AN ORDER ESTIMATION BASED APPROACH TO IDENTIFY RESPONSE GENES
FOR MICROARRAY TIME COURSE DATA

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

ZHIHENG LU

In partial fulfilment of requirements

for the degree of

Doctor of Philosophy

September, 2008

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ISBN: 978-0-494-47605-5
Our file Notre référence
ISBN: 978-0-494-47605-5

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ABSTRACT

AN ORDER ESTIMATION BASED APPROACH TO IDENTIFY RESPONSE GENES FOR MICROARRAY TIME COURSE DATA

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Microarray time course experiments have been widely used to investigate temporal patterns of gene expression profiles. These expression profiles provide a unique opportunity to examine genome-wide signal processing and gene responses. A fundamental issue in microarray experimental design is that the treatment condition can only be controlled to the cell level rather than to the gene level. Given that some genes depend on other genes to detect changes in external conditions and that this kind of dependency is not fully deterministic and may vary across genes and treatment conditions, the expression of each gene is potentially affected by two confounding effects: the treatment effect and the gene context effect arising from the regulatory interaction structure among genes. This gene context effect is hard to isolate. Neither can it be simply ignored. Instead, this gene context information which is different under different treatment conditions is of primary biological interest and thus demands attention of statistical analysis.

We introduce an approach which provides a way to deal with the confounding effects and takes into account the uncontrollable gene context effect. Our method is developed to estimate the number of hidden states which is also referred to as the order of a hidden Markov model (HMM) for each gene. The observed gene expressions are modeled by gamma distributions determined by the corresponding hidden state at each time point. Those genes showing evidence for more than one hidden state can be categorized as the signaling genes, or in a wider sense, as the response genes which are coordinated by a cell system in reaction to a specific external condition. These response genes can be used in the comparison of different treatment conditions, to investigate the gene context effect under different treatments. Our method also provides flexibility in adjusting type I error rates to find response genes at different response intensity levels. Both simulated data and real microarray time course data are analyzed to demonstrate our method.

Acknowledgements

I sincerely thank my advisor Dr. Brian Allen for his insightful guidance, support and help during my study. I would like to thank my other advisory committee members, Dr. T. Desmond, Dr. R. Lu, Dr. G. Darlington and Dr. J. Horrocks for their treasured advice and help. Dr. A. Canty deserves special thanks for very extensive and constructive suggestions which have greatly improved this thesis.

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Chapter 1

INTRODUCTION

Microarray technology makes it possible for researchers to simultaneously assess the expression of thousands of genes (Schena et al., 1995; Lockhart et al., 1996; Richmond et al., 1997; Eisen et al., 1998). A huge amount of data that represents the expression patterns of potentially all of the genes in a cell under various treatments are generated by the scientific community and becomes available for statistical analysis. See Craig et al. (2003) for a more detailed review. In this chapter, we will first briefly introduce microarray technology in section 1.1. We then provide a description of microarray time course experiments in section 1.2 and explain the motivation and the goal of our order estimation analysis. In section 1.3, we briefly introduce microarray data pre-processing.

1.1 Microarray Technology

According to the central dogma of molecular biology (Snustad and Simmons, 2003), many key biological functions of cells are performed by proteins. The production of proteins is controlled by genes, which are coded in DNA sequences and passed along generations of cells by the DNA replication process. Protein production from genes involves two stages, known as transcription and translation, as shown in Figure 1.1. A single strand of messenger RNA or mRNA is first copied from the DNA sequence of the coding gene. After transcription, mRNA is used as a template to assemble a chain of amino acids to produce the protein during the translation stage. Since most of the functions of cellular biological processes are related to the changes of mRNA levels for some genes, systematic investigation of mRNA abundance on a genome-wide scale is critical in understanding cell systems. Microarray technology makes it possible to examine gene expression for any number of genes simultaneously, and hence for the first time in history, the comprehensive measurement of a large system as complex as a cell becomes available. The understanding of microarray data, however, turns out to be much more challenging than expected.

The Central Dogma of Molecular Biology

Figure 1.1, A schematic of the role of RNA in gene expression and protein production.

Graphics from <http://www.accessexcellence.org>.

There are two different microarray technologies, spotted arrays (also referred to as cDNA arrays) (Schena et al., 1995) and oligonucleotide arrays (Lockhart et al., 1996). The images for these two types of microarrays are shown in Figure 1.2 and Figure 1.3 respectively. To quantify gene expression levels in a cell or organism, microarray technology utilizes the natural affinity of single stranded DNA to bind with its

complementary sequence (either DNA or RNA sequence). There are four bases in a DNA sequence: A (adenine), T (thymine), G (guanine) and C (cytosine). In an RNA sequence, the base T is replaced by U (uracil). According to molecular biology, base A pairs with base T (or base U in the case of RNA sequences) and base C pairs with base G. A large number of known DNA sequences are first attached to the surface of an array chip or slide. These sequences are referred to as probes. The probes from the same sequence are attached to a small region referred to as a spot on a microarray slide surface. Then the genetic material (i.e. mRNA) is extracted from a cell or an organism. In some situations, mRNAs are further reverse-transcribed into cDNA. These sequences are referred to as targets. Then targets from a specific biological sample are labeled with a fluorescent dye and allowed to be hybridized to the probes. A laser scanner is used to measure the color intensity of the dye fluorescence for each spot on a slide. Higher fluorescence intensity indicates larger amounts of hybridized targets, which further indicates a higher expression level for the corresponding gene. The scanned slide images are shown as in Figure 1.2 and Figure 1.3.

Figure 1.2, cDNA Array Image. Graphics adapted from presentation “Statistical Issues in the Design of Microarray Experiment”, Jean Yee Hwa Yang, University of California, San Francisco, <http://www.biostat.ucsf.edu/jean/>.

Figure 1.3, GeneChip Affymetrix Array Image. Graphics adapted from presentation “Statistical Issues in the Design of Microarray Experiment”, Jean Yee Hwa Yang, University of California, San Francisco, <http://www.biostat.ucsf.edu/jean/>.

Targets are labeled slightly differently between spotted arrays and oligonucleotide arrays. For spotted arrays, samples from two different treatment conditions are prepared. The targets are labeled with two fluorescent dyes: Cy3 (green) or Cy5 (red). Then these targets are mixed together and hybridized competitively to the probes. The images scanned for these spotted arrays have potentially two colors for each spot as shown in Figure 1.2. The major steps in a microarray experiment are illustrated in Figure 1.4. The oligonucleotide array is prepared in a similar way to cDNA arrays.

Oligonucleotide arrays use only one color for each spot. The targets are labeled with a single color and hybridized to the probes. For the most widely used Affymetrix GeneChip arrays, normally 11-20 pairs of probes, of length 25 bases, are attached to a GeneChip for each known sequence. In each pair, one is the probe with known DNA sequence, known as perfect match (PM). The other is a sequence with the middle base (the 13th base of the 25 bases) changed, known as mismatch (MM). The MMs are used to control for experimental variation and to measure nonspecific binding of targets from other genes. Since the data analyzed in chapter 6 are from Affymetrix oligonucleotide microarrays, our subsequent discussion will focus on this type of microarray, and we further focus our discussion on time course microarrays performed for the purpose to investigate the regulatory relationship among genes.

Figure 1.4, cDNA microarray scheme. Graphics from <http://www.accessexcellence.org>.

1.2 Microarray Time Course Experiments

Microarray time course experiments with genome-wide gene expression profiles provide a unique opportunity to study biological signal processing among genes at the transcription level. In response to different environmental conditions, a cell system alternates the transcription of different genes to coordinate its adaptation process based on programmed logic stored in its genome. These organized responses provide a unique signature of cellular response to a specific environmental condition at the transcription level and hence is of major biological interest. Since the signals sent from a specific gene may only be present for a short period of time, time course microarrays provide very

important information to study cellular signaling processes. As an example, the temporal expression profiles for 8 genes in an experiment to examine the effect of infection of *A. phagocytophilum* on polymorphonuclear leukocytes (PMNs or neutrophils) (Borjesson et al., 2005) are shown in Figure 1.5. This data set is analyzed in chapter 6.

There is increasing interest in microarray time course experiments and several statistical models have been proposed for time course data in recent years, among them the two-way ANOVA model (Park et al., 2003), B-spline model based time curve fitting (Bar-Joseph et al., 2003; Luan and Li, 2004), multivariate empirical Bayes method (Tai and Speed, 2006), and hidden Markov models (HMMs). Schliep et al. (2003, 2004, 2005) used partial supervised learning HMMs to cluster gene time course profiles. Their method uses a group of HMMs to represent clusters estimated from the temporal profiles of a known group of genes, then iteratively assigns unknown genes into these clusters and refines the clusters. The order (i.e. the number of hidden states) for the HMM in each cluster is either predetermined or can be inferred from the genes in a training set. Yuan and Kendziorski (2006) proposed another HMM in which the order is specified as a function of the number of treatment conditions. Existing HMM methods assume that the order is either already determined by some imposed criterion or is the same as those of known genes. This may not always be the case. The biological processes generating the observed expression for different genes are not known and may potentially provide important biological insight for cell systems. The order can not be assumed to be a known parameter; instead needs to be investigated. The estimated order under one particular treatment condition may provide important information about regulatory interactions among genes.

Figure 1.5, Gene expression profiles for 8 genes at time points 0, 1.5, 3, 6, 9, 12 and 24 hours.

To investigate cell-wide gene expression profiles for a large number of genes, biologists usually measure gene expressions repeatedly over time. This kind of experimental setting is described in Figure 1.6. Our further discussion will focus on the situation where the same type of cells are prepared under different treatment conditions. In other situations, the treatment condition may be kept the same, while different types of cells are measured. We discuss the first situation here only since it is a typical microarray experimental setting.

Figure 1.6, Microarray time course experiment under one specific treatment condition.

As shown in Figure 1.6, individual cells from the same biological type under one specific treatment condition are lysed at a given time point. Their gene expression levels

are measured for a given list of genes. Normally, the gene list is fixed throughout the whole time course experiment. This list is usually determined by the design of the microarray to cover most important genes in the genome. The list typically consists of a large number of genes. At a particular time point, the same microarray experiments are performed several times. The expression levels of the genes are recorded as replicates for a specific time point. The replicates here are the biological replicates because the replicates come from different cells as shown in Figure 1.6. The number of time points is normally chosen to be 5 to 10. The number of replicates could be different across time points.

One important effort for experimentalists is to synchronize the cell so that at each time point, the cells undergo the same stage of the biological process. The biological process which generates a gene's expression profile is of primary interest and is also the essential motivation for the development of our analysis method. As commonly agreed, the interaction relationship among genes is usually represented by a gene regulatory network. The structure of this network, however, may change along with treatment conditions and over time. To illustrate the cause and effect structure, two simplified conceptual diagrams for a cell system consisting of only six genes are presented in Figure 1.7 and Figure 1.8. They are used to represent two treatment conditions as an example.

Figure 1.7, First category of assumptions: the effect of treatment condition is assumed to be able to reach every gene of a cell. The same treatment is assumed to have constant effect on each gene across time. Under the constant treatment effect over time, the change of the expressions is usually attributed to the time effect for a gene.

There are at least two different categories of assumptions about the underlying biological process which generates the gene expressions. The first one, as illustrated in Figure 1.7, is the most often used. It assumes that every gene can be affected directly by an external treatment condition controlled by a human experimentalist. The arrows in Figure 1.7 represent the effects exerted from the cause to the affected items. Different treatment conditions are assumed to be able to reach each gene directly in the same way. The influence from a treatment condition to a gene is commonly assumed to be the same across time. Under the same treatment, the change of expression of a gene is regarded

sometimes as the result of a time effect. Unfortunately, this category of assumptions is not supported by existing biological evidence. The well observed regulatory interactions among genes make those analyses based on these assumptions problematic.

Instead, the gene regulatory network discovered so far strongly suggests the second category of assumptions, as shown in Figure 1.8. Under this category of assumptions, under one treatment condition, each gene can be influenced by the external condition of a cell directly, or by the effect of other genes or a mixture of both. For example, in Figure 1.8, at time point 0 under treatment condition 1, gene 1 is affected directly by treatment condition 1. Gene 3 is not directly affected by treatment condition 1 since there is no arrow connecting treatment 1 to gene 3. However, it is indirectly affected by treatment condition through the influence of gene 1. Gene 2 receives both direct and indirect influences. The causal relationship among genes can also change with treatment conditions, and with time under the same treatment condition. As shown in Figure 1.8, the arrows and their connected genes may be different at time 0 between treatment 1 and treatment 2. Under the same treatment condition, the arrows and their connected genes can be different between time point 0 and time point 1. In other words, at the same time point, different treatment conditions may affect different genes. In case a fixed set of genes is used by cells to sense different treatment conditions, their activated genes could be different. Furthermore, these activated genes may be different from time to time.

Figure 1.8, Second category of assumptions: the treatment condition is not able to reach all the genes in a cell. Different treatment conditions may affect different genes. The genes affected by a specific gene may be different from time to time.

The key difference between the above two types of assumptions is that it is acknowledged only in the second category of assumptions that a treatment condition can only be controlled to the cell level instead of to the gene level. In a real situation, which gene is affected directly by treatment condition is not known. Given a treatment, which gene activates another gene is not known either. Because this kind of knowledge is of primary biological interest, the analysis assuming the interaction is ignorable is inappropriate. Moreover, how a controllable experimental condition affects the regulatory

relationship is not known and thus is not controllable. The fact that genes are not equally affected by one particular external condition of the cell, and that some genes depend on other genes for information about external conditions is well established in biology. In addition, the cascading of cellular signals also results in different times at which a treatment condition affects particular genes through other genes. Regulatory interactions, such as the turning on or turning off of the expression of a gene, are not fully deterministic and uncertainty can be reasonably assumed. Hence, the observed gene expression level is affected potentially by two confounding effects. The first effect is the controllable treatment condition. The second one is the uncontrollable gene regulatory effect. The effect produced from other genes that changes the expression of a specific gene is referred to as the gene context effect. For a particular gene, the gene context effect is potentially gene specific under the same treatment. It may also be different for the same gene under different treatments.

In Figure 1.8, the arrows coming from the treatment condition represent the controllable treatment effects. The arrows between the genes inside each cell represent the uncontrollable gene context effects. The gene context effect is not deterministic. In particular, the higher expression of one specific gene may not be due to a treatment condition. Instead, it may have resulted from random activation by one of its regulatory genes. The regulatory network among genes is not fully understood yet and is of primary research interest, making the first category of assumptions which ignores the effect of interaction among genes undesirable.

An often-used approach of microarray analysis is aimed to compare the expression levels between different treatment conditions and to find those genes which are differentially expressed. For the same consideration, most time series microarray analysis is aimed to compare the expression profiles between treatments to see whether the temporal expression pattern is different or not. This kind of analysis is problematic because it is based on the first category of assumptions. To see the problem, suppose the activation (activation means a higher level expression) of gene 1 will lead to the activation of gene 3 as shown in Figure 1.8. Because the regulatory relationship is not deterministic, the probability that gene 3 is activated, given that gene 1 is activated, is less than 1.

$$P(\text{gene 3 activated} \mid \text{gene 1 activated}) < 1 \quad (1.1)$$

This means that there is a non-zero probability that gene 1 fails to activate gene 3 under a given treatment condition 1. If gene 1 is always activated under treatment condition 1, each realization of gene 1 can be used to characterize treatment condition 1. However, each realization of gene 3 is not guaranteed to come from its activated state because the probability given gene 1 is activated is less than 1. Therefore, the expression measured for a gene like gene 3 under treatment condition 1 can not be guaranteed to come from its activated state in each realization. For this kind of gene, the comparison of the expression levels between two treatments can not be used as a characterization of treatment condition 1 because its expressions may be generated when it is activated or inactivated under a specific treatment condition.

To further illustrate this, let us assume there are two expression states for gene 1 and gene 3. Also let us assume that the activated state is the state with higher expression

levels and the inactivated state has lower expression levels for both genes. If the realized expressions for gene 1 under treatment 1 are always from the activated state (higher expressions), the realized expressions for gene 3 under treatment 1 can come from either the activated state or the inactivated state because the probability in (1.1) is less than 1. In other words, in some situations gene 1 fails to activate gene 3 even though they are always under treatment condition 1. The comparison of the expressions for gene 3 between the two treatment conditions is not meaningful because the replicates under each treatment condition are potentially a mixture from both activated and inactivated states. In a real situation, because we do not know a gene's position in the pathway for a treatment condition, statistical comparisons conducted to look for the differential expressions may simply generate misunderstanding.

