MASTER'S THESIS

Clustering Algorithms for Microarray Data Mining

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ABSTRACT

Title of Thesis:	CLUSTERING ALGORITHMS FOR MICROARRAY DATA MINING
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This thesis presents a systems engineering model of modern drug discovery processes and related systems integration requirements. Some challenging problems include the integration of public information content with proprietary corporate content, supporting different types of scientific analyses, and automated analysis tools motivated by diverse forms of biological data.

To capture the requirements of the discovery system, we identify the processes, users, and scenarios to form a UML use case model. We then define the object-oriented system structure and attach behavioral elements. We also look at how object-relational database extensions can be applied for such analysis.

The next portion of the thesis studies the performance of clustering algorithms based on LVQ, SVMs, and other machine learning algorithms, to two types of analyses – functional and phenotypic classification. We found that LVQ initialized with the LBG codebook yields comparable performance to the optimal separating surfaces generated by related SVM kernels.

We also describe a novel similarity measure, called the unnormalized symmetric Kullback-Liebler measure, based on unnormalized expression values. Since the Mercer criterion cannot be applied to this measure, we compared the performance of this similarity measure with the log-Euclidean distance in the LVQ algorithm.

The two distance measures perform similarly on cDNA arrays, while the unnormalized symmetric Kullback-Liebler measure outperforms the log-Euclidean distance on certain phenotypic classification problems.

Pre-filtering algorithms to find discriminating instances based on PCA, the Find Similar function, and IB3 were also investigated. The Find Similar method gives the best performance in terms of multiple criteria.

CLUSTERING ALGORITHMS FOR MICROARRAY DATA MINING

by

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CHAPTER 1: INTRODUCTION

1.1 Outline of the Thesis

The thesis is organized as follows.

Chapter 2 gives an overview of genetics and gene expression. It discusses DNA structure and its role in protein synthesis processes. It also covers representative mechanisms of the control of gene expression for the interpretation of genetic expression data.

Chapter 3 details large-scale expression analysis using DNA microarrays. Two popular technologies – oligonucleotide and cDNA arrays, are explained. Based on the overview in Chapter 2, the nature and analysis of microarray data for gene functional classification are discussed.

In Chapter 4, we attempt to model the integration of the high-throughput microarray technology and genetics databases in drug discovery and development processes. We give a brief overview of traditional and modern drug discovery. UML use cases are developed to model the requirements of a pharmaceutical discovery/analysis system. The overall system architecture is described using package and class diagrams, including the behavioral elements.

Chapter 5 gives an overview of supervised and unsupervised clustering algorithms for expression analysis. We outline two similarity measures – the log-Euclidean distance and the unnormalized symmetric Kullback-Liebler measure - and their application in the (supervised) learning vector quantization (LVQ) algorithm. We also give a brief

description of other learning techniques like support vector machines (SVM) and instance-based learning.

In Chapter 6, we compare different algorithmic implementations of important use cases in microarray analysis from Chapter 4. Two types of analyses are considered – functional and phenotypic classification. We give a description of the data sets, the methods and performance measures used, and a summary of the results.

CHAPTER 2: GENETICS AND GENE EXPRESSION

The biological information in an organism is contained in the DNA molecule, which is present in all cells. Cellular processes such as growth, replication, differentiation, and response to environmental conditions, are controlled by the DNA sequence data and the interaction of DNA with cellular compounds.

2.1 DNA Structure and Function

In this section, we elaborate on how the structure of the DNA molecule plays a vital role in regulating biochemical activities, and discuss mechanisms by which this is carried out.

The DNA molecule consists of two strands of nucleotide sequences forming a double-helical structure. Individual strands are composed of repeating blocks of deoxyribose sugar and phosphate subunits forming the exterior backbone of the molecule, and a nucleotide base on the interior. The two strands are held by hydrogen bonding between the nucleotide bases, as shown in Fig. 2.1.

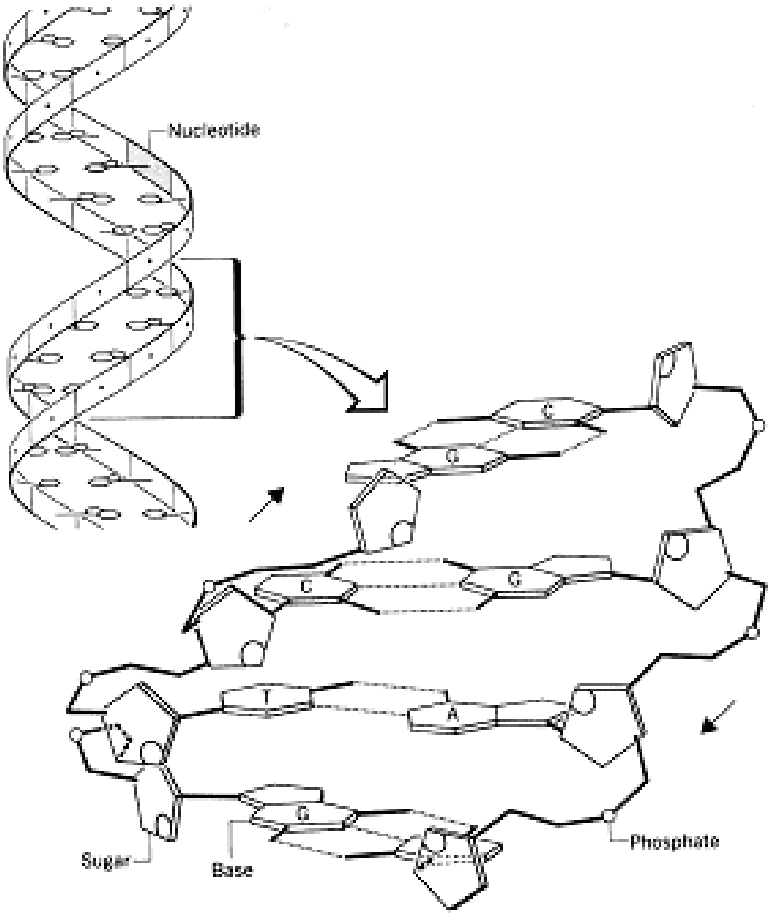


Fig. 2.1 Schematic Diagram of DNA Structure [10]

Due to the pairing properties of deoxyribose to the phosphate subunit, the ends of each strand have different chemical properties. The two strands run anti-parallel to each other, thus imposing directionality to the DNA molecule.

The nucleotide subunits in DNA are derived from a four-letter alphabet, viz., adenine (A), guanine (G), cytosine (C), and thymine (T). An additional constraint on DNA structure is illustrated by the complementary base-pairing rule; the nucleotide bases line up in such a

way that adenine on one strand corresponds to thymine on the other, and guanine corresponds to cytosine.

2.2 Protein Synthesis

The nucleotide sequences in a DNA molecule act as a template for synthesizing proteins; enzyme molecules catalyze these reactions. Most of the enzymes are proteins themselves, with structural properties that render them suitable for specific cellular processes. The order of reaction events in a cell is determined by a combination of sequence information and the presence of enzymes.

Sections of the DNA molecule called genes contain the information for synthesizing specific proteins, which are essentially amino-acid sequences. The information for protein synthesis is organized in nucleotide triplets called codons, defined by the four-letter DNA alphabet. Codons act as the template for 20 different amino acids, as well as start and stop markers for protein synthesis.

Gene expression is the physiological manifestation of the genetic makeup of an organism. At a finer level, it is the process by which information on a gene is used for protein synthesis. It takes place in two steps. During transcription (Fig. 2.2), a single-stranded ribonucleic acid (RNA) molecule is synthesized based on the complementary genetic sequence, in the presence of the RNA polymerase enzyme. The RNA molecule is structurally similar to the DNA molecule and is composed of the four-letter alphabet AUGC, with uracil (U) in place of the thymine (T) base.

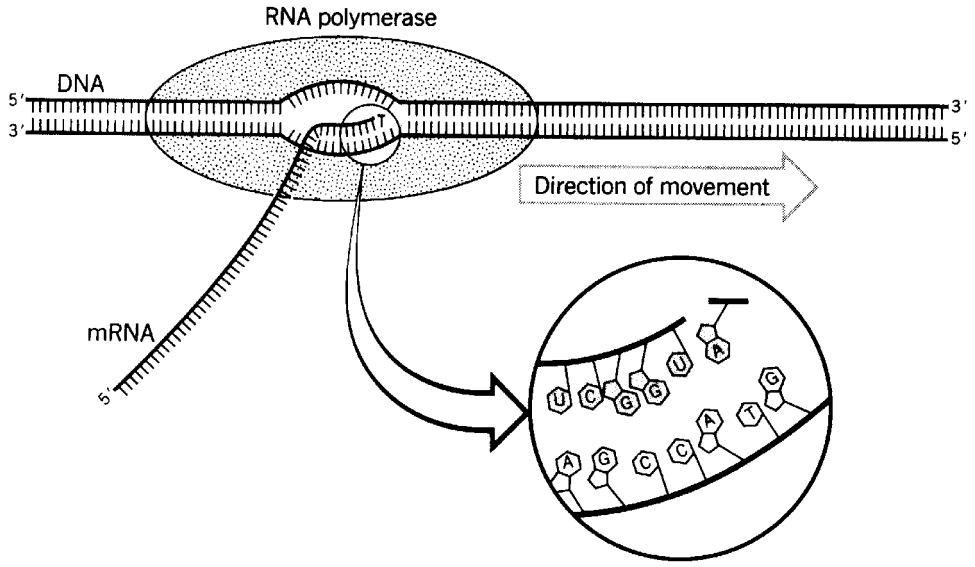


Fig. 2.2 Synthesis of mRNA during Transcription [10]

The RNA molecule from transcription, also called mRNA (messenger RNA), attaches to ribosomes in the cytoplasm of the cell. In the second step called translation (Fig. 2.3), a molecule called transfer RNA (tRNA) with a nucleotide triplet complementary to any of the mRNA codons forms a complex with specific amino acids in the presence of the aminoacyl-tRNA synthetase enzyme. By sequential alignment of codon-specific tRNA molecules, polypeptide chains of amino acids are constructed to form proteins from the start to the stop codons on the mRNA molecule.

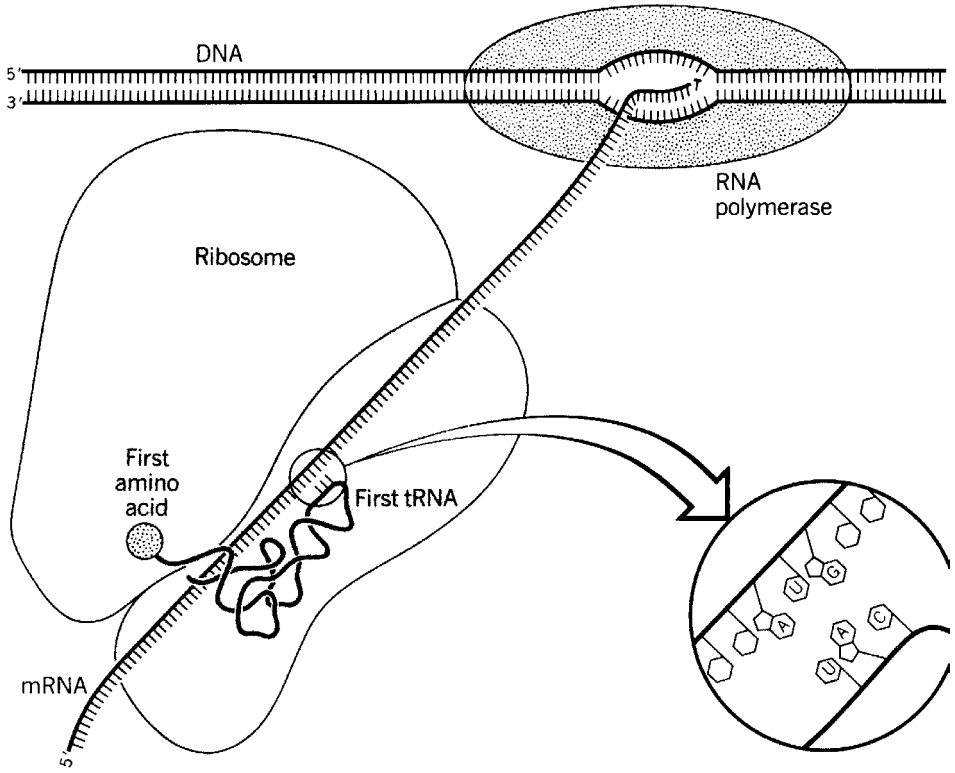


Fig. 2.3 Amino Acid Synthesis during Translation [10]

The entire DNA sequence information in DNA is not useful for protein synthesis. A significant portion is composed of non-coding regions, such as those that form regulatory elements or garbage sequences between coding regions. Bacterial genomes, for example have a very high codon density. However, an increase in genome size does not necessarily indicate an increase in efficiency or quality.

2.3 Regulation of Gene Expression

Protein synthesis as described above, is a fundamental and relatively uncomplicated process. However, in order for the cell to respond to the environment and initiate higher-level processes like growth and differentiation, complex regulatory schemes exist. Control

can occur at various stages of the protein synthesis process. In this section, we illustrate a few well-known mechanisms of gene expression regulation and relate them to gene function.

2. 3.1 Chromatin Structure

The chromatin is the fibrous complex of DNA and proteins within the nucleus. The physical structure of the DNA in the chromatin can vary in differentiated cells in an organism, and result in enhancing or repressing the expression of specific genes. For instance, the presence of compounds like histones, might affect the ability of RNA polymerase and transcriptional regulatory proteins to access specific genes on the DNA.

2.3.2 Transcriptional Control

Repression is a transcriptional control mechanism to turn specific genes on or off and is explained by the operon model of regulation in prokaryotic cells (cells having no nuclear membrane). The model states that groups of genes coding for related proteins exist close to each other on the DNA and are controlled by a single promoter region, where the transcriptional enzyme RNA polymerase attaches itself. The operator region separates the upstream promoter site from the genes. In the case of the lac operon, the constituent genes code for enzymes to break down lactose. In the absence of lactose, a regulatory gene upstream of the promoter codes for a repressor protein that binds to the operator region and inhibits transcription initiation. When lactose is present, it forms a complex with the repressor protein and detaches it from the operator site.

Attenuation is the mechanism by which the abundance of a protein inhibits its own transcription. This occurs in genes that code for energy-consuming processes like amino-acid production.

In activation, the binding of enhancer proteins near promoter and upstream regions of the DNA enable the RNA polymerase enzyme action, thereby initiating transcription.

2. 3.3 Processing-level Control

This refers to the relation between the coding scheme of genes and proteins. Proteins are often encoded by members of a multigene family. A multigene family of genes arises by undergoing modifications during evolution from a single ancestor. Such a set can code for homologous proteins with similar functions.

2.3.4 Translational Control

Translation of mRNA can be enhanced or suppressed by the amount of the specific protein in the cell. For instance, iron is stored in the protein ferritin. When iron levels are low in the cell, a repressor molecule binds to the mRNA for ferritin inhibiting synthesis. When iron levels in the cell rise, iron binds to the mRNA-repressor complex and detaches the repressor protein, thereby enhancing the synthesis of ferritin for storage.

2.3.5 Post-translational Factors

Protein expression varies even after translation. The presence of an inhibitor in the environment can repress protein function. Most proteins exist in an inactive state after translation and need to undergo polypeptide cleavage to become active. Some proteins may

also require activation through a combination with another molecule. The operation of enzymes in the presence of a cofactor is an example.

Many other mechanisms of gene regulation at various levels of biochemistry exist, and these may be specific to organisms. However, it is important to note that not all changes are observable in gene expression experiments. Hence, knowledge of the metabolism, phylogeny, and careful experimentation is required to draw meaningful results about gene functional characteristics.

CHAPTER 3: GENE EXPRESSION ANALYSIS

Based on the background on gene expression, we give a brief overview of the state-of-the-art in microarray technology, and two different types of microarrays. We then discuss the nature and applications of various gene microarray data. In the section on expression analysis, we explain how microarrays can be related to molecular biology and gene function.

3.1 Microarray Technology

Genetic analyses have traditionally been based on single-gene experiments in order to estimate the preferential expression of the gene in multiple experiments. With the availability of the complete genome sequence information for some organisms, it is now possible to simulate and study cellular control at the level of genetic interactions. The DNA microarray is an experimental tool that combines genome information with chip technology, and allows us to monitor specifically, the gene expression of thousands of genes at the same time, in different environmental conditions designed by the investigating biologist.

DNA microarrays give a quantitative measure of gene expression from all genes in a tissue sample, under a variety of conditions. To explore various genetic properties, experimental methods need to be designed to map them to expression values.

Microarrays measure the ability of DNA or RNA sequences from a sample to bind (or hybridize) to their complementary DNA sequences (cDNAs) laid out on a chip. Because of

complementary base pairing, measurement of the degree of hybridization between nucleic acids provides good sensitivity and specificity in detection. This basic idea remaining the same, two popular techniques exist to measure gene expression on microarrays. They differ in the manner in which the sequences are prepared initially and are described in the sections below.

3.1.1 Oligonucleotide Arrays

Oligonucleotides are nucleotide sequences that are 5-25 bases long. The oligonucleotide array was the first microarray product developed by Affymmetrix. In a procedure similar to semiconductor manufacturing, it uses photolithography techniques to synthesize nucleotide sequences.

The entire chip is initially covered with the photolithographic mask. The laser exposes precise locations on the chip. The particular amino acid solution is passed over the chip and binds nucleotides at these locations.

The masking agent is applied again and the process is repeated until sequences up to 25 base pairs are generated. Finally, when the fluorescently tagged DNA sequences are treated with the oligonucleotides, the degree of hybridization is measured by the amount of fluorescent emission following laser excitation.

A unique feature of oligonucleotide arrays compared to other microarray techniques is their high degree of accuracy. They hybridize multiple independent oligonucleotides with different segments of the same RNA. Two sets of (usually) ten probes, called the perfect match (PM) and mismatch (MM) probe sets, are used with each pair differing in a single

base ([7][5]). The MM probes, which act as the control, are supposed to display a much lower signal compared to the PM probes. This kind of redundancy leads to more accurate results as averaging and outlier detection can be performed prior to quantitative evaluation. Since the hybridization process is simple, these arrays have high reproducibility.

To generate oligonucleotide arrays, clearly, we need to know the entire sequence information of genes and non-coding regions involved in the experiment. However, once the sequence is known, it can be used in genotypic analysis ([20]). For example, resequencing known DNA by inserting minor modifications in the complementary oligonucleotides can detect single nucleotide polymorphisms (SNPs), which are point mutations in DNA found in a part of the population. Similarly, such mutations can help in identifying multiple forms of existence of longer sequences by partial matching. Another advantage of the technique is that since the sequence lengths are small, it is possible to construct high-density chips monitoring relatively larger number of genes.

However, array synthesis is slow and expensive as it uses a large amount of photolithographic mask reagent during synthesis. These problems are overcome in cDNA microarrays discussed below.

3.1.2 cDNA Microarrays

cDNA microarrays were first prepared by the Brown Lab of Stanford University. They improve upon the oligonucleotide arrays by changing the layout strategy in a fundamental way. Using purified mRNA transcripts from tissues, the reverse-transcription polymerase chain reaction (RTPCR) is performed to obtain a large number of gene-specific

polynucleotide clones. Thus, after purification of RNA samples and PCR amplification, the clones are spotted on the array using a non-contact method similar to ink jet printing as shown in Fig. 3.1.

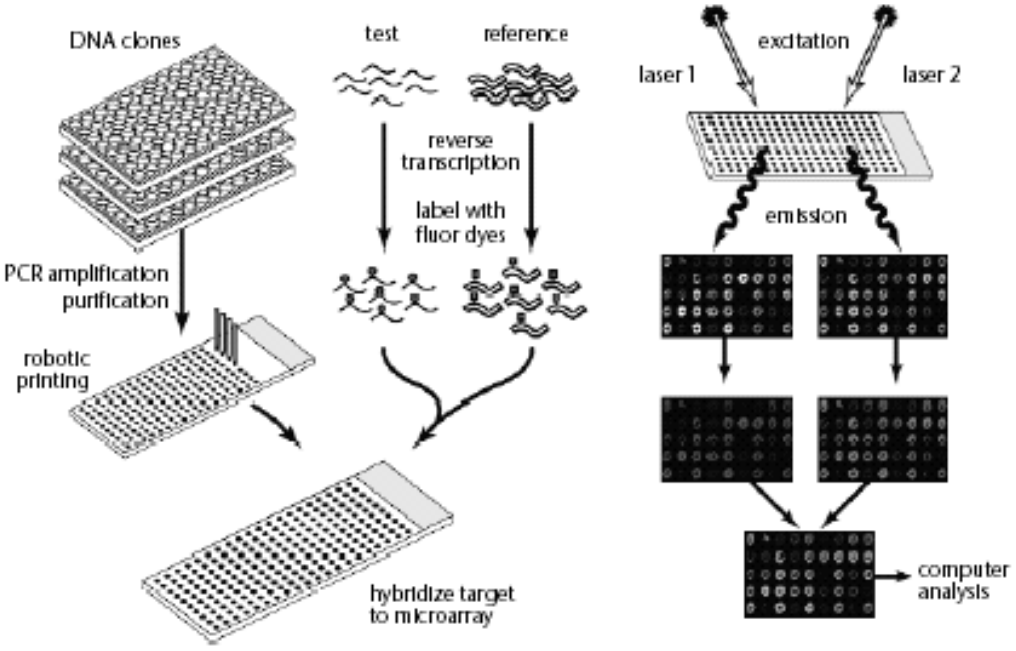


Fig. 3.1 cDNA Microarray Manufacturing [6]

RNA samples obtained from two samples - a test case and a reference/control case, are converted to cDNA sequences by reverse transcription. These sequences are then labeled with two cyanine dyes Cy5 (test) and Cy3 (control). At this point, the array is simultaneously hybridized with the fluorescently labeled cDNA from both samples. The expression values are given by the relative degree of hybridization, which is calculated by image processing software. For example, the two-color hybridization would yield red (ratio

Cy5/Cy3>1) when the gene is induced, or green when it is repressed, or yellow when there are no changes.

The cDNA method of fabrication is quick and less expensive compared to oligonucleotide arrays, and allows the production of oligonucleotides longer than 500 base pairs. The precise arrangement of spots leads to accurate signal measurement. The individual expression values are normalized with respect to extracted subsets of closely related samples.

One chief disadvantage of cDNA microarrays is that it monitors the expression of relatively fewer genes. Since hybridization ratios are not reliable when gene expression is compared across chips, this poses an obstacle for large genomes.

Another problem with cDNA microarrays is that they are limited by the availability of clones for the solid phase and the purity of RNA samples derived from tissues. Further, cDNA microarrays require a large quantity of RNA (usually 50-200 micrograms) per hybridization [6].

