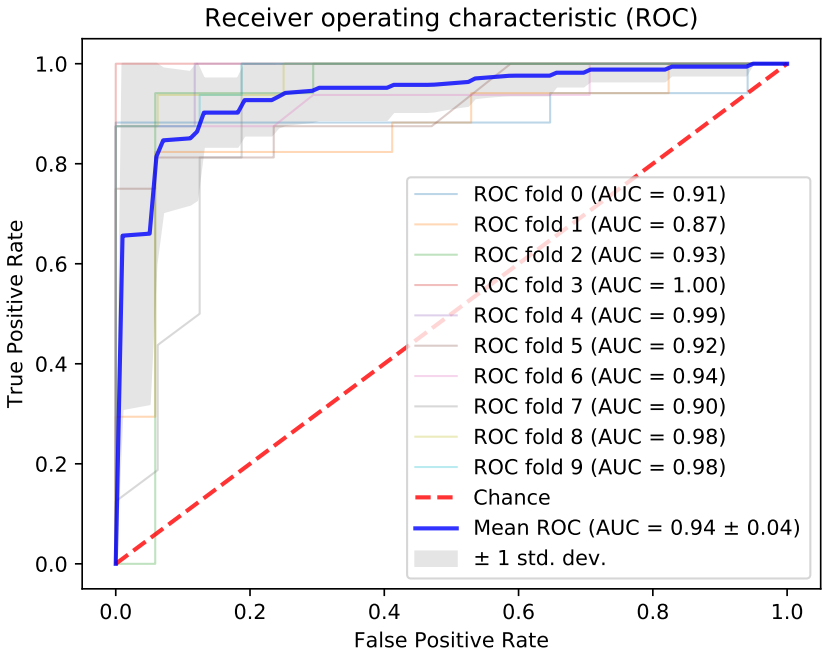


Figure A.1: Baseline ROC Curve for Random Forest on Hepmark data sets.

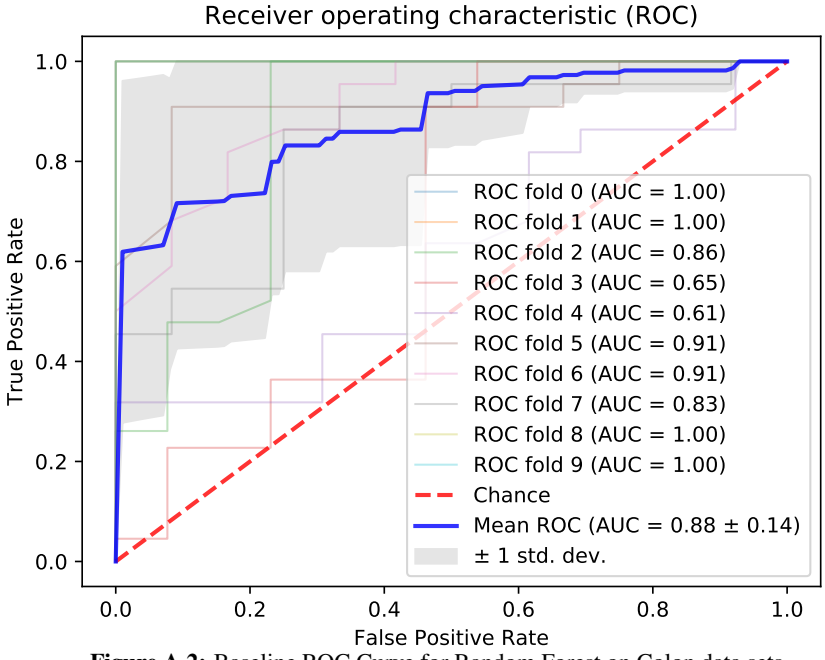


Figure A.2: Baseline ROC Curve for Random Forest on Colon data sets.

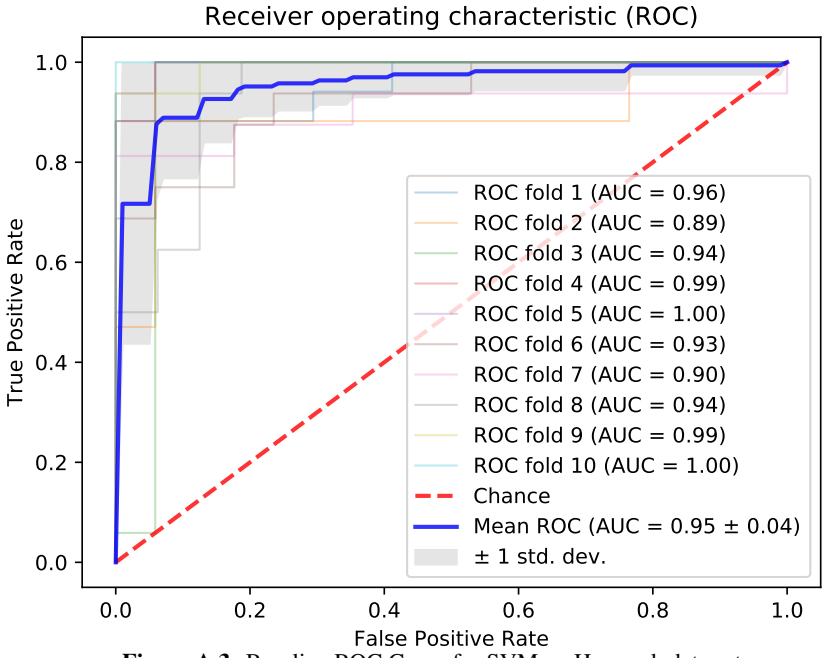


Figure A.3: Baseline ROC Curve for SVM on Hepmark data sets.