For the same reason, the temporal expression curves or the state sequences estimated based on time course expression profiles should not be used in the comparison of treatments. Without knowing the position in a pathway for a particular gene, the comparison for the expression at each time point among treatments has the same problem as mentioned above. That is, the realization of expressions at specific time points may arise from either of the states.

From above we can see that whether the realizations of a gene's expression comes from more than one state depends on its position in a pathway or depends on how it relates to other genes. The probability of visiting more than one state could be different for different genes, and it may differ with treatment conditions. This is the reason why the effect is referred to as the gene context effect. The gene context effect thus can be

thought of as a modifier of the effect from a specific treatment condition, making it different for different genes. Understanding the gene context effect will lead us to discover the regulatory relationships among genes and this is also the primary goal of our method.

To tackle the challenging problem caused by gene context effects, we propose a new approach for the statistical inference on microarray time course data. We propose to identify the response genes first. These response genes could be genes which are involved in sending out regulatory signals or performing certain biochemical functions or a mixture of both. As response genes, they all have a common characteristic: their expressions are generated from different states over time. We define response genes as those genes which have shown evidence that they have occupied different states of expression. The detailed definition of expression state will be discussed in the following chapters. Those genes influenced by the gene context effect may show expressions realized from different states. By extracting this kind of gene, we can obtain information about those genes which interact with each other under a specific treatment. This knowledge, accumulated across many different treatment conditions, can lead us to discover the gene regulatory network.

The different states (for example, activated state or inactivated state) have their own molecular biological meaning too. Based on current biological knowledge, the transcriptional responses are all performed by employing molecular association to targeted molecular complexes, that is, by binding to certain sites. In a general sense, binding means molecular binding, which is the association of two or more molecules or

molecular complexes. Molecular binding is an attractive interaction between two molecules or molecular complexes (formed by association of two or more molecules). The molecules that can participate in molecular binding include proteins, nucleic acids, carbohydrates, lipids and other organic molecules of a cell. The formation of binding during gene interaction and cellular signaling processes suggests the following characteristics. First, the binding or other responses (e.g. no binding, binding or some different types of binding) are achieved by changing the expression levels. For example, the shift from a lower expression to a higher one may result in an activation signal sent to the genes it regulates. Note that a specific expression level itself such as a higher expression does not always correspond to activation for different genes. Second, the responses or the regulatory signals that can be communicated among genes are discrete in nature, and the number of different states or responses is limited. The status of binding or different types of binding is less likely to be continuous. Third, without accurate expression level detection and control mechanisms in cells, the binding or release of binding in a cell system can only be achieved probabilistically rather than deterministically. Of course, the certainty of a signal is increased if the expression level change is sufficiently large from its original expression intensity. However, the expression level change may not be necessarily large enough to meet a statistical significance level imposed by analysts.

The focus of our proposed method is to identify the set of response genes that change state under a treatment condition. We will show that state differentiation will lead us to find those genes that are used by a cell system in response to a specific treatment condition. In the following analysis, the temporal expression is conceptually represented

by a hidden Markov model (HMM). The HMM is specified for each gene under each treatment condition separately. We postulate that there is a finite number of hidden states representing the different molecular binding status, such as binding, not binding or different kinds of binding. The status of binding can not be directly observed from microarray measures and therefore is represented by the hidden states of the model. To make the signal detectable for other genes or to alter an on-going biochemical process, the states need to be stochastically distinguishable. At each time point, the observed expressions are regarded as being generated from a distribution which depends on the corresponding state at that time point. Different genes under different treatment conditions may employ different hidden states to perform biological functions. These states are not directly comparable. Instead, the differentiation of the states provides the only basis for identification. The estimation of the number of hidden states, also referred to as the order of HMMs, provides the most important information for us to understand gene context effects.

There are two dependence structures in our hidden Markov model. First, at each time point, the gene's expression level depends only on the corresponding hidden state. This dependency is modeled by the so called emission distribution. Second, at each time point, the state is influenced only by its immediate previous state. This temporal dependency is modeled by the transition probabilities. For the reasons discussed above, we assume that the Markov chain of the hidden states has a finite and discrete parameter space. The underlying Markov process is also assumed to have a stationary probability when we perform the order estimation. The reasons of using a stationary probability will be discussed below.

Those genes with more than one state consist of the subset of genes which are used by a cell in response to a particular treatment condition. For each treatment condition, our analysis provides a subset of response genes which show evidence of having different hidden states. The rest of the genes are in the non-response gene subset. Our method provides essential information to compare the response gene set (or the non-response gene set) across any number of treatment conditions without the need to align the time points. Those genes having high frequency to appear together may be involved in the same or closely related biological pathways.

Estimating the order is also the most straightforward way to find the response genes. Estimating the state sequence on the other hand requires that more parameters be estimated, making it less efficient for microarray analysis where a typical limited sample size is always the case. Hence, we suggest estimating the order instead of estimating the state sequence to find the response gene set. In addition, because the state sequences can not be used for the comparison between treatments, it does not provide any informational gain beyond the order in characterization of the gene context effect.

For each gene, we further assume that the observed expressions at a specific time point come from the same hidden state for a specific treatment condition. Usually one of the objectives of experimentalists is to keep all cells involved in time course microarrays synchronized so that the cells are reasonably regarded as undergoing the same stage of a biological process. With a limited number of replicates and time points, it is really

difficult to find the number of hidden states in a non-synchronized situation. We focus on the synchronized situations hereafter and assume this condition is satisfied.

The limited sample size and the limited number of time points are the reasons why microarray experiments are used as a screening tool to narrow down a subset of genes which can be further investigated in more detail. Our proposed method is developed according to this purpose. The subset of response genes provides the genes for more detailed examination. In addition, our method is an estimating method. It therefore provides flexibility to identify response genes at different response intensity levels.

1.3 Microarray data pre-processing

In most situations, before a statistical analysis is performed for microarray data, the raw expressions need to be pre-processed to reduce the unwanted variation resulting from the multiple steps in a microarray experiment. The quantification of the fluorescence intensities consists of multiple steps such as locating the spot and measuring the spot color intensity using image analysis techniques (Schadt et al., 2000; Yang et al., 2000; Nguyen et al., 2002).

At the first step, each spot needs to be located. The intensity of either one color for oligonucleotide arrays or two colors for spotted arrays is measured. The background color intensity around the spot is also measured. The background color intensity is usually subtracted from the spot intensity to estimate the actual intensity for each spot.

When there is more than one spot corresponding to the same probe, the intensity measures of these spots are averaged. These averaged intensity values are commonly used as the input data for the statistical analysis of gene expression.

The background color subtraction sometimes results in a negative intensity value, indicating the spot measured may have some quality issues. Two common practical ways to deal with this are either to discard the data for these spots or to set these values to an arbitrary constant slightly bigger than zero.

Another important consideration in microarray data pre-processing is to choose the sampling distribution to describe the data. There are two common choices for the intensity based on the well observed skewness of intensity data: log-normal distribution and gamma distribution (Kerr et al., 2000; Newton et al., 2001; Lee et al., 2000; Black and Doerge, 2001). For the log-normal distribution, a log transformation of the data is sometimes used. The log transformation is used quite often when inference is targeted on the fold change between two treatment conditions. The transformation techniques were developed with a focus on variance stabilization so that the statistical inference of fold changes could be expected to be more efficient. As discussed above, the approaches for conducting statistical tests on fold change are based on the first category of assumptions. These assumptions imply that the mean expression level can be used as a proper characterization of a treatment condition. Such simplification may not be valid when the gene context effect and treatment effect both have potential impacts on the expression intensity.

Since our focus here is to model the expression distribution directly, we choose to follow the direction of Newton et al. (2001) and use the gamma distribution without any data transformation. The gamma distribution is used by many researchers in modeling microarray data (Chen et al., 1997; Ideker et al., 2000; Newton et al., 2001; Kendzierski et al., 2003). Its fitting is examined by Newton et al. (2004) using QQ plots of the expressions of genes with similar mean expressions. The result confirms that the gamma distribution is a good choice for microarray data.

An additional step to remove other potential sources of variation is referred to as normalization (Nadon and Shoemaker, 2002). In addition to background intensity, there are other variation sources, caused by differences in hybridization conditions, such as temperature or humidity, when the experiments are conducted at different times. Additionally, laser power differences during the scanning may also produce differences in the intensity readings. It is therefore considered a necessary step to normalize the data across arrays when different arrays are used in an experiment. Typical approaches for normalization focus on standardizing overall intensity by fitting a loess curve and looking at the residuals and the MA plots (an MA plot is a plot of log-ratio of two expression intensities versus the mean log-expression of the two) (Dudoit et al., 2002). An alternative approach is the quantile normalization method (Bolstad et al., 2002), which transforms each of the array-specific intensity distributions so that they all have the same quantiles. The data set we analyze in chapter 6 is pre-processed and normalized by GeneSpring package version 6.0. The expressions among probe pairs are first averaged and the mean expression is obtained for each probe set. The expressions for different

probe sets are normalized for each array to produce the input data for our statistical analysis.

The pre-processed data from the study of Dorjesson et al. (2005) are used in the analysis in chapter 6. The data pre-processing and normalization were performed using GeneSpring package 6.0 in the original study. The GeneSpring package from Silicon Genetics provides data pre-processing and analysis tools for both Affymetrix microarray and cDNA microarray data. The normalization procedures provided in GeneSpring are similar to those of Affymetrix MAS 5. For the data set analyzed in chapter 6, the averaged difference between a perfect match and mismatch for each probe set is normalized by a per-chip normalization using the distribution of all probe sets on each chip. Similar to the global scaling procedure of MAS 5, per-chip normalization centers the intensities of each chip to a constant to control for the chip-wide variations. In addition, the per-gene normalization is applied using the median intensity of each gene to control for differences in detection efficiency among spots.

Pre-processed microarray data are then inputted for further statistical analysis. As discussed above, because of the confounding of treatment effect and gene context effect, the usual statistical tests for differential expression are questionable. Instead, we propose to estimate the order to find the subset of response genes. In chapter 2, we introduce our general model and notation. In chapter 3, the empirical Bayes estimation of gene specific coefficient of variation (CV) and the shape parameter of the gamma component distribution is discussed. In chapter 4, the order is estimated for each gene. The simulation study of order estimation is discussed in chapter 5. In chapter 6, we analyze a

real microarray time course data set GDS1428. This experiment investigates the effect of infection of *A. phagocytophilum* on Polymorphonuclear Leukocytes. We find that the order is both gene specific and treatment specific. The comparison of response gene set between the two treatment conditions is also described. In chapter 7, we include conclusions and further discussion.

Chapter 2

GENERAL MODEL

Hidden Markov models are widely used in modeling various stochastic processes over time (Rabiner, 1989; MacDonald and Zucchini, 1997). The underlying biological process produces the observed gene expressions. This process is potentially gene specific based on the second category of assumptions introduced in previous chapter. The expression level is obviously continuous, while the underlying biological responses can be reasonably assumed to be finite and discrete in nature.

The hidden Markov model has two stochastic components. One is the hidden Markov chain, which has temporal dependency structure described by the transition probabilities. The other component is the observed expression levels which are assumed to depend on the hidden state occupied at the corresponding time point. The observed gene expressions at a time point are thought of as being generated from a gamma

distribution whose mean parameter is determined by the hidden state at the corresponding time point. The gene specific shape parameter is assumed to be the same across time under one particular treatment condition. Letting the mean parameter depend on the corresponding hidden state is because the signaling or the response of a gene is usually achieved by alternating the expression levels based on available biological evidence. Note that a specific hidden state alone (whether it represents a high expression level or a lower expression level) does not indicate a definitive function. And the activation (or a higher expression level) has different functions for different genes. Thus the state itself alone does not provide useful information to characterize a gene's behavior. Instead, a response as defined as a change of states can be used in characterizing a treatment condition.

The shape parameter for a specific gene represents the biological variation across individual cells from the same cell type under a treatment condition. It is observed that some certain type of cells under a specific external condition can display higher variability so that the variation among observed gene expression replicates could be large. However, we have no evidence showing that this kind of variation changes dramatically over the time under the same treatment condition. Accordingly, the shape parameter is assumed to be the same across time for each gene. To account for potential gene to gene differences, we allow the shape parameter to differ among genes.

The number of hidden states for each gene (also referred to as the order of the hidden Markov chain) provides important information to characterize gene context effect under a treatment condition. The estimation of the order for microarray time course data,

to our knowledge, has not been investigated so far. The number of hidden states can also provide information in understanding the nature of biological signals in a cell system. If the expression profile is believed to be generated from a single distribution, the gene is probably a gene that does not send out a signal or does not respond to an ongoing process. Those genes with more than one state are probably responding or signaling and thus can be used to investigate the interaction relationship among genes. The set of response genes are those genes which are used by a cell to organize its response to a specific treatment condition. These genes exchange signals with each other to perform certain biological functions so that the cell can adapt to its environment. Some of these response genes can be the same for different treatment conditions because a cell may employ a portion of a pathway repeatedly to respond to different conditions. This is also supported by biological evidence that certain essential biological functions are performed under different environmental conditions.

Furthermore, the whole set of response genes is the characterization of the response for a cell. The information about interactions among genes is contained in the response set in terms of co-occurrence of other genes. Thus the information regarding gene interaction is contained not only in the appearance of a single gene but also in co-occurrence with other genes in the response set. The signal or response of one gene is characterized also by the response of its regulated genes. In other words, the signal sent out by a gene should be judged by the response of its regulated genes instead of by a criterion from human observers. In this sense, the set of response genes as a whole is the signature of a cell's response toward an external condition. It is therefore important for us to report the whole set of response genes when a real data set is analyzed.

The notation for a typical microarray time series experiment can be described as follows: there are G genes measured in one microarray under a specific treatment condition. For each gene, there are T time points measured. T is usually the same for all genes under one condition. The replicates at different time points need not be equal, but for notational convenience, we will assume that for gene g , at a time point t , we have R replicates. Our method does not require equal replicates. Let $\mathbf{Y}_{gt} = (y_{gt1}, y_{gt2}, \dots, y_{gtR})^T$ denotes the observed expressions for gene g at time point t with R replicates. The total number of observations is n . In case R is equal for all time points, the total number of observations n is equal to RT .

Let Z_{gt} be the hidden state at time t for gene g . The conditional distribution of $\mathbf{Y}_{gt}$ given Z_{gt} does not depend on t . The $\{Z_{g1}, Z_{g2}, \dots, Z_{gt}, \dots, Z_{gT}\}$ denotes the sequence of hidden states in a discrete state space. The subscript g indicates that the hidden states are gene specific, which can be defined differently for different genes. Since our HMM and its order are always estimated for each gene separately, we drop the subscript g hereafter. The observed expressions at a specific time point can be thought of as being generated from the corresponding emission distribution determined by the hidden state. The expression replicates at one specific time point are generated from the corresponding distribution independently.

The following conditions are assumed to be satisfied for the HMM considered subsequently when the order estimation is our primary objective:

1. The hidden state Z_t has stationary probabilities π_k , for $k = 1, 2, \dots, K$.

2. Conditioning on Z_t , the observed expression collection Y_t at time point t is independent of observations at other time points: $Y_1, Y_2, \dots, Y_{(t-1)}, Y_{(t+1)}, \dots, Y_T$ and is also independent of the hidden states at other time points: $Z_1, Z_2, \dots, Z_{(t-1)}, Z_{(t+1)}, \dots, Z_T$.
3. The replicates at one specific time point $Y_t = (y_{t1}, y_{t2}, \dots, y_{tR})^T$ are independent of each other, given the corresponding hidden state Z_t .

In estimating the number of hidden states or the order of an HMM for each gene, the stationary probabilities for the hidden Markov chain are denoted by $\pi = (\pi_1, \pi_2, \dots, \pi_K)^T$, where K is the order of hidden Markov model. This stationary distribution is commonly assumed in order estimation for HMMs by existing methods (Poskitt and Zhang, 2005; Lindgren, 1978; Leroux and Puterman, 1992; Ryden, 1995; MacKay, 2002). Poskitt and Zhang (2005) argued that the theoretical analysis based on the joint density of observed data and hidden states is extremely difficult, while there are substantial computational gains if inference is based on the marginal distribution of observations using the stationary probability. Here we follow this direction to assume a stationary distribution for the unobserved Markov chain and estimate the order based on the marginal distribution of observed data.