3.2 Nature of Microarray Data

Since microarray expression data are going to be the basis for gene function prediction in many applications, we list some of the limitations of microarrays and their role in experimental design.

1. The quality of microarray data depends on its mRNA source. Tissue samples used in *in vivo* experiments might be composed of inseparable cell types, and might show large variability during replication of experiments.

2. With regard to time-series data, it is important to note that individual cycle times of individual processes have order-of-magnitude differences. Expression analysis can be used to reveal interactions at the gene-to-gene level but not at the level of cellular processes/mechanisms. Based on the knowledge of biochemistry of the experiment, sampling should be carefully designed to enunciate valid and significant interactions.

    a. Unwinding of the helix ~ microseconds

    b. Transcription ~ seconds

    c. Translation ~ minutes

    d. Life of a protein ~ hours

3. A microarray dataset represents a snapshot of particular cell lines. This cell 'state' varies significantly based on the environmental conditions, the stage of the cell cycle, etc. Hence, it is essential to collect multiple data points for each gene and base inferences on average values.

4. Measurement of mRNA transcript levels after hybridization might not be a true indicator of protein levels due to post-transcriptional factors (See Sec. 2.3.5). If the proteins are synthesized, they sometimes might not have any physiological consequence in the experiment. In such cases, a combination of the knowledge of

protein interactions and gene expression values might be a good indicator of gene function.

CHAPTER 4: USE-CASE MODELING OF MICROARRAY ANALYSIS

4.1 Introduction

In this chapter, firstly, a brief description of processes in pharmaceutical drug development is given. The impact of the high-throughput microarray technology on processes in pharmaceutical research and development is explained. A UML systems engineering model of an analysis system for modern drug development is developed, that captures the high-level requirements. In the UML use cases, the main actors and their interaction with the system are studied to build a structural model. From the UML model, we construct a database schema for microarray data mining. Finally, we look at alternate system and data architectures for pharmaceutical analysis in an enterprise.

4.2 Overview of Drug Discovery and Development

The discovery and development of drugs involves several stages, and careful planning and allocation of large investments and time. A drug research plan might attempt to target an untreated disease, or improve upon an existing drug using a novel approach. The decision to pursue any project is based on criteria such as the immediate medical requirements, the effectiveness of current products, etc.

According to the 2000-2001 statistics from Pharmaceutical Research and Manufacturers of America (PhRMA), for every 5000 medicines tested, 5 of them pass on to undergo clinical trials, of which only one is accepted ([32]). Considering that the average development cost

for a single drug costs $500 million and 12-15 years and the fact that only 30% of marketed drugs generate revenues in excess of development costs, it is imperative for pharmaceutical companies to investigate the integration of new genomic technology in dealing with their lifecycle cost breakdown.

4.2.1 Traditional Drug Discovery

Fig. 4.1 shows an approximate distribution of the times involved in the stages of traditional drug development [34].

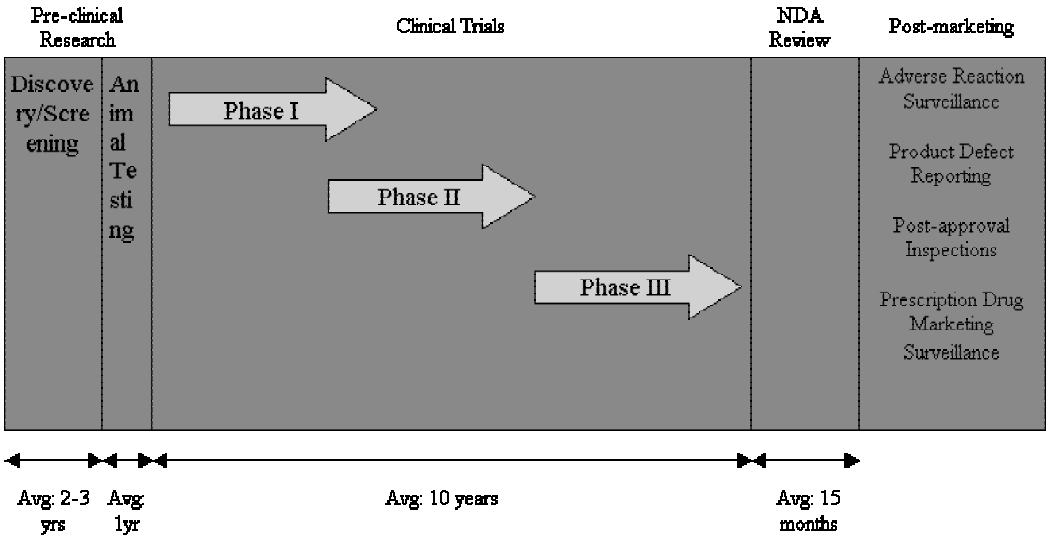


Fig 4.1 Traditional Drug Discovery Life Cycle

Drug discovery is a complex process with repetition and is characterized by many trials. The process is initiated by investigating the biochemistry of the disease. Drugs based on biochemistry produce their desired effects by acting on small protein molecules called receptors located in the cell membrane. Receptors monitor extra-cellular activity and are normally activated by hormones, whereby they undergo shape modifications and trigger

cellular responses. Since receptors are connected with signaling pathways, they are able to swiftly affect cellular mechanisms by reacting with drug molecules.

The molecular biologist uses biochemical pathways participating in the disease pathophysiology to form a hypothesis about the chemical reactions involved. Common drug targets chosen are those that code for enzymes, transporters, and hormone receptors, since they can be easily controlled by small external molecules.

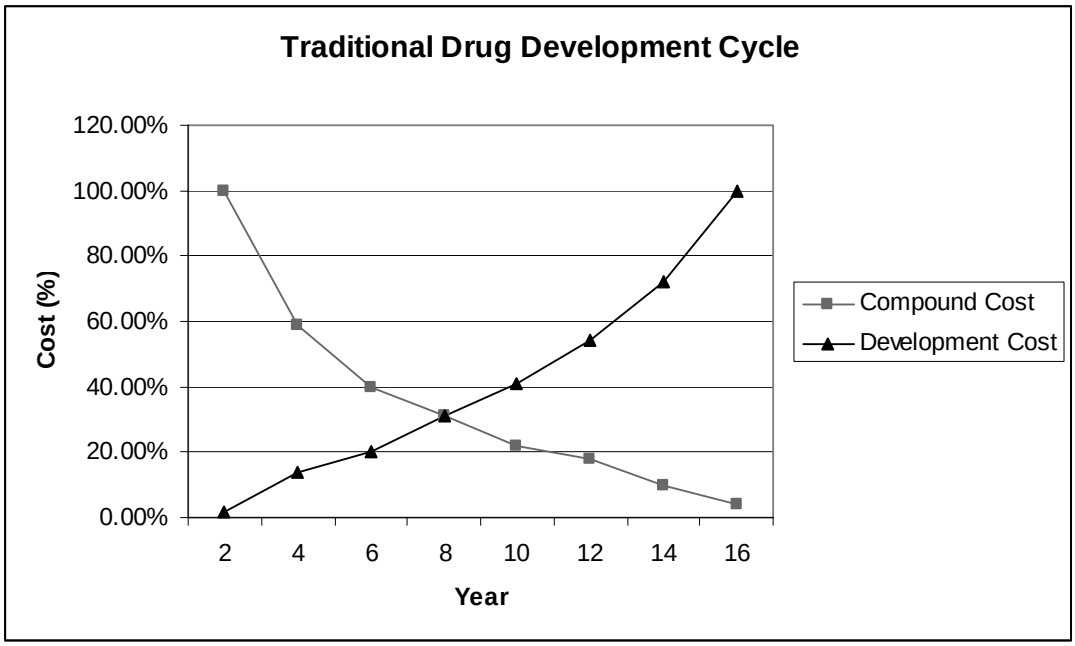
Feasible lead compounds are selected based on the knowledge of their structure and action. A majority of these lead compounds arise from natural extracts, which have been discovered and proven effective previously. The targets are purified and screened against a variety of lead compounds. The lead compounds are filtered based on their effectiveness on the drug target. They are then optimized by combinatorial chemistry techniques to form new compounds with greater specificity. Pre-clinical testing involves *in vitro* testing on tissue samples and *in vivo* testing on animal models (when available) for compound toxicity. This set of compounds is filtered further to evaluate their side effects, dosage, etc. on a larger population during the long and expensive clinical trials process.

There are many drawbacks and implicit limitations in the above procedure, in the current context. The selection of targets is limited by the knowledge of their molecular function. The proteins that some gene targets encode, like transcription factors, are not easy to modulate. In the case when the molecular nature of the target is not known, random screens are performed against thousands of lead compounds, which consume resources, time and expenses. By having a large number of compounds after screening, the cost of testing is

carried over to the expensive development and clinical trials phases. In cases where pre-clinical testing can be carried out in animal models alone, the same lead compounds might not be effective in human tissues, as some receptors are very species-specific; this risk is carried on to the expensive clinical trials process.

Research and pre-clinical testing are the steps where automation and new technology can play an important role in reducing process times and carry-over costs. The sequencing of the human genome, miniaturization and automation of key biological processes, high-throughput techniques like microarrays and the increasing integration of public information can dramatically reduce the time, risk, and expenses involved in the drug development life cycle.

Using microarrays, it is possible to screen lead compounds against all known genes and filter out fewer compounds with greater accuracy and possibility of success. This is illustrated below in Fig. 4.2 by the percentage expenditure in terms of compound and development costs involved in these steps.



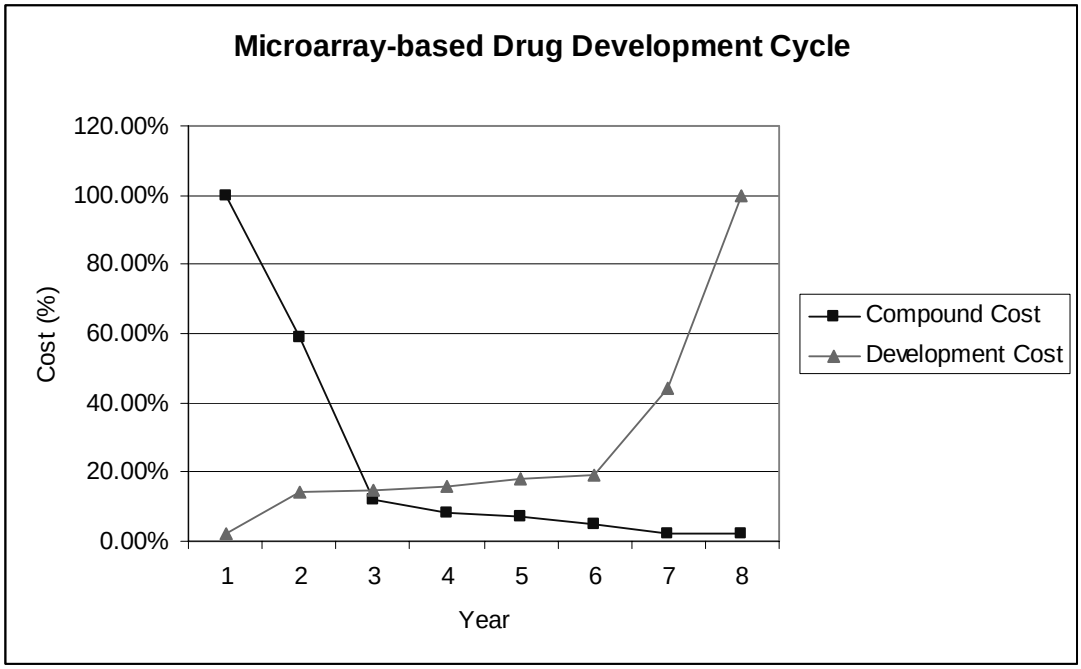


Fig 4.2 Comparison of Life-cycle Cost Variation

In comparison with Fig. 4.1, the following is an estimate of how micro-array data processing affects process costs and times for each stage of the life cycle, by bringing down the number of pre-selected compounds ([33, 35]).

- Research & Pre-clinical Testing: Average 18 months
- Clinical Trials (on human subjects): Average 5 years.

Some examples of the use of new technologies in drug discovery are listed below.

1. Sequence information opens up a large number of new feasible drug targets. It is possible to conduct genome-wide experiments with microarrays, which have much lower turnaround times compared to traditional polymerase chain reaction (PCR) techniques. Gene sequencing from the human genome project is expected to increase the number of gene targets for drug innovation from 500 to 3000-10000 [8].

2. Diseases like lymphoma and viral infections require drugs that can target the transcriptional mechanism. Genes related to such cases can be targeted using gene expression profiling.

3. A single-nucleotide polymorphism (SNP) is a point mutation that represents a subset of a large population. SNPs are strong markers that can be used in association studies to identify correlations between the presence of a chromosomal region and any trait such as a disease phenotype. Microarrays can be used to study drug response in diverse genotypes in clinical trials.

4. Pre-clinical trials can make use of microarrays to conduct toxicology experiments and to study *in vitro* testing.

5. Since gene expression is a clear indicator of function, functional prediction of target genes can lead to rational drug design during the lead identification phase.

6. Gene expression in different experiments and across time points is a fair indication of gene function. However, in some cases, mRNA levels might not give an indication of protein levels, due to post-translational factors (Sec. 2.3.5).

4.2.2 Modern Drug Discovery

Using scenarios of the use of microarrays in modern drug development, a detailed description of the processes involved in drug discovery is given below. Based on this, the flow of events is illustrated in the activity diagram of Fig. 4.3.

1. **Target Identification and Validation**

Target identification is an exploratory phase that involves hypothesizing disease-causing genes with evidence that can arise from multiple sources. The molecular biologist uses the knowledge of biochemistry of the disease and associates known targets with new genes of unknown function through information about DNA sequence, single nucleotide polymorphisms (SNPs), and population genetics. In cases where little prior knowledge is available, studies can be based on parallel results from model organisms, or differential expression profiling of normal and diseased tissues. Information on pathways involving these targets and sequence homology is also used to suggest alternate genes that can be attacked.

In the target validation step, microarray-based experiments are conducted on the individual genes to determine their molecular function and interaction with others under different cellular conditions. They are then filtered by their cellular response and marked as potential drug targets.

2. Lead Identification and Validation

Biochemical assays of target gene products are developed in a closely similar environment for *in vitro* testing. Since the target function may be determined or unknown, they are screened 'rationally' or through random screens against compound library. The compound library is composed of thousands of synthetic chemicals and natural products.

Cell-based assays, on the other hand, represent animal and cellular models of the disease. They are used for *in vivo* testing, and provide more accurate information on drug action inside the body. While biochemical assays identify lead compounds for a threshold level of drug action in relevant pathways, cell-based assays also test their potency in being able to act on cellular models.

These lead compounds are filtered further by studying their specificity, cellular response, toxicity, and other pharmacological and chemical properties. These validated leads are characterized by structural properties, which can be found from databases like MDL/ISIS. Using combinatorial chemistry techniques, they are further optimized by synthesizing lead compounds with these properties and improved activity on the drug targets.

3. Pre-clinical Testing

Pre-clinical testing determines the toxic effects of a particular drug on secondary drug targets, similar to the lead validation phase. Proteome analysis can be used to determine if the cell is in a natural state, or showing a specific response mechanism, or an unspecified response. The subset of proteins showing the response can be analyzed further. These results can support future characterization of lead compounds during the previous phase.

4. Clinical Trials

In this phase, the drug discovery process is reviewed and clinical trial experiments are designed to be implemented in the following order.

a. Phase I: Determine potential side effects and dosage of the drug by administering on 20-80 healthy volunteers.
b. Phase II: Determine effectiveness on a small number of volunteers with the disease.
c. Phase III: Determine large-scale effectiveness on 1000-3000 patients with the disease.
d. Regulatory Review and Approval by the FDA.
e. Post-marketing surveillance: Medical practitioners continue to monitor the drug's safety and efficacy over a much larger population with the disease.

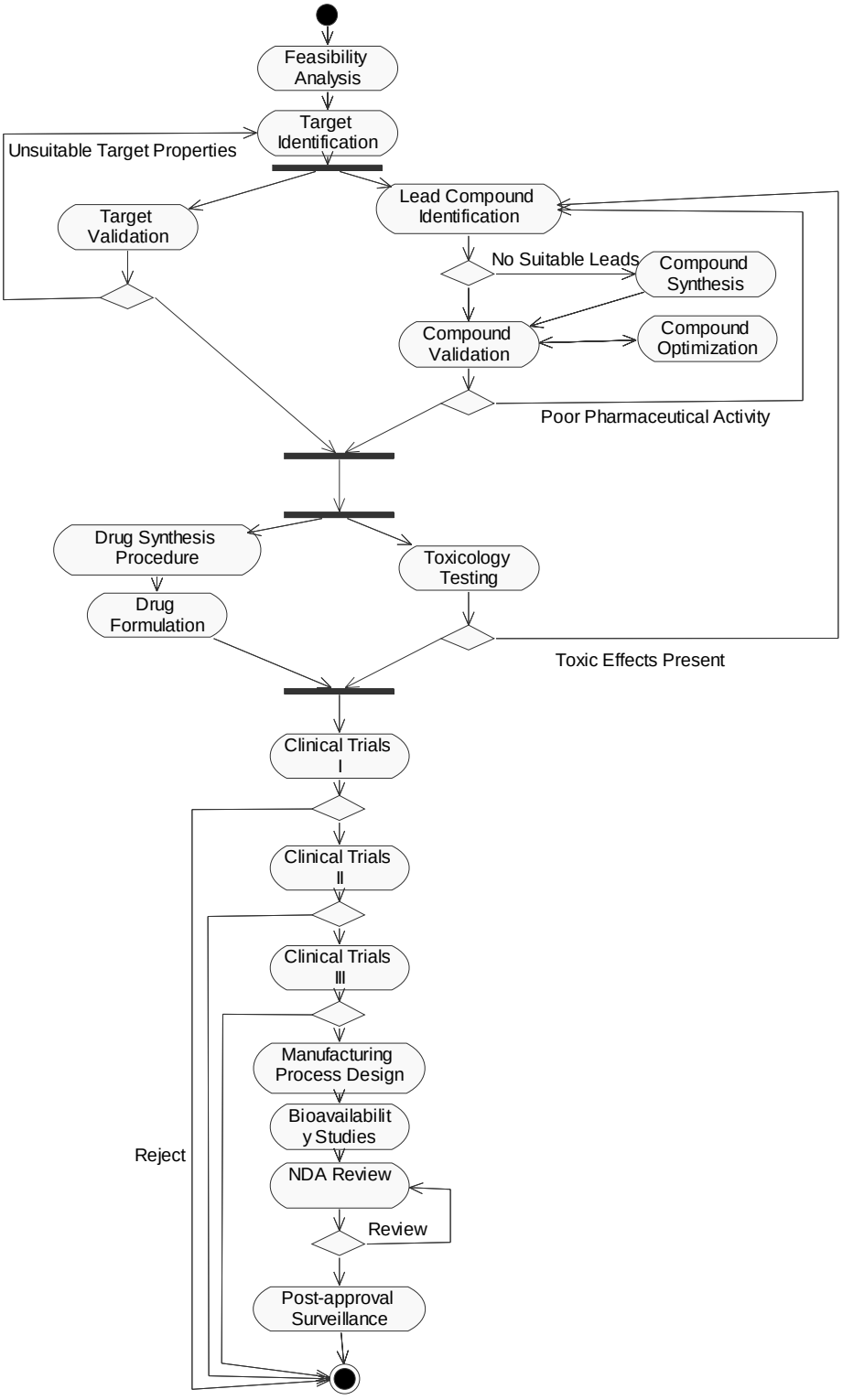


Fig. 4.3 Business Process Model

4.3 System Description and System Requirements

A pharmaceutical corporation typically consists of hundreds of users of different backgrounds such as biologists, chemists, bioinformaticians, clinical scientists, program managers, and administrators. Users conduct analyses on project-related (transactional) data such as from experiments, previous analyses, processes, etc. and aggregations of project data (analytical) at the corporate-level. The applications implementing business logic are handled by computing on distributed hardware. The broad requirements of an analysis system within such an enterprise for the modern drug development process can be listed as follows:

1. Data mining across distributed public and corporate databases.
2. Storage and retrieval of user-specific analyses.
3. Access to archived and current project data such as process status, materials, analyses, etc. for tracking and prediction in research.
4. (Restricted) Corporate-wide access ability for departmental data stored in a pre-defined schema/format.
5. Controlled access to different users and customized interfaces for visualization/data mining.
6. Ability to integrate modules of new functionality with minimal configurational changes to the system.
7. Database-independent data and results transfer.

4.4 UML Requirements Modeling

In this section, we model the high-level requirements of the pharmaceutical analysis system with UML use cases. These use cases represent unit-transaction scenarios where the users (actors) interact with system components during different phases of the drug development life cycle. Knowing the nature of these interactions allows us to create the system structure in terms of the data model for database system design. This process is an iterative one, where the structure is related back to the initial requirements model and modified if any conditions are ambiguous or not met.

The following use cases attempt to model the requirements of a pharmaceutical corporation in terms of microarray data processing as rigorously as possible. However, when we derive the class structure from the UML model, some schema elements such as lead compound properties, storage of results from different public databases, and so on are deliberately left ‘masked’ or undetermined to keep the implementation from becoming too specific while keeping the model as accurate as possible.

The main actors of the model include the product development manager, computational biologist/chemists, molecular biologists, pharmacologists, chemists, clinical scientists, technical managers, system developers, and lab managers. They relate to the drug development processes that are shown in Fig. 4.3. The use cases are grouped by the users involved in these major processes. Entities like the corporate knowledge base, public databases, and transactional databases in the use cases denote subsystems, and are denoted as actors and abstract entities themselves. They have a detailed structure for different phases of the process, which will be derived in Section 4.5 on system structure.

4.4.1 Use Cases Associated with a Biologist

4.4.1.1 FORMULATE TARGET HYPOTHESIS: This use case deals with the biologist's research on feasible targets and results reported to the transactional database. The biologist queries public databases and the corporate knowledge base about the biochemistry of the disease. The system collates information and returns the pathways involved, disease categories, and related targets (genes, receptors, enzymes, and other proteins). It also retrieves experimental data from normal and diseased cells, and treatments with several compounds, references, and so on. The biologist stores the results of the search, including the target, its type, associated diseases, and pathways involved, in the transactional database.

4.4.1.2 IDENTIFY AND VALIDATE TARGET GENES: The use case provides shared behavior for the specific use cases such as Find Similar, Find Discriminating, Pre-filter and Cluster, and Search. It reports the results of experimental findings on genes from (4.4.1.1) to the transactional database.