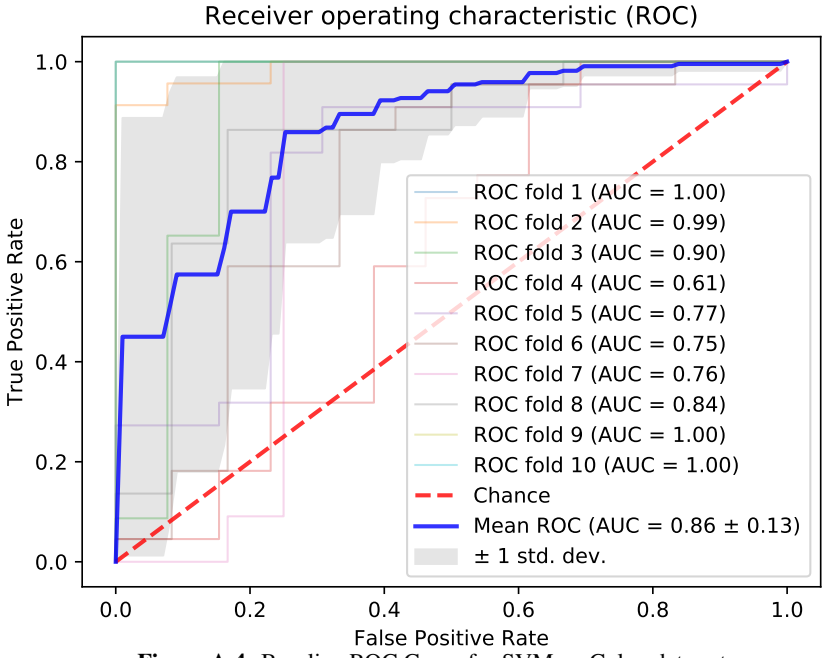


Figure A.4: Baseline ROC Curve for SVM on Colon data sets.

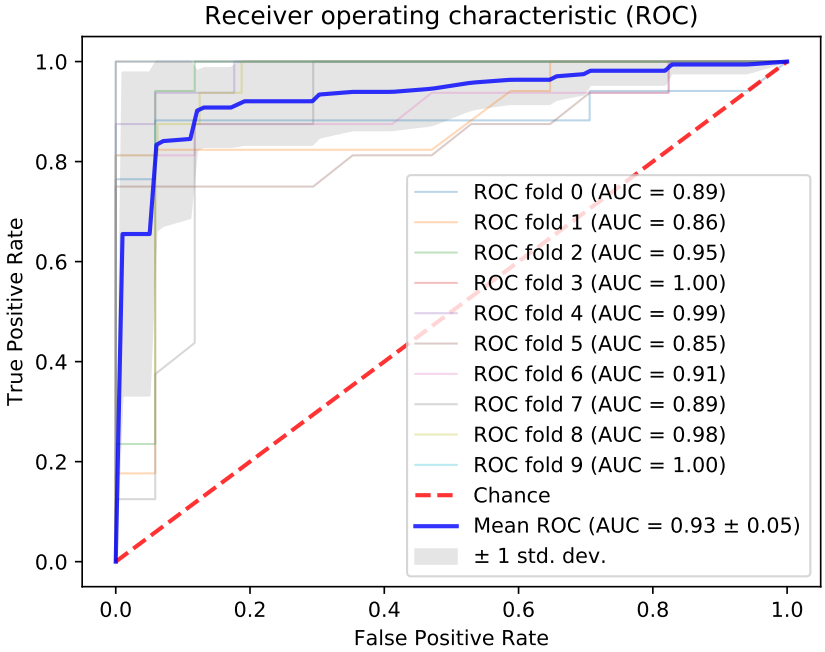


Figure A.5: Baseline ROC Curve for Random Forest on Hepmark data sets with filling of missing features.

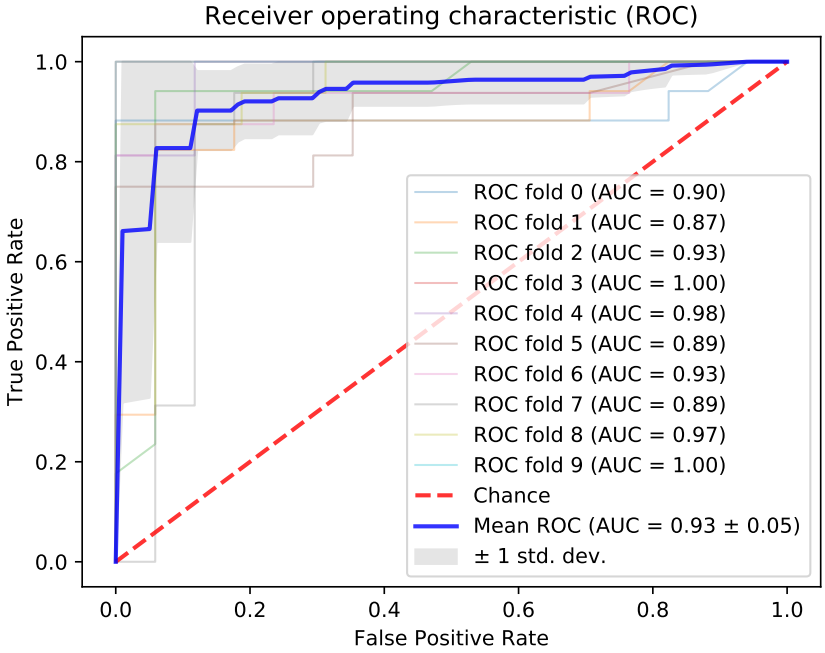


Figure A.6: Feature Selection ROC Curve for Random Forest on Hepmark data sets.