Under above assumptions, the observation Y_{ir} can be thought of as coming from a mixture density function where the stationary probability corresponds to the mixing proportion of the finite mixture model:

$$f_K(y_{ir} | \underline{\pi}, \underline{\mu}, \alpha) = \sum_{k=1}^K \pi_k h(y_{ir} | \mu_k, \alpha) \quad (2.1)$$

where $h(y_t | \mu_k, \alpha)$ is a family of gamma distributions defined in (2.2),

$\underline{\pi} = (\pi_1, \dots, \pi_K)^T$ and $\underline{\mu} = (\mu_1, \dots, \mu_K)^T$. It is also referred to as the emission distribution in the context of HMM's. The gene specific shape parameter α will be estimated by an empirical Bayes approach in the next chapter. The value of μ in a one dimensional parameter space, is allowed to vary across time and is expected to be different for different genes. For the emission density or the mixture component $h(y_t | \mu_k, \alpha)$, the mean parameter μ_k is determined by the corresponding hidden state Z_t at time point t , while the shape parameter α is the same across all time points. Different genes may have a different shape parameter α .

As a stationary probability is assumed in estimating the order of a HMM, our method does not require the equal time interval in the microarray time course data. The simulation data sets in chapter 5 are also generated from finite mixture models.

The gamma distribution used as the mixture component distribution for each gene at time point t is parameterized as follows:

$$h_t(y_t | \alpha, \mu_k) = \frac{1}{\Gamma(\alpha)} \left(\frac{\alpha}{\mu_k} \right)^\alpha y_t^{\alpha-1} \exp\left(-\frac{y_t \alpha}{\mu_k} \right) \quad (2.2)$$

where μ_k is the mean at a specific time point t corresponding to state k . Different hidden states have different μ_k values which depend on the corresponding hidden state Z_k . α is the shape parameter. With this setting, $E(y_t) = \mu_k$, $Var(y_t) = \mu_k^2 / \alpha$ and $CV = 1 / \sqrt{\alpha}$. The CV is the gene specific coefficient of variation. $\alpha = 1 / CV^2$ can be thought of as a squared stability indicator.

Another parameterization of the gamma distribution, which will be also used in the following chapters, is

$$h_t(y_t | \alpha, \beta_k) = \frac{1}{\Gamma(\alpha)\beta_k^\alpha} y_t^{\alpha-1} \exp\left(\frac{-y_t}{\beta_k}\right) \quad (2.3)$$

where β_k is the scale parameter at a specific time point t . Different hidden states have different β_k values which depend on the corresponding hidden state Z_t . α is the shape parameter. With this parameterization, $E(y_t) = \mu_k = \alpha\beta_k$, $Var(y_t) = \alpha\beta_k^2$, $CV = 1/\sqrt{\alpha}$. The above two parameterizations of the gamma distribution are used for the empirical Bayes estimation of the shape parameter in chapter 3 and order estimation in chapter 4.

Chapter 3

EMPIRICAL BAYES ESTIMATION OF THE SHAPE PARAMETER

Empirical Bayes and Bayesian hierarchical models have become popular approaches in recent years for microarray analysis. The typical small sample size both for non-time course experiments and time course data is probably the reason. Trying to take advantage of the large number of genes measured, Bayesian models seem to provide a possible way to borrow information from other genes. Another reason to consider a Bayes approach is the well observed heterogeneous variances among genes with different expression levels. To use a common variance for all genes is obviously problematic, while gene specific variance estimation is very difficult with such a small sample size. Many suggest borrowing information across genes in estimating expression means or variances. This strategy is hard to justify because the mean or variance for different genes may differ dramatically without any evidence that they share any common pattern. Information borrowing requires the assumption that the genes are sharing a common pattern in terms of mean or variance. Without the support of experimental evidence that

a common pattern is shared based on the mean or variance, the information borrowing approach only results in the introduction of irrelevant information.

There is no evidence indicating that the mean or variance shares a common pattern. However, for the ratio of the standard deviation over mean or the coefficient of variation (CV), there does exist evidence that a similar CV is shared across different genes (Chen et al., 1997; Ideker et al., 2000; Baggerly et al., 2001; Li and Wong, 2001; Rocke and Durbin, 2001; Theihaber et al., 2001; Tsodikov et al., 2002; Newton et al., 2001; Baldi et al., 2001; Newton et al., 2004). This is also one of our considerations to use a gamma distribution to model the expressions because the gamma distribution takes into account that the variance increases as mean increases. The gamma model also has past success in modeling continuous abundance data as suggested by Dennis and Patil (1984). In addition, as discussed by Durbin et al. (2002), at higher expression levels, the standard deviation of expression intensity varies linearly with the mean, at low expression levels, log transformed data have a potential inflation effect on the variance. The log-normal distribution may have the same problem at low expression levels.

The original use of the gamma distribution in microarray data assumed the same shape parameter for all the genes in data set. This may be too strict and can be relaxed by using a hierarchical Bayesian model as proposed by Lo and Gottardo (2007). Instead of assuming the same shape parameter or CV for all genes, we allow the shape parameter to be different across genes. As proposed by Lo and Gottardo (2007), we postulate that the shape parameters of different genes are generated from a prior distribution. Because there is no evidence indicating that the sample variability (described by CV or shape parameter)

changes over time for the same gene, the CV is assumed to be the same across time but allowed to be different for different genes.

The shape parameter α is directly related to the CV through $\alpha = 1/CV^2$ or $CV = 1/\sqrt{\alpha}$. The CV is a unitless variation measure. The shape parameter accordingly can be thought of as a characterization for the stability or variability among the replicates sampled at a specific time under a treatment condition. Since, there is no biological evidence indicating the variability changes over time under the same treatment, we choose to assume it is constant across time. The shape parameter can also be interpreted as the inverse of the measure of variability among replicates at a specific time point. The shape parameter thus can be estimated by combining the variability information across time. The realized variability measure can only be obtained when the sample has two or more replicates at a specific time point. We use the estimated sample mean and sample variance to estimate the variability and treat the estimates at different time points as multiple realizations from the same underlying CV or shape parameter. In detail, for gene g at time point t , the squared sample mean (m_{gt}^2) over sample variance (S_{gt}^2) can be used as the realized inversed variability measure $\hat{\alpha}_{gt} = \frac{m_{gt}^2}{S_{gt}^2}$ at time point t for a specific gene g .

Using non-time series data, Lo and Gottardo (2007) showed the log-normal prior provides a good fit for the shape parameter. Non-time course data can be thought of as a special case with only one time point. The log-normal prior for time course data works well. The shape parameter is modeled by a log-normal distribution with mean parameter

τ and variance parameter σ , i.e. $\alpha_{gt} \sim \text{log-normal}(\tau, \sigma)$, where the log-normal distribution is defined as follows,

$$p(\alpha_g | \tau, \sigma) = \frac{1}{\alpha_g \sigma \sqrt{2\pi}} \exp\left(-\frac{(\log(\alpha_g) - \tau)^2}{2\sigma^2}\right) \quad (3.1)$$

As suggested by Lo and Gottardo (2007), the method of moment estimators for the two parameters in the lognormal prior are used here,

$$\hat{\tau} = \frac{\sum_{g=1}^G \sum_{t=1}^T \log(\hat{\alpha}_{gt})}{GT} \quad (3.2)$$

$$\hat{\sigma}^2 = \frac{\sum_{g=1}^G \sum_{t=1}^T (\log(\hat{\alpha}_{gt}) - \hat{\tau})^2}{GT} \quad (3.3)$$

After the hyper-parameters (τ, σ) are estimated from the data, the posterior distribution can be used to estimate the shape parameter for each gene. The posterior distribution for the shape parameter is as follows for the situation with K distinct hidden states over T time points with R replicates at each time point:

$$L_k(\alpha | y_{11}, \dots, y_{TR}, \underline{\mu}, \tau, \sigma) = \frac{p(y_{11}, \dots, y_{TR}, \underline{\mu} | \alpha) p(\alpha | \tau, \sigma)}{\int_{\alpha} p(y_{11}, \dots, y_{TR}, \underline{\mu} | \alpha) p(\alpha | \tau, \sigma) d\alpha} \\ \propto p(y_{11}, \dots, y_{TR}, \underline{\mu} | \alpha) p(\alpha | \tau, \sigma) \quad (3.4)$$

where $p(y_{11}, \dots, y_{TR}, \mu_1, \dots, \mu_K | \alpha)$ is the joint density of $y_{11}, \dots, y_{TR}$ given α , and

$$\underline{\mu} = (\mu_1, \dots, \mu_K)^T$$

The posterior without normalization for α conditional on y is shown in (3.5). The same number of replicates R at each time point is assumed. The total number of observations $n=RT$.

$$L_k(\alpha | y_{11}, \dots, y_{TR}, \underline{\mu}, \tau, \sigma) \propto \Gamma^{-n}(\alpha) \alpha^{n\alpha} \prod_{t=1}^T \prod_{r=1}^R \{y_{tr}^{\alpha-1} \sum_{k=1}^K \pi_k \mu_k^{-\alpha} \exp\left(\frac{-y_{tr}\alpha}{\mu_k}\right)\} \\ \frac{1}{\alpha\sigma\sqrt{2\pi}} \exp\left(\frac{-(\log(\alpha) - \tau)^2}{2\sigma^2}\right) \quad (3.5)$$

This un-normalized posterior requires K , the number of hidden states, which is unknown at this stage. The estimation of the shape parameter can not be performed based on (3.5) directly. This problem can be circumvented if the hidden states are defined according to the time points. As discussed in the first chapter, time series microarray experiments can be reasonably assumed to be synchronized so that at each time point the same stage of a biological process can be assumed. In other words, the expression intensities at each time point can be thought of as being generated from the same hidden state. At each specific time point t , the expressions are generated independently from a different $gamma(\mu_t, \alpha)$ distribution. Assuming the distributions across time have different mean parameters, the distribution for the observations with R replicates at the time point t is:

$$f_{tR}(y_{t1}, y_{t2}, \dots, y_{tR} | \mu_t, \alpha) = \prod_{r=1}^R h(y_{tr} | \mu_t, \alpha) \quad (3.6)$$

where $h(y_{tr} | \mu_t, \alpha)$ is the gamma distribution characterized by μ_t as defined in (2.2).

Because we now know which distribution generates the observations, the complexity of

(3.5) can be reduced immediately. This way the estimation of the shape parameter does not require information about the order K .

For each gene, the expressions at different time points are independent of each other given that the corresponding state is known, or equivalently, given the corresponding mean parameter μ_t . Hence the distribution for all expressions over time for one gene is as follows:

$$f_{TR}(y_{11}, \dots, y_{TR} \mid \mu_1, \dots, \mu_T, \alpha) = \prod_{t=1}^T \prod_{r=1}^R h(y_{tr} \mid \mu_t, \alpha) \quad (3.7)$$

where $h(y_{tr} \mid \mu_t, \alpha)$ is the gamma distribution characterized by μ_t as defined in (2.2).

Based on (3.7), the un-normalized posterior function can be formulated as follows:

$$\begin{aligned} L_{TR}(\alpha \mid y_{11}, \dots, y_{TR}, \mu_1, \dots, \mu_T, \tau, \sigma) \\ \propto \left[\prod_{t=1}^T \prod_{r=1}^R h(y_{tr} \mid \mu_t, \alpha) \right] \frac{1}{\alpha \sigma \sqrt{2\pi}} \exp\left(-\frac{(\log(\alpha) - \tau)^2}{2\sigma^2}\right) \\ \propto \Gamma^{-n}(\alpha) \alpha^{n\alpha} \left[\prod_{t=1}^T \prod_{r=1}^R y_{tr}^{\alpha-1} \mu_t^{-\alpha} \exp\left(-\frac{\alpha y_{tr}}{\mu_t}\right) \right] \frac{1}{\alpha \sigma \sqrt{2\pi}} \exp\left(-\frac{(\log(\alpha) - \tau)^2}{2\sigma^2}\right) \end{aligned} \quad (3.8)$$

This way, the shape parameter of each gene can be estimated without knowing the true number of hidden states. Above (3.8) can be thought of as a saturated model with the number of mean parameters larger than the true number of hidden states. In this sense, the order estimation conducted later is to decide which mean parameters are estimating the same true mean. Because the shape parameter is related to the variance, with mean parameter estimated separately for each time point, the fitting could be improved and more accuracy is expected for the variance estimate. Moreover, the observed variability (i.e. the realized shape parameter value) can be obtained from a sample at a specific time

point. Combining two samples together has the risk that they may come from different states. There is no risk if we do not combine two samples together when they actually come from the same population. On the other hand, combining any of the samples may introduce bias because whether those samples combined come from the same distribution or not is not known at this point. Estimating the shape parameter independent of the order and treating the distribution at different time points to be different can make the estimation of shape parameter more robust to potential error in the order estimation.

The sample mean, which is the MLE of the mean parameter for the gamma distribution, is used in (3.8) to estimate μ_t . Since microarray analysis typically involves a large number of parameters to be estimated, it is a common to estimate some parameters based on the estimated value of other parameters. Under the condition that the distributions are all different in terms of mean across time, the information about the mean can be only obtained based from the data at the corresponding time point.

With all other parameters estimated, the shape parameters can be estimated. Since the estimation for the shape parameter needs to be performed for each time one at a time, to reduce computational burden, the mode of the un-normalized posterior is chosen for the estimator. Since there is no closed form solution for the mode of (3.8), we use numeric methods to find the mode for each gene. Of course, the mean, median or mode of the posterior can be considered in the estimation. We chose the mode here mainly due to computational considerations.

The difference between a typical mixture model specification and above distribution in (3.7) is that the allocation identity for each observation is known in the latter situation. In other words, for each data point, we know exactly which state it belongs to when the state is defined by time point. Hence, it is no longer a mixture model. The estimation of the shape parameter is accordingly reduced to the situation where we have several samples taken over time and we estimate the shape parameter by combining the information of multiple samples together.

Chapter 4

ORDER ESTIMATION

The problem of estimating the number of hidden states (referred to as the order estimation problem) has been widely discussed in both the finite mixture model and hidden Markov model literature. Although there are quite a few approaches proposed, this problem has not been satisfactorily solved yet. In this chapter, we first review current literature for order estimation in both finite mixture models and HMMs in section 4.1. We then discuss our proposed method with a focus on the microarray data, in section 4.2. Next we discuss related issues in subsequent sections. The computation algorithm is discussed in section 4.3 and the choice of threshold values is discussed in section 4.4. The identifiability issue is briefly discussed in section 4.5.

4.1 Literature Review of Order Estimation for HMMs and Finite Mixture Models

The order of a hidden Markov model can be defined as the number of distinct hidden states. Or as suggested by Cappe et al. (2004), the order of a HMM is the minimum size of the hidden state space of an HMM that can generate the observations. Estimation of the parameters of an HMM when the true order is known has been extensively studied. The consistency and asymptotic normality of the maximum likelihood estimates were established by Leroux (1992) and Bickel et al. (1998) respectively. As discussed above, the stationary probability is commonly assumed in estimating the order for HMMs, making the order estimation of HMMs the same as that of finite mixture models.

The problem of estimating the order of HMMs or finite mixture models has not been fully solved yet. The Akaike information criterion (AIC) and Bayes information criterion (BIC) approaches were adapted as proposed estimation methods by Leroux and Puterman (1992), Hughes and Cuttorp (1994), Albert et al. (1994) and Wang and Puterman (1999). These applications of AIC or BIC criteria did not establish that statistical properties such as consistency held (MacDonald and Zucchini, 1997). One important approach for order estimation is penalized likelihood methods. Baras and Finesso (1992) developed a consistent estimator of the order when the observations are discrete. Leroux and Puterman (1992) and Ryden (1995) analyzed the problem using model selection techniques. Ryden (1995) established that information criteria such as AIC and BIC do not under-estimate the true number of states asymptotically. Csiszar and Shields (2000) investigated the asymptotical property for both over-estimation and under-estimation. They established the consistency of BIC for order estimation. Following this

path, MacKay (2002), and Poskitt and Zhang (2005) proposed two new estimators based on penalized maximum likelihood methods. They established the consistency property for penalized likelihood estimators. In addition to penalized maximum likelihood estimation of the order of HMMs, likelihood ratio testing is another option to find certain information about the order. Hansen (1992) and Hamilton (1996) provided initial studies of this problem. Hamilton (1996) also provided several tests of model misspecification. Heckman, Robb and Walker (1990) developed a test statistic based on the method of moments. Feng and McCulloch (1996) proposed the likelihood ratio statistic. Chen, Chen and Kalbfleisch (2001) also provided a modified likelihood ratio test for homogeneity in finite mixture models.