With the leads from (4.4.1.1), namely genes involved and corresponding experimental data, the biologist performs different kinds of analyses. The biologist also specifies experimental protocols to obtain differential expression data on the activity of the feasible targets in normal and disease cells. The system retrieves analysis results based on the criteria and receives analysis reports about the resultant set of genes, experiments analyzed, similarity measure used, feature analyzed, threshold similarity (if applicable), and data values used (raw values, normalized logarithmic values, and so on)

4.4.1.2.1 FIND DISCRIMINATING: The biologist tries to find (gene or chip) expression profiles are able to discriminate two classes of profiles the most. The system retrieves these classifier-boundary instances to examine their properties further in context.

4.4.1.2.2 FIND SIMILAR: This is a high-level case that can be further specialized by specifying qualifiers such as similarity in compound structure, gene sequence, target structure, or microarray expression profile. The system uses appropriate or specified analysis algorithms for retrieval.

4.4.1.2.2.1 FIND SIMILAR PROFILE: The biologist tries to find similar expression profiles over an experiment's chip set. He can also pick an interesting profile, such as those obtained from (1.2.1) and find profiles, which are closest to it.

4.4.1.2.2.2 FIND SIMILAR TARGET: The computational biologist queries structure databases like MDL/ISIS to find targets with similar structural and functional features to a known one.

4.4.1.2.2.3 FIND SIMILAR SEQUENCE: The biologist queries for genes with similar sequence to a given sequence. The system can return, for example, E-values from BLAST searches for genetic sequences.

4.4.1.2.3 PRE-FILTER AND CLUSTER: This case builds on the previous two use cases.

The biologist queries genes with a chosen threshold activity or other criteria. This is done to eliminate redundant or irrelevant features and to increase the efficiency of clustering. He then partitions (the algorithm, similarity measure, and number of clusters can be specified) co-expressed profiles. He further analyzes the results by

clustering over genes or chip profiles. The system executes server-side algorithms, retrieves the results, and displays them in a visualization tool.

4.4.1.2.4 SEARCH: Like (4.4.1.2.2), this is also a generic use case, which can be specialized for searches on various criteria.

The biologist queries several public databases and the corporate knowledge base and executes tools to find genes with high similarity in terms of sequence, structure, or genetic linkage, to the available genes. For instance, the results of a BLAST query on sequence similarity can be stored in the form of analysis type, gene sequence, BLAST E-value, result set, and other parameters. The system collates information from disparate databases and returns the results. The analysis reports stored by the system will also have similarity measures based on multiple criteria along with those described in (4.4.1.2).

4.4.1.3 BUILD DISEASE MODELS: The biologist accesses corporate/in-house and public references, and builds disease models to simulate or predict the target's response to different compounds, if the function of every gene in all pathways where the target gene is involved, is known. He stores his model in the transactional database. The results from the target validation phase are submitted for the approval of the product development manager.

4.4.2 Use Cases Associated with a Chemist

4.4.2.1 IDENTIFY LEAD COMPOUNDS: The chemist obtains the list of probable drug targets from (4.4.1.3). He queries the in-house and public compound libraries and references for target structure and previous results of effective structural (this

methodology is called rational drug design) and other properties of compounds for targets with known molecular function. For example, these include queries on structural databases like MDL / ISIS. The system retrieves results and stores the hypothesis on feasible lead compounds, listing the compound, its structure, the target and its structure, target type, references, related diseases, and other compound properties.

4.4.2.1.1 PREDICT TARGET STRUCTURE: If the target site function is not known in (4.4.2.1), the chemist and the computational chemist query the compound library for functional groups with a wide range of structural properties and activities, and perform experiments on the target by repeated addition of these groups (function site mapping). The system executes algorithms to predict function and returns the compound set. The results are stored in the form of the target, its predicted structure, and its geometric and chemical properties. Then, suitable compounds are found as described in (4.4.2.1).

4.4.2.2 PREPARE AND TEST WITH BIOCHEMICAL ASSAYS: The chemist prepares the protocol for biochemical (*in vitro*) and cell-based (*in vivo*) assays in normal and diseased cells, specifying genes, compounds to be tested, organism, experimental conditions, cell stage, etc. A request is submitted to the laboratory subsystem. The system retrieves and stores the experiment information, the assay protocol, and the data in the transactional database. The chemist filters compounds in biochemical assays based on a minimum level of drug activity over at least a chosen proportion of target genes. Further, he filters the compounds in *in vivo* testing, based on cross-validation with action on regulatory pathways and toxicity measurements. The system retrieves information on pathways of the tested targets and other genes in the

assay. The analysis results stored include the resultant compound set, experiments analyzed, activity level, and threshold activity.

4.4.2.3 OPTIMIZE LEAD COMPOUNDS: The chemist studies the structural properties of the lead compounds and synthesizes new compounds using computer models of the reaction mechanisms and combinatorial chemistry tools. He documents the rationale, synthesis procedure and uses the same assay protocol for testing. The screen results are submitted for validation.

4.4.2.4 FORMULATE DRUG SYNTHESIS AND DOSAGE: The chemist implements and records the procedure to make any novel candidate lead compound and tests the purity of the product.

4.4.3 Use Cases Associated with a Pharmacologist/Toxicologist

4.4.3.1 VALIDATE COMPOUNDS: This use case provides shared behavior for toxic testing in the lead validation and pre-clinical testing phases.

The toxicologist filters compounds in biochemical assays based on a minimum level of drug activity over at least a chosen proportion of target genes. Further, he filters the compounds in *in vivo* testing, based on cross-validation with action on regulatory pathways and toxicity. The system retrieves information on pathways of the tested targets and other genes in the assay. The analysis results stored include the resultant compound set, experiments analyzed, activity level, cellular response, pharmacological and chemical properties, and threshold activity.

4.4.3.2 DETERMINE TOXIC EFFECTS: The pharmacologist and toxicologist perform toxic studies on primary and secondary drug targets as described in (4.4.2.3) on animal cells. They query proteome information to determine the nature of cell state. They document characteristics such as cellular response and drug selectivity, potency, and toxicity.

4.4.4 Use Cases Associated with a Clinical Scientist

4.4.4.1 DETERMINE STUDY PARAMETERS: In the clinical trials phase, the clinical scientist determines parameters for drug experimentation such as normal dose ranges, expected values, measurement techniques, and equipment required.

4.4.4.2 DEVISE EXPERIMENTAL PROTOCOL: The scientist prepares a case report form to study the drug effects on patients, prepares schedules, dosage, etc.

4.4.5 Use Cases Associated with a Lab Manager

4.4.5.1 IMPLEMENT EXPERIMENT PROTOCOLS: The lab technician obtains the experimental protocol for microarray and assay development from the drug discovery team. He co-ordinates and documents procedures for sample preparation, hybridization, normalization, quality check, etc. using a LIMS (Laboratory Information Management System) tool. The system stores the raw experimental data in the transactional database.

4.4.6 Use Cases Associated with a Product Development Manager

The Product Development Manager oversees the progress of different project groups working in the firm.

4.4.6.1 CONDUCT FEASIBILITY ANALYSIS: The manager picks a preliminary research area based on the current demand, knowledge of competing brands, etc. He queries the corporate knowledge base for availability and potential of compounds in the

company's compound libraries for new products, and the performance and viability of similar projects. He then initiates a research project. The system collates information across projects, analysis results, financial and other corporate data for business decisions.

4.4.6.2 ALLOCATE RESOURCES FOR PROJECTS: With simultaneous drug development projects in progress, the manager allocates personnel to specific project phases. He makes decisions on manufacturing or purchasing resources such as chemical compounds, assays, etc.

4.4.6.3 MONITOR THE PERFORMANCE OF PROJECT GROUPS: On the basis of the performance of ongoing and past projects, the manager can allow or revoke the continuation of a particular project phase. For instance, this might be in the form of the following queries: 'Which projects have been more productive in terms of the number of leads?'

4.4.6.4 CONDUCT PEER REVIEW: The peer review team, involving the product manager, reviews the analyses results at different checkpoints during the drug discovery life cycle. They approve the transfer of new results at the end of individual sub phases into the corporate database, and allow other research teams to make use of these results.

4.4.7 Use Cases Associated with a Computational Biologist/Chemist

4.4.7.1 FIND SIMILAR:

4.4.7.1.1 FIND SIMILAR TARGET: (As in 4.4.1.2.2.2)

4.4.7.1.2 FIND SIMILAR COMPOUND: The computational chemist tries to find compounds with similar activity and physical properties to a compound known to

produce desired therapeutic response on a given target. He performs a large number of experiments with a wide variety of compound chemistries. The system runs correlation methods like QSAR (Quantitative Structure Activity Relationship) and stores the results in the form of the target, the compound, its QSAR activity score, and its structure.

4.4.7.2 PREDICT TARGET STRUCTURE: (As described in (4.4.2.1.1))

4.4.7.3 DESIGN LIBRARIES: The computational chemist uses combinatorial chemistry techniques to determine compounds with high activity scores on a chosen target. The results obtained are similar to (4.4.7.1.2)

4.4.7.4 PREDICT COMPOUND PROPERTIES: (Similar to 4.4.7.1.2) The system runs correlation methods like QSPR (Quantitative Structure Property Relationship) to predict the chemical properties given the compound structure.

4.4.8 Use Cases Associated with a Technology Manager

4.4.8.1 ORGANIZE REQUIREMENTS: The technology manager represents the domain experts from different areas of research and testing in the corporation. He studies new technology and current shortcomings in the system, and prioritizes new requirements from different users. He communicates with the system developer to assess and improve the structure and functionality of the system.

4.4.9 Use Cases Associated with the System Developer

4.4.9.1 OBTAIN REQUIREMENTS: The developer obtains requirements from the technology manager, and interacts with him to understand how the system will be used. Changes to the system are to be made incrementally, after new requirements come in.

4.4.9.2 DESIGN KNOWLEDGE BASE: The developer designs the database to organize current as well as archived data and results. He creates a client-server model of microarray analysis, and designs the interfaces for different users.

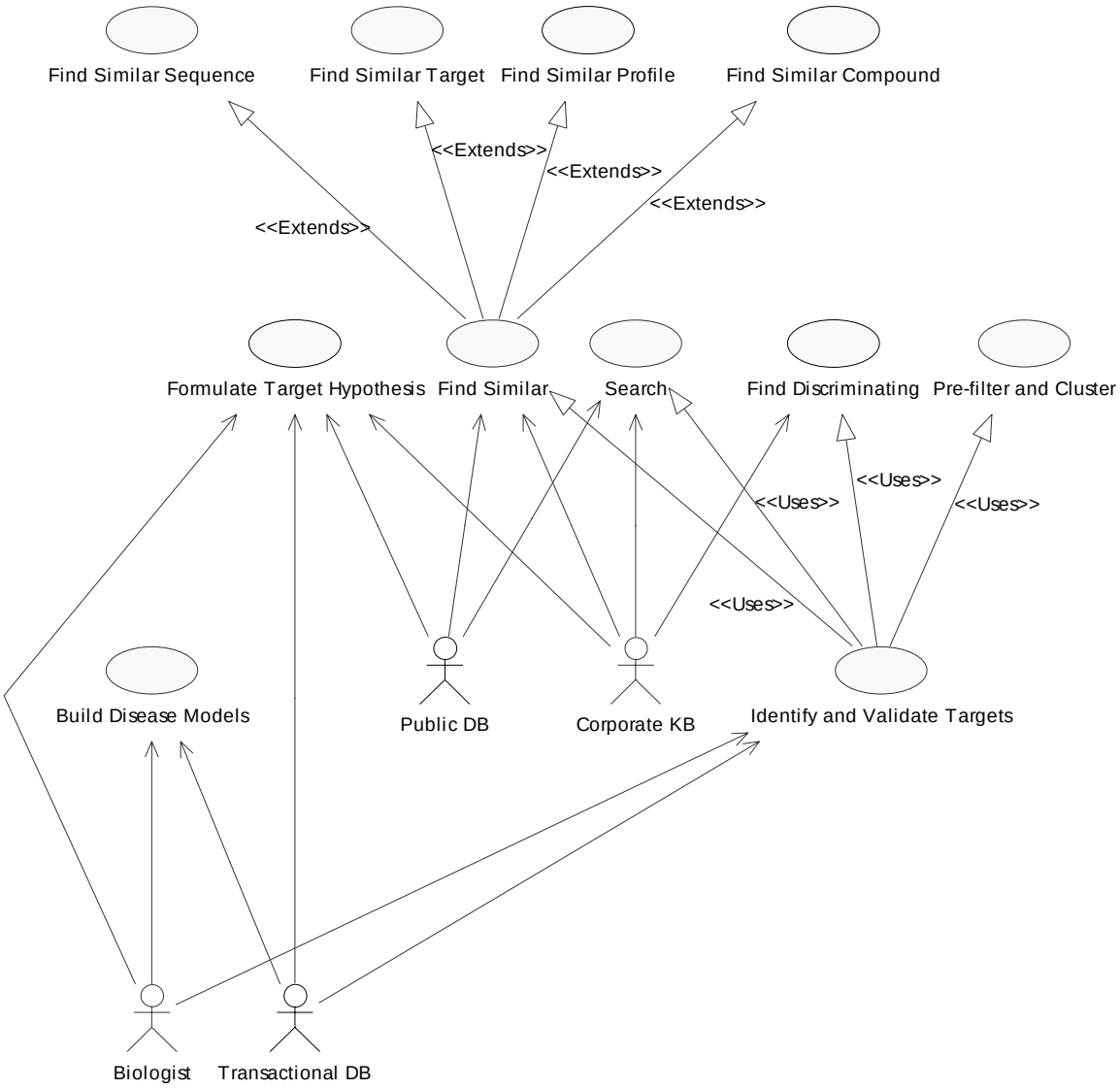


Fig. 4.4 Use Cases for Target Identification and Validation

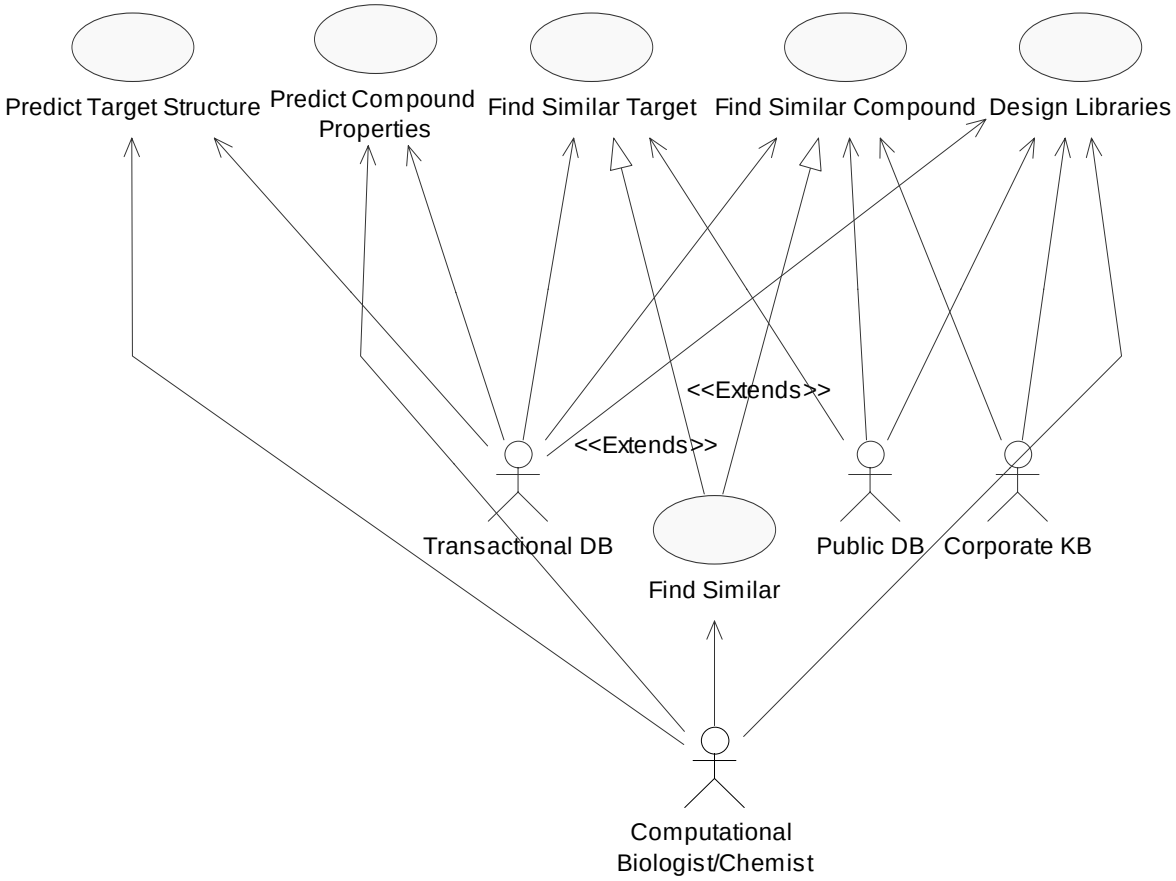


Fig. 4.5 Use Cases for the Computational Biologist/Chemist

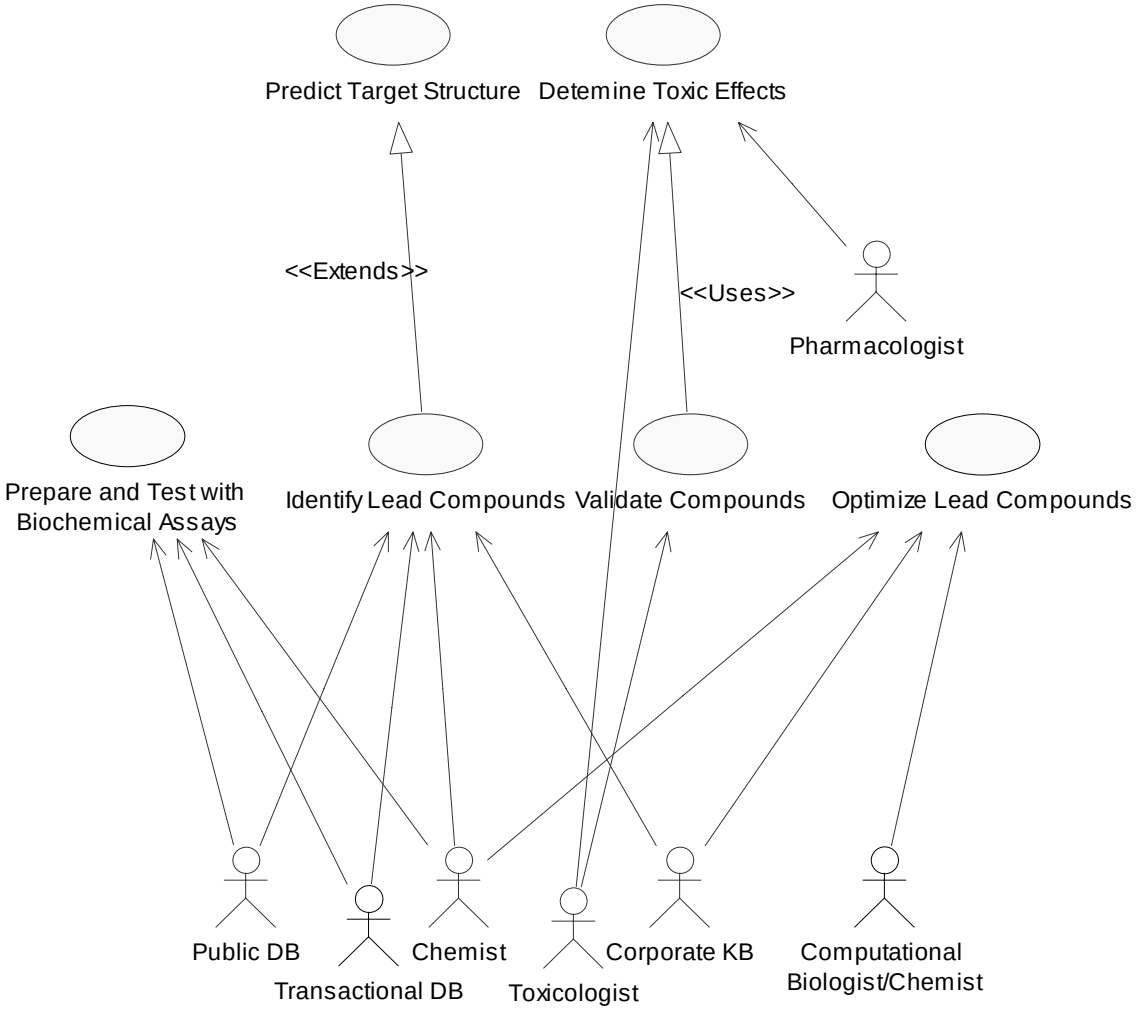


Fig. 4.6 Use Cases for Compound Identification and Validation, and Pre-clinical Testing

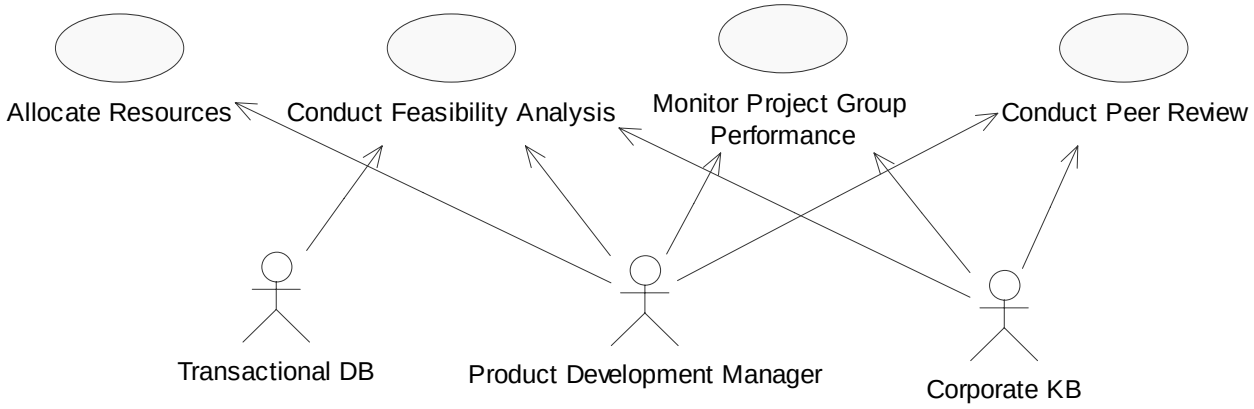


Fig. 4.7 Use Cases for the Product Development Manager

4.5 System Structure

The UML use case model described in the previous section used very abstract definitions of the system components that the actors interact with, like the TransactionDB and the CorporateKB. This section goes into more details by first grouping use cases based on their interaction with the analysis system and deriving a package diagram for system architecture. The individual classes are then defined to create the system structure.

4.5.1 Package Architecture

The following lists the mapping between related use cases and the packages. Some of the packages can directly translate to database schema, if all the constituent objects are persistent.