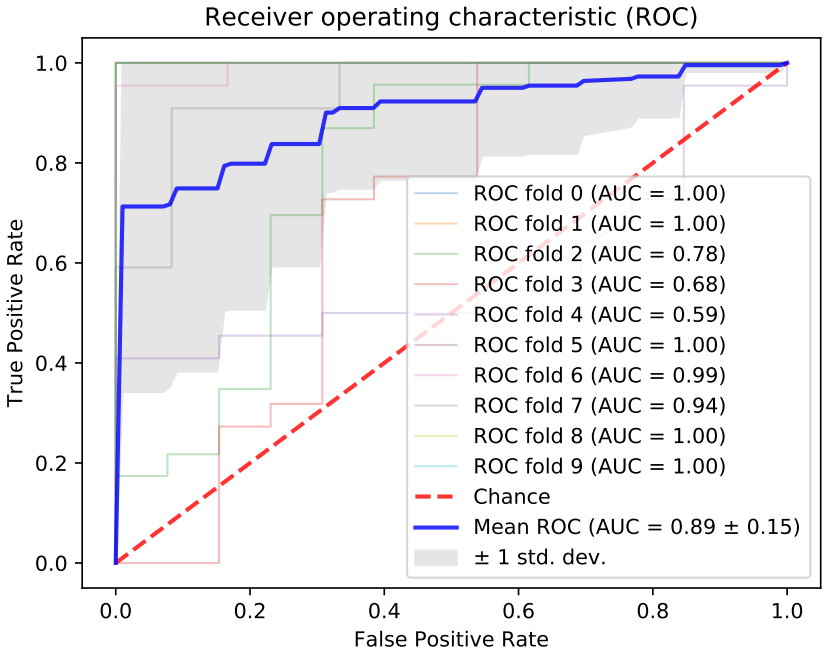


Figure A.7: Baseline ROC Curve for Random Forest on Colon data sets with filling of missing features.

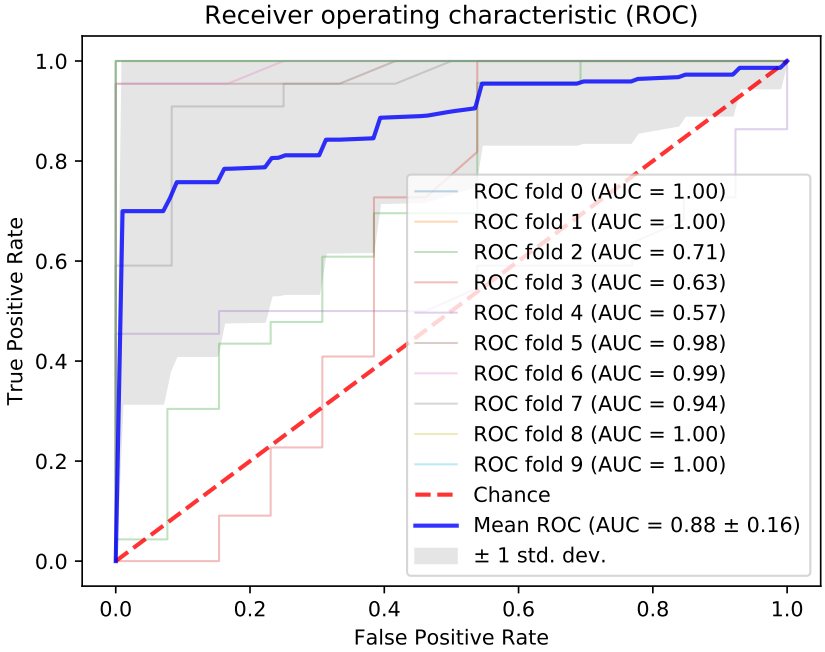


Figure A.8: Feature Selection ROC Curve for Random Forest on Colon data sets.

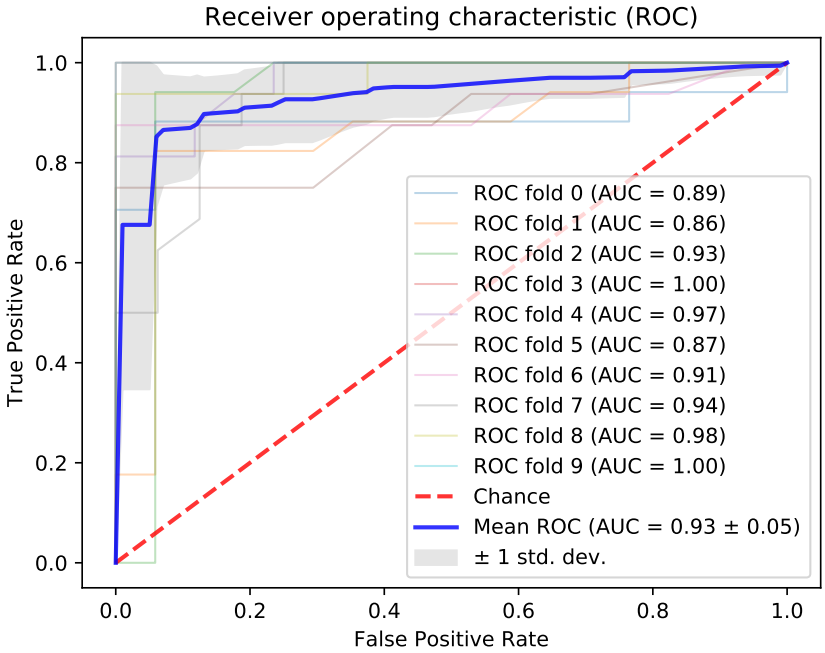


Figure A.9: Full parameter search ROC Curve for Random Forest on Hepmark data sets.