Poskitt and Zhang (2005) further argued that order estimation of HMMs can be based on the marginal distribution based on a stationary HMM. Order estimation of HMMs in this way is the same problem as the order estimation of finite mixture models. In finite mixture model situations, the order is defined as the number of unique mixture component distributions.

For finite mixture models, the number of distinct component distributions is also referred to as the order of the mixture model. Hereafter, the order is referred to as the order of both finite mixture models and the stationary HMMs. The problem of order estimation has not been solved in finite mixture models either (McLachlan and Peel, 2000). Roeder and Wasserman (1997) have shown that when a normal mixture model is used to estimate a density, the density estimate that uses BIC to select the number of components in the mixture is consistent. Other proponents for the use of AIC or BIC in

this situation are discussed in Biernacki et al. (1998), Cwik and Koronacki (1997) and Solka et al. (1998). Richardson and Green (1997) also provided a Bayesian treatment of the order estimation problem.

As suggested by MacLachlan and Peel (2000), “it is therefore sensible in practice to approach the question of the number of components in a mixture model in terms of an assessment of the smallest number of components in the mixture compatible with the data.” And the order can be defined to be the smallest value of K in (2.1) such that all the components $h_k(y_{ir} | \mu_k, \alpha)$ are different and all the associated mixing proportions π_k are nonzero. These two conditions require the components to be distinct. They are also important to maintain the model’s identifiability as we will discuss below.

In this thesis, we develop our method based on the penalized maximum likelihood estimation approach. The difficulty of order estimation in stationary HMMs (or equivalently order estimation for finite mixture models) mainly comes from the monotonic increase of the likelihood as the order increases. The methods developed by Chen and Kalbfleisch (1996) and James et al. (2001) try to minimize the distance between a nonparametric curve based on the sample of data and the fitted model. Poskitt and Zhang (2005) proposed to use a penalized quasi-likelihood estimator and investigated its asymptotic properties. Here we adopt the penalized maximum likelihood method with two penalty terms proposed in Chen and Khalili (2005). The consistency of the penalized maximum likelihood estimators are established by Chen and Khalili (2005) and Poskitt and Zhang (2005). Our focus here is on the small sample situation as it is quite typical for microarray time course data.

There are two penalty terms in our proposed penalized likelihood. The second penalty term was first proposed by Chen and Kalbfleisch (1996) and was also used by MacKay (2002) in estimating the order of HMMs. The first penalty term is based on the development of the least absolute shrinkage and selection operator (LASSO) function (Tibshirani, 1996) and the smoothly clipped absolute deviation (SCAD) function (Fan and Li, 2001). We extend this two-term penalized likelihood method and propose a measure of the distance between adjacent emission distributions for the first penalty term, which we shall refer to as separation probability. This way, we have a probabilistic measure of how different adjacent components are and therefore provide a way to compare the distance between multiple adjacent component distributions. Chen and Khalili (2005) developed the two-term penalized maximum likelihood method to estimate the number of mixture components when the variance of normal component distributions are known and equal to 1, while the mean is the only unknown parameter. Our method extends theirs to the situation where the mean of gamma mixture distributions is unknown, while the variances are different and also unknown.

4.2 Order Estimation

According to Chen and Khalili (2005), an upper bound for the order, denoted as K_u , needs to be specified at the starting point. It is required to be at least as large as the true order. For microarray time course analysis, we propose to start with the upper bound K_u as 5. This is because an order that is too large requires that the means of the mixture

components be spread out in a wide range which is probably not the case for microarray data. For a reasonable separation probability, starting with an upper bound of five is usually sufficient for microarray data. Because the biological evidence regarding possible different responses is mostly between binding and non-binding, it does not suggest a large number of states. Although starting with a higher upper bound with data having more time points and replicates is possible, an upper bound of 5 can be a reasonable starting point based on our analysis.

The log-likelihood function of the stationary hidden Markov model (or equivalently the mixture models) based on gamma mixture components with K hidden states for gene g is defined as follows, where $f_K(y_{tr} | \underline{\pi}, \underline{\mu}, \alpha)$ is defined as in (2.1),

$\underline{\pi} = (\pi_1, \dots, \pi_K)^T$ with $\sum_{k=1}^K \pi_k = 1$, and $\underline{\mu} = (\mu_1, \dots, \mu_K)^T$.

$$l_K(K, \underline{\pi}, \underline{\mu}, \alpha) = \sum_{t=1}^T \sum_{r=1}^{R_t} \log(f_K(y_{tr} | \underline{\pi}, \underline{\mu}, \alpha)) \quad (4.2.1)$$

To prevent two types of over-fitting, two penalty terms are included in the following penalized log-likelihood:

$$\tilde{l}_K(K, \underline{\pi}, \underline{\mu}, \alpha) = l_K(K, \underline{\pi}, \underline{\mu}, \alpha) - \sum_{k=1}^{K-1} p(\eta_k) + C_k \sum_{k=1}^K \log \pi_k \quad (4.2.2)$$

The function $p(\eta_k)$ is a non-decreasing function of η_k on $(0, +\infty)$ and $p(0) = 0$.

It is twice differentiable for η_k except for a finite number of points. The first penalty term is used to penalize the likelihood if the means of some mixture components are too

close to each other. It is designed so that if any η_k has a small fitted value without the first penalty term, its fitted value with the first penalty term has a positive chance to be zero. Note that the mean parameter is the only parameter depending on the hidden state at corresponding time point. The second penalty term, originally developed by Chen and Kalbfleisch (1996) is used to penalize the likelihood for small values of mixing proportions. C_k is a constant. For a given upper bound K_u , the means of component distributions first need to be arranged in increasing order as

$\mu_{(1)} < \mu_{(2)} < \mu_{(3)}, \dots, \mu_{(K_u)}$ and define the distance measure for adjacent means as:

$$\eta_k = \mu_{(k+1)} / \mu_{(k)} - 1 \quad (4.2.3)$$

for some $K_u \geq 2$.

The first penalty is calculated based on this distance between two adjacent means of mixture components using a penalty function called the hard penalty function. It is defined as follows. The λ is the threshold for η .

$$p(\eta) = \lambda^2 - (|\eta| - \lambda)^2 I(|\eta| \leq \lambda) \quad (4.2.4)$$

Fan and Li (2001, 2002) discussed other possible penalty functions and provided detailed comparisons of their performance. We choose to use the hard penalty term mainly for computational considerations since the algorithm needs to run for each gene one at a time in a microarray.

In the computational implementation, a simplified procedure can be used as proposed by Fan and Li (2001, 2002). The procedure is: when the penalized value is

smaller than the threshold, simply set the value to zero. This is based on the observation that once the penalized value falls under a threshold value, the penalized likelihood function will never produce a value larger than the threshold; when the penalized value is larger than a threshold, an approximation function can be used to derive the updated parameter values for different penalty functions. This simplified procedure actually unifies the implementation of different penalty functions. As defined above $\eta_k = \mu_{(k+1)} / \mu_{(k)} - 1$, η_k is the parameter used in the hard penalty term. When η_k is smaller than a threshold value, it will be set to zero by our algorithm. This is equivalent to setting $\mu_{(k+1)} = \mu_{(k)}$, which leads to fewer distinct mean parameters, and accordingly the order estimated is reduced by 1.

For a given threshold λ in the hard penalty term, the penalized log-likelihood (4.2.2) is reduced by a constant λ^2 when $\eta > \lambda$. Because of the constant, the penalized MLE does not produce additional bias, compared to the original MLE. On the other hand, when $\eta \leq \lambda$, the first penalty term is smaller (and hence the likelihood is larger) and the penalty value becomes smaller as η tends to zero.

Note that inference about K is conducted indirectly. The likelihood function and the hard penalty function do not include K . Instead, the K is indirectly inferred based on the information about all mean parameters. Because the order is defined as the number of distinct hidden states or equivalently the number of distinct mean parameters, those means with the same value will be counted only once in the final step for the estimated order. The hard penalty term does not directly work on the hidden states either. Instead, it looks at the distance or difference between two adjacent means. Once they move closer to

each other than a threshold, the two corresponding states will be regarded as the same. And K will be reduced by 1. In this sense, our procedure is to merge those means once they are closer to each other below a specified threshold. Because this is an indirect approach, the likelihood (4.2.2) does not need to include hidden states or the order K , making the likelihood much simpler and thus more suitable for small sample situations such as microarray data.

Starting from the upper bound $K_u = 5$, our algorithm first sets initial mean parameter values to be spread over the range of observed expressions. With each iteration, the estimates of mean parameters change their values to increase the likelihood. Our algorithm then sets those mean estimates equal when they are close to each other and sets the mixing proportions to zero when they are sufficiently small. Because the estimated order is the number of unique mean values, setting two means equal is equivalent to reducing the order by 1. The condition $\sum_1^K \pi_k = 1$ is also maintained in each iteration.

We further propose to use separation probability p_η as an alternative distance measure between two adjacent means, in addition to η . Accordingly a separation threshold, p_λ , can be specified in the same way as choosing a λ value for η . Expressing the distance of two adjacent means in a probability measure may help us to understand how strong a biological signal in terms of probability measure. On the other hand, these two distance measures have the same function in the penalty term. Therefore either one can be used.

Our model setting allows the shape parameter to be common for all the gamma mixture components while the means differ across time. The distance between two adjacent means can be defined as in (4.2.3) which takes into account the possible different variances involved for different pair-wise distances. For ease of discussion we assume that the means of the mixture components are always arranged in ascending order hereafter. Because distances of all pairs of adjacent means need to be compared with a pre-determined threshold λ value, these distances obtained for different pairs should be comparable. As we allow the variance of mixture components to be different, the variances of two adjacent mixture components can potentially affect the distance of means. Therefore the distance needs to be adjusted for the variance effect across different adjacent pairs. Hence, we define the distance between two adjacent means as the ratio of adjacent means minus 1, or equivalently, as the difference between two adjacent means divided (or standardized) by the smaller mean. A threshold λ value then can be specified for the standardized distance between all adjacent pairs.

The above standardized distance across different adjacent pairs can be expressed in our proposed separation probability measure. We will show that the standardized distance measure results in the same separation probability across different adjacent pairs for gamma distributions. We explain this with an example of three gamma mixture components. Suppose there are three mixture component distributions with common shape parameter α but different mean parameters in increasing order as

$$\mu_{(1)} < \mu_{(2)} < \mu_{(3)} \text{ with the corresponding cumulative distribution functions } F_{(1)}, F_{(2)}, F_{(3)}.$$

The separation probability between $F_{(1)}$ and $F_{(2)}$ is defined as $F_{(1)}(x_0)$, where x_0 is the value that maximizes $F_{(1)} - F_{(2)}$, with $x_0 \in R^+$ for the gamma distribution. As defined in

this way, if the separation probability between the first and second mixture component is the same as the separation probability between the second and third distribution, the ratio of two adjacent means is a constant; that is $\frac{\mu_{(2)}}{\mu_{(1)}} = \frac{\mu_{(3)}}{\mu_{(2)}}$ and vice versa. The separation threshold has a one-to-one relationship with the threshold of the distance measure λ for a given shape parameter. Note that the relationship may not be valid when the shape parameter is not the same across component distributions.

Defined this way, the separation probability can be interpreted biologically as follows. For a given separation probability s between two states characterized by distributions $F_{(1)}, F_{(2)}$ for gene A, there is a probability of $1-s$, that the first state is mistakenly understood to be the second state by those regulated genes of gene A.

Here we prove that the separation probability between the first and second mixture component is the same as the separation probability between the second and third distribution if and only if the ratio of two adjacent mean parameters or the ratio of the two scale parameter is constant $\frac{\mu_2}{\mu_1} = \frac{\mu_3}{\mu_2} = \frac{\beta_2}{\beta_1} = \frac{\beta_3}{\beta_2} = \text{Const.}$, where μ_1, μ_2 and μ_3 are the mean parameter for the gamma distribution functions $F_{(1)}, F_{(2)}$ and $F_{(3)}$ respectively and β_1, β_2 and β_3 are the scale parameters for the gamma distribution functions $F_{(1)}, F_{(2)}$ and $F_{(3)}$ respectively.

Figure 4.2.1. A gamma mixture of three component distributions, with probability density functions $f_{(1)}$, $f_{(2)}$ and $f_{(3)}$.

Figure 4.2.1 presents three cumulative distribution functions $F_{(1)}$, $F_{(2)}$ and $F_{(3)}$ with increasing mean values but the same shape parameter. Corresponding to the cumulative distribution functions $F_{(1)}$, $F_{(2)}$ and $F_{(3)}$ are the density functions $f_{(1)}$, $f_{(2)}$ and $f_{(3)}$. At x_1 , $f_{(1)}(x_1) = f_{(2)}(x_1)$ and at x_2 , $f_{(2)}(x_2) = f_{(3)}(x_2)$.

We first show that at x_1 , $F_{(1)} - F_{(2)}$ is maximized. That is, x_1 is the point at which $F_{(1)}(x_1)$ defines the separation probability between $F_{(1)}$ and $F_{(2)}$. To find the point where the function $F_{(1)} - F_{(2)}$ is maximized, we need to take the derivative with respect to x and let it equal to zero.

$$\frac{d}{dx}(F_{(1)}(x) - F_{(2)}(x)) = 0 \quad (4.2.5)$$

which is:

$$\frac{d}{dx}(F_{(1)}(x) - F_{(2)}(x)) = \frac{d}{dx} \left(\int_0^x f_{(1)}(z) dz - \int_0^x f_{(2)}(z) dz \right) = \frac{d}{dx} \int_0^x f_{(1)}(z) dz - \frac{d}{dx} \int_0^x f_{(2)}(z) dz = 0 \quad (4.2.6)$$

thus:

$$f_{(1)}(x) = f_{(2)}(x) \quad (4.2.7)$$

We denote this point as x_1 as shown in Figure 4.2.1. Therefore according to the definition of separation probability above, the separation probability between $F_{(1)}$ and $F_{(2)}$ is $F_{(1)}(x_1)$. Similarly, the separation probability between $F_{(2)}$ and $F_{(3)}$ is $F_{(2)}(x_2)$.

Next, we would like to show that if $\frac{\mu_2}{\mu_1} = \frac{\mu_3}{\mu_2} = \frac{\beta_2}{\beta_1} = \frac{\beta_3}{\beta_2} = a$, where a is an

arbitrary constant, then the separation probability between $F_{(1)}$ and $F_{(2)}$ and $F_{(2)}$ and $F_{(3)}$ is the same; that is, $F_{(1)}(x_1) = F_{(2)}(x_2)$. As proved above: $f_{(1)}(x_1) = f_{(2)}(x_1)$ and $f_{(2)}(x_2) =$

$f_{(3)}(x_2)$. And $\frac{\mu_2}{\mu_1} = \frac{\mu_3}{\mu_2} = \frac{\beta_2}{\beta_1} = \frac{\beta_3}{\beta_2} = a$ indicates $\beta_2 = a\beta_1$, $\beta_3 = a\beta_2 = a^2\beta_1$.

Therefore we have

$$\frac{1}{\Gamma(\alpha)\beta_1^\alpha} x_1^{\alpha-1} \exp\left(\frac{-x_1}{\beta_1}\right) = \frac{1}{\Gamma(\alpha)(a\beta_1)^\alpha} x_1^{\alpha-1} \exp\left(\frac{-x_1}{a\beta_1}\right) \quad (4.2.8)$$

$$\frac{1}{\Gamma(\alpha)(a\beta_1)^\alpha} x_2^{\alpha-1} \exp\left(\frac{-x_2}{a\beta_1}\right) = \frac{1}{\Gamma(\alpha)(a^2\beta_1)^\alpha} x_2^{\alpha-1} \exp\left(\frac{-x_2}{a^2\beta_1}\right) \quad (4.2.9)$$

Simplifying the above two equations, we then have

$$\beta_1^{-\alpha} \exp\left(\frac{-x_1}{\beta_1}\right) = (a\beta_1)^{-\alpha} \exp\left(\frac{-x_1}{a\beta_1}\right) \quad (4.2.10)$$

$$(a\beta_1)^{-\alpha} \exp\left(\frac{-x_2}{a\beta_1}\right) = (a^2\beta_1)^{-\alpha} \exp\left(\frac{-x_2}{a^2\beta_1}\right) \quad (4.2.11)$$

By dividing each side of (4.2.10) by the corresponding side of (4.2.11), the following can be obtained:

$$\left(\frac{1}{a}\right)^{-\alpha} \exp\left(\frac{-x_1}{\beta_1} + \frac{x_2}{a\beta_1}\right) = \left(\frac{1}{a}\right)^{-\alpha} \exp\left(\frac{-x_1}{a\beta_1} + \frac{x_2}{a^2\beta_1}\right) \quad (4.2.12)$$

Simplifying the above, we get $x_2 = ax_1$.