1. Use cases such as Formulate Target Hypothesis (4.4.1.1), Find Similar Target (4.4.1.2.2.2), and Predict Target Structure (4.4.2.1.1), chiefly involve querying the in-house knowledge base on target properties such as the geometrical structure and relevant pathways. This is characterized by the Target subsystem. The attributes and operations of the Target class can be further specialized for individual target types such as receptors, enzymes, and other proteins.

2. In a similar manner, Identify Lead Compounds (4.4.2.1), Optimize Lead Compounds (4.4.2.3), and Find Similar Compound (4.4.7.1.2) use cases make extensive use of a compound's structural and chemical properties. The Chemical Compound subsystem handles these features.

3. A large number of use cases such as those involved with target or compound identification and validation retrieve and query archived experimental data. These characterize the Experiment subsystem, consisting of diverse data like raw data, normalized expression values, etc. for many protocols, and array and experiment types.

4. Many of the hypothesis-related use cases also use public genomic and structural databases. The PublicData subsystem can consist of collated information from these databases like pathways, structure, sequence, and homology information, which can also be retrieved on-demand. It can also provide access to Internet-based tools like BLAST for sequence comparison.

5. Frequent upgrades of the public databases, archiving validated information or completed projects, as well as access control privileges and maintenance is handled by the DB Administration subsystem.

6. The LIMS subsystem deals with laboratory techniques and protocols in the acquisition and preparation of diverse samples, assays, and microarrays.

7. Finally, the documentation of analysis steps and results from use cases in the target and lead compound identification and validation phases, are stored in the Analysis subsystem. This might also consist of archived results from use cases like Predict Target Structure (4.4.7.2), Design Libraries (4.4.7.3), Predict Compound Properties (4.4.7.4), Conduct Feasibility Analysis (4.4.6.1), and so on. The storage of data in this subsystem can be similar to a data warehouse and is used by all the major users of the system, making it the most important component of the system. It may be further specialized for target and compound analyses.

Fig. 4.8 shows the high-level package diagram for the analysis system. The arrows indicate the dependency of packages on each other. The following section discusses the individual classes in each subsystem.

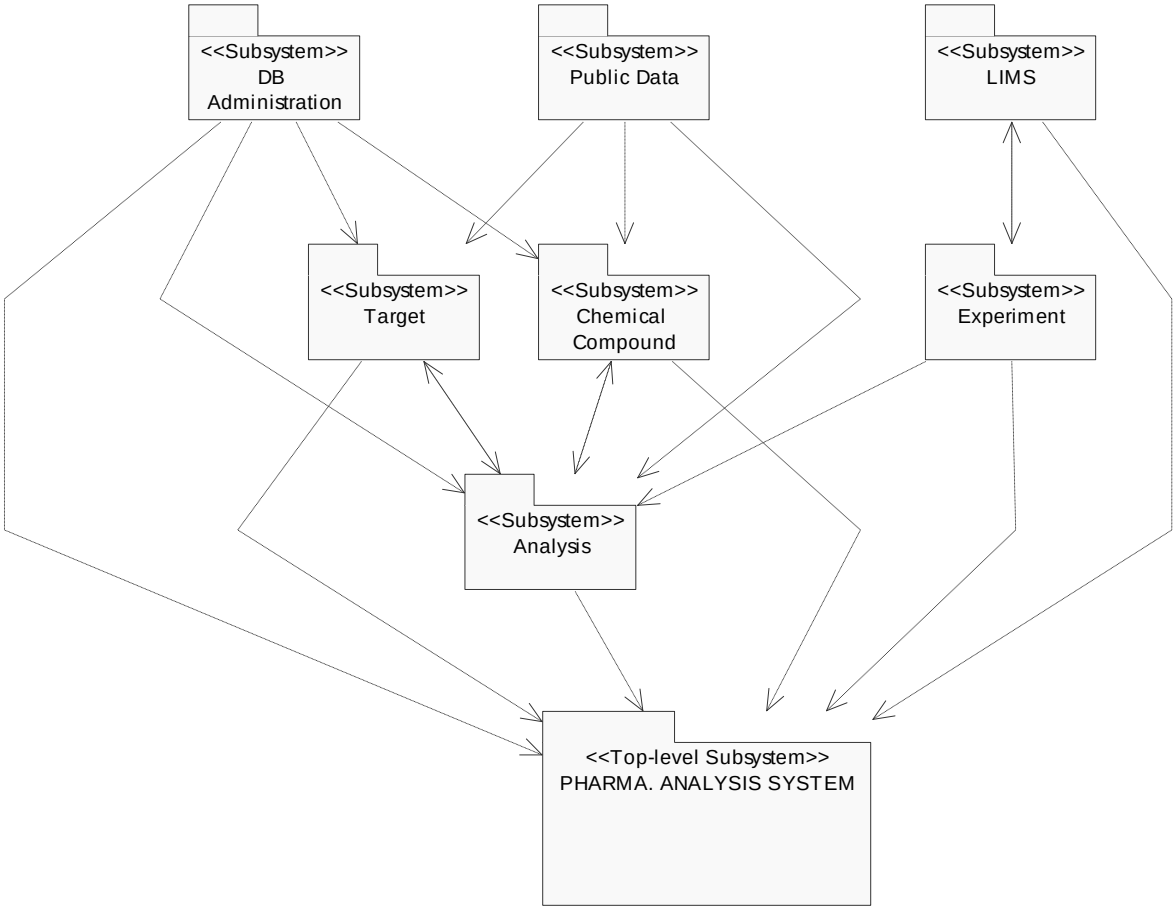


Fig. 4.8 Package Diagram

4.5.2 Class Diagrams

Figures 4.9-4.11 show the class models for the subsystems outlined in Sec. 4.5.1. In this section, we will be concerned about the data model alone. The operations defining the behavior of classes are discussed in Sec. 4.6.

A representative structure of classes in the Public Data subsystem is shown in Fig. 4.9. This might involve collated information from knowledge bases like the characteristics of a

disease in Disease_Phenotype and the MYGD (MIPS Yeast Genome Database). Or, it might include data from primary databases like KEGG pathways, and related genomic information shown in Pathway, Pathway_Map, and related classes.

Fig. 4.10 shows the Target, Compound and Analysis subsystems. The classes Target and Compound allow indexing across different chemical and physical properties. They can also be linked to an external structural database in the Public Data subsystem. The Analysis class shown here stores a report of a scientist's study. This leads to its documentation in Analysis_Steps, of the experiments, methods used, and their parameters. As a specific example in the case of target and compound validation, the TC_Analysis class extends the Analysis_Steps class to include specific functions like finding significant genes/compounds and search. The TC_Analysis also permits the execution of ad hoc queries through the Random_Query interface. This extends the functionality to use cases like Conduct Feasibility Analysis (4.4.6.1) for other users.

Finally, Fig. 4.11 shows the Administration and Experiment subsystems. The former merely shows the relation between large projects with many project groups and users. Details regarding user restrictions and other maintenance criteria are not discussed further here. The Experiment subsystem links the User class with experiments conducted by an individual. Each Experiment class object corresponds to many individual chips (environmental conditions) and each chip is defined by a protocol and parameter set.

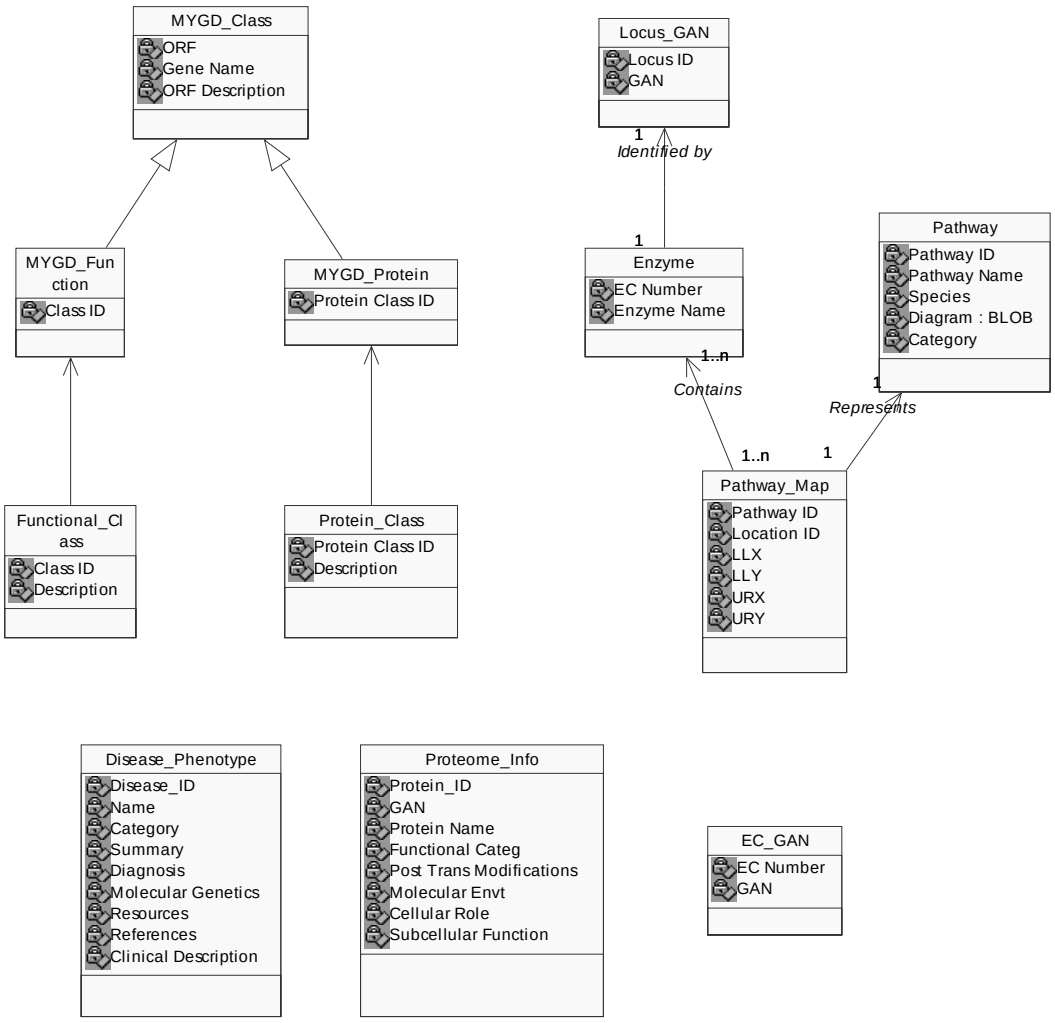


Fig. 4.9 Public Data Subsystem Class Diagram

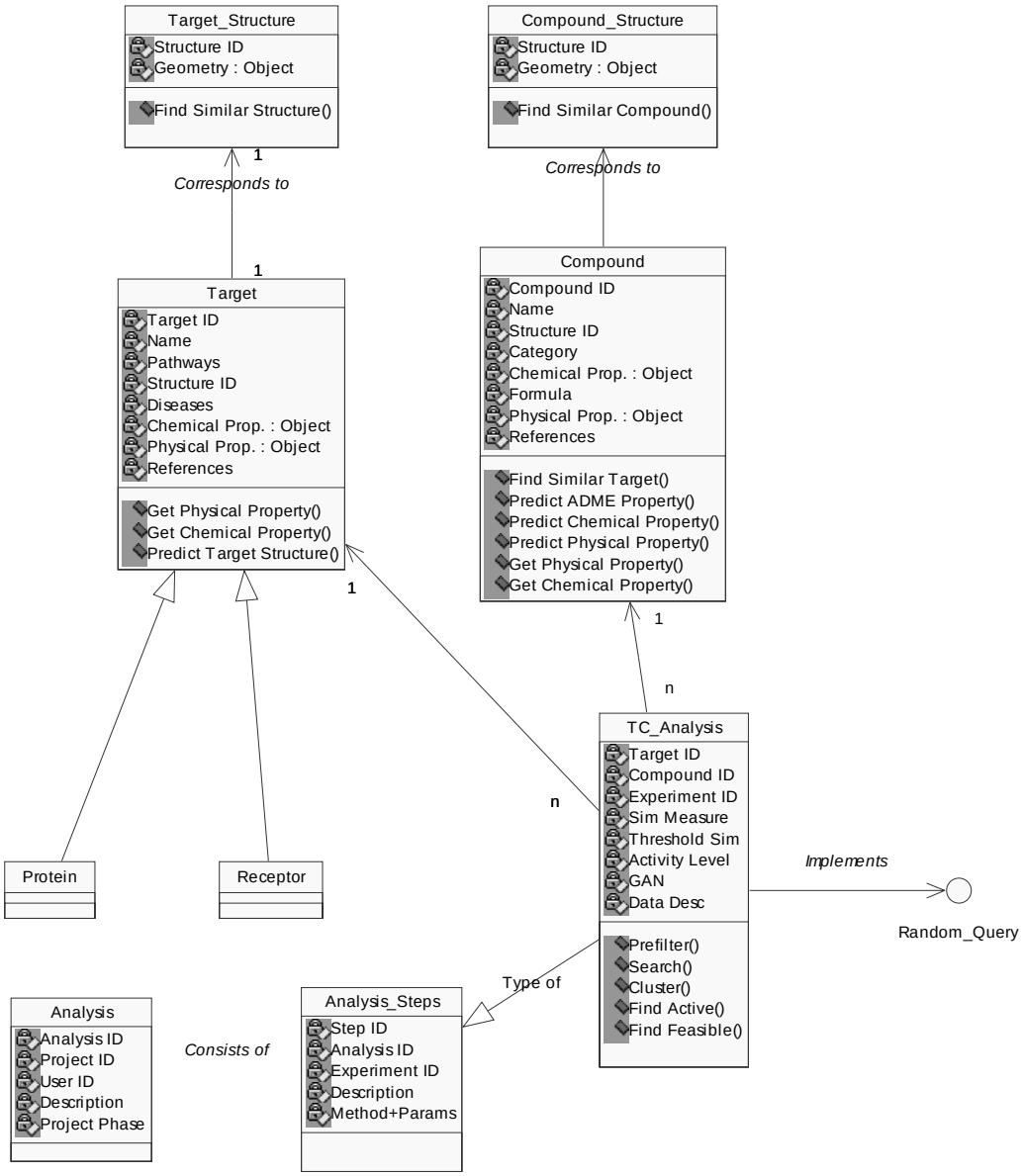


Fig. 4.10 Target, Compound, and Analysis Subsystem Class Diagram

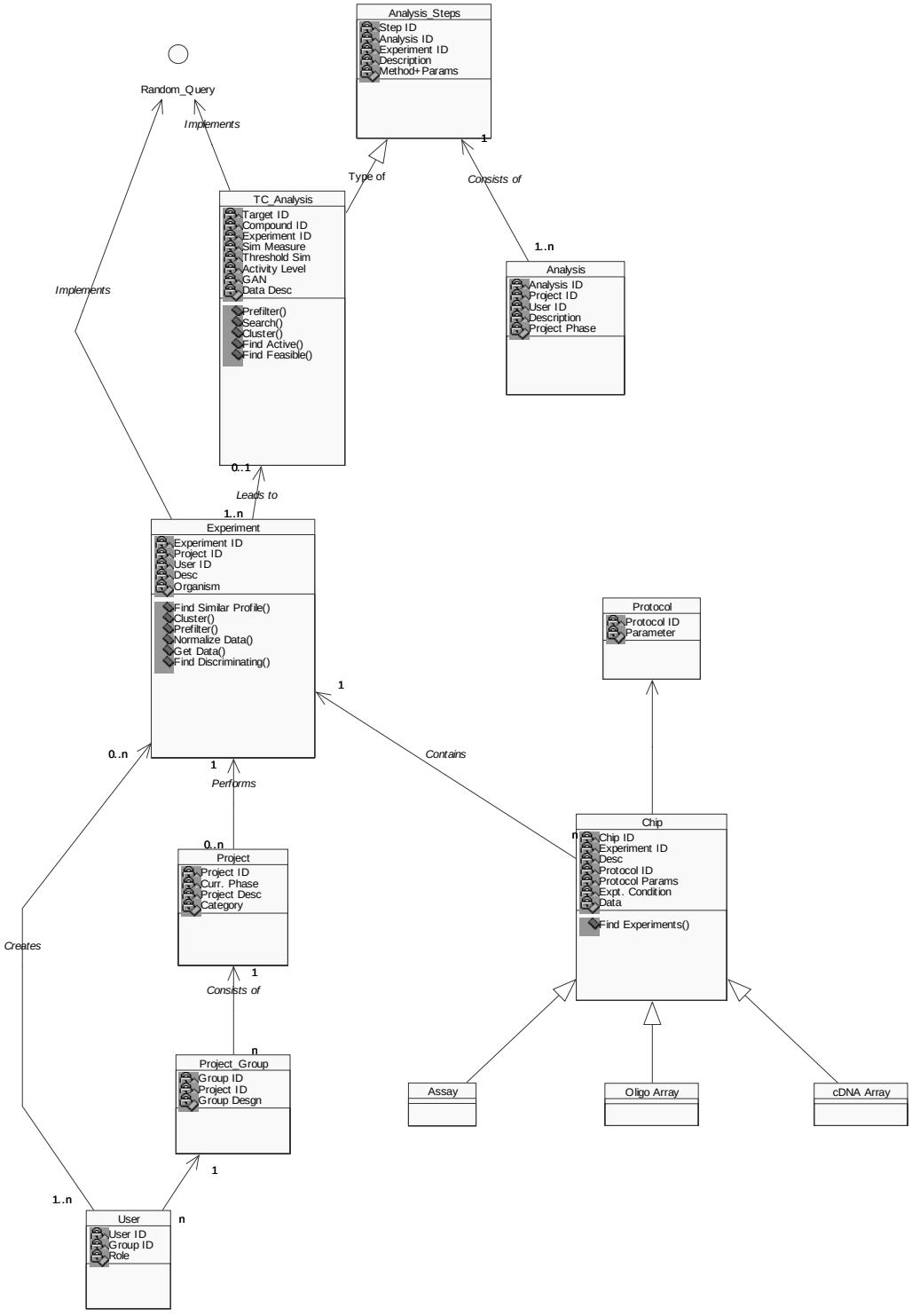


Fig. 4.11 Experiment and Administration Subsystem Class Diagram

4.6 System Behavior

Modeling system behavior allows us to capture what the system does, without specifying the actual implementation used. It thus allows us to simulate time-dependent execution of different scenarios and study them for correctness and efficiency.

In Sec. 4.3, we defined the system requirements that specify behavior at the highest level. In this context, we now define the low-level functions, their inputs and outputs, and their ordering through functional flow block diagrams, as applied to common microarray data processing.

Fig. 4.12 shows the functional flow block diagram (FFBD) for microarray data analysis, as a state-chart diagram. Each block indicates a state, and defines its main action. The arrows indicate transitions between states, which might be based on selection criteria, be concurrent (AND condition), or be optional (OR condition). The corresponding input-output diagram for each function is shown in Fig. 4.13. The order of operations is explained below.

The scientist imports generated microarray data and other experimental data from the archives in Function 1. The input to the function can be a gene or compound involved in the Experiment database, or any other description. Expanding this function in Fig. 4.14, the system should combine diverse experimental data into a single, standard format.

In the next step, the scientist tries to build a data model by applying transformations to the data, and fitting various models to it. This might involve the use of information from

external sources like sequence and structure databases, and the use of visualization tools as detailed in Fig. 4.15.

He/she then picks clustering methods and applies the data transform appropriate to the data model (Functions 6,7). A variety of filtering steps can be applied at this point to the data, to eliminate redundant or noisy features and reduce the dimension of the dataset (Function 8). Fig. 4.16 expands this function including filtering based on fold-variation, significant features, and function. The output of this step is a significant subset of features, which is used in clustering or classification in the next steps (Functions 9, 11).

The scientist analyzes the clusters obtained by unsupervised clustering using contextual information from queries to external information sources (Fig. 4.17), while he uses the results of supervised classification directly for performance evaluation. For each type of dataset, he/she tries different algorithms and varies their parameters. He/she then generates a report of the findings and conclusions of the analysis, which can be made available to other groups within the organization.