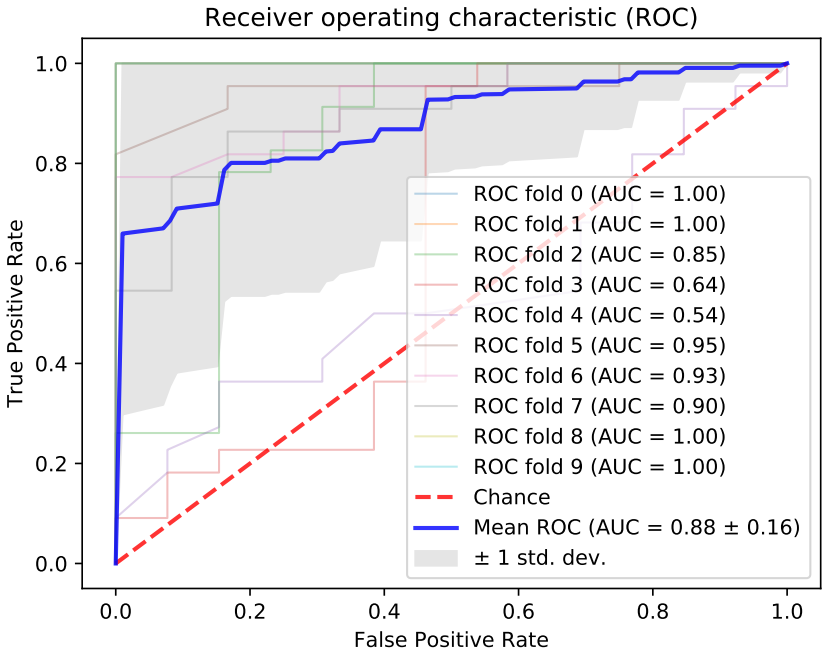


Figure A.10: Full parameter search ROC Curve for Random Forest on Colon data sets.

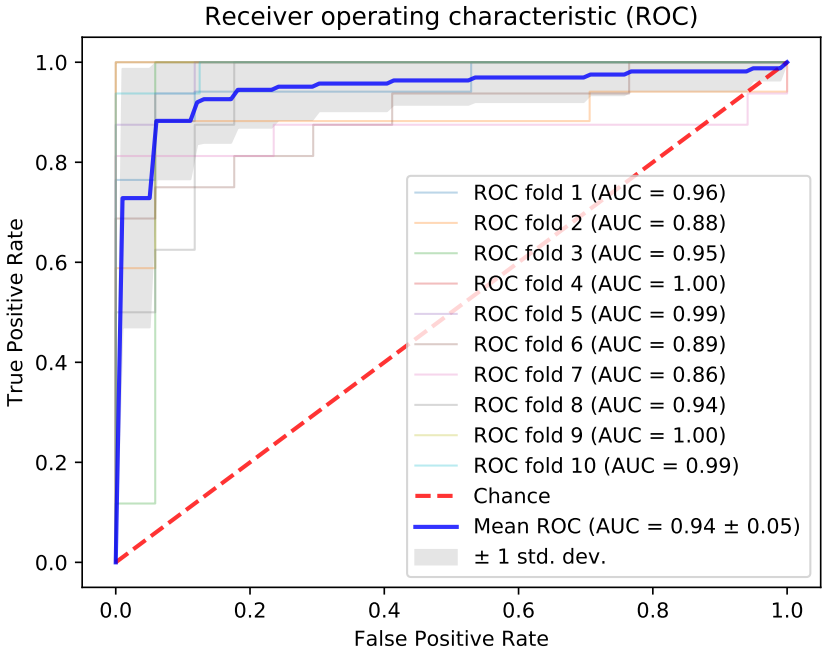


Figure A.11: Full parameter search ROC Curve for SVM on Hepmark data sets.

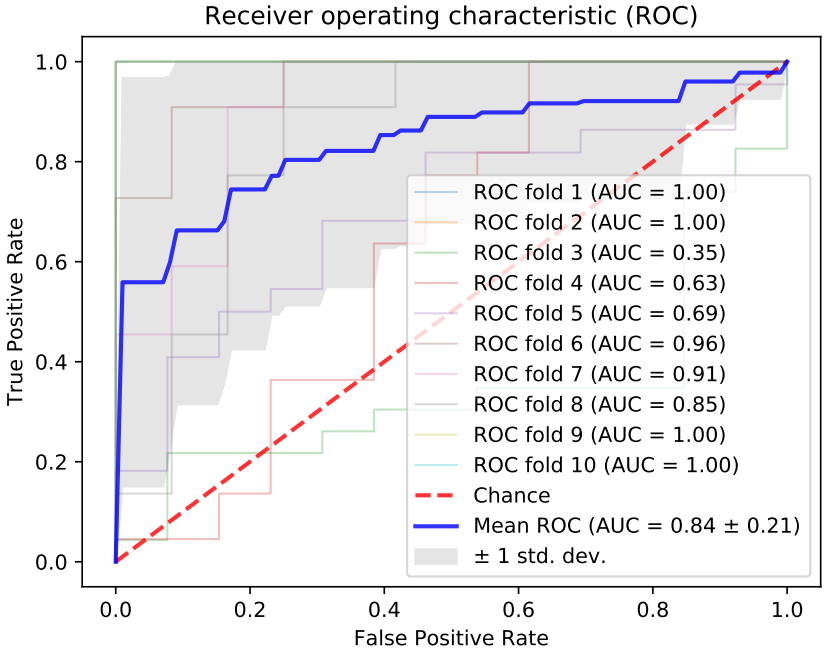


Figure A.12: Full parameter search ROC Curve for SVM on Colon data sets.