For a gamma distribution, it is true that if $X \sim \text{gamma}(\alpha, \beta_1)$, then $aX \sim \text{gamma}(\alpha, a\beta_1)$ or equivalently, $aX \sim \text{gamma}(\alpha, \beta_2)$ where $\beta_2 = a\beta_1$. At x_1 , the separation probability is defined as $F_{(1)}(x_1) = P_{(1)}(X < x_1)$. Then we have:

$$F_{(1)}(x_1) = P_{(1)}(X < x_1) = P_{(1)}(aX < ax_1) = P_{(1)}(aX < x_2) = P_{(2)}(aX < x_2) = F_{(2)}(x_2) \quad (4.2.13)$$

This means the separation probability between $F_{(1)}$ and $F_{(2)}$ and between $F_{(2)}$ and $F_{(3)}$ is the same.

Next, we prove that if $F_{(1)}(x_1) = F_{(2)}(x_2) = p$, where p is a given arbitrary positive constant, then $\frac{\mu_2}{\mu_1} = \frac{\mu_3}{\mu_2} = \frac{\beta_2}{\beta_1} = \frac{\beta_3}{\beta_2}$.

For a given separation probability p , we have $F_{(1)}(x_1) = P_{(1)}(X < x_1) = F_{(2)}(x_2) = P_{(2)}(Y < x_2) = p$. As the shape parameter α is the same for the three distributions, we can

assume the density functions for $f_{(2)}$, $f_{(3)}$ have the form $f_{(2)} = \text{gamma}(\alpha, h\beta)$ and

$f_{(3)} = \text{gamma}(\alpha, k\beta)$. We want to show $h = k/h$, i.e. $k = h^2$ if $F_{(1)}(x_1) = F_{(2)}(x_2) = p$ is true.

As proved above, $f_{(1)}(x_1) = f_{(2)}(x_1)$ and $f_{(2)}(x_2) = f_{(3)}(x_2)$. Then, the following two equations can be obtained:

$$\frac{1}{\Gamma(\alpha)\beta^\alpha} x_1^{\alpha-1} \exp\left(\frac{-x_1}{\beta}\right) = \frac{1}{\Gamma(\alpha)(h\beta)^\alpha} x_1^{\alpha-1} \exp\left(\frac{-x_1}{h\beta}\right) \quad (4.2.14)$$

$$\frac{1}{\Gamma(\alpha)(h\beta)^\alpha} x_2^{\alpha-1} \exp\left(\frac{-x_2}{h\beta}\right) = \frac{1}{\Gamma(\alpha)(k\beta)^\alpha} x_2^{\alpha-1} \exp\left(\frac{-x_2}{k\beta}\right) \quad (4.2.15)$$

Simplifying the above two equations, we then have

$$\beta^{-\alpha} \exp\left(\frac{-x_1}{\beta}\right) = (h\beta)^{-\alpha} \exp\left(\frac{-x_1}{h\beta}\right) \quad (4.2.16)$$

$$(h\beta)^{-\alpha} \exp\left(\frac{-x_2}{h\beta}\right) = (k\beta)^{-\alpha} \exp\left(\frac{-x_2}{k\beta}\right) \quad (4.2.17)$$

Dividing each side of (4.2. 16) by the corresponding side of (4.2.17), the following can be obtained:

$$\left(\frac{1}{h}\right)^{-\alpha} \exp\left(\frac{-x_1}{\beta} + \frac{x_2}{h\beta}\right) = \left(\frac{h}{k}\right)^{-\alpha} \exp\left(\frac{-x_1}{h\beta} + \frac{x_2}{k\beta}\right) \quad (4.2.18)$$

Based on the function we assumed above for $f_{(2)}$ and $f_{(3)}$, which is

$f_{(2)} = \text{gamma}(\alpha, h\beta)$ and $f_{(3)} = \text{gamma}(\alpha, k\beta)$, we know that if $X \sim f_{(1)}(x): \text{gamma}(\alpha, \beta)$,

then let $Y = hX$, and $Y \sim f_{(2)}(y): \text{gamma}(\alpha, h\beta)$. Because $P(Y < x_2) = P(hX < x_2)$ (according to

the relationship between X and Y we just defined); while $P(hX < x_2) = P(X < (x_2/h))$ (use the probability function of X); $P(Y < x_2) = P(X < (x_2/h)) = P(X < x_1)$ (use the condition we assumed at the beginning); and $P(X) = F_{(1)}(x)$ is a monotonic increasing function.

Therefore $x_2 = hx_1$. Then (4.2.19) becomes:

$$\left(\frac{1}{h}\right)^{-\alpha} \exp\left(\frac{-x_1}{\beta} + \frac{hx_1}{h\beta}\right) = \left(\frac{h}{k}\right)^{-\alpha} \exp\left(\frac{-x_1}{h\beta} + \frac{hx_1}{k\beta}\right) \quad (4.2.19)$$

And further we can obtain:

$$\left(\frac{1}{h}\right)^{-\alpha} = \left(\frac{h}{k}\right)^{-\alpha} \exp\left(\frac{-x_1}{h\beta} + \frac{hx_1}{k\beta}\right), \text{ for arbitrary positive values of } \alpha, \beta, x_1. \text{ The solution}$$

leads to $k = h^2$. That is, the ratio of two adjacent mean parameters or scale parameters is constant.

The relationship between the separation probability and the standardized distance between adjacent means, as we shown above, is closely related. Their function in the first penalty is the same. They provide a measure which makes the distances between different pairs of adjacent component distributions comparable. The threshold value, either the standardized distance threshold η or the separation threshold p_η , can be chosen in a similar way. Actually in our model setting, they have a one-to-one relationship.

Our order estimation method is developed with two considerations in mind. The first is our primary goal to analyze microarray time course data. The typical limited sample size and limited time points make it very difficult to estimate the order precisely. These limitations are well understood by the biological community and hence microarray

analysis is used as a screening tool to help to narrow down the candidate genes to a small subset of genes. These genes may need to be further examined using other experimental techniques. Our method is developed mainly to address this objective. We have designed a type I error rate based approach to specify the threshold value for the first penalty term so that the threshold can be determined by simulated data. Of course our simulation is based on the information of sample size, the shape parameter values and the desired type I error rate level for a specific microarray data set. Details will be discussed in section 4.4. The advantage of this approach is that it does not require pre-knowledge of the separation probability so that the microarray time course can be immediately analyzed. Another advantage is that we can control the accuracy to divide the genes into a response set with order as one and another set with order two or above. The disadvantage is that the accuracy of order estimation among those genes with order above 2 in the response set can not be controlled. However, we believe that identifying those response genes with order as two or above is sufficient to serve the screening function in microarray analysis. In addition, our method provides a way to characterize the type I error rate so that the accuracy can be measured and controlled. We suggest that further order estimation for the multiple order genes needs to be performed with more detailed experimental measures rather than the limited data from a microarray. With microarray data alone, with such a limited sample size and no pre-knowledge of separation probability, it is not realistic to expect the higher order can be quantified reliably.

The secondary goal of our method is to provide some methodological discussion for the situation where a sufficiently large sample is available. The large samples may be obtained by more detailed measurements of the response genes identified with our

method. Given a sufficiently large sample size, the threshold for the first penalty can be obtained by two data driven methods as discussed by Chen and Khalili (2005). Note that the estimation of the exact order requires pre-knowledge of the threshold values for the hard penalty term. This knowledge certainly is not available for microarray data because microarray experiments are typically conducted before other experiments. Since microarray analysis is our primary goal in this thesis, we focus on the discussion of order estimation for small sample situations. We will only outline the key direction for exact order estimation for medium to large sample sizes without going into detail.

4.3 Computational Algorithm

The algorithm for the above computation is a version of the EM algorithm (Dempster et al., 1977) and can be summarized in the following steps. There is some revision of the M step.

1. Specify initial values for the upper bound of order, mean parameters and stationary probabilities. Specify the separation threshold value for the first penalty term and the constant for the second penalty term.
2. E step: Calculate the conditional expectation as shown in (4.3.1) for (4.2.2) conditioning on the current estimated parameter values.
3. M step: Update the stationary probability by maximizing the conditional expectation function in (4.3.1). Update the mean parameters by maximizing (4.3.1). If the separation

probability is less than the specified threshold, merge the two adjacent means so that they have the same estimated mean in all subsequent iterations. Go to step 2.

4. The iteration between step 2 and step 3 stops when the increase of the penalized likelihood is sufficiently small. Alternatively, a sufficiently large number of iterations can be used.

The initial values of the mean parameters and the initial values of the mixing proportions need to be first specified. Our simulation shows that these initial values are not critical as long as the means are not too close to one another or concentrate in a small range. An equal mixing proportion based on an upper bound of the order can be chosen at the start. We normally choose the initial means such that they spread out equally within the range of expression levels.

In detail, the EM algorithm maximizes (4.2.2) iteratively in two steps as follows.

E step: Let $\Psi^{(m)}$ be the estimate of the parameters after the m th iteration. The E step first calculates the conditional expectation of (4.2.2) given the observed data and the parameter estimates from the previous iteration. For a particular gene with a total of n

observations, using the standardized distance $\eta_k = \frac{\mu_{k+1}}{\mu_{k1}} - 1 = \frac{\beta_{k+1}}{\beta_{k1}} - 1$ as an example, the

conditional expectation is

$$\begin{aligned} Q(\Psi; \Psi^{(m)}) = & \sum_{i=1}^n \sum_{k=1}^K w_{ik}^{(m)} \log\{f(y_i | \beta_k)\} - \sum_{k=1}^{K-1} p(\eta_k) \\ & + \sum_{i=1}^n \sum_{k=1}^K \left\{w_{ik}^{(m)} + \frac{C_k}{n}\right\} \log \pi_k \end{aligned} \quad (4.3.1)$$

where

$$w_{ik}^{(m)} = \frac{\pi_k^{(m)} f(y_i | \beta_k^{(m)})}{\sum_{l=1}^K \pi_l^{(m)} f(y_i | \beta_l^{(m)})}, k = 1, 2, \dots, K. \quad (4.3.2)$$

is the conditional expectation of the probability of each observation belonging to a mixture component given the observed data and the current estimated parameters. The gamma distribution for each component distribution is parameterized as $\text{gamma}(\alpha, \beta_k)$ for the corresponding $\text{gamma}(\alpha, \mu_k)$, with $\mu_k = \alpha \beta_k$. The parameter α is treated as known for each gene based on the estimation performed in Chapter 3. (4.3.1) is the conditional expectation of (4.2.2). The values of mean parameters (or equivalently the values of scale parameters) determine the hidden states. The order can be obtained by counting the distinct mean or scale parameters once the iteration is completed. The same procedure is used in Chen and Khalili (2005) to estimate the number of mixture components.

M step: update the parameter values for $(m+1)$ iteration by maximizing $Q(\Psi | \Psi^{(m)})$ in (4.3.1). First $\pi_k^{(m+1)}$ is updated by maximizing the expectation with respect to the mixing proportions π_k . The derived new estimates are:

$$\pi_k^{(m+1)} = \frac{\sum_{i=1}^n w_{ik}^{(m)} + C_K}{n + KC_K}, k = 1, 2, \dots, K \quad (4.3.3)$$

(4.3.3) is the result of the following maximization steps:

1. Maximize $Q(\Psi | \Psi^{(m)})$ in (4.3.1) with respect to π_k , which is the same as maximizing

$$\sum_{i=1}^n \sum_{k=1}^K \{w_{ik}^{(m)} + \frac{C_k}{n}\} \log \pi_k \text{ with respect to } \pi_k.$$

2. Under the constraint that $\sum_{k=1}^K \pi_k = 1$, the Lagrange multiplier technique is used. Set

$$A = \sum_{i=1}^n \sum_{k=1}^K \left\{ w_{ik}^{(m)} + \frac{C_k}{n} \right\} \log \pi_k + \delta \left(\sum_{k=1}^K \pi_k - 1 \right) \quad (4.3.4)$$

where δ is a constant.

3. Let $\frac{\partial A}{\partial \pi_k} = 0$ for $k=1, 2, \dots, K$. We will get K equations. As an example for k th equation

we have:

$$\frac{\partial A}{\partial \pi_k} = \sum_{i=1}^n \left(w_{ik}^{(m)} + \frac{C_k}{n} \right) \frac{1}{\pi_k} + \delta = \left(n\pi_k + \frac{C_k}{n} \right) \frac{1}{\pi_k} + \delta = n + \frac{C_k}{\pi_k} + \delta = 0 \quad (4.3.5)$$

so that we have:

$$n\pi_k + C_k + \delta\pi_k = 0 \quad (4.3.6)$$

4. Add above (4.3.6) for $k=1, \dots, K$ together, we have

$$n + KC_K + \delta = 0 \quad (4.3.7)$$

therefore:

$$\delta = -(n + KC_K) \quad (4.3.8)$$

5. Using (4.3.8) in the second portion of equation (4.3.5), we then have:

$$\sum_{i=1}^n \left(w_{ik}^{(m)} + \frac{C_k}{n} \right) \frac{1}{\pi_k} + \delta = \sum_{i=1}^n \left(w_{ik}^{(m)} + \frac{C_k}{n} \right) \frac{1}{\pi_k} - (n + KC_K) = 0 \quad (4.3.9)$$

Rearranging (4.3.9), we obtain the updating equation (4.3.3)

Next, we need to update $\mu_k^{(m+1)}$ or $\beta_k^{(m+1)}$, $k=1, \dots, K$ by maximizing the conditional expectation with respect to each scale parameter β_k of the mixture

component. The conditions imposed on the penalty function $p(\cdot)$ require that $p(0) = 0$ and that it be a non-decreasing function on $(0, +\infty)$. Thus $p(\cdot)$ is not differentiable at $\eta_k = 0$. The updated values can not be obtained using the derivatives. Fan and Li (2001) suggested using the following approximation of $p(\cdot)$ when the penalized values are bigger than the threshold.

$$p^*(\eta_k; \eta_k^{(m)}) = p(\eta_k^{(m)}) + \frac{p'(\eta_k^{(m)})}{2\eta_k^{(m)}} (\eta^2 - \eta_k^{(m)^2}) \quad (4.3.10)$$

When the penalized values are less than the threshold, they suggest simply setting the value to zero which is equivalent to setting two adjacent means to the same value in our particular case. For the hard penalty term, when the penalized value is bigger than the threshold, above (4.3.10) becomes zero because the slope for a constant is zero.

Therefore, the update of $\mu_k^{(m+1)}$ or $\beta_k^{(m+1)}$, $k = 1, \dots, K$ can be obtained as in a normal EM algorithm. That is, find the update of $\beta_k^{(m+1)}$, $k = 1, \dots, K$ by maximizing following equation:

$$\sum_{i=1}^n w_{i1}^{(m)} \frac{\partial}{\partial \beta_1} \log\{f(y_i | \beta_k)\} = 0 \quad (4.3.11)$$

The solution of the following equation produces the update values of $\beta_k^{(m+1)}$, $k = 1, \dots, K$ for the next iteration.

$$\frac{\partial}{\partial \beta_k} \sum_{i=1}^n w_{ik}^{(m)} \log\{f(y_i | \beta_k)\} = -\frac{n\alpha}{\beta_k} + \frac{\sum_{i=1}^n y_i}{\beta_k^2} = 0 \quad (4.3.12)$$

$$\text{therefore: } \beta_k^{(m+1)} = \frac{\sum_{i=1}^n y_i}{n\alpha} \text{ or } \mu_k^{(m+1)} = \frac{\sum_{i=1}^n y_i}{n}.$$

The algorithm starts with an initial set of parameter values, then iterates between the E step and M step until the conditional expectation converges. After all iteration completed, the number of distinct values of the scale (or mean) parameters is obtained as the estimated order for a particular gene. This procedure runs on each gene one at a time. The threshold value of first penalty term is specified for each gene according to the shape parameter value and its sample size. The constant for the second penalty term is chosen for all genes of an experiment. The details of how to choose the threshold for the penalty terms is discussed in next section.