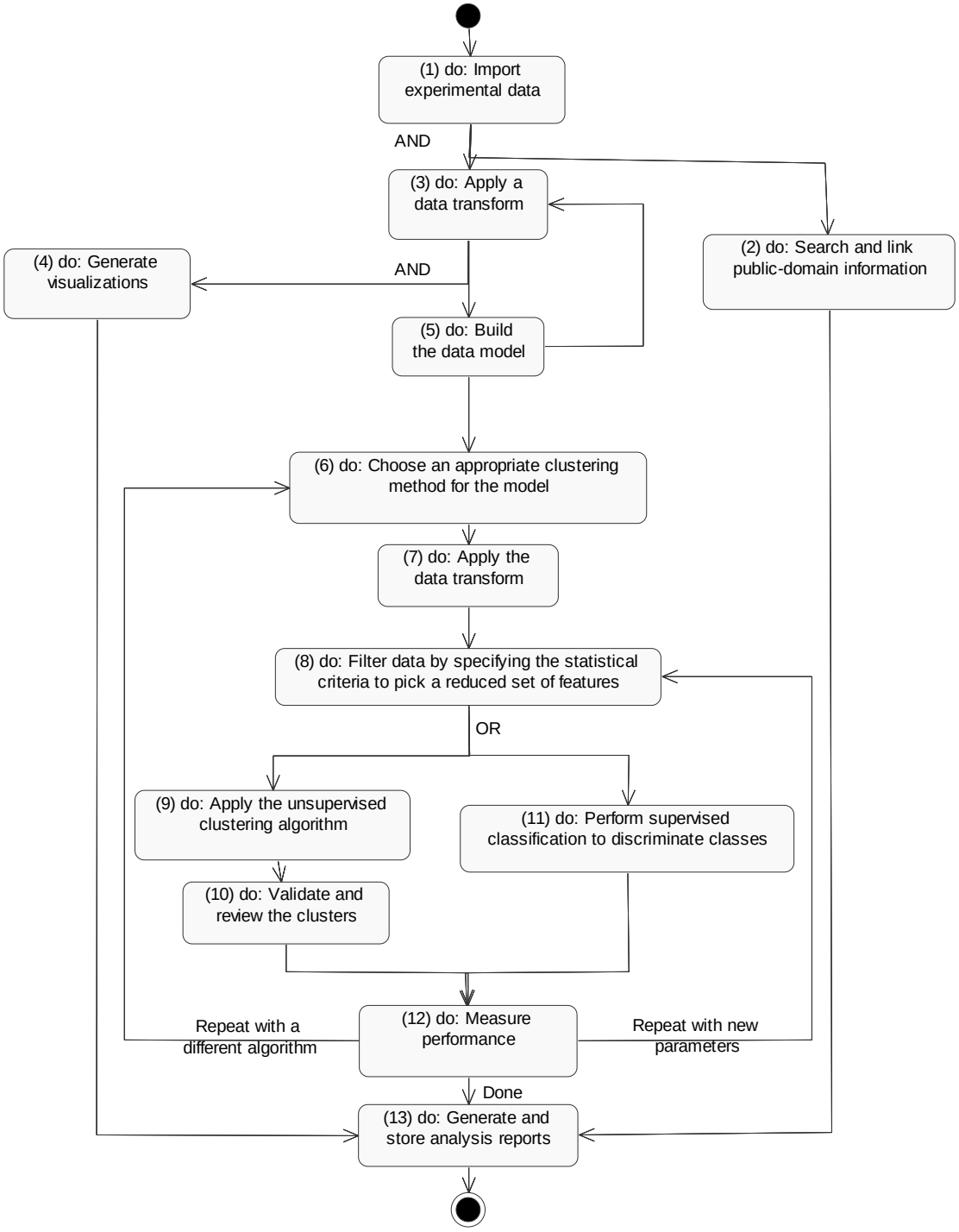


Fig. 4.12 State-chart Diagram for Behavior

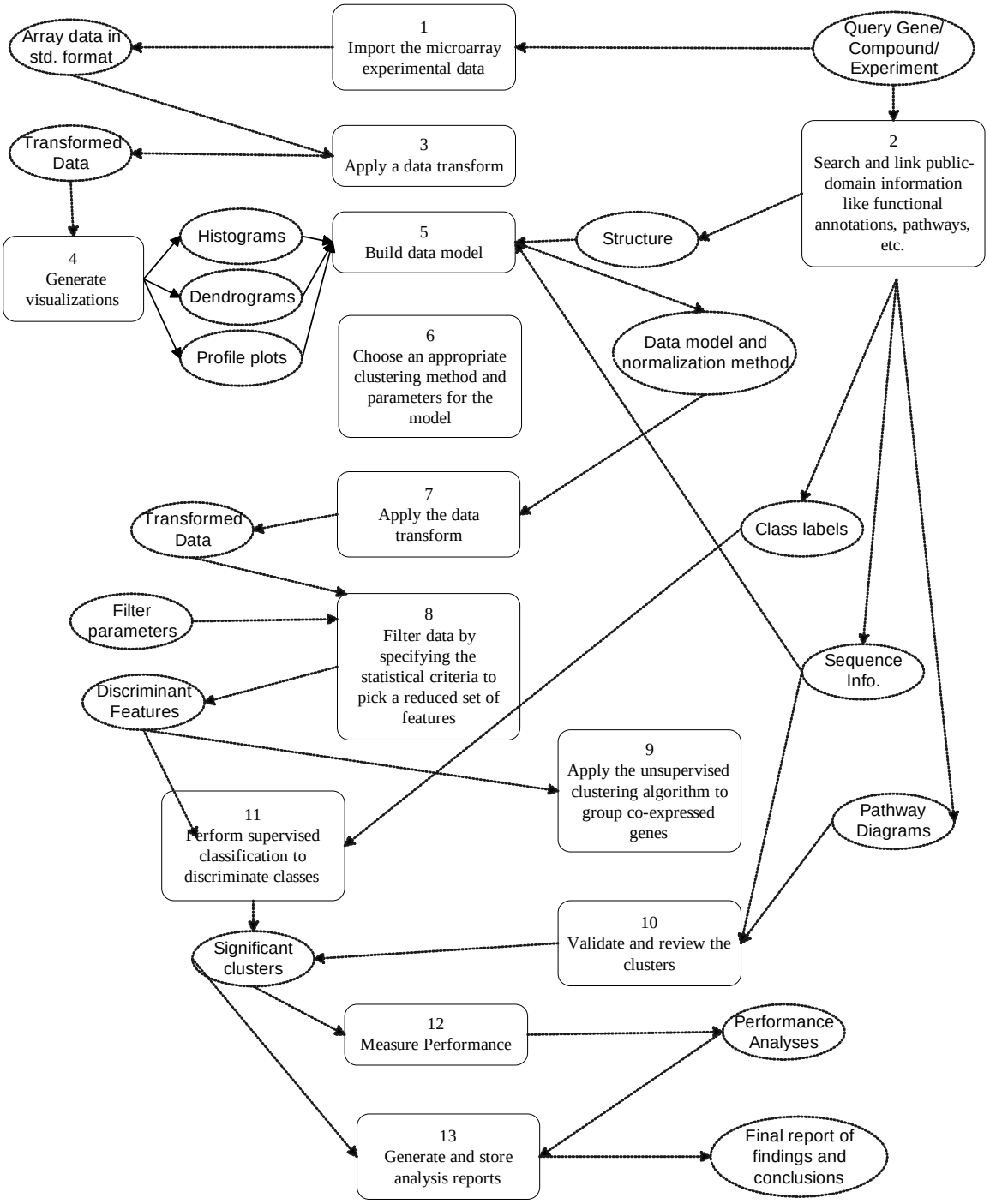


Fig. 4.13 Input-output Diagram for Behavior

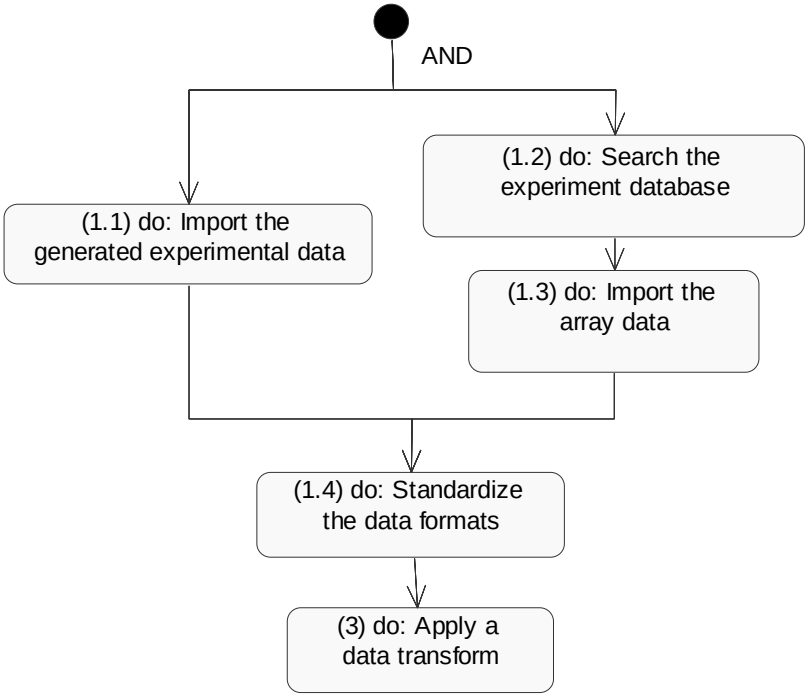


Fig. 4.14 Lower-level FFBD for Function 1: Import Experimental Data

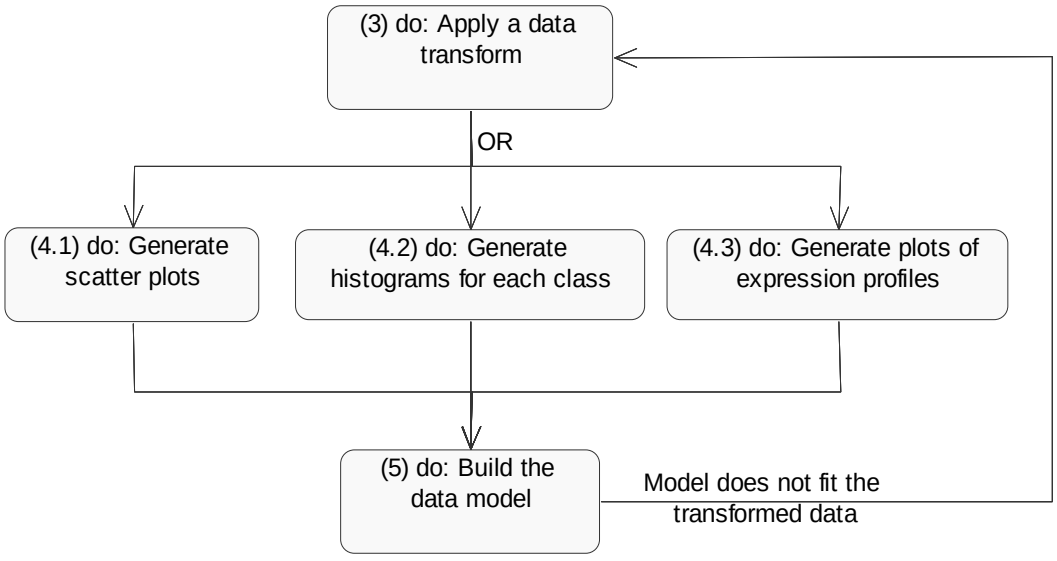


Fig. 4.15 Lower-level FFBD for Function 4: Generate Visualizations

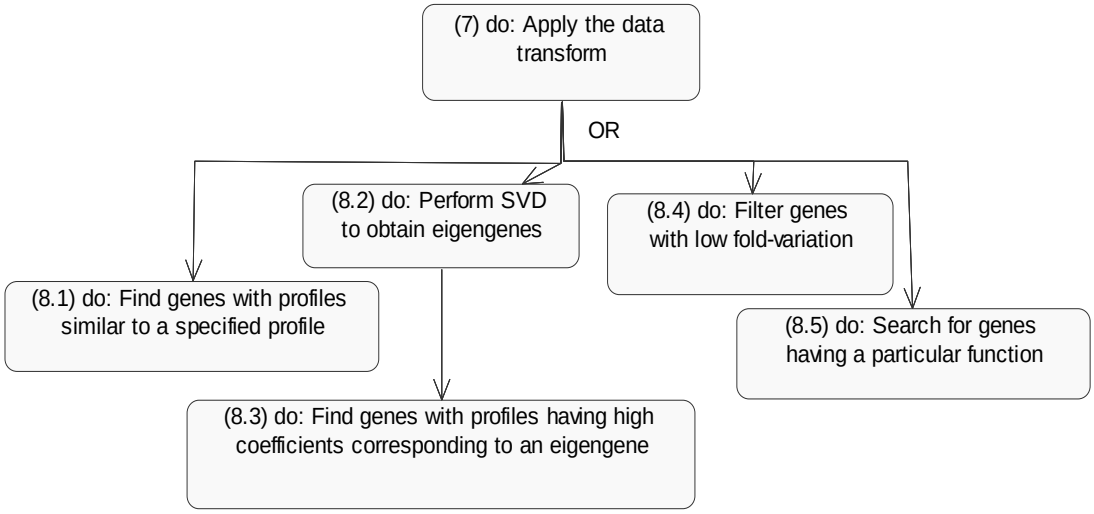


Fig. 4.16 Lower-level FFBD for Function 8: Filter Data

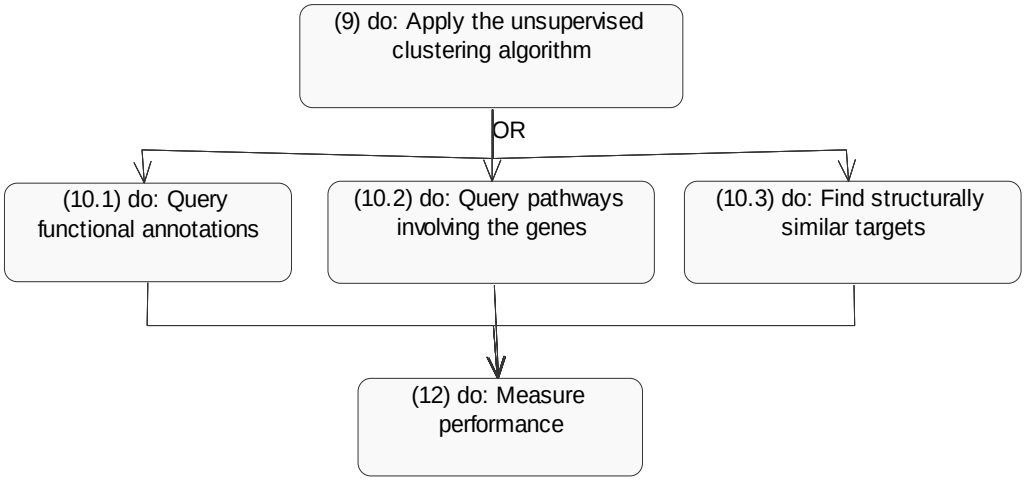


Fig. 4.17 Lower-level FFBD for Function 10: Validate and Review Clusters

We now map the overall system behavior as the operations associated with the classes in Figs. 4.9-4.11. We illustrate the mapping between the behavior and the structure with

examples from the class diagrams. By indexing over target structure, it is possible to obtain targets with high structural similarity to a known one. This is defined in the FindSimilarStructure() operation of the Target_Structure class. Other related functions are FindSimilarCompound() in the Compound_Structure class, and FindSimilarProfile() in the Experiment class.

The operations on the Compound class like PredictADMEProperties() are derived from the use case scenarios for the computational biologist/chemist. Such queries might also involve other subsystems and require indexing across heterogeneous databases.

It is also important to note that the class diagrams above show only persistent object structure, and their constraints and relationships. By associating persistent object behavior with the database, the processing of array data can be handled by the DBMS itself. The advantage of this technique is that processing can be carried out using the DBMS optimizations in parallel with the application.

ORDBMS products like Oracle 8 emulate database server behavior by allowing the operations to be coded in a database programming language like PL/SQL. The implementation can also be performed in modules containing the class methods, such as Oracle NCS Cartridges and Informix Data Blades. The features of the object-relational model and database extensions are discussed in Sec. 4.8. Here, we list some of the common data mining queries and the corresponding system responses derived from the UML models:

(i) What are the characteristics of a disease? (pathways, active compounds, known targets, disease phenotype, references)

(ii) What are the properties of known targets? (target type, structures, function, chemical and geometric properties)

(iii) Which experiments in MicroarrayDB involve the known targets and corresponding active compounds? (ExperimentID, data format, expression data)

(iv) Which genes have shown activity similar to known targets?

(v) Which genes are good discriminating instances across all experiments in a dataset?

(vi) Which genes are likely to be drug targets from a set of experiments, given their sequence and structure?

(vii) Which compounds are likely to show good activity, specificity and pharmacological properties (for ADME tests) on these targets? (QSPR)

(viii) What compounds in the libraries are likely to give a comparable performance to an existing product?

(ix) How will the activity of a compound be on a particular target given their structure? (QSAR)

(x) Which drug research categories have high lead times?

4.7 System Architecture Summary

In this section, a variety of system architectures are studied, to determine how they meet the high-level requirements outlined in Sec. 4.3.

A simple two-tier client-server architecture (Fig. 4.18) distributes the functions of user interface (session tracking, console, display, etc.) and database management (application execution). Both tiers share the process management functions such as process development, monitoring, and resources. Business logic is usually implemented as stored procedures and triggers in the database management server. This architecture is advantageous in offloading computational load on the client's side and reducing the amount of data transfer over the network. However, there are several shortcomings with this approach. When the application resides on the client machine, it is difficult to maintain application versions and integrate new applications. Further, the implementation of business logic, which is data-intensive, through stored procedures on the database server is limited by the server's processing power as the complexity of computation or the number of applications or users increases. When processing occurs on the server, its link with the client is kept alive through 'hello' messages, increasing network load. Typically, the performance of a two-tier architecture deteriorates beyond one hundred users ([36]).

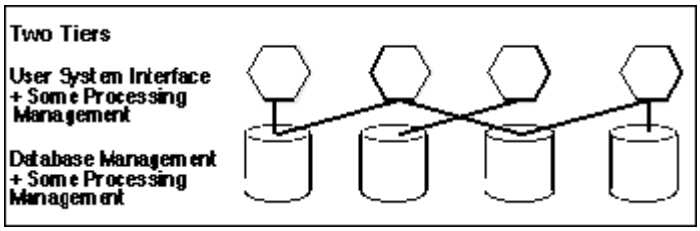


Fig. 4.18 Two-tier Client/Server Architecture [36]

The three-tier client-server architecture (Fig. 4.19) overcomes some of these limitations by incorporating an additional application services layer as the middle tier. It handles

application execution and queuing, allowing the client to detach itself from the database server during processing. Now, each client application is linked with multiple applications and upgrades can be performed with minimal configuration changes.

The three-tier architecture can be extended for example, by considering further abstractions of application management at the level of project phases or scientific analyses, leading to a n-tier architecture. Though such architectures have desirable properties in terms of scalability, reusability, and abstraction, the structure causes the application code design to become very complex. This is especially true in handling data transfer operations while collating heterogeneous databases and communication between applications in different programming environments.

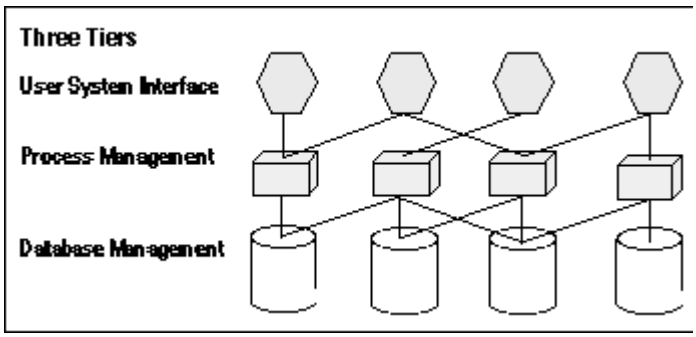


Fig. 4.19 Three-tier Client/Server Architecture [37]

The distributed-object computing model incorporates an object-oriented approach in handling such problems. It partitions applications into components interacting with particular elements of the architecture. For instance, an algorithm mining microarray

expression data can be divided into database-intensive and algorithmic components, and these can be executed on specialized hardware.

Distributed computing architectures like CORBA (Common Object Request Broker Architecture) provide the abstract IDL (interface definition language) utility, which allows communication between heterogeneous objects such as applications written in different programming languages, different database management systems, and platforms. The CORBA architecture (Fig. 4.20) also provides services like object lifecycle management, naming, and persistence.

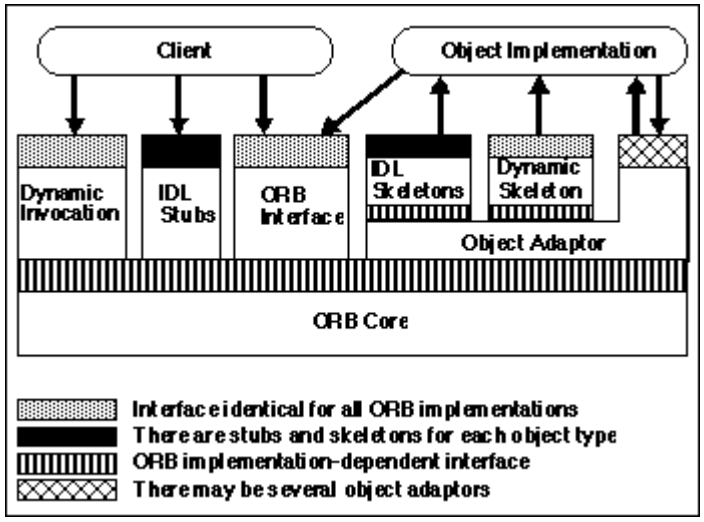


Fig. 4.20 CORBA Architecture [38]

4.8 Data Architecture and Database Extensions

Bioinformatics applications involve heterogeneous data, such as pathway diagrams, nucleotide sequences, molecular structures, and diverse microarray data formats. A large number of queries derived from the use cases collate information across different data

types and finding patterns in uncommon data types like molecular structures, gene expression, and so on.

The traditional relational data model is restrictive in modeling the structure of data and such queries based on particular properties would need to be implemented as part of the application behavior, thereby increasing complexity and execution time.

The object-relational data model provides several extensions to the relational and object-oriented models:

- The ability to implement restrictions on data types as subtypes.
- Encapsulated data types, which can be extending from the base types, and can be implemented by separate indexing and access methods.
- Complex objects and collections like nested tables, typed columns, references, and function indices.
- More complex rule mechanisms compared to relational triggers.

The following examples show how object-relational database extensions can be designed to handle bioinformatics queries:

- Operator and function notation in place of indices. E.g. NearestNeighbor()
- User-Defined Aggregates: E.g. Centroid (similarity)
- User-defined Comparison Operators: E.g. MoreSpecificThan()

CHAPTER 5: LEARNING ALGORITHMS

5.1 Introduction

In this chapter, we discuss a few algorithms that implement by the system behavior described in the previous chapter. We begin with a description and properties of a new similarity measure for gene expression data based on raw, un-normalized values, called the Symmetric Kullback-Liebler similarity measure. In the later sections, we describe the analysis methods.

5.2 Similarity Measures

The database behavior described in Chapter 4 required several algorithms involving search, indexing, and clustering procedures. With a variety of microarray manufacturing techniques, this requires the development of appropriate measures of similarity of gene expression.

5.2.1 Microarray Expression Data

In particular, we look at two types of microarrays - cDNA and oligonucleotide arrays. An expression value from a cDNA array represent the lognormal ratio of the amount of RNA present in an experiment sample to that measured in a control sample. Oligonucleotide array expression values indicate the average difference between (approximately 10) replicate probe pairs containing the perfect match (PM) and mismatch (MM) values [7]. Because of manufacturing technique and standardization procedures, oligonucleotide arrays have a very high reproducibility compared to cDNA arrays as explained in Sec. 3.1.1.

5.2.2 Probabilistic Models of Microarray Data

With common microarray technologies, it has been noted that the log-values of expression data for a functional class or a phenotype, roughly follow the Gaussian distribution ([9]). In our analysis, we also implicitly assume conditional independence. This is a reasonable assumption since we focus on classification based on co-expression and are not trying to infer higher-level genetic interactions. Thus the likelihood of a set of *m* genes $\{g_i\}$ with expression values $D = \{x_{ij}\}$ over *n* experiments, where j indicates the experiment index, belonging to a class k is given by -

$$P(D \mid \mu_k, \sigma_k^2) = \prod_{i=1}^{m} P(y_i \mid \mu_k, \sigma_k^2),$$
$$y_i = \{x_{ij}\},\ j = 1, \ldots, n$$

It has been shown in numerous experiments ([1], [2], [8], and [23] for example) that the log-Euclidean distance over expression values has been successful in classification problems.

5.2.3 Unnormalized Symmetric Kullback-Liebler Measure

In this section, we discuss the formulation and properties of a novel similarity measure for microarray data, which was developed by A. S. Baras and J. S. Baras in [24,25] and utilized in [23,25,26,27].

This measure is based on the relative entropy or the Kullback-Liebler (KL-) distance from information theory, which measures the error (in terms of the excess number of bits

needed) to represent a random variable with a distribution q, given that its true distribution is p. For a random variable x, the KL-distance is given by

$$D(p \,||\, q) = \sum_{x \in X} p(x) \log_2 \frac{p(x)}{q(x)} = E_p \log_2 \frac{p(x)}{q(x)}$$

The KL-distance is also known as relative entropy([30]). The relative entropy is asymmetric and measures the deviation between the two distributions.