A.5.2 Heatmaps

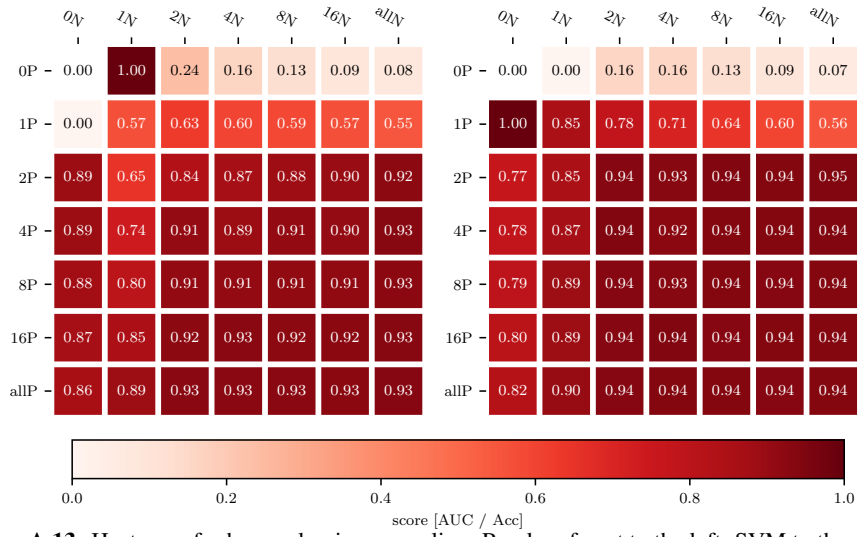


Figure A.13: Heatmaps for hepmark minmax scaling. Random forest to the left. SVM to the right.

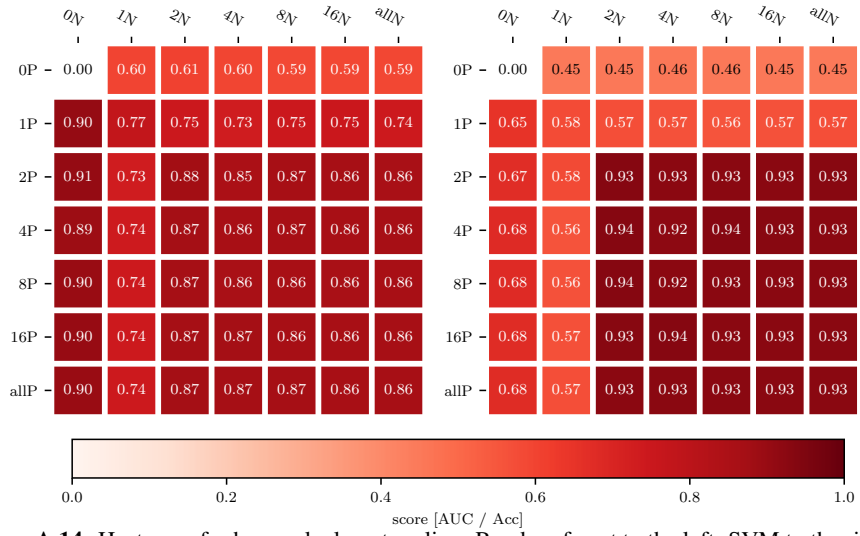


Figure A.14: Heatmaps for hepmark closest scaling. Random forest to the left. SVM to the right.

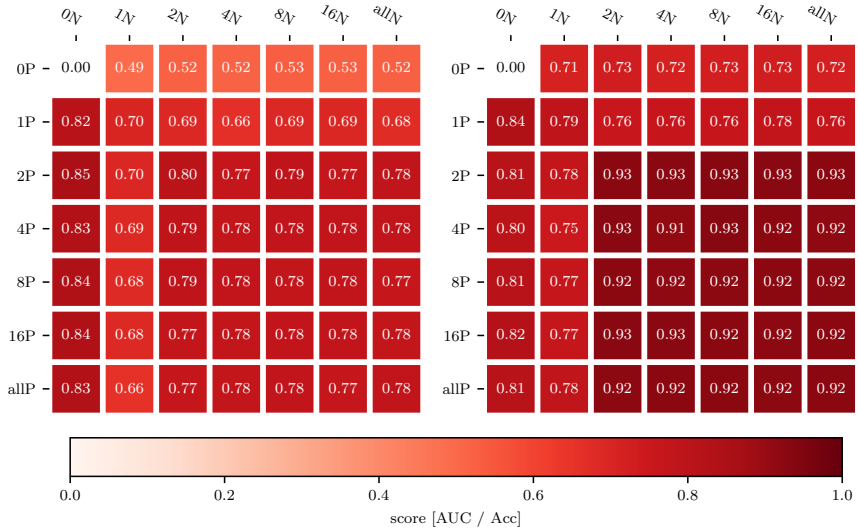


Figure A.15: Heatmaps for hepmark unscaled. Random forest to the left. SVM to the right.

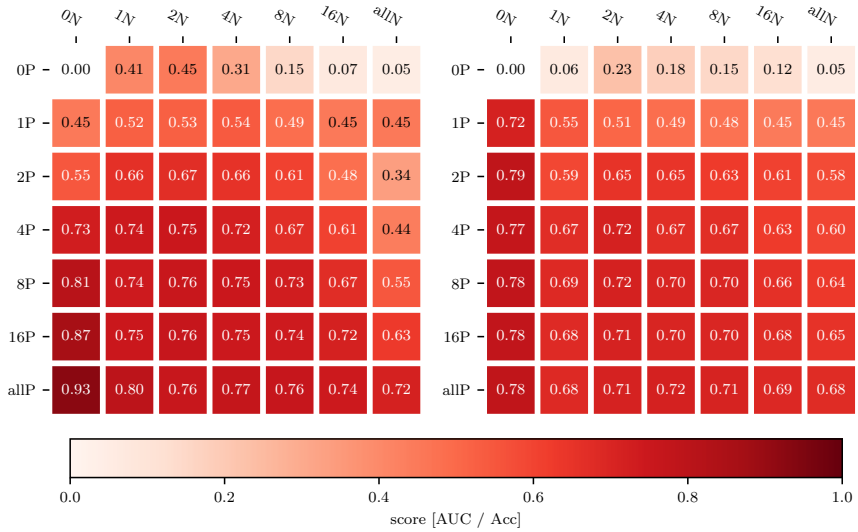


Figure A.16: Heatmaps for colorectal minmax scaling. Random forest to the left. SVM to the right.