4.4 Choices of the Threshold Parameters

For the algorithm to run, the two threshold parameters need to be specified at the beginning. For C_K , both Chen et al. (2001) and Chen and Khalili (2005) reported that the choice of its value is not crucial, which is the case found in our computation. Here, we adopt the specification of MacKay (2002) to let $C_K = 0.01 \frac{\log(n)}{\sqrt{n}}$. The sample size is the only information needed. Because normally all genes measured in a microarray experiment share the same sample size, the constant is specified once for all genes within an experiment.

As pointed out by Chen and Khalili (2005), the specification of the threshold for the first penalty term is theoretically difficult. Current development only provides some guidance on the choice of threshold to achieve consistency, which requires the threshold

to shrink as the sample size increases. This guidance may be too general to help in real data analysis situations.

With the typical small sample size, the selection of the threshold becomes even more difficult. Based on this situation, we would like to propose a simulation-based approach specially designed for microarray data. We will discuss two possible ways to specify the penalty threshold for the first penalty term. The first one is developed specifically for microarray time course data analysis. The second one is for more general purposes and requires a larger sample size. As our focus is obviously the first one, we will only provide a brief discussion for the second one.

Microarray time course data has very limited sample size and even fewer time points. For these kinds of data, it is difficult to provide enough information for both order estimation and threshold specification. Although the number of genes is large, the separation threshold is gene specific and should not be determined by combining information from other genes. Besides the possible biological variation involved in an experiment, many other sources of technical variation may potentially affect the underlying separation of the expressions. On the other hand, it is generally understood that microarray analysis is designed to obtain a subset of genes for further investigation. Therefore, the primary goal of our analysis is to separate the set of genes with order one from those genes with order two or above. The set of genes with order two or above is the response set which is of major biological interest. For the response genes, whether their order is two or three or above is not critical, not only because the estimation has to be based on a very limited sample size and thus its accuracy is difficult to control, but also due to the fact the resulting response gene set needs to be verified by further detailed

quantitative experimental measurements. Based on these considerations, we develop an approach suitable for microarray time course data analysis. We refer to it as the simulation based approach, which enables us to specify the threshold by simulation.

The focus for our method is to divide the genes into the set with order one from the set with multiple hidden states. The response genes are characterized by their change of state during a certain period of time. The identification of genes of order greater than one is sufficient for extracting the response gene set. For this purpose, as we have shown below, typical microarray time course data provide enough information. On the hand other, the goal of finding the exact number of hidden states may not be achievable using microarray data only. Even if we can obtain an estimate, how reliable it is by looking at only several biological replicates is still unknown.

The simulation based approach to specify a threshold as we introduce here also makes it possible to control for a desired type I error rate. When the shape parameter of a gene and the sample size are given, samples with a single underlying gamma distribution can be generated and our estimating method can be applied to find the order. In this way the falsely discovered multiple order samples can be identified and the accuracy of the order estimates can be obtained. We then can find a threshold value that has the corresponding type I error rate that we wish. The scale parameter value is not critical for maintaining a certain level of type I error rate based on our simulation. A few scale parameter values can be tried with a given shape parameter and sample size to evaluate the averaged number of falsely discovered samples. We will demonstrate this approach using the simulations in chapter 5 and using the real data in chapter 6.

In applications where the estimate of the exact number of hidden states is of interest, cross validation (Stone, 1974) and generalized cross validation (Craven and Wahba, 1979) are often used as data-driven approaches to choosing the threshold values. To use these cross-validation approaches, the data set needs to be randomly divided into two subsets, typically referred to as the training set and test set. There is no fully objective criterion developed for the threshold specification to date. These two data driven approaches still have some problems. The threshold found in this way is a random variable which may not satisfy the asymptotic properties required for the penalty terms. To ensure the validity of the asymptotic results, normally a restriction is imposed on the values found by cross-validation approaches (James et al., 2001). In addition, these approaches normally require intensive computation and make it too difficult to be applied on the large number of genes from microarray data.

All above computations are implemented using R language version 2.3.1 and later versions. The R code is attached in Appendix B.

4.5 Identifiability

To estimate the parameters for the model (4.2.2), we need to first make sure that the model parameters Ψ are identifiable. According to McLachlan and Peel (2000), in general, a parametric family of densities $f(y_i | \Psi)$ is identifiable if distinct values of the parameters Ψ determine distinct family of models

$$\{f(y_i | \Psi) : \Psi \in \Omega\},$$

where Ω is the parameter space. That is

$$\text{if } \Psi \neq \Psi^*, \quad (4.5.1)$$

$$\text{then } f(y_i | \Psi) \neq f(y_i | \Psi^*) \quad (4.5.2)$$

For parametric distributions such as the normal distribution and gamma distribution, the parameters are identifiable in the single distribution situation. However, the identifiability becomes an issue in the mixture model situation when we do not know which component distribution generates an observation. The identifiability problem is discussed here mostly in a mixture model situation.

In mixture distribution situation, the identified parameters for the component distributions can be re-labeled using any permutation of the labels. The same group of parameters can be regarded as different when labeled differently. To avoid this problem, in the mixture distribution situation, one normally imposes an ordering restriction, as demonstrated by Aitkin and Rubin (1985), to order the parameters. After ordering, the parameters can be compared to see whether the condition of identifiability is satisfied. Two groups of parameters with the same values after ordering are always the same. The identifiability problem discussed below is for the situation where the lack of identifiability can not be resolved only by ordering the parameters.

Typically, lack of identifiability occurs in estimating parameters for mixture distributions, due to over fitting. That is, a larger than true order is used in estimating the parameters. In such a situation, one of following is expected to happen:

1. One of the mixing proportions is actually estimating a zero proportion.
2. Two different component distributions are actually estimating the same distribution.

These two situations cause an identifiability problem in estimation of the mixture models.

The parameters for a component whose proportion is zero can be estimated in many different ways. Similarly, for two estimated components which are actually estimating the same distribution, their mixing proportions can be estimated in many ways. The two penalty terms used in (4.2.2) are developed to prevent these two possible situations of over fitting. The first penalty term in (4.2.2) is designed to prevent two mixture components from being estimated for the same distribution. The second penalty term is designed to prevent any mixing proportion from being estimated to be too close to zero.

There is an important condition for the lack of identifiability. That is, lack of identifiability occurs only when we have limited information from the sampled data. Asymptotically, the above two possible estimation problems can be avoided. Lack of identifiability is a problem introduced by the limited information from a finite sample. Under such information limitation, there may be at least two different parameter sets providing the same likelihood measurement for a given set of observed data.

Chapter 5

SIMULATION STUDY

The objective of the simulation study in this chapter is to evaluate the performance of our proposed method with a focus on our primary goal. The primary objective of our analysis and simulation is to demonstrate how the threshold for the first penalty term can be specified using simulated data. The simulations also provide evaluation of the performance of our method for microarray data. The secondary objective is to provide some evaluations for the situation where we know the separation threshold among mixture components, which is not the case for microarray experiments. Therefore these simulations are designed to reflect general goals instead of specifically for microarray data. Accordingly, the data are simulated directly from the conditions we used in the development of our model rather than for the microarray situation. We also include simulations based on hidden Markov models.

We first use our simulation based approach to find a value for the separation threshold. This is consistent with our primary objective in this thesis. Our proposed order estimation combined with the simulation based approach for threshold specification makes it especially suitable for analyzing microarray time course data.

Because microarray experiments are used as screening tools, it is usually the case that we do not have any information about the separation threshold. Even if we do know the threshold information for some genes, with such small sample size, the order estimation can not be expected to be very reliable. This is the case as we will show using simulation with small samples. Therefore, the characterization of the exact order for a gene has to be conducted in two steps. First, narrow down the collection of genes to a subset of genes which potentially have two or more states. Second, perform more detailed measurements on those genes selected in the first stage, with sufficiently large sample size, using some other experiments. Here our focus is on the first step. We only include a brief discussion of estimating the exact order for situations with sufficiently large sample size in the simulation. Furthermore, in our opinion, to estimate the exact order using microarray data is not achievable and thus is not an objective of our method.

The detailed situation is illustrated in Table 5.1. The classification of genes into those with order one and those with order two or above can be achieved for both small samples and medium to large samples. The estimation of the exact order only performs well with medium to large samples. Hence, our simulation will be performed mainly for the estimation aimed to identify response genes with order of two or above. We also

provide some simulations and a brief discussion for the situations where one is estimating the exact order for medium to large sample sizes.

Table 5.1, The four situations of order estimation.

	Separate order of one from the order of two or above	Find the exact order
Small sample size	Achievable	Not Achievable
Medium to large sample size	Achievable	Achievable

A common approach to deal with the typical small sample sizes in microarray literature is to use the Bayesian method to borrow information from other genes. This can not be used here because as we explained in the first chapter, the gene context effect is always gene specific and need to be investigated first. Even if a group of genes shares the same order, this does not guarantee their data are combinable. This is because the shared number of hidden states can result from completely different shape parameter and mean parameter values. Their underlying separation probability can be also very different. Supporting evidence found from biological experiments indicates that different genes have different basal expression levels, indicating that their smallest mean parameters are potentially different. In real situations, without knowing whether the shape parameter, mean parameters and the separation probability are similar or not, combining information from different genes is difficult to justify.

For a given sample size and shape parameter, a separation threshold can be chosen to maintain a type I error rate level. The procedure is:

1. Find the sample size and shape parameter for a specific gene from the analysis of microarray time course data found in previous chapters.

2. Choose several mean parameter values within the expression range of the gene. The scale parameters can be chosen accordingly with a known shape parameter. These values, as we shall discover from our simulations, are not critical.

3. Simulate a large number of samples (we use 500 samples per simulation) of a single gamma distribution with the mean (or scale parameter), shape parameter and sample size as specified as in step 2.

4. Run our order estimation algorithm with a threshold value and find how many samples are found to have order estimated as two or above. The proportion of falsely discovered high order samples (order equals two or above) is recorded for each simulation.

5. For a desired type I error rate value, find the corresponding separation threshold which leads to the desired type I error rate value. As an example, the threshold probability for a 3% type I error rate is in the range from 68% to 84% as shown in Table 5.2.

The proportion of falsely discovered samples (i.e. the number of samples found to have more than one hidden state) is compared to a targeted level. We expect on average that the proportion of falsely discovered response genes is under a specified level if the same situation is repeated many times.

As shown in Table 5.2, the type I error rate can be effectively controlled for a specific shape parameter and a range of mean parameter values. We generate 500 samples from a single gamma distribution with its shape and scale parameters as specified in each row of Table 5.2. The mean parameter (not shown) is the product of the shape and scale parameters. The sample size of each of the 500 samples is chosen as 20 to represent a typical microarray time course situation. At each specification of the shape and scale parameter, three separation threshold values are chosen as examples. Our algorithm then computes the order and finds the proportion of samples with more than one hidden state, as listed in Table 5.2. Since all of the samples are generated from a single underlying gamma distribution, the number recorded in the table divided by 500 provides the proportion of the falsely discovered samples whose order is higher than 1. Using 3% type I error rate as an example, we mark the corresponding separation threshold values with a star which have the type I error rate controlled at about 3% level.

For different genes, because the estimate of shape parameter is different, the simulation needs to be performed again in the same way. The type I error rate value can also be chosen at a different type I error rate level for different genes. Here we use the same type I error rate level across all genes for ease of illustration. The threshold values with a star in Table 5.2 are the separation threshold values that we used in the real data analysis in chapter 6.

Table 5.2. Type I error rate at three separation thresholds for various single gamma distributions.

True Order	gamma (shape α , scale β parameter)	Separation Threshold	False Discovered proportion among 500 samples
1	(60, 10)	73%	0.016
1	(60, 10)	*72%	0.026
1	(60, 10)	71%	0.044
1	(60, 15)	73%	0.008
1	(60, 15)	*72%	0.02
1	(60, 15)	71%	0.028
1	(60, 30)	73%	0.012
1	(60, 30)	*72%	0.016
1	(60, 30)	71%	0.03
1	(40, 10)	73%	0.004
1	(40, 10)	*72%	0.028
1	(40, 10)	71%	0.05
1	(40, 15)	73%	0.012
1	(40, 15)	*72%	0.026
1	(40, 15)	71%	0.048
1	(40, 30)	73%	0.01
1	(40, 30)	*72%	0.018
1	(40, 30)	71%	0.03
1	(20, 10)	74%	0.01
1	(20, 10)	*73%	0.022

1	(20, 10)	72%	0.04
1	(20, 15)	74%	0.01
1	(20, 15)	*73%	0.022
1	(20, 15)	72%	0.04
1	(20, 30)	74%	0.016
1	(20, 30)	*73%	0.034
1	(20, 30)	72%	0.048
1	(15, 10)	75%	0.006
1	(15, 10)	*74%	0.02
1	(15, 10)	73%	0.038
1	(15, 15)	75%	0.002
1	(15, 15)	*74%	0.01
1	(15, 15)	73%	0.018
1	(15, 30)	75%	0.004
1	(15, 30)	*74%	0.016
1	(15, 30)	73%	0.028
1	(10, 10)	76%	0.01
1	(10, 10)	*75%	0.028
1	(10, 10)	74%	0.038
1	(10, 15)	76%	0.01
1	(10, 15)	*75%	0.02
1	(10, 15)	74%	0.036
1	(10, 30)	76%	0.012
1	(10, 30)	*75%	0.018
1	(10, 30)	74%	0.024

1	(8, 10)	76%	0.01
1	(8, 10)	*75%	0.024
1	(8, 10)	74%	0.048
1	(8, 15)	76%	0.01
1	(8, 15)	*75%	0.03
1	(8, 15)	74%	0.048
1	(8, 30)	76%	0.016
1	(8, 30)	*75%	0.032
1	(8, 30)	74%	0.06
1	(5, 10)	78%	0.01
1	(5, 10)	*77%	0.026
1	(5, 10)	76%	0.036
1	(5, 15)	78%	0.01
1	(5, 15)	*77%	0.016
1	(5, 15)	76%	0.032
1	(5, 30)	78%	0.012
1	(5, 30)	*77%	0.022
1	(5, 30)	76%	0.046
1	(3, 10)	80%	0.014
1	(3, 10)	*79%	0.028
1	(3, 10)	78%	0.038
1	(3, 15)	80%	0.022
1	(3, 15)	*79%	0.028
1	(3, 15)	78%	0.048
1	(3, 30)	80%	0.014

1	(3, 30)	*79%	0.02
1	(3, 30)	78%	0.058
1	(2, 10)	82%	0.016
1	(2, 10)	*81%	0.022
1	(2, 10)	80%	0.046
1	(2, 15)	82%	0.02
1	(2, 15)	*81%	0.04
1	(2, 15)	80%	0.064
1	(2, 30)	82%	0.016
1	(2, 30)	*81%	0.02
1	(2, 30)	80%	0.038
1	(1, 10)	87%	0.014
1	(1, 10)	*86%	0.024
1	(1, 10)	85%	0.036
1	(1, 15)	87%	0.012
1	(1, 15)	*86%	0.02
1	(1, 15)	85%	0.042
1	(1, 30)	87%	0.026
1	(1, 30)	*86%	0.032
1	(1, 30)	85%	0.052

The type I error rate of our method is an important tool to control the performance of the procedure for distinguishing genes with only one state from those genes with multiple states. Further characterization of the number of states for the multiple state

genes, can be investigated in more detail by employing other biological techniques. The above simulations demonstrate that the separation threshold can be determined to effectively control the type I error rate for a specific sample size. The type I error rate in the above table is also an indicator of the accuracy that our proposed method can achieve.

As shown in Table 5.2, for a given shape parameter, a corresponding separation threshold level can be found to keep the type I error rate at a desired level for various values of the scale parameter or mean parameter. This confirms that for a given shape parameter, the separation threshold can be used to consistently control the type I error rate for various values of the scale parameter or mean parameter. On the other hand, as the shape parameter varies, the separation probability needs to be adjusted to maintain a specified type I error rate. A larger separation threshold value is needed for a smaller shape parameter. In Table 5.2, a series of separation probability thresholds are obtained for different shape parameter values for the analysis of real microarray time course data in chapter 6. We expect to keep the type I error rate near or under 3% for all genes with different shape parameters in the real data analysis in the next chapter.