The unnormalized symmetric Kullback-Liebler measure quantifies the dissimilarity between two expression indexes (over genes or experiments), so that it is symmetric and there is no need for normalization of the expression data; its novelty is the latter property. It is based on the un-normalized expression indexes like the average difference values in oligonucleotide arrays and the fluorescence ratio of Cy5 to Cy3 in cDNA arrays. We follow [24,25] in describing it here.

Definition ([24,25]): The unnormalized symmetric Kullback-Liebler measure between two expression vectors *x* and *y* of length N is defined as

$$D(x \,||\, y) = \sum_{i=1}^{N} \frac{1}{2} \left[x_i \frac{\log(x_i)}{\log(y_i)} + y_i \frac{\log(y_i)}{\log(x_i)} \right]$$
$$x_i, y_i > 0, i = 1, ..., N$$

Properties ([24,25]):

1. $D(x \,||\, y) \geq 0$, if $x_i, y_i > 0, i = 1, ..., N$

$D(x \| y) = 0$, iff $x_i = y_i \forall i$

2. $D(x \| y)$ is convex in the pair (x, y).

$D(\lambda x_1 + (1-\lambda)x_2 \| \lambda y_1 + (1-\lambda)y_2) \leq \lambda D(x_1 \| y_1) + (1-\lambda)D(x_1 \| y_1)$, where $0 \leq \lambda \leq 1$

3. There exists a unique centroid c of a set $S = \{x_k\}$, k=1,...K of vectors of length N based on the unnormalized symmetric Kullback-Liebler measure given by the solution of the following equation ([24,25]).

$$\ln \frac{c_i}{x_i} = \frac{\tilde{x}_i}{c_i} - 1, i = 1, ..., N \text{, where}$$

$\bar{x}_i = \frac{1}{K}\sum_k x_i^k$ is the arithmetic mean, and $\tilde{x}_i = \exp\left[\frac{1}{K}\sum_k \ln(x_i^k)\right]$ is the geometric mean. Further, the centroid is bounded by the two means:

$$\tilde{x}_i \leq c_i \leq \bar{x}_i, i = 1, ..., N$$

The unnormalized symmetric Kullback-Liebler measure describes quantitatively the dissimilarities between two expression vectors x and y, assuming either is the true distribution. It combines the value difference and fold variation (log-values) between the two vectors, and can lead to a more accurate prediction across classes where genes have widely varying levels of expression. With reference to the planar separating surfaces formed by the log-Euclidean distance during classification, we note that the unnormalized symmetric Kullback-Liebler measure leads to non-linear hyper-surfaces.

5.3 Learning Algorithms

In this section, we look at specific algorithms for different types of gene expression analyses. The chief properties of such algorithms should include:

- Minimum inductive bias.
- Ability to handle noisy and irrelevant attributes, and outliers.
- Ability to handle different similarity measures.
- Good initial hypothesis.
- Ability to generalize well with limited training data.

5.3.1 LBG/LVQ Algorithm

The Linde-Buzo-Gray algorithm (LBG) or the Generalized Lloyd algorithm [19] is a signal approximation algorithm, which generates a codebook of centroid vectors. We use LBG for unsupervised clustering in a tree-structured manner ([15]). The algorithm starts with a representative pattern of gene expression for the entire dataset and partitions the pattern space by applying small perturbations at each successive node. At the same time, it iteratively optimizes the codebook at each level until the centroids at the level are stationary. The stopping rule for LBG is based on the percentage decrease in the overall distortion. The algorithm steps are listed below.

1. Given the input matrix X_{MxN}, initial learning rate ε, fix the number of codevectors K = 1 and the tree depth L=1.

$$c_1 = centroid(X)$$

$$Err_1 = \frac{1}{NM}\sum_{n=1}^{N} dist(x_n, c_1) = \left\{ \begin{array}{l} \frac{1}{NM}\sum_{n=1}^{N} \| x_n - c_1 \|^2, \text{Euclidean distance} \\ \frac{1}{NM}\sum_{n=1}^{N}\sum_{m=1}^{M}[(x_{nm} - c_{1m})(\log(x_{nm}) - \log(c_{1m}))], \\ \text{unnormalized symmetric Kullback-Liebler} \\ \text{measure} \end{array} \right\}$$

2. Node splitting: Set $K = 2K$, L=1, and for k=1,2,…K, initialize the codevectors at the next level by

$$c_k^0 = (1-\varepsilon)c_k$$
$$c_{K+k}^0 = (1+\varepsilon)c_k$$

3. Set the iteration index i=0 and $Err_L^{(i)} = Err_1$.

(i) Find the closest centroid to each data vector x_l and the associated class $C(x_l)$ as

$$C^i(x_l) = \arg\min_k (dist(x_l, c_k^i)).$$

(ii) Update the codevector for the K classes. For k=1,…,K,

$$c_k^{i+1} = centroid(\{x : x \in X, C^i(x) = k\}).$$

Set i = i + 1 and evaluate the new error Err_L^i.

(iii) If $(Err_L^{i-1} - Err_L^i)/Err_L^{i-1} > \varepsilon$, go to step (i). Otherwise, for k=1,…,K, set $c_k = c_k^i$.

4. Repeat steps 2 and 3 until the desired number of levels is reached.

Learning vector quantization (LVQ) [18,28,29] is a neural network learning method, which uses the nearest neighbor rule to train the codebook. Its inductive bias simply states that the code vectors of a class are closer to its instances.

Each cluster from the LBG algorithm is labeled with the class that has the maximum percentage proportion of members in it. This codebook forms the input to supervised LVQ training. The code vector closest to the training instance is moved at a rate proportional to the gradient of the distance, as per the LVQ1 competitive learning rule ([21]). The learning rate is decreased uniformly with time. The LVQ algorithm steps are listed below.

1. Set the iteration index $iter = 1$ and the learning rate $\alpha = \frac{\alpha_0}{iter}$, where α_0 is the initial learning rate.

2. Pick a random instance *x* and determine its nearest code vector c^o. Update the centroid by

$$c^n = \begin{Bmatrix} c^o + \alpha \nabla \text{distance}(x, c^o), & h(x) = C(x) \\ c^o - \alpha \nabla \text{distance}(x, c^o), & h(x) \neq C(x) \end{Bmatrix}$$, where $0 \leq \alpha \leq 1$, *h(x)* is the class of the nearest centroid and *C(x)* is the true class of *x*.

3. Repeat 1 and 2 until the centroids converge.

Being a gradient-descent algorithm, LVQ can converge to local minima. The performance of LVQ strongly depends on the initial codebook vectors. Hence, we expect that systematic initialization by LBG should outperform a random initialization of K centroids.

The class boundaries formed by LVQ are composed of finite hyperplanes with the Euclidean distance and of finite hypercurves with unnormalized symmetric Kullback-Liebler measure.

5.3.2 Support Vector Machines

Support vector machines (SVM)[12] are classifiers based on statistical learning theory, which map the input space to a high-dimensional space of non-linear features. This mapping is implicitly made via a kernel function and all the computation is performed with the input space vectors.

The SVM training algorithm generates hyperplanes in the feature space, whose margin is optimized to obtain good generalization. Consider a data set composed of N expression vectors $<\boldsymbol{x_i}, y_i>$, where y_i denotes the label for the data vector $\boldsymbol{x_i}$. Using the notation from [11], the problem of finding the weight vector $\boldsymbol{w}$ can be formulated as minimizing the function

$$L(w) = \frac{1}{2} \| w \|^2 \text{, subject to } y_i \left[w.\phi(x_i) + b \right] \geq 1, i = 1, ..., N.$$

Here, the function $\phi(x)$ maps the input vector to the feature vector. The dual formulation is given by maximizing

$$Q(\alpha) = \sum_{i=1}^{N} \alpha_i - \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} y_i y_j \alpha_i \alpha_j K(x_i, x_j) \text{ subject to}$$

$$\sum_{i=1}^{N} y_i \alpha_i = 0 \text{ and } 0 \leq \alpha_i \leq C, i = 1, ..., N.$$

The α_i's are the Lagrange multipliers and C is the regularization parameter. Non-negative α_i's correspond to support vectors. $K(x_i, x_j) = \phi(x_i).\phi(x_i)$ denotes the kernel function satisfying Mercer's theorem, which states that

"For all vectors $x, z \in D$ (here, *D* is the input domain), there exists a mapping ϕ to the feature space *F*, iff the function $K(x, z)$ is symmetric and positive semi-definite [12]."

Kernel functions used in Chapter 6 are listed in Table 5.1 below.

TYPE OF KERNEL	KERNEL FUNCTION
Linear Kernel	$K(x_i, x_j) = < x_i, x_j > + c$
Polynomial Kernel	$K(x_i, x_j) = (< x_i, x_j > + c)^d$
Gaussian Kernel	$K(x_i, x_j) = \exp\left[-\left(\frac{< x_i - x_j >^2}{\sigma^2}\right)\right]$

Table 5.1 SVM Kernel Functions

The generation of SVM surfaces assumes no prior probability distributions and the solution obtained is the global optimum. Thus, the accuracy of SVMs provides an upper bound for gradient-descent methods like LVQ when similar hypothesis spaces are considered. They

also have good generalization properties because SVM training minimizes the empirical risk for a chosen hypothesis space.

Choosing a particular kernel function imposes a restriction bias on the analysis. Another limitation is illustrated by the fact that the unnormalized symmetric Kullback-Liebler measure does not satisfy Mercer's conditions and cannot be represented as a kernel function.

5.3.2 IB3 Algorithm

The IB3 algorithm developed by Aha *et al.* ([13]) is an instance-based learning algorithm. It classifies new instances in a lazy manner, using a set of representative stored instances called the concept description. The nearest neighbor rule is used to associate the test data to the class of the closest stored instance.

IB3 eliminates the high computational cost involved in the testing phase of instance-based methods by storing instances with good predictive strengths. In the training phase, the algorithm stores misclassified instances in the concept description and associates a classification accuracy for all instances. The algorithm performs a statistical test to accept or reject new instances based on the classification accuracy and the prevalence of the instance's true class, thus eliminating noisy data. The final concept description generated after one epoch is thus likely to contain instances a little farther from the class boundary than methods like IB2, which store all misclassified instances. The algorithm steps are described below.

1. Initiate the concept description $CD = \phi$.

2. Loop over the training instances $x \in D$

(i) For each $y \in CD$, evaluate its distance dist(x, y).

(ii) If there exists an acceptable instance in *CD*,

$$y_{\max} = \arg\min_{y} \left[dist(x, y) \right], y \in AcceptableSet(CD)$$

Else:

Pick a random number *i* of instances from *CD* and set y_{max} to the i-th most similar instance.

(iii) If $class(y_{\max}) \neq class(x)$, add x to *CD*.

(iv) Update classification records:

For all y whose $dist(y) \leq dist(y_{\max})$, update the classification record.

If the upper bound of y's accuracy $accuracy^{ub}(y, \alpha_{reject}) \leq prevalence(class(y))$, discard y.

Else if the lower bound of y's accuracy $accuracy^{lb}(y, \alpha_{accept}) \geq prevalence(class(y))$, accept y.

In [13], Aha *et al.* have shown that the concept description set stores instances with high classification accuracy, robustness, noise insensitivity, and high utilization (in terms of classifying other instances during training).

IB3 has a restriction bias towards convex concepts. It shows poor performance with sparse data sets, and is very sensitive to irrelevant attributes. However, it has minimal inductive bias as it can approximate class boundaries with piecewise linear surfaces (with the Euclidean distance). This feature makes it suitable, for example, to learn informative instances in pre-filtering steps. Compared to IB3, decision trees have a stronger bias as their separating surfaces are hyper-rectangles.

CHAPTER 6: RESULTS

6.1 Objectives

The aims of the experiments described in this chapter are as follows.

- Evaluate the performance of supervised classification of the LVQ algorithm with LBG codebook initialization and SVMs.

- Compare the unnormalized symmetric Kullback-Liebler measure against Euclidean distance in similarity-based algorithms with oligonucleotide arrays (the Find Similar function).

- Evaluate the performance of the similarity measures in retrieving informative instances (the Find Discriminating function).

- Conduct experiments on two applications of expression analysis - gene functional classification and phenotype classification.

6.2 Description of Microarray Data

The three datasets used in testing the algorithms denote distinct applications in microarray expression analysis, namely, phenotype classification and functional classification.

6.2.1 Lymphoma Dataset

The lymphoma cDNA array dataset comprises gene expression patterns of genes involved in different classes of lymphoma and normal cell lines from Alizadeh et al [1].

The mechanism of cancer is characterized by uncontrolled growth and proliferation brought about by mutations to vital genes. Cancer diagnosis has traditionally been carried out based on clinical and molecular evidence such as cell and tissue type, and heredity. However, such information is mostly incomplete for evaluation or prognosis. It also leads to re-validation or re-classification in some cases of cancer. By studying the phenotype or the genetic signature of the set of relevant genes for a particular condition, using microarray gene expression data, it is possible to understand the mechanism at the genetic level. Such analysis is related to pharmacogenomic studies to design customized drugs.

In [1], Alizadeh et al collect the gene expression in three classes of lymphoid malignancies: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and chronic lymphocytic leukemia (CLL), and genes relevant to lymphocyte and/or cancer biology, as shown in Table 6.1. The notation is given below.

CELL TYPE	**DESCRIPTION**
DLBCL	Diffuse Large B-cell Lymphoma
GCB	Germinal Center B
NLT	Normal Lymph Node/Tonsil
APB	Activated Peripheral B
RAT	Resting/Activated T
TCL	Transformed Cell Line
FL	B-cell Follicular Lymphoma
RPB	Resting Peripheral B
CLL	Chronic Lymphocytic Leukemia

Table 6.1 Lymphoma Tissue Types

The microarray dataset consists of expression values from 4026 genes involved in 96 subpopulations. Each data point X_{ij} represents the logarithm of the Cy5/Cy3 fluorescence ratio for gene j in tissue sample i. The distribution of tissue types in the sample dataset from [1] is shown in Table 6.2.

	DLBCL	**GCB**	**NLT**	**APB**	**RAT**	**TCL**	**FL**	**RPB**	**CLL**
#Arrays	46	2	2	10	6	6	9	4	11
Cancerous Tissue?	Y	N	N	N	N	Y	Y	N	Y

Table 6.2 Tissue Sample Distribution

In the case of a disease or malignancy, pharmacological studies have found that functional genes are likely to have a binary mode of operation.[†] Thus, in the identification of drug targets, the biologist is only concerned with genes that exhibit discrepancies in the signature. That is, their expression is either unaffected by the cellular condition, or they are inhibited.

6.2.2 Yeast Data

The second dataset consists of the genome-wide expression in budding yeast in response to different cell cycle-related processes like the diauxic shift, sporulation, pressure and reducing shocks. Since the expression values are representative of well-known cellular processes, co-expression analysis is expected to yield genes that are regulated by a common upstream transcription factor, belonging to a common metabolic pathway, or coding similar proteins.

This is an example of a case where genome-wide expression data is necessary to make sound predictions about gene function. The yeast data from cellular processes hides complex underlying mechanisms, which can be understood only by looking at the global genome expression.

The microarray measures the expression of 6221 genes in the yeast genome, collected at 79 time points during the diauxic shift (shift from anaerobic to aerobic respiration), division cycle, sporulation, and temperature and reducing shocks. A data point X_{ij} represents the logarithm of the expression of gene i at time point j, as compared to a control.

In [4], Eisen et al. used pairwise-linkage clustering to show that genes of five functional classes cluster together well based on expression data alone. These classes correspond to the MYGD (MIPS Yeast Genome Database) functional classes of tricarboxylic acid, respiration (TCA), cytoplasmic ribosomes, proteasomes, helix-turn-helix proteins (HTH), and histones. The HTH protein group is not expected to cluster well in this experiment, and is included as a control group. Brown et al. [2] showed superior results in accurately classifying these functional classes using support vector machines. The distribution of genes across these classes is shown in Table 6.3.

FUNCTIONAL CLASS	**# SAMPLES**
Histones	
HTH Proteins	16
Proteasomes	35
Respiration	27

[†] Private discussions with Novartis Pharmaceuticals

Ribosomes	120
TCA	14

Table 6.3 Distribution of Yeast Functional Classes

6.2.3 Leukemia Data

These experiments from Golub et al. ([5]) attempt to differentiate between two types of acute leukemias – acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). Traditional identification techniques for leukemia have been based on factors like the morphology and the course of clinical trials. Microarray experiments have been shown to provide a systematic understanding and make accurate prediction feasible as described in [5].

There are two data sets available for training (class prediction) and testing (class discovery). The training data consists of 38 bone marrow samples of adult leukemia patients, of which 27 are of the ALL type and 11 of the AML type. The test data set is chosen independently, and consists of 24 bone marrow and 10 peripheral blood samples.

The supervised learning algorithms are trained on a portion of the training set during cross-validation experiments and tested on the remainder (class prediction). The separating surfaces generated during training are also used to identify the type of leukemia in the independent testing data set (class discovery).

6.3 Methods

6.3.1 Supervised Classification

Supervised classification with LBG/LVQ, SVM, IB3, and C4.5 algorithms, is performed using 3-fold cross-validation (except where indicated). Multiple runs with the data sets have been used to determine parameters like the LVQ learning rate, number of epochs in training, and the tree height in LBG.

The SVMTorch support vector machine software [31] used here is Ronan Collobert's implementation for large data sets and uses sequential minimal optimization (SMO). For multi-class problems, SVMTorch trains each class with a one-over-all mechanism.

The IB3 algorithm implementation uses 90% and 68% confidence intervals for accepting and rejecting instances respectively, when compared to the corresponding class frequency.

The C4.5 decision tree algorithm performs classification by repetitively choosing attribute/value pairs, which minimize the overall entropy after partitioning the data space. Quinlan's information gain (or gain ratio to compensate diverse populations among classes) can be used to select attributes at each step. The decision tree is then post-pruned based on a user-specified threshold similarity, to avoid over-fitting.

6.3.2 Similarity-based Clustering

The log-Euclidean and the unnormalized symmetric Kullback-Liebler measures are compared using a simple nearest-neighbor algorithm. We do not expect to see significant differences in the performance with cDNA arrays, as against oligonucleotide arrays.

6.3.3 Comparison of Algorithms

In all methods described, we generate the confusion matrices and measure the overall accuracy and the accuracy of individual classes.

The performance of two learning algorithms is compared by a t-test. The average error rate of two learning algorithms L_1 and L_2, trained and tested on the same data sets S_k and T_k is given by

$$\bar{\delta} = \frac{1}{K}\sum_{k=1}^{K}\left[err_{T_k}(L_1(S_k)) - err_{T_k}(L_2(S_k))\right].$$

The N% confidence interval for $\bar{\delta}$ to be an estimate of the true error difference is given by

$\bar{\delta} \pm t_{N,K-1} s_{\bar{\delta}}$,

where $s_{\bar{\delta}} = \sqrt{\frac{1}{K(K-1)}\sum_{k=1}^{K}(\delta_k - \bar{\delta})^2}$

and $t_{N,K-1}$ is the t-test value for two-sided confidence intervals [14].

6.4 Experiments

6.4.1 Lymphoma Data

Two types of classification are studied with the lymphoma data:

- Binary classification between cancerous and non-cancerous samples.
- Tissue type classification based on global gene expression.

In Cai et al.[3], differential analysis based on genes known to be involved in B-cell lymphoma, has been shown to have no significant impact on the classifier's ability to recognize cancerous tissues. Hence, in both cases, we do not distinguish between the selected genes based on prior knowledge. The C4.5 algorithm is expected to classify cancerous tissues if the genetic signature of a subset of genes is a sufficient indicator.

Using the performance measure defined above, the results from the LVQ algorithm are compared with those from other supervised learning techniques in [3], viz., SVM and C4.5 decision tree, using 10-fold cross-validation.

6.4.1.1 Binary Classification of Cancerous/Non-cancerous Tissues

For both cancer detection and tissue type identification (Section 6.4.1.2) experiments, the initial codebook generated by the LBG algorithm consists of 16 code vectors and the LVQ algorithm is trained for over 2 epochs.

Table 6.4 shows the typical performance of LVQ with the log-Euclidean distance for cancer detection. These results on error average and variance are compared with results from Cai *et al.* ([3]) using a linear-kernel SVM and a C4.5 decision tree. In Table 6.5, the results from the LBG/LVQ algorithm are based on 10 cross-validation experiments, and compare closely to the globally optimal SVM method. The LBG/LVQ algorithm gives a much better and more stable performance compared to the C4.5 decision tree algorithm.

LVQ CLASSIFICATION	TRUE POSITIVE	TRUE NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE
Cancerous Tissues	72	22	2	0
Non-cancerous Tissues	22	72	0	2

Table 6.4 Log-Euclidean LVQ Classification Error for Cancer Detection using 10-fold Cross-validation

	LVQ	SVM	C4.5
Average Error Rate	2.08%	1.04%	18.55%
Error Rate Std. Devn.	0.01%	0.03%	13.83%

Table 6.5 Classification Error Comparison for Cancerous/Non-cancerous Cells using 10-fold Cross-validation

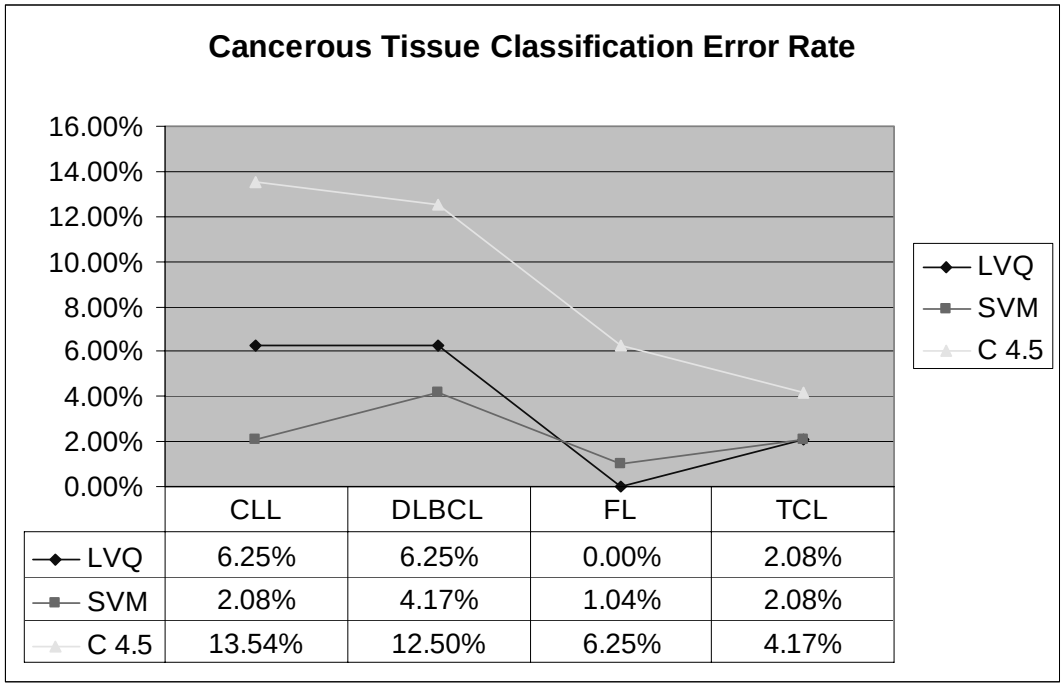
6.4.1.2 Tissue Type Classification of Cancerous Cells

Table 6.6 and Fig. 6.1 show the classification of tissue types of cancer cells by the three algorithms with 10-fold cross-validation. As in the binary classification case, we see that the LVQ algorithm initialized with LBG closely follows the performance of the SVM, and both outperform the decision tree algorithm by a large margin.