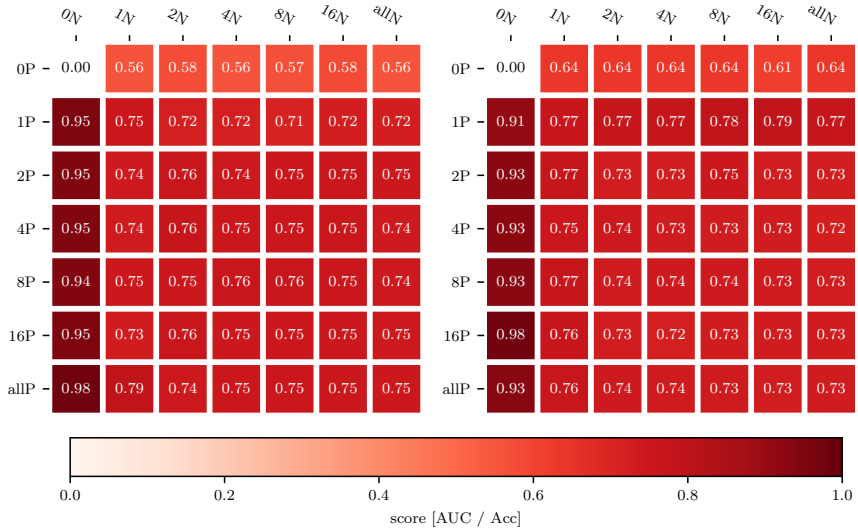


Figure A.17: Heatmaps for colorectal closest scaling. Random forest to the left. SVM to the right.

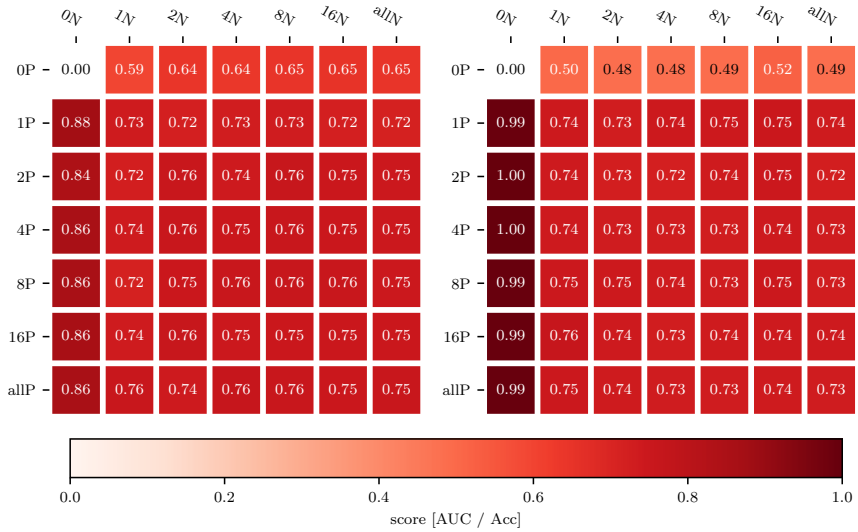


Figure A.18: Heatmaps for colorectal unscaled. Random forest to the left. SVM to the right.

A.5.3 Density Plots of Feature Scaling

These illustrates the effects the different feature scaling done in this project.

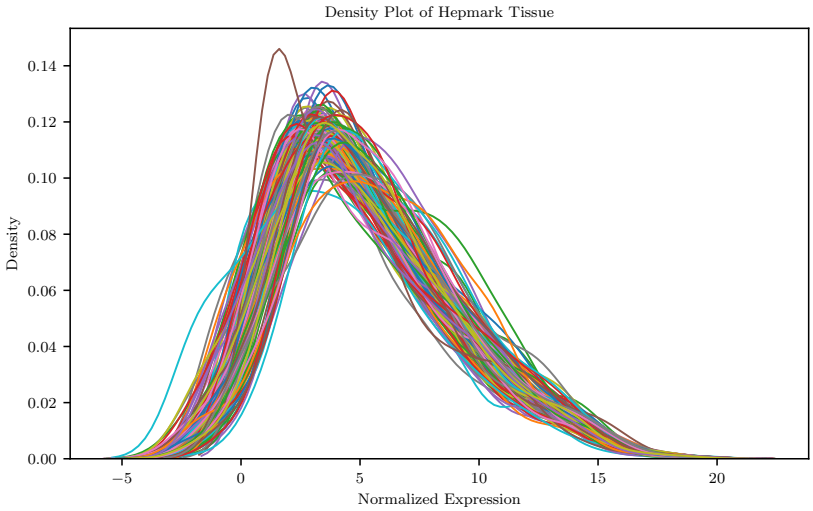


Figure A.19: Density plot over hepmark tissue data set that is unscaled.

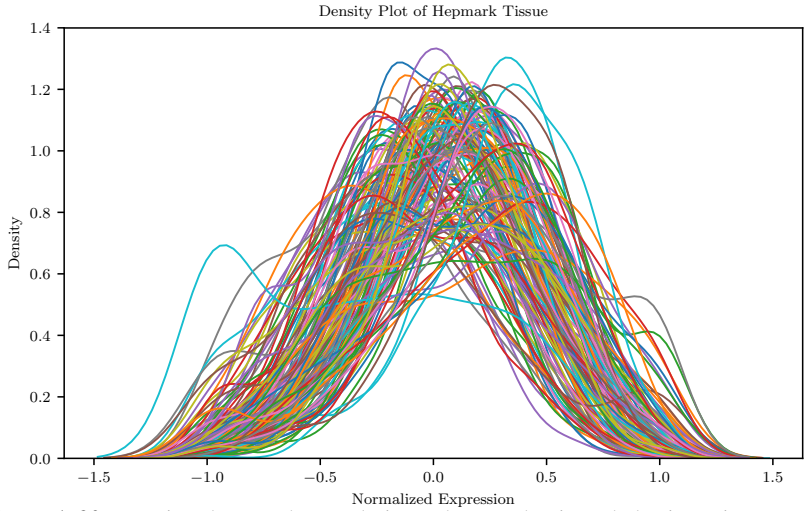


Figure A.20: Density plot over hepmark tissue data set that is scaled using minmax scaling.