Here we also provide some evaluations for the situation where the exact number of states is of interest. In such cases, our order estimation procedure can be used to find out the order of a hidden Markov model or a finite mixture model. As we discussed above, the separation probability for microarray data is unknown. The mixing proportion is also unknown. Simulations are unable to be performed for real microarray data. Instead, simulations are performed for general situations where sufficiently large samples are

available and separation probability is known. The data are also assumed to come from stationary HMMs or a finite mixture models and homogeneous HMMs.

We use a small sample size of 20 and a medium sample size of 300 to show that exact order estimation requires a sufficiently large sample size, given a known separation threshold. The difficulty of exact order estimation comes from not only the lack of sophisticated theoretical tools but also the lack of information contained in a given sample. We briefly present simulations in Table 5.4 where the true order is 2 and in Table 5.5 where the true order is 3 with both sample size of 20 and 300. Each run in Table 5.4 and Table 5.5 consists of 500 samples. We selected the separation probability in the range from 72% to 99%, as examples.

Table 5.3 explains how the data are generated for Table 5.4 and Table 5.5. The first three columns specify the sample size, the true order and the separation probability for the mixtures. The distance between two adjacent gamma distributions is defined by the separation probability in the third column. The mixing proportion is specified in the fourth column. The parameters of the gamma mixture components are listed in the fifth and sixth columns.

Table 5.3. The specifications for the simulation of mixtures of two gamma distributions.

The sample size, true order, separation threshold, mixing proportion and the parameters of each gamma distribution with 500 samples are listed in each row. The estimated order is given in Table 5.4 and Table 5.5.

Sample size	True order	Separation prob.	Mixing proportion	First gamma Dist. (shape α , scale β)	Second gamma Dist. (shape α , scale β)
20	2	72%	0.4, 0.6	(40,15)	(40, 17.85)
20	2	82%	0.4, 0.6	(40,15)	(40, 19.8)
20	2	92%	0.4, 0.6	(40,15)	(40, 23.1)
20	2	73%	0.4, 0.6	(20,15)	(20, 19.2)
20	2	83%	0.4, 0.6	(20,15)	(20, 22.35)
20	2	93%	0.4, 0.6	(20,15)	(20, 28.2)
20	2	76%	0.4, 0.6	(10,15)	(10, 22.05)
20	2	86%	0.4, 0.6	(10,15)	(10, 28.05)
20	2	96%	0.4, 0.6	(10,15)	(10, 43.05)
20	2	79%	0.4, 0.6	(5,15)	(5, 27.15)
20	2	89%	0.4, 0.6	(5,15)	(5, 39.75)
20	2	99%	0.4, 0.6	(5,15)	(5, 112.05)
300	2	72%	0.4, 0.6	(40,15)	(40, 17.85)
300	2	82%	0.4, 0.6	(40,15)	(40, 19.8)
300	2	92%	0.4, 0.6	(40,15)	(40, 23.1)
300	2	73%	0.4, 0.6	(20,15)	(20, 19.2)
300	2	83%	0.4, 0.6	(20,15)	(20, 22.35)
300	2	93%	0.4, 0.6	(20,15)	(20, 28.2)
300	2	76%	0.4, 0.6	(10,15)	(10, 22.05)
300	2	86%	0.4, 0.6	(10,15)	(10, 28.05)
300	2	96%	0.4, 0.6	(10,15)	(10, 43.05)
300	2	79%	0.4, 0.6	(5,15)	(5, 27.15)
300	2	89%	0.4, 0.6	(5,15)	(5, 39.75)
300	2	99%	0.4, 0.6	(5,15)	(5, 112.05)

The data in Table 5.4 and Table 5.5 are generated according to the listed specifications and the following steps:

1. Use the multinomial distribution with the specified mixing proportions to generate the number of observations for each gamma component.

2. Generate the observations for each component distribution with the specified shape and scale parameters and with the number of observations decided by step 1.
3. Mix the data points together.

This data generation procedure is used to generate gamma mixtures with order 2 and 3 for the evaluations in Table 5.4 and Table 5.5.

In Table 5.4 and Table 5.5, the data are generated from mixture models (or equivalently from a HMM with a stationary probability) specified as in each row of the table. The estimated order is reported as a proportion of 500 samples. Assume that the underlying separation probability is known. A separation threshold value is chosen as an example. For illustration purposes, we select a value slightly smaller than the true underlying separation probability to account for sample variation. The probability threshold used in Table 5.4 and Table 5.5 is among many possible values that can be specified. When the threshold is specified bigger than the true threshold, the correct estimation proportion could be very low and close to zero (data not shown). The threshold value is picked as an example. There are many possible threshold values which could be chosen in Table 5.4 and Table 5.5. The values used serve only as an example.

Table 5.4. Simulation evaluations for the mixture models with 2 components.

Sample size	True order	Sep. Threshold	Sep. prob.	Mixing proportion	First gamma Dist. (shape α , scale β)	Second gamma Dist. (shape α , scale β)	Among 500 repeats, the proportion of order <2	Among 500 repeats, the proportion of order =2	Among 500 repeats, the proportion of order >2
20	2	60%	72%	0.4, 0.6	(40,15)	(40, 17.85)	0.276	0.578	0.146
20	2	62%	82%	0.4, 0.6	(40,15)	(40, 19.8)	0.074	0.682	0.244
20	2	68%	92%	0.4, 0.6	(40,15)	(40, 23.1)	0.032	0.60	0.368
20	2	63%	73%	0.4, 0.6	(20,15)	(20, 19.2)	0.304	0.574	0.122
20	2	63%	83%	0.4, 0.6	(20,15)	(20, 22.35)	0.088	0.666	0.246
20	2	63%	93%	0.4, 0.6	(20,15)	(20, 28.2)	0.004	0.53	0.466
20	2	66%	76%	0.4, 0.6	(10,15)	(10, 22.05)	0.332	0.558	0.11
20	2	66%	86%	0.4, 0.6	(10,15)	(10, 28.05)	0.072	0.64	0.288
20	2	66%	96%	0.4, 0.6	(10,15)	(10, 43.05)	0	0.552	0.448
20	2	69%	79%	0.4, 0.6	(5,15)	(5, 27.15)	0.3	0.568	0.132
20	2	69%	89%	0.4, 0.6	(5,15)	(5, 39.75)	0.038	0.676	0.286
20	2	69%	99%	0.4, 0.6	(5,15)	(5, 112.05)	0	0.534	0.466
300	2	66%	72%	0.4, 0.6	(40,15)	(40, 17.85)	0.15	0.79	0.06
300	2	70%	82%	0.4, 0.6	(40,15)	(40, 19.8)	0.082	0.74	0.178
300	2	76%	92%	0.4, 0.6	(40,15)	(40, 23.1)	0.086	0.822	0.092
300	2	66%	73%	0.4, 0.6	(20,15)	(20, 19.2)	0.114	0.722	0.164
300	2	71%	83%	0.4, 0.6	(20,15)	(20, 22.35)	0.106	0.712	0.182
300	2	76%	93%	0.4, 0.6	(20,15)	(20, 28.2)	0.016	0.796	0.188
300	2	70%	76%	0.4, 0.6	(10,15)	(10, 22.05)	0.108	0.726	0.166
300	2	74%	86%	0.4, 0.6	(10,15)	(10, 28.05)	0.168	0.636	0.196
300	2	79%	96%	0.4, 0.6	(10,15)	(10, 43.05)	0	0.814	0.186
300	2	74%	79%	0.4, 0.6	(5,15)	(5, 27.15)	0.098	0.664	0.238

300	2	77%	89%	0.4, 0.6	(5,15)	(5, 39.75)	0.198	0.562	0.24
300	2	82%	99%	0.4, 0.6	(5,15)	(5, 112.05)	0	0.82	0.18

Similar to above simulation, data was also generated from a mixture when the true order is 3. The result of the estimation is shown in Table 5.5.

Table 5.5. Simulation evaluations for the mixture models with 3 components.

Sample size	True order	Separation Threshold	Separation prob.	Mixing proportion	First gamma Dist. (shape α , scale β)	Second gamma Dist. (shape α , scale β)	Third gamma Dist. (shape α , scale β)	Among 500 repeats, the proportion of order<3	Among 500 repeats, the proportion of order=3	Among 500 repeats, the proportion of order>3
20	3	56%	72%	0.2, 0.3, 0.5	(40,15)	(40, 17.85)	(40, 21.24)	0.55	0.33	0.12
20	3	60%	82%	0.2, 0.3, 0.5	(40,15)	(40, 19.8)	(40, 26.14)	0.376	0.48	0.144
20	3	68%	92%	0.2, 0.3, 0.5	(40,15)	(40, 23.1)	(40, 35.57)	0.276	0.632	0.092
20	3	58%	73%	0.2, 0.3, 0.5	(20,15)	(20, 19.2)	(20, 24.58)	0.57	0.356	0.074
20	3	61%	83%	0.2, 0.3, 0.5	(20,15)	(20, 22.35)	(20, 33.3)	0.37	0.506	0.124
20	3	66%	93%	0.2, 0.3, 0.5	(20,15)	(20, 28.2)	(20, 53.02)	0.06	0.65	0.29
20	3	58%	76%	0.2, 0.3, 0.5	(10,15)	(10, 22.05)	(10, 32.41)	0.538	0.33	0.132
20	3	60%	86%	0.2, 0.3, 0.5	(10,15)	(10, 28.05)	(10, 52.45)	0.32	0.462	0.218
20	3	0.66	96%	0.2, 0.3, 0.5	(10,15)	(10, 43.05)	(10, 123.55)	0.022	0.52	0.458
20	3	58%	79%	0.2, 0.3, 0.5	(5,15)	(5, 27.15)	(5, 49.14)	0.45	0.328	0.222
20	3	60%	89%	0.2, 0.3, 0.5	(5,15)	(5, 39.75)	(5, 105.34)	0.148	0.51	0.342
20	3	68%	99%	0.2, 0.3, 0.5	(5,15)	(5, 112.05)	(5, 837.01)	0.006	0.402	0.592
300	3	66%	72%	0.2, 0.3, 0.5	(40,15)	(40, 17.85)	(40, 21.24)	0.414	0.496	0.09
300	3	70%	82%	0.2, 0.3, 0.5	(40,15)	(40, 19.8)	(40, 26.14)	0.058	0.62	0.322
300	3	76%	92%	0.2, 0.3, 0.5	(40,15)	(40, 23.1)	(40, 35.57)	0	0.744	0.256

300	3	66%	73%	0.2, 0.3, 0.5	(20,15)	(20, 19.2)	(20, 24.58)	0.27	0.532	0.20
300	3	71%	83%	0.2, 0.3, 0.5	(20,15)	(20, 22.35)	(20, 33.3)	0.14	0.712	0.148
300	3	80%	93%	0.2, 0.3, 0.5	(20,15)	(20, 28.2)	(20, 53.02)	0	0.892	0.108
300	3	68%	76%	0.2, 0.3, 0.5	(10,15)	(10, 22.05)	(10, 32.41)	0.228	0.616	0.156
300	3	76%	86%	0.2, 0.3, 0.5	(10,15)	(10, 28.05)	(10, 52.45)	0.03	0.80	0.17
300	3	82%	96%	0.2, 0.3, 0.5	(10,15)	(10, 43.05)	(10, 123.55)	0	0.932	0.068
300	3	72%	79%	0.2, 0.3, 0.5	(5,15)	(5, 27.15)	(5, 49.14)	0.214	0.666	0.12
300	3	80%	89%	0.2, 0.3, 0.5	(5,15)	(5, 39.75)	(5, 105.34)	0.02	0.84	0.14
300	3	82%	99%	0.2, 0.3, 0.5	(5,15)	(5, 112.05)	(5, 837.01)	0	0.858	0.142

In both Table 5.4 and Table 5.5, the percentage of correct estimates ranges from 32% to 93% and the proportion of correct estimates improves as the sample size increases. For sample size of 20, the order is poorly estimated, especially when the true order is high. With a sample size of 300, a threshold specified slightly less than the true separation probability may produce a good percentage of correct estimates. If the threshold specified is very different from the true separation probability, the correctly estimated percentage can be much lower, even close to zero. It is obviously important to know the threshold value even for large samples. Moreover, the above simulations also indicate that for a small sample size, even with a correctly specified threshold there may still be limited capability to obtain a high accuracy in estimating the exact order. Again, small sample sizes impose serious limitations on the performance of order estimation. Based on the small sample sizes that occur with microarray data, the information for discovering the underlying order is too limited. This is the reason that we suggest

identifying the response genes with order above 1 for microarray data rather than estimating the exact order.

In the simulations above, the data were generated from the mixture density (2.1). To evaluate the performance of our method when the observations are generated from hidden Markov models, we generate data from a homogeneous hidden Markov model with two states. As shown in Table 5.6, we evaluated the HMMs with two gamma emission distributions. These two emission distributions are specified in the same way as in Table 5.4. The stationary distribution is (0.4, 0.6) for the two states corresponding to the two gamma emission distributions in Table 5.6. A homogeneous Markov chain is assumed, with transition matrix: $A = \begin{bmatrix} 0.7 & 0.3 \\ 0.2 & 0.8 \end{bmatrix}$. The total number of time points is 5.

For sample size of 20 or 300, we generated 4 or 60 runs of observations respectively with five observations in each run. This data generation procedure is repeated 500 times to get the 500 samples for each row of Table 5.6. Our algorithm was then run on the 500 samples to estimate the order for each row, as shown in Table 5.6. The order estimation proportions are recorded in Table 5.6. Comparing the result between Table 5.4 and Table 5.6, the performance of our algorithm is similar for data generated from finite mixture models and homogeneous hidden Markov models. Our simulation results also provide certain support to the suggestion proposed by Poskitt and Zhang (2005).

Table 5.6. Simulation evaluations for hidden Markov models with 2 hidden states. The sample size, true order, separation threshold, the true separation probability between the two emission distribution and the parameters of the two emission distributions are specified in following table.

Sample size	True order	Sep. Threshold	Sep. prob.	Initial Transition Dist.	First gamma Dist. (shape α , scale β)	Second gamma Dist. (shape α , scale β)	Among 500 repeats, the proportion of order<2	Among 500 repeats, the proportion of order=2	Among 500 repeats, the proportion of order>2
20	2	60%	72%	0.4, 0.6	(40,15)	(40, 17.85)	0.282	0.546	0.172
20	2	62%	82%	0.4, 0.6	(40,15)	(40, 19.8)	0.098	0.566	0.336
20	2	68%	92%	0.4, 0.6	(40,15)	(40, 23.1)	0.06	0.564	0.376
20	2	63%	73%	0.4, 0.6	(20,15)	(20, 19.2)	0.29	0.506	0.204
20	2	63%	83%	0.4, 0.6	(20,15)	(20, 22.35)	0.13	0.64	0.23
20	2	63%	93%	0.4, 0.6	(20,15)	(20, 28.2)	0.014	0.572	0.414
20	2	66%	76%	0.4, 0.6	(10,15)	(10, 22.05)	0.35	0.53	0.12
20	2	66%	86%	0.4, 0.6	(10,15)	(10, 28.05)	0.096	0.622	0.282
20	2	66%	96%	0.4, 0.6	(10,15)	(10, 43.05)	0.004	0.51	0.486
20	2	69%	79%	0.4, 0.6	(5,15)	(5, 27.15)	0.37	0.50	0.13
20	2	69%	89%	0.4, 0.6	(5,15)	(5, 39.75)	0.084	0.57	0.346
20	2	69%	99%	0.4, 0.6	(5,15)	(5, 112.05)	0	0.41	0.59
300	2	66%	72%	0.4, 0.6	(40,15)	(40, 17.85)	0.166	0.756	0.078
300	2	70%	82%	0.4, 0.6	(40,15)	(40, 19.8)	0.122	0.68	0.198
300	2	76%	92%	0.4, 0.6	(40,15)	(40, 23.1)	0.082	0.73	0.188
300	2	66%	73%	0.4, 0.6	(20,15)	(20, 19.2)	0.13	0.686	0.184
300	2	71%	83%	0.4, 0.6	(20,15)	(20, 22.35)	0.124	0.68	0.196
300	2	76%	93%	0.4, 0.6	(20,15)	(20, 28.2)	0.004	0.718	0.278
300	2	70%	76%	0.4, 0.6	(10,15)	(10, 22.05)	0.1	0.716	0.184

300	2	74%	86%	0.4, 0.6	(10,15)	(10, 28.05)	0.194	0.584	0.222
300	2	79%	96%	0.4, 0.6	(10,15)	(10, 43.05)	0	0.706	0.294
300	2	74%	79%	0.4, 0.6	(5,15)	(5, 27.15)	0.2	0.62	0.18
300	2	77%	89%	0.4, 0.6	(5,15)	(5, 39.75)	0.162	0.582	0.256
300	2	82%	99%	0.4, 0.6	(5,15)	(5, 112.05)	0	0.766	0.234

Chapter 6

ANALYSIS OF POLYMORPHONUCLEAR LEUKOCYTE MICROARRAY TIME SERIES DATA

In this chapter, the methods presented in chapter 3 and 4 are applied to a microarray time course data set which is available on the NCBI GEO web site (<http://www.ncbi.nlm.nih.gov/geo/>). This data set is published on GEO with the data set ID of GDS1428 (Borjesson et al., 2005). The experiment uses Affymetrix oligonucleotide arrays. It is designed to investigate the effect of infection of *A. phagocytophilum* on polymorphonuclear leukocytes (PMNs or neutrophils). We choose to analyze two of the treatment conditions from this experiment: PMNs treated with *A. phagocytophilum*, and PMNs treated by the control condition (i.e. without any infection). There are seven time points with 2 to 6 replicates at each time point, under each treatment condition. The intensity values analyzed are the normalized values computed using GeneSpring version 6.0 software. For further details, see Borjesson et al. (2005).