The overall error rate of LVQ is more than two times that of the SVM, in tissue type classification as shown in Table 6.7.

CANCER TISSUE CLASSIFICATION		**TP**	**TN**	**FP**	**FN**	**ERROR RATE**
CLL	LVQ	8	82	3	3	6.25%
	SVM	11	83	2	0	2.08%
	C 4.5	4	79	6	7	13.54%
DLBCL	LVQ	42	48	2	4	6.25%
	SVM	43	49	1	3	4.17%
	C 4.5	38	46	4	8	12.50%
FL	LVQ	9	87	0	0	0.00%
	SVM	9	86	1	0	1.04%
	C 4.5	5	85	2	4	6.25%
TCL	LVQ	6	88	2	0	2.08%
	SVM	6	88	2	0	2.08%
	C 4.5	4	88	2	2	4.17%

Table 6.6 Classification Error for Cancerous Tissues using 10-fold Cross-validation



	CLL	DLBCL	FL	TCL
LVQ	6.25%	6.25%	0.00%	2.08%
SVM	2.08%	4.17%	1.04%	2.08%
C 4.5	13.54%	12.50%	6.25%	4.17%

Fig. 6.1 Tissue Classification Error Comparison

TISSUE CLASSIFICATION	LVQ	SVM	C4.5
Average Error Rate	9.72%	4.17%	29.17%

Table 6.7 Overall Error for Tissue Type Detection using 10-fold Cross-validation

6.4.2 Gene Functional Classification from yeast data

The following simulation runs involve genes in the six functional classes mentioned in the data description. The objective is to test the ability of the decision surfaces generated by the algorithms and similarity measures to separate these functional classes. In this step, we compare the ability of the LVQ and SVM algorithms in supervised functional classification. The LVQ algorithm is initiated with a codebook of 16 vectors and converges after training over one epoch. We test the SVM method for three types of kernels – linear,

Gaussian, and second degree polynomial kernels. In all cases, 3-fold cross-validation is used.

Table 6.8 gives a description of typical training and test data distributions in the six functional classes.

CLASS ID / SIZE	DESCRIPTION	TRAINING	TESTING	TOTAL
1	Diverse	1493	746	2239
2	TCA	7	7	14
3	Respiration	17	10	27
4	Ribosomes	80	40	120
5	Proteasomes	25	10	35
6	Histones	8	3	11
7	HTH	12	4	16

Table 6.8 Yeast Data Set Distributions

Fig. 6.2 shows the estimated overall error rate and the 95% confidence intervals with different algorithms. We can clearly see that the LVQ algorithm performs as well as the SVM, though there is no significant difference between the two similarity measures. This is also illustrated by the similarity in representative confusion matrices for the two distances in Tables 6.9 and 6.10. The SVM algorithm with the Gaussian kernel has the least average error rate, and also outperforms the unnormalized symmetric Kullback-Liebler LVQ algorithm with 95% confidence.

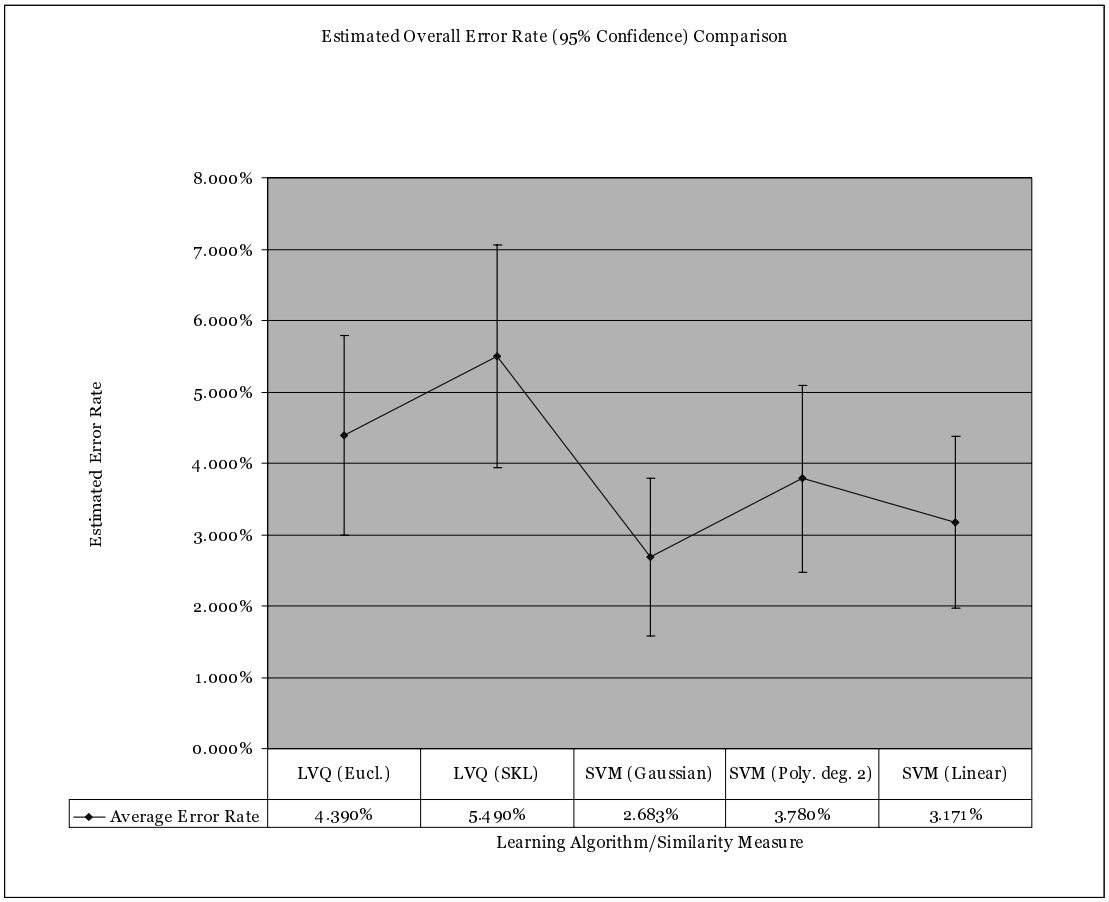


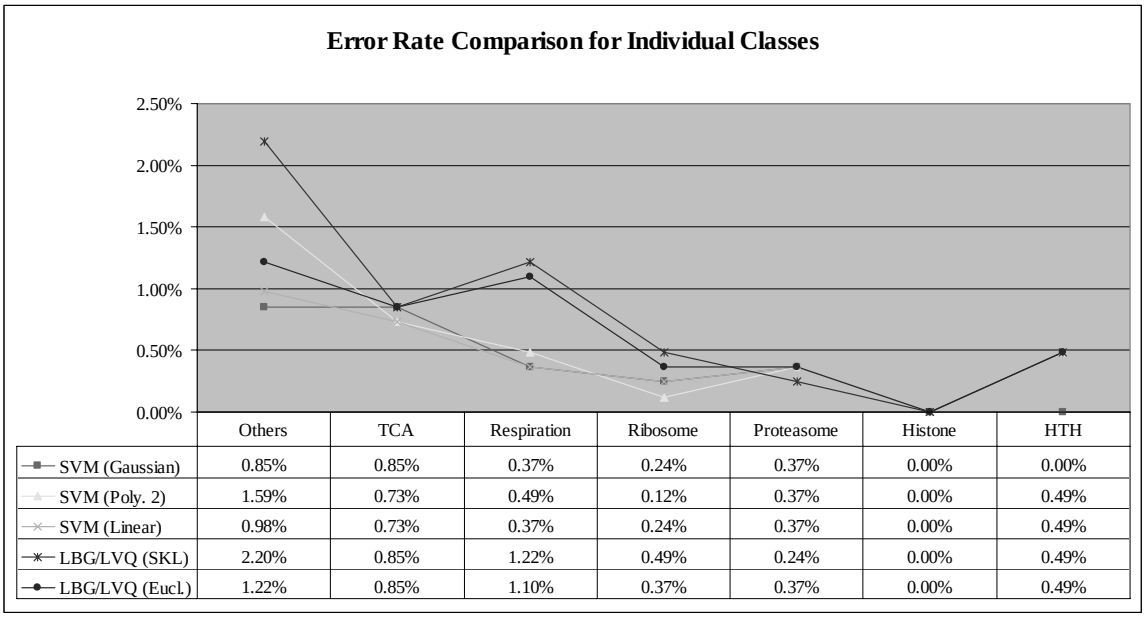
Fig. 6.2 Overall Error Rate Comparison with 3-fold cross-validation

CONFUSION MATRIX (TESTING) WITH SKL LBG/LVQ ALGO							
PREDICTED/ACTUAL	A1	A2	A3	A4	A5	A6	A7
P1	728	7	10	4	2	0	4
P2	0	0	0	0	0	0	0
P3	0	0	0	0	0	0	0
P4	5	0	0	36	0	0	0
P5	13	0	0	0	8	0	0
P6	0	0	0	0	0	3	0
P7	0	0	0	0	0	0	0
	746	7	10	40	10	3	4
PREDICTED/ACTUAL	A1	A2	A3	A4	A5	A6	A7
P1	0.9759	1	1	0.1	0.2	0	1
P2	0	0	0	0	0	0	0
P3	0	0	0	0	0	0	0
P4	0.0067	0	0	0.9	0	0	0
P5	0.0174	0	0	0	0.8	0	0
P6	0	0	0	0	0	1	0
P7	0	0	0	0	0	0	0

Table 6.9 Confusion Matrix for LVQ Testing with the log-Euclidean Distance

CONFUSION MATRIX (TESTING) WITH EUCL. LBG/LVQ ALGO							
PREDICTED/ACTUAL	A1	A2	A3	A4	A5	A6	A7
P1	736	6	9	3	3	0	4
P2	0	0	0	0	0	0	0
P3	0	1	1	0	0	0	0
P4	6	0	0	37	0	0	0
P5	4	0	0	0	7	0	0
P6	0	0	0	0	0	3	0
P7	0	0	0	0	0	0	0
	746	7	10	40	10	3	4
PREDICTED/ACTUAL	A1	A2	A3	A4	A5	A6	A7
P1	0.9866	0.8571	0.9	0.075	0.3	0	1
P2	0	0	0	0	0	0	0
P3	0	0.1429	0.1	0	0	0	0
P4	0.008	0	0	0.925	0	0	0
P5	0.0054	0	0	0	0.7	0	0
P6	0	0	0	0	0	1	0
P7	0	0	0	0	0	0	0

Table 6.10 Confusion Matrix for LVQ Testing with the Unnormalized Symmetric Kullback-Liebler Measure



	Others	TCA	Respiration	Ribosome	Proteasome	Histone	HTH
SVM (Gaussian)	0.85%	0.85%	0.37%	0.24%	0.37%	0.00%	0.00%
SVM (Poly. 2)	1.59%	0.73%	0.49%	0.12%	0.37%	0.00%	0.49%
SVM (Linear)	0.98%	0.73%	0.37%	0.24%	0.37%	0.00%	0.49%
LBG/LVQ (SKL)	2.20%	0.85%	1.22%	0.49%	0.24%	0.00%	0.49%
LBG/LVQ (Eucl.)	1.22%	0.85%	1.10%	0.37%	0.37%	0.00%	0.49%

Fig. 6.3 Comparison of Error Rates for Individual Classes using 3-fold Cross-validation

Fig. 6.3 shows the error rate components for individual functional classes. The helix-turn-helix proteins are the control group, while the other five named classes are known to respond to the conditions. We see that taking the standard deviation into consideration, the methods are indistinguishable in all classes. All classes are able to classify histones, ribosomes and genes in the tricarboxylic acid (TCA) cycle consistently.

6.4.3 Cancer Classification and Discovery in Leukemia data

Two types of experiments are conducted with the leukemia data – leukemia class prediction with the training dataset and class discovery with the independent testing dataset. The oligonucleotide array data used here measures the average difference values for each gene

in an experiment against a control. The pre-processing step involves setting negative expression values to 20; we do not exclude genes marked absent in certain experiments.

We primarily study the Find Discriminating function (Use Case 1.2.1 in Sec. 4.4), which tries to filter a small subset of genes, which are sufficient to predict a disease phenotype, and which can be used to classify new phenotypes. We compare four different methods using the following performance measures:

(i) Cross-validation error with the training set.

(ii) Overall error with the independent testing set.

(iii) Number of genes required obtaining certain prediction accuracy with the independent testing set.

6.4.3.1 Prefiltering with the IB3 Algorithm

As in the method described in [5], we choose the ideal expression pattern as the one which is uniformly high in the ALL experiments and low in the AML experiments, and vice-versa. We then pick genes {g}, which have a close resemblance to this pattern c', based on the following similarity measure:

$$P(g,c') = \frac{\mu_g^{ALL} - \mu_g^{AML}}{\sigma_g^{ALL} + \sigma_g^{AML}}$$

We picked an arbitrary absolute threshold value of 0.5 to prefilter leading to 1331 'active' genes.

The class prediction tests now involve several steps:

- Cluster with the unsupervised LBG algorithm and label the genes based on the initial codebook.
- Pick informative instances from the clusters using the IB3 algorithm.
- Filter acceptable stored instances with high predictive accuracy exceeding its class frequency by a large margin.
- Use this set of ‘informative’ genes in clustering across experiments using the LVQ and SVM algorithms.

Here, we note that we use the nearest neighbor rule to predict the class and do not take a weighted vote from all the informative genes, as in [5].

The LBG algorithm divides the 1,331 genes into 4 clusters and the IB3 algorithm picks 59 and 66 instances of high-accuracy, with the log-Euclidean and the unnormalized symmetric Kullback-Liebler measures respectively. The training data composed of expression measurements of these genes across 38 types of leukemia, are used to train LVQ with 4 code vectors.

In a similar manner, the set of informative genes from the log-Euclidean distance based filtering, is used to train SVMs.

The error rates from 3-fold cross-validation experiments with these training methods are shown in Fig. 6.4. The log-Euclidean LVQ algorithm shows the best average error rate of all algorithms.

The performance of LVQ algorithms before pre-filtering shown in Fig. 6.5 shows that IB3 is able to capture certain discriminative instances.

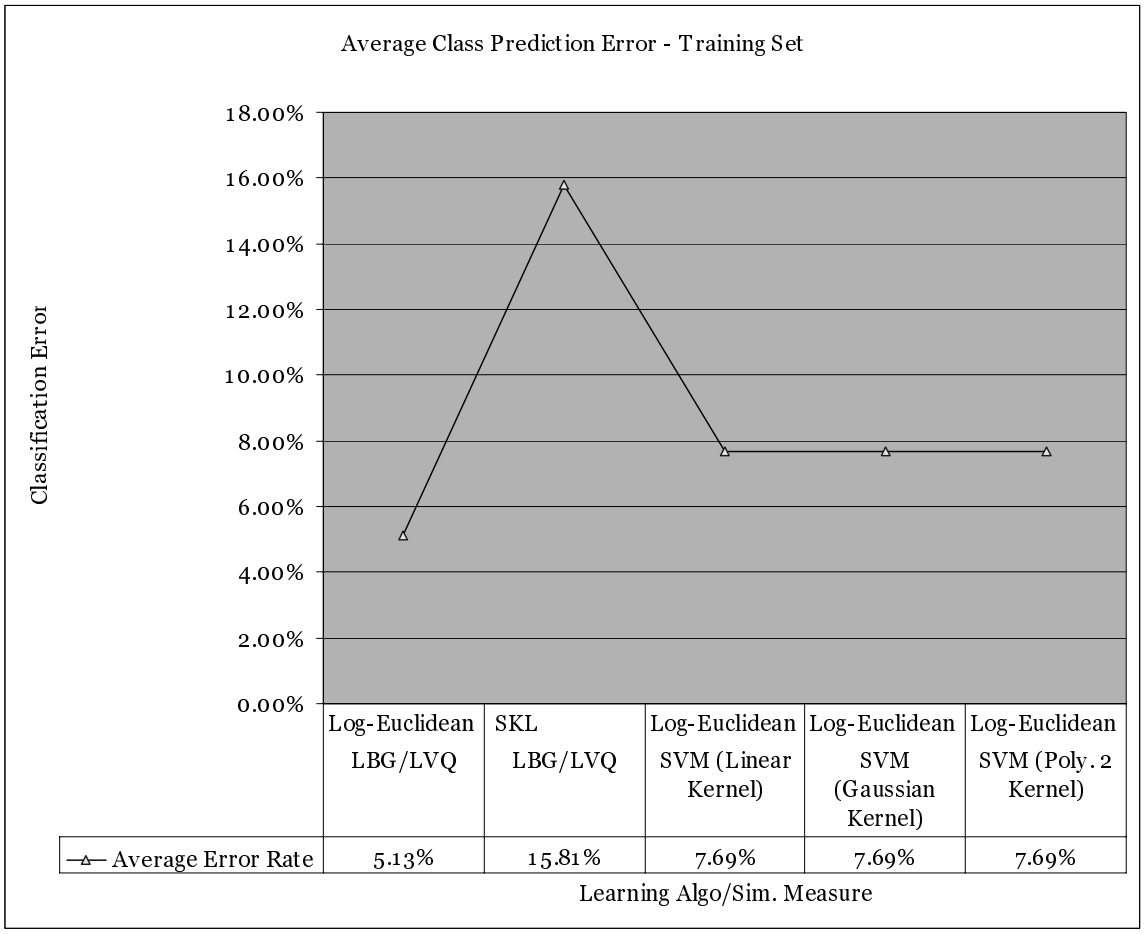


Fig. 6.4 Average Class Prediction Error over the Training Set

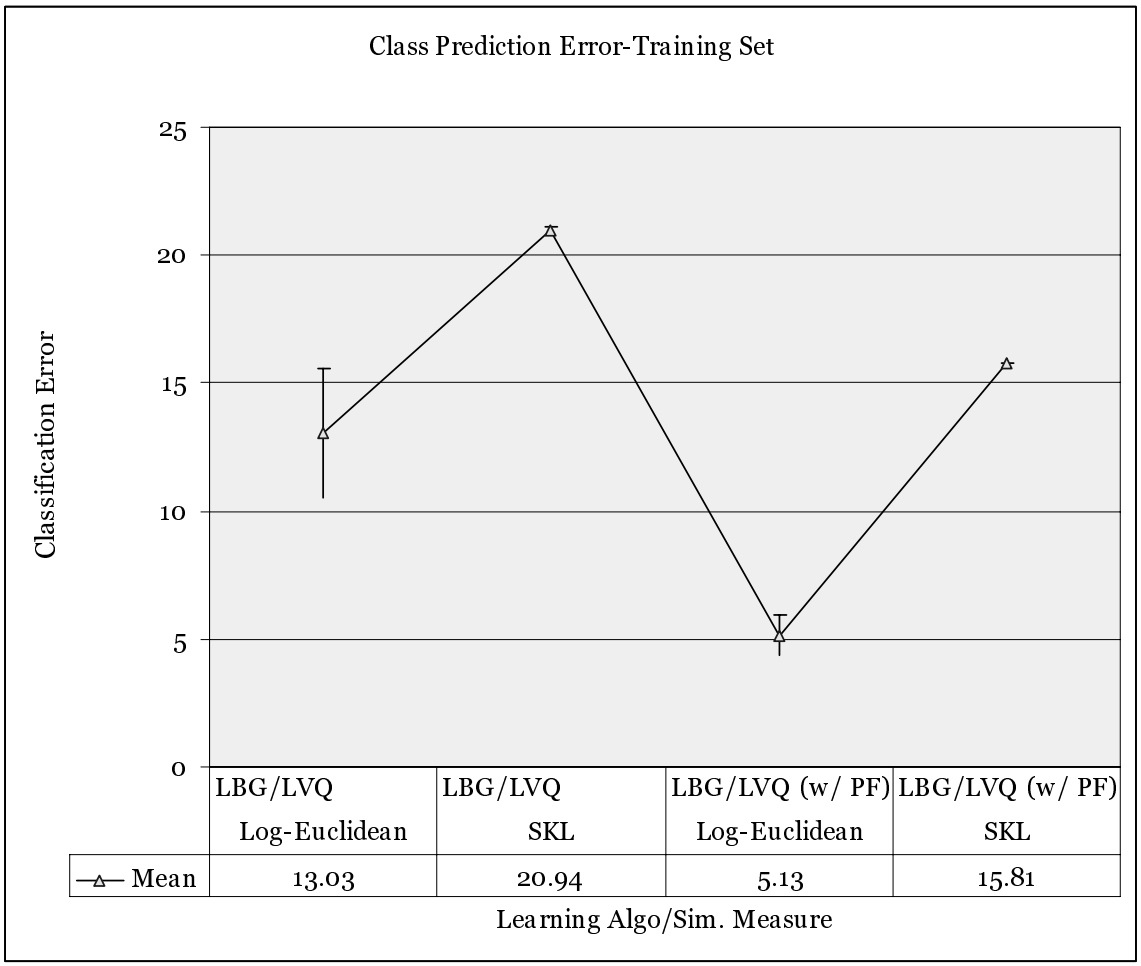


Fig. 6.5 Decrease in Classification Error with IB3 Discriminative Instances

Fig. 6.6 shows the performance of the learning algorithms on the independent testing data set using the same set of discriminating genes as in the training set. We find that both LVQ and SVM algorithms fail badly on the testing data compared to results from [5], indicating a defect in the pre-processing steps and the retrieval of stored instances from IB3. Further, we also note that the unnormalized symmetric Kullback-Liebler measure leads to a significantly poorer performance compared to the log-Euclidean distance on this data set.