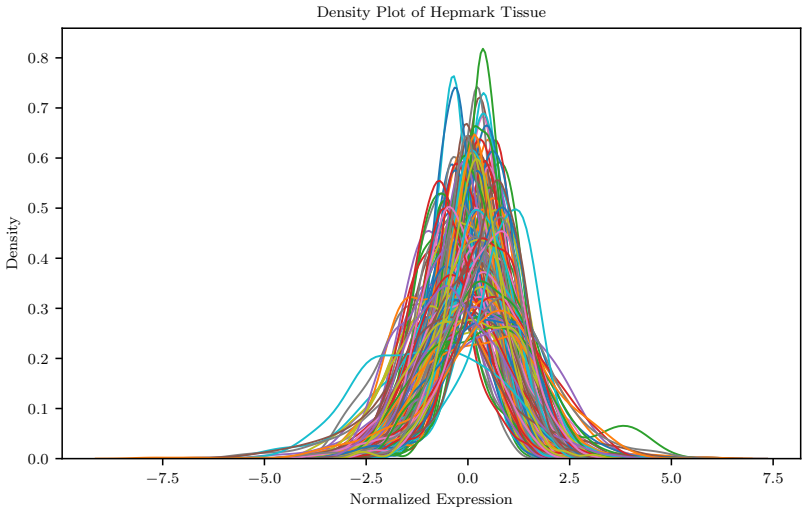


Figure A.21: Density plot over hepmark tissue data set that is using standardization.

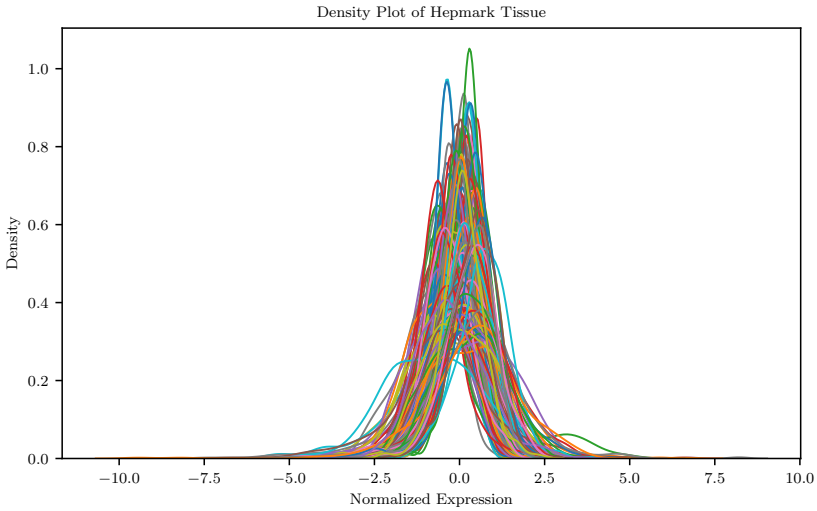


Figure A.22: Density plot over hepmark tissue data set that is scaled using robust scaling.

A.5.4 PCA Plots

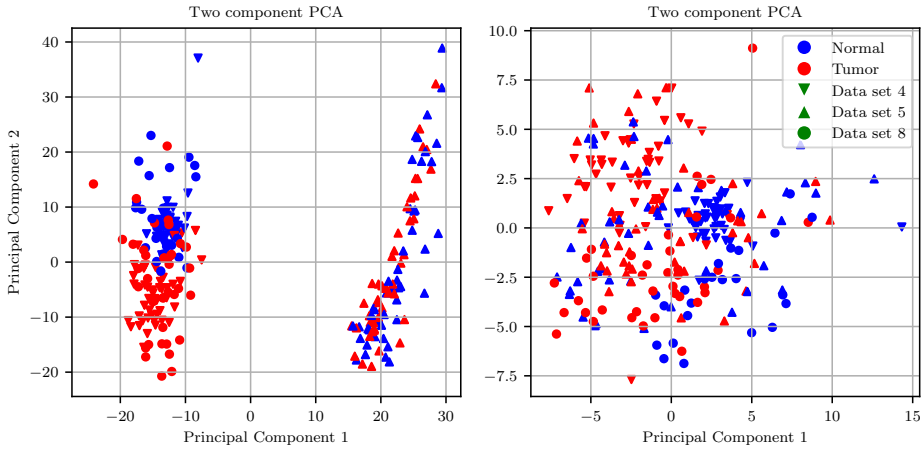


Figure A.23: Principal component analysis for colorectal data sets D_4, D_5 and D_8. To the left the data sets are unscaled and to the right each data sets has been scaled using minmax scaling.