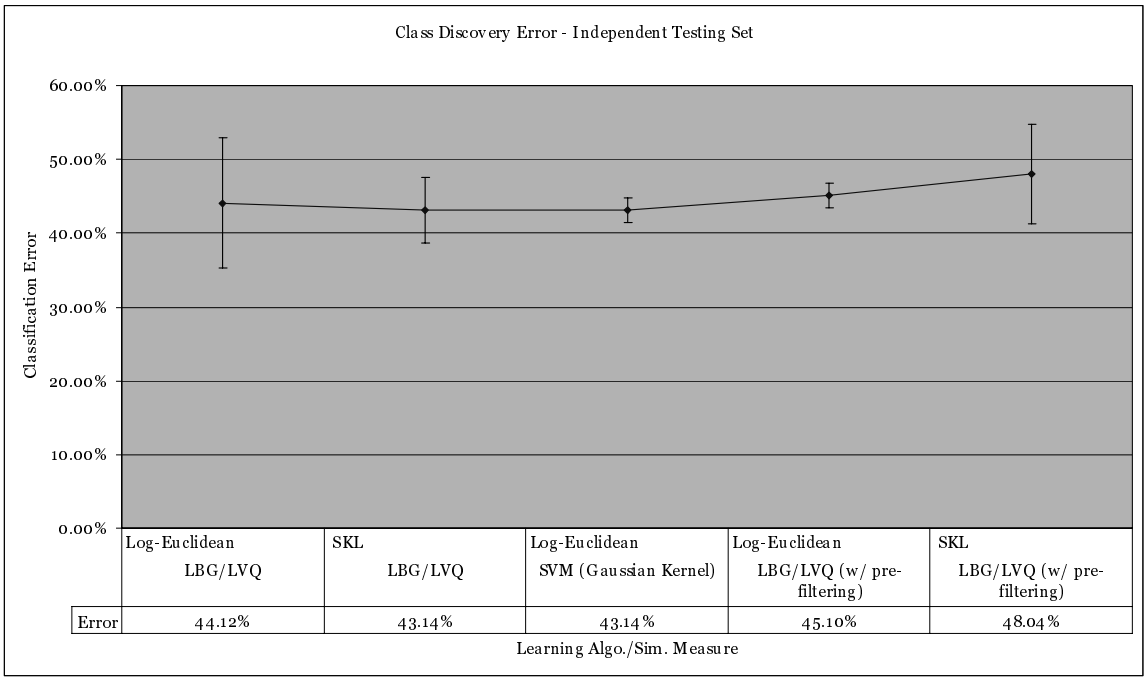


Fig. 6.6 Classification Error Variation on the Independent Testing Data Set

6.4.3.2 Pre-filtering with the Relative Distance

In this method, we compare the relative distance between the expression profiles of a gene in the 27 ALL and 11 AML data sets, to filter 'interesting' genes. The objective is to identify genes that have uniformly high dissimilarity between the two classes. The steps are listed as described below.

(i) Eliminate gene profiles that are constant or show low variation (This filters 483 genes).

(ii) Randomly select 100 combinations of 11 ALL experiments corresponding to each gene and evaluate their distance with the corresponding AML profile.

(iii) Sort the genes by the coefficient of variation $\frac{\mu}{\sigma}$ of the distances. Pick *N* genes with the least value.

(iv) Apply the LBG/LVQ algorithm to determine cross-validation error on the training dataset, and perform unsupervised clustering with the LBG algorithm on the independent testing dataset.

Fig. 6.7 shows the results of the method with the log-Euclidean and SKL similarity measures. We find that by filtering to a much smaller subset of genes (*N* = 30-70) gives an improved performance over classification with the independent testing dataset. The SKL similarity gives a better performance with 30 and 50 discriminating genes compared to the log-Euclidean similarity over the testing dataset.

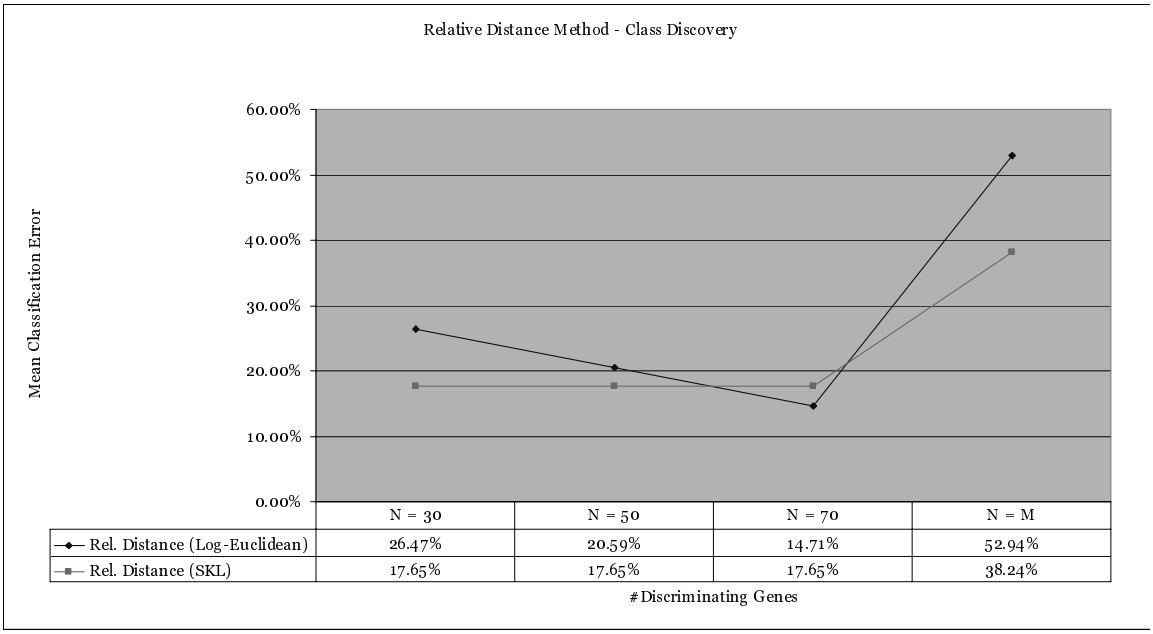


Fig. 6.7 Class Discovery Error with the Relative Distance Method

Table 6.11 shows the best performance of the algorithm using 30 and 50 discriminating genes; we find that both similarity measures perform similarly with this training set.

Find_Discriminating Error	Mean/N = 50	Std. Dev.	Mean/N = 30	Std. Dev.
Rel. Distance (Log-Euclidean)	23.29%	12.95%	31.41%	20.02%
Rel. Distance (SKL)	24.15%	17.01%	29.06%	5.34%

Table 6.11 Average Class Prediction Error with the Relative Distance Method and N = 50

6.4.3.3 Pre-filtering with Principal Components Analysis

Principal components analysis (PCA) is a second-order statistical method that finds linear components of genes, called eigen-genes, which explain the maximum amount of variance. PCA is inherently applicable to Gaussian data, and hence we use log-AvgDiff values based on the assumption stated in 5.2.2 and [9].

Using singular value decomposition (SVD), it is possible to obtain K $(= \min(M, N))$ combinations of genes from a (N genes x M experiments) dataset, which contribute to the total variance in decreasing order. We use the profiles of these eigen-genes to determine the discriminating genes as follows:

(i) Eliminate gene profiles that are constant or show low variation and center the dataset consisting of the log-values of Average Difference measurements.

(ii) Perform SVD on the centered dataset.

(iii) Pick the principal component PC_i that explains a large proportion of the variance and is closest to the ideal reference vector (uniformly high in one class and low in the other).

(iv) Sort the genes that are closer to PC_i than other principal components by their correlation with PC_i.

(v) Pick the top $N/2$ genes with high positive and negative correlations to obtain the discriminating genes.

(v) Apply the LBG/LVQ algorithm to determine cross-validation error on the training dataset, and perform unsupervised clustering with the LBG algorithm on the independent testing dataset.

The results of PCA pre-filtering and classification with the two similarity measures are shown in Fig. 6.8. We find that by filtering to a much smaller subset of genes (N = 30-70) gives an improved performance in classification with the testing dataset. The SKL similarity gives a marginally better performance with 30 and 50 discriminating genes compared to the log-Euclidean similarity over the testing dataset. However, the best training cross-validation error obtained in Table 6.12 with 50 genes indicates that the principal component obtained from the training set is able to discriminate the experimental profiles in the training dataset. There is no significant difference in their performance of the similarity measures as expected (Sec. 6.4.2), since the same set of genes is used to train the LBG/LVQ algorithm.

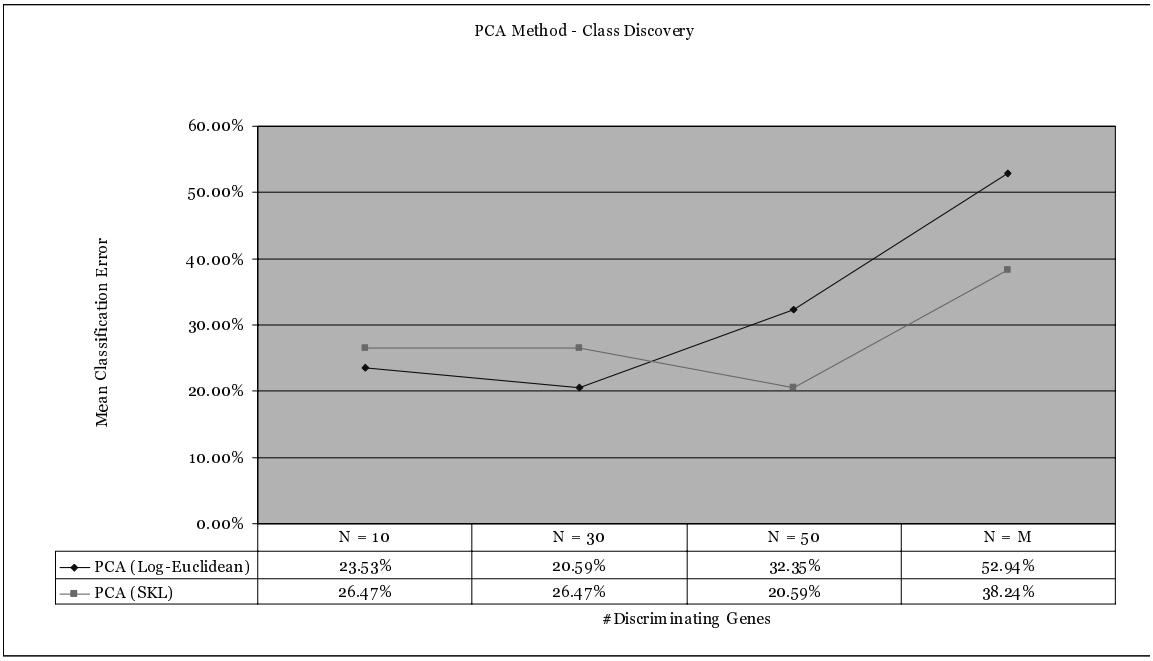


Fig. 6.8 Average Class Prediction Error with the PCA Method

Find_Discriminating Error	Mean Error	Std. Dev.
PCA (Log-Euclidean)	5.13%	4.44%
PCA (SKL)	5.13%	8.88%

Table 6.12 Class Discovery Error with the Relative Distance Method

6.4.3.4 Pre-filtering with the Find Similar Method

The Find Similar method simply takes the ideal reference vectors for both the ALL and AML cases, about the centroid of the expression profiles. It then finds the top $N/2$ genes that are closest to the ideal vector according to the similarity measure.

Fig. 6.9 shows the performance of the method with the two similarity measures. The SKL similarity classifies all experiments in the independent testing dataset, with fewer (30) discriminating genes, compared to the best subset (of 50 genes) with the log-Euclidean

similarity. Fig. 6.10 shows the performance on the training data; we find that a subset of 30 genes gives the least cross-validation error with both similarity measures. This shows that the SKL similarity is able to give the least errors in class prediction and discovery, with a fewer number of discriminating genes, than the log-Euclidean distance.

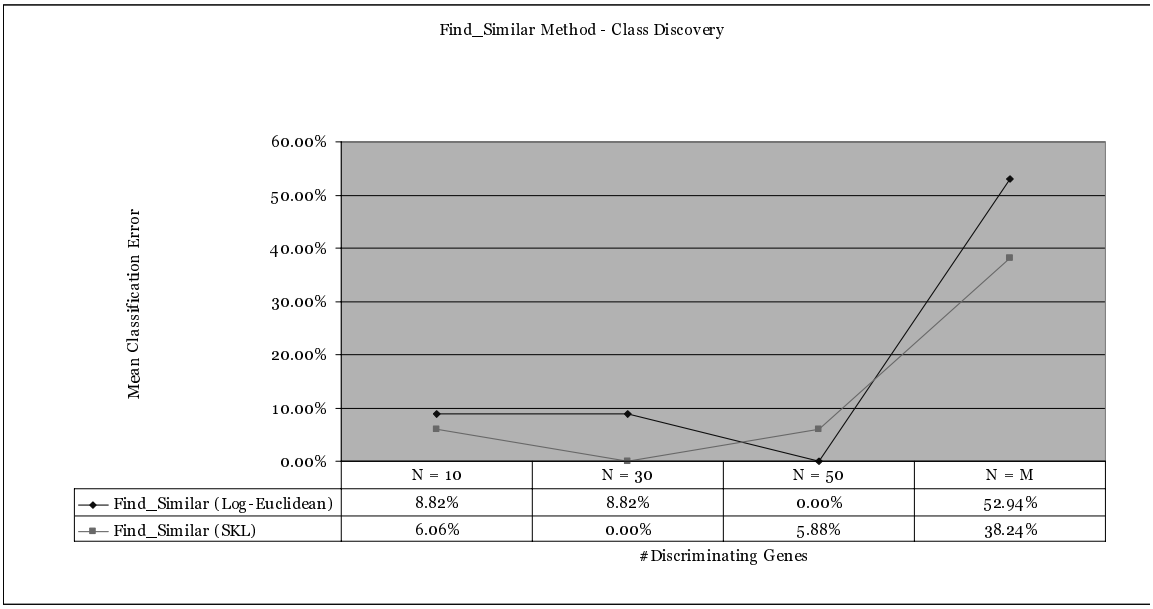


Fig. 6.9 Average Class Discovery Error with the Find_Similar Method

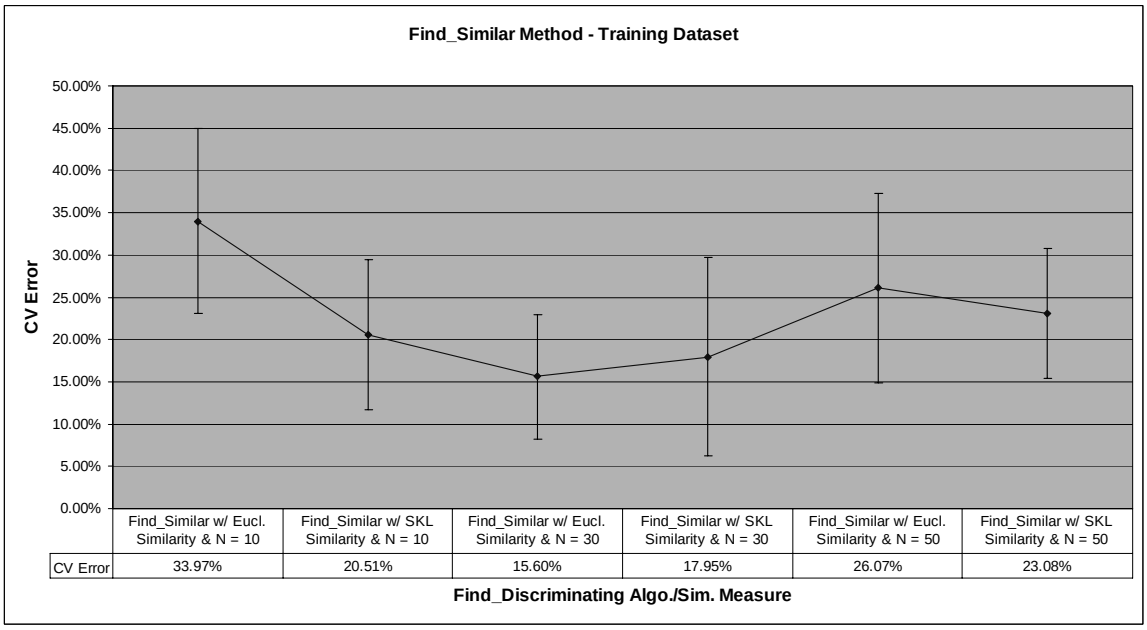


Fig. 6.10 Class Prediction Error with the Find_Similar Method

6.4.3.5 Comparison of Find Discriminating Methods

Fig. 6.11 shows a chart comparing the class prediction and discovery error rates of different Find Discriminating pre-filtering algorithms and the basic LBG and LVQ algorithms run on the entire dataset.

All these methods show a much-improved performance in detecting new disease phenotypes against the base case, with a very few number of genes. The Find Similar method, which compares the profiles in the gene set with an ideal gene vector, shows the best performance in terms of class prediction, class discovery, and the number of discriminating genes. Significantly, the SKL similarity measure is able to perfectly discriminate the two classes in the independent testing dataset, with 30 genes. It outperforms the log-Euclidean distance with the LBG algorithm.

The relative distance method captures genes with large and relatively constant separation between the expression profiles in the two types of experiments. Due to the large range of expression values in this dataset and noise, this might exclude significant genes.

The PCA method suffers from similar problems as the base case. Since the gene subset is obtained by the correlation with eigen-genes of the training data, it shows poor generalization with the testing dataset.

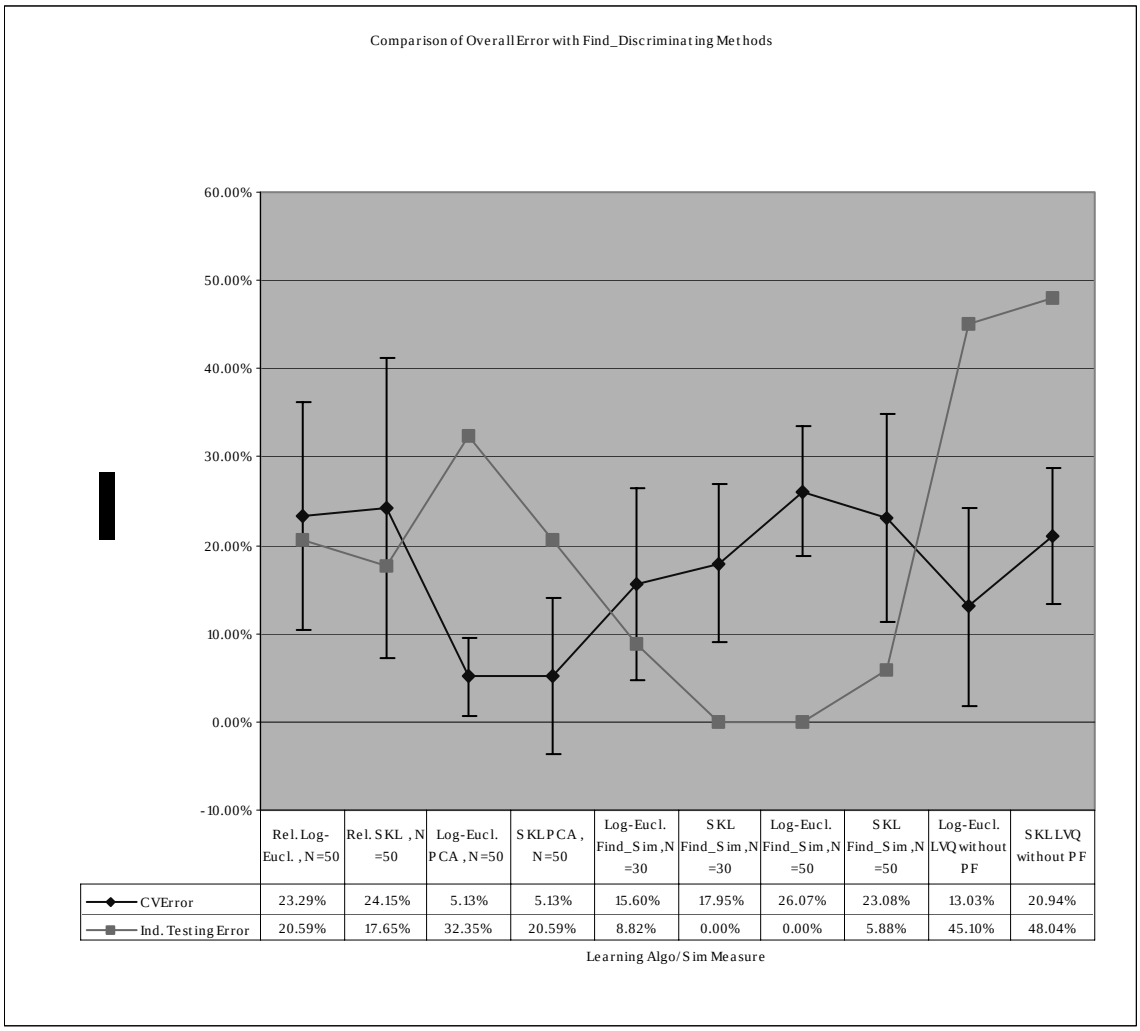


Fig. 6.11 Comparison of Class Prediction and Discovery Error Rates with Find_Discriminating Methods

6.5 Conclusions

In this thesis, we investigated processes in drug discovery through formal modeling techniques from systems engineering. We built a UML use case model to capture the requirements of a modern drug discovery analysis system and the related systems integration issues. We developed system structure and behavior, and discussed alternate methods of implementation. The discovery of efficient clustering algorithms for any particular type of microarray data will lead to efficient indexing mechanisms over very large experimental data sets. We also looked at database extensions that can handle new data types and how they can be applied to biological data.

The next major focus of the thesis was the application of algorithms from signal processing and machine learning, to the analysis of gene expression data. We investigated two different types of analyses – functional and phenotypic classification, and found that the supervised LVQ algorithm, with the LBG codebook initialization, shows very good performance and is comparable to support vector machines, which are globally optimal classifiers. We also applied an instance-based learning algorithm IB3, and found good results with the identification of yeast functional classes.

We also described a novel similarity measure for clustering based on unnormalized expression data, called the unnormalized symmetric Kullback-Liebler measure, based on the concept of relative entropy, and originally developed in [24,25]. Though there was no significant difference between the unnormalized SKL measure and log-Euclidean distance with cDNA arrays (as expected), we found that the unnormalized SKL measure is able to provide better performance with a leukemia oligonucleotide array data set. Different pre-

filtering methods for finding discriminating genes based on the Find Similar function, PCA, relative distances, and the IB3 instance-based learning algorithm, were studied. All methods except IB3 showed a large improvement in performance with pre-filtering. The Find Similar pre-filtering method with the unnormalized SKL measure and the LBG/LVQ algorithm together gave the best performance in class prediction and class discovery, with the fewest number of discriminating genes.

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