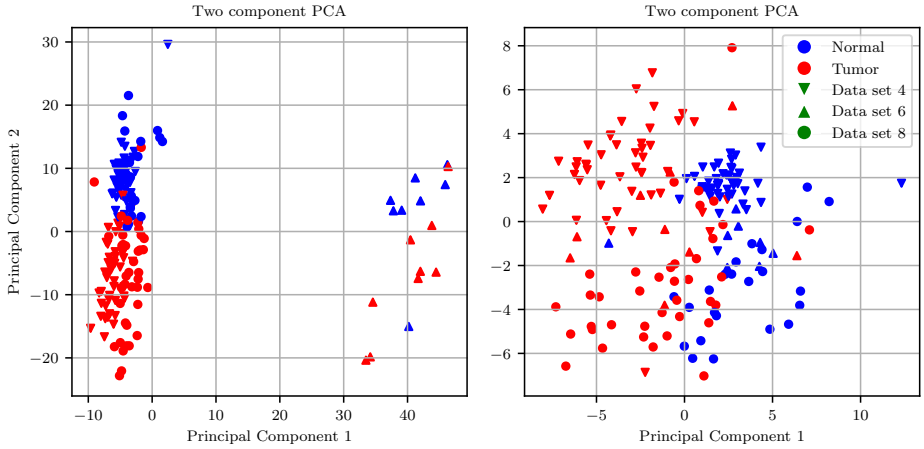


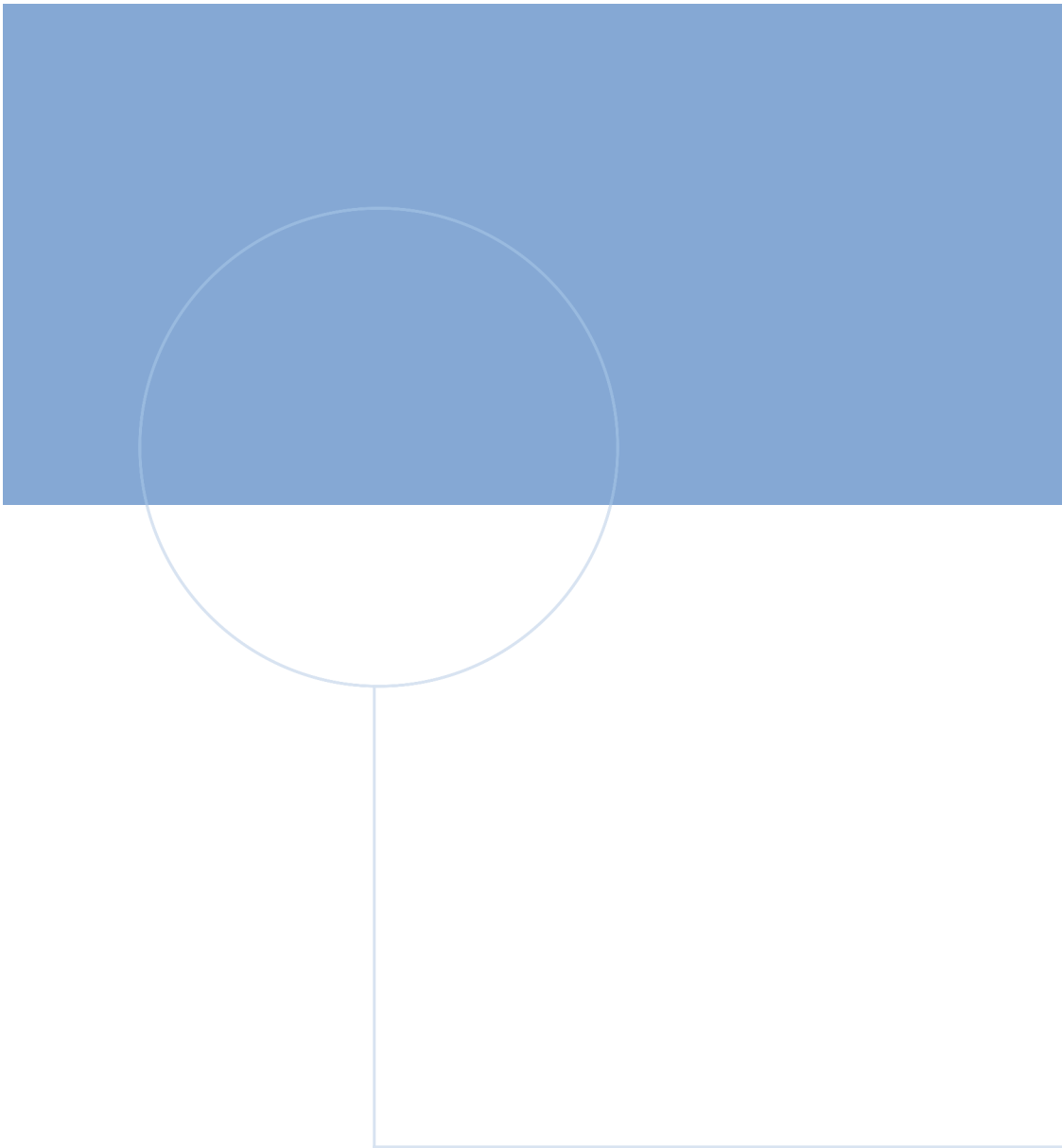
Figure A.24: Principal component analysis for colorectal data sets D_4, D_6 and D_8. To the left the data sets are unscaled and to the right each data sets has been scaled using minmax scaling.

A.6 Code Snippets

A.6.1 extract miRNAs R

```
dup.cor <- duplicateCorrelation(count.voom,
    design=design, block=block)
fit <- lmFit(count.voom, design=design,
    block=block, correlation=dup.cor$consensus.correlation)
fit <- eBayes(fit)
topTab <- topTable(fit, coef=2, p.value=0.05, number="inf",
    sort.by="p")
```

duplicateCorrelation estimates the correlation between duplicate spots or between technical replicates from a series of arrays. *lmFit* fits a linear model for each gene given a series of arrays. *eBayes* is given a microarray linear model fit, then computes moderated t-statistics, moderated F-statistics, and log-odds of differential expression by empirical Bayes moderation of the standard errors towards a common value. *topTab* sorts the table by p-value.



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