The data are analyzed using the methods of chapter 3 and 4, for each treatment condition separately. We use gamma distributions to model the expression intensities at each time point for each gene. For the GDS1428 data set, the normalized intensity measurements after pre-processing are used as the input data for all subsequent analysis. As described in chapter 3, empirical Bayesian analysis is first performed to find the gene specific shape parameter. This shape parameter and the CV are computed for each gene. These values are assumed to be the same across time points for one specific gene. The histograms for the estimated CV and shape parameter values are plotted in Figure 6.1 and Figure 6.2.

Figure 6.1 Histogram of CV values for control and treatment condition of GDS1428 (n=21775).

Figure 6.2 Histogram of shape parameter values for control and treatment condition of GDS1428 (n=21775).

As we discussed in chapter 3, the shape parameters of all genes under the same treatment condition are generated from a log-normal prior distribution. If we think of the CV as a unitless measurement of variation, the shape parameter of the gamma distribution can be thought of as the squared stability measurement for a specific gene. The estimated hyper-parameters using (3.2) and (3.3) for the two treatment conditions are: $\hat{\tau} : 2.182803$ and $\hat{\sigma} : 1.955049$ for the GDS1428 control group; and $\hat{\tau} : 2.295019$ and $\hat{\sigma} : 1.840149$ for the GDS1428 treatment group. These histograms suggest that the assumption made here and by Lo and Gottardo (2007) that the shape parameter follows a lognormal distribution is a reasonable claim. The shape parameter is estimated based on the posterior distribution (3.7) for each gene.

With the shape parameter estimated and treated as known in later stage analysis, the order is estimated by the penalized maximum likelihood method as discussed in

chapter 4. The initial upper bound for order is set to 5, with corresponding starting mixing proportions set to 0.2 for each of the five components. The separation threshold values chosen are those identified in chapter 5, as the values with stars shown in table 5.2. These separation threshold values are used to control the type I error rate at about 3%, corresponding to different shape and scale parameter values. Choosing a 3% type I error rate level here is only for illustration purposes. From the analysis of several other microarray time course data sets (results not shown), when the threshold value is above 0.9, there is usually no gene showing more than one state. This suggests that genes do not use very high separation probabilities to increase signal certainty. Perhaps the biological cost is prohibitively high for statistically significant separation. Instead, implementing some check points across cell growth stages may be more cost effective.

For the GDS1428 data set, we found 1644 response genes which responded under the control treatment condition, and 1902 genes which responded under the treatment condition. As shown in Table 6.1, the total number of genes measured in both the control and treatment microarray is 13996. The number of genes which did not respond under the control treatment is thus 12352, and is 12094 for the treatment condition. In terms of the percentage of response genes, 11.75% and 13.59% of genes respond under the control and treatment conditions, respectively. After the 3% type I error rate adjustment, the expected response gene percentages are 11.40% and 13.18% for control and treatment groups, respectively. The total number of probes are 21775 for both control and treatment conditions. According to the Affymetrix gene chip design, normally more than one probe is used for each gene, making the number of probes bigger than the number of genes. Our discovered response genes are annotated first based on their probe ID.

Table 6.1. The number of response genes for each treatment condition for the GDS1428 data.

	GDS1428 Control	GDS1428 Treatment
Number of Response Genes	1644	1902
Number of non-Response Genes	12352	12094
Total Number of Probes	21775	21775
Total Number of Genes	13996	13996

In Table 6.1, the annotation platform of hgu133A from Affymetrix is used. The probe ID is first used to identify the response genes. Then based on Affymetrix's annotation database, the corresponding gene ID is identified. Since there are typically several probe ID's matching one gene ID, the number of probes is bigger than the number of gene ID. We choose to report gene ID in Table 6.1 and in the tables of Appendix A for ease of biological interpretation. The full list of the response genes under one treatment condition is the summary of cellular response under one external condition. The information maintained in each response set is not only about the appearance of a single gene but also about the co-appearance with other genes in that set. Thus, the full response set as a whole provides a signature for the cellular response.

The sets of response genes are presented in Table A.1 and Table A.2 of Appendix A. There are 894 genes which are responded under both the control and treatment conditions. This common set of genes is listed in Table A.3 of Appendix A. The genes that responded only for the control group are listed in Table A.4, and the genes which

responded only under the treatment condition are listed in Table A.5 of Appendix A. It is biologically reasonable that certain genes are commonly used by a cell to respond to different external conditions. This situation is similar to those house keeping genes which are activated across different treatment conditions.

The comparison of the response set of genes under different treatment conditions can provide us with more information regarding regulatory relationships among the genes. And as the number of gene sets extracted from experiments increases, the relationship inferred can be more reliable. We identify the commonly activated gene set for the GDS1428 treatment and control group. When many treatment conditions are compared, if we find one gene's appearance has a high probability to co-appear with another gene, they are probably involved in the same or a closely related regulatory pathway. As an example, as shown in Table A.3, among the common response gene set for both control and treatment conditions, we find genes: NFKB1, NFKB2, NFKBIE, EIF2AK3. Current biological knowledge indicates that NFKB1 and NFKB2 may both respond under various treatment conditions. This is confirmed by our result. NFKB1 and NFKB2 encode for the nuclear factor dimmer protein that regulates the transcription of genes involved in immune and inflammatory responses, stress remediation, cell growth and apoptosis. In normal situations, the protein encoded by the NFKB1 and NFKB2 genes are associated with the inhibitor (IKB) and only presented in cytoplasm. In response to certain conditions, IKB will be degraded and will release NFKB1 and NFKB2 proteins into the nucleus and induce a series of gene activations.

There is a complicated mechanism that controls the release of NFkB proteins and different activation processes when they enter the nucleus. The IKK (IKB kinase) is one kinase that contributes to the degradation and the release of IKB from NFkB proteins. We found that NFkB1 and NFkB2 genes responded under both the control and treatment conditions. In addition, NFkBIE (or IKBE) also responds under both conditions, which means the proteins of NFkB1 or NFkB2 are inhibited by IKBE and kept in the cytoplasm under both conditions. On the other hand, the gene IKBKAP responds only in the control group. This gene encodes a scaffold protein which assists to form IKKs into an active kinase complex, which in turn may lead to the release of NFkB proteins into the nucleus.

The gene EIF2AK3 responds under both the control and treatment conditions. This gene is related to the reaction of impaired protein folding in the endoplasmic reticulum (i.e. ER stress). ER stress is one of several mechanisms involving NFkB mediated regulation. Phosphorylation of another translation related protein by EIF2AK3 results in general reduction of translation, which allows the cell more time to correct the impaired protein folding problem caused by ER stress. In the mean time, the phosphorylation also contributes to the activation of NFkB protein by a mechanism that involves release, but not degradation of IKB. Both response sets have the co-occurrence of NFkB1, NFkB2 and EIF2AK3, indicating that they probably work together in activation of the pathway for ER stress. For more information, please see Jiang et al. (2003) and Liang et al. (2006).

However, the common response set of 894 genes we found for both control and treatment conditions does not necessarily indicate a deterministic causal relationship between any pair of the genes in this set. Genes in this set may belong to several parallel pathways within which the casual relationship is found to be more significant. Moreover, even within the same pathway, the regulatory relationship is still governed by probabilistic rules. If two genes always appear together under many experimental conditions, the probability of the causal relationship among them is much higher than that under a few experimental conditions. Hence, the statistical inference of the causal relationship among genes can be dramatically improved as the number of experiments analyzed increases.

To illustrate the difference between our approach and the direct expression comparison approaches, we compare our response gene lists with the differentially regulated gene list identified in the original study (Borjesson et al., 2005). Those genes identified by Borjesson et al. are defined as differentially regulated genes, as listed in Table 1 of their complementary material (Borjesson et al., 2005). Their approach compares the gene expression levels between the treatment and the control group. Those genes with at least 1.5 fold changes over the control group are defined as differentially regulated genes (including both up-regulated genes and down-regulated genes). The gene expression comparisons were conducted between treatment and control at the time points 1.5 hours, 3 hours, 6 hours, 9 hours, 12 hours and 24 hours. To make the comparison of the gene lists from both studies possible, the overall differentially expressed gene list from their study is used. This gene list is referred to as gene set A hereafter. Gene set A consists of those genes with 1.5 or higher fold changes at one or more time points

between treatment and control group. The total number of these genes in gene set A is 810, which is the number of unique gene IDs with at least one corresponding probe set found to meet the criterion. Because the response gene lists in our study are defined differently from theirs, we use two of the most relevant gene sets in the comparison. The first one is the response gene set found under the treatment condition, which is referred to as gene set B hereafter. Gene set B has 1902 response genes. The second gene list consists of those genes found to respond only under the treatment condition, which is referred to as gene set C. Gene set C has 1008 genes. The difference between gene set B and C is that those genes found to respond under the control condition are excluded from gene set C.

The comparison is shown in Table 6.2. There are 232 genes found in both sets A and B. 578 genes are in gene set A but not B. There are 1670 genes identified only in gene set B. For gene set A and C, there are 80 genes in common. 730 genes appear only in gene set A and 928 genes are identified only in gene set C.

Table 6.2. The comparison of gene lists identified by our proposed method and the method described in the original study (Borjesson et al., 2005). Gene Set A contains the differentially regulated gene set based on the comparison between treatment and control condition as defined in the original study. Gene Set B is the response gene set under treatment condition. Gene Set C contains the genes which respond only under treatment condition. Gene Set B and C are found using our proposed method.

	Gene Set A: Differentially Regulated Genes Between Treatment and Control, total 810 genes		
	Number of genes in both set	Number of genes in Set A only	Number of genes in Set B/C only
Gene Set B: Response Genes Under Treatment, total 1902 genes	232	578	1670
Gene Set C: Response Gene Under Treatment Only, total 1008 genes	80	730	928

As shown in the above comparison, the two approaches are quite different in terms of their identified gene list. This is probably due to the difference in analysis methods used and the definition of the targeted genes. Detailed gene lists for the above comparisons are included in Table A. 6 to Table A.11 of Appendix A.

Chapter 7

CONCLUSIONS AND FURTHER DISCUSSION

We introduce a method based on order estimation to deal with the confounding problem in microarray time course data. Because the treatment effect is confounded with the gene context effect, the commonly used statistical comparisons and tests are inappropriate. The fact that a treatment condition can only be administrated to the cell level and that it affects different genes in a gene specific way requires more attention to the analysis. The influence of a treatment condition on a gene potentially depends on other genes in a way which is unknown and could not be simply ignored. In such a situation, the ANOVA models and similar linear models incorporating a correlation structure are not sufficient. Results obtained from this kind of analysis, especially those aimed at identifying genes with differential expressions, are incorrect. Because the regulatory relationship is not deterministic, the expressions of a specific gene under the same treatment condition do not necessary come from the same underlying state each

time. The comparison of means in such a situation can not provide conclusive information on whether the gene behaves the same or not. For the same reason, the comparison of the state sequence between two treatment conditions can not provide information about whether the gene behaves the same or not either. Since the comparison for non-time course experiments is only a special form of time course experiments with a single time point, the most direct informative statistic under this situation is the order, which provides the essential information about the differentiation of states instead of a particular state. The differentiation in expressions rather than the expression itself can be used to characterize the behavior of a gene for a treatment condition.

The method we proposed here is developed to first extract the transcription level response. The response genes are used by a cell either to communicate a signal to other genes or to perform certain biological functions. The identified response genes are expected to be those genes coordinated by the cell to react to a specific external condition. A cell response is much more complicated than a single gene response. This kind of response thus can be characterized by the full set of response genes.

The response genes are identified separately under each experimental condition. We developed the method to estimate the order when the mean and shape parameters are all unknown. Our penalized maximum likelihood estimation coupled with the simulation based approach to find the threshold provides a way to estimate the order, particularly suitable for microarray time course data. The sample size is typically small and there is no previous quantification for the separation probability.

To apply our method, certain conditions are required to ensure proper inferences.

Our approach requires the following conditions to be satisfied:

1. The time course experiments are synchronized.
2. There are replicates at each time point.
3. There are at least three time points measured.

The number of time points is very important because more time points provide better chance to detect response genes, if the different states do exist. The time intervals are not required to be equal, based on the stationary Markov process assumed for order estimation. The above conditions are satisfied for most microarray time course experiments.

To deal with the limited sample size of microarray data, we propose to target the separation between genes with order one and the genes with order two or above. Our method is designed specially for microarray time course data and takes into account the typical small sample size. One of the reasons that the order estimation is a difficult problem is that it is related to the sample size and the information available for the separation of components at a given sample size. This perspective has not been discussed much by the existing literature, which focuses on the asymptotic properties in the large sample situation.

Another issue of order estimation for HMMs is the assumption used for the transition probabilities. Interestingly, although existing frequentist methods always adopt the stationary probability assumption, there is no formal justification in the literature. Poskitt and Zhang (2005) provided some reasons for computational gain. Perhaps the

order estimation for HMMs requires more clear definition. Before the order of a HMM is determined, there is no way to tell whether the underlying process is a homogeneous or heterogeneous. A different order may also change a homogenous process to a heterogeneous one. In other words, a sufficiently large order will always produce a homogeneous process. Without knowing the order, the states can not be defined. Without the definition of the states, we do not even have a criterion to judge whether a process as heterogeneous or homogeneous. The order estimation therefore should not be influenced by such a subjective decision. Hence a stationary probability can be reasonably assumed for the order estimation.

The identified response gene set as a characterization for a particular treatment condition has two fold meaning. First, each response gene plays a certain role in the response to a treatment condition. Second, the regulatory interaction with other genes is maintained in the response set by the gene's appearance together with other genes in the set. This co-appearance of genes in a response set provides information concerning the regulatory relationships among genes.

There are several possible further developments. For each gene, the shape parameter is assumed to be the same across the time points. This can be examined by performing statistical tests to find out whether it is the case for every gene. The shape parameter then can be estimated according to the test results. Theoretically it is possible to let the number of hidden states depend on both the shape and mean parameters. However, this modeling setting still needs supporting biological evidence, especially on whether the shape parameter changes its value across time.

For hidden Markov models and finite mixture models, our order estimation method relies on the specification of the threshold. Finding a proper threshold value is critical and could be another possible further development. As gene expression data is accumulated, the knowledge about the separation probability for different genes may become available and can be used in further analysis of new time course data. However, as we begin our proposed analysis, this kind of information is very difficult to find. Thus our proposed simulation based approach provides a way to start. There is certainly much room for further improvement.

References

- Albert, P. S., McFarland, H. F., Smith, M. E., and Frank, J. A. (1994). Time series for modeling counts from a relapsing-remitting disease: application to modeling disease activity in multiple sclerosis. *Statistics in Medicine* **13**, 453-466.
- Aitkin, M. and Rubin, D. B. (1985). Estimation and hypothesis testing in finite mixture models. *Journal of the Royal Statistical Society, Series B* **47**, 67-75.
- Baggerly, K. A., Coombes, K. R., Hess, K. R., Stivers, D. N., Abruzzo, L. V., and Zhang, W. (2001). Identifying differentially expressed genes in cDNA microarray experiments. *Journal of Computational Biology* **8**, 639-659.
- Baldi, P. and Long, A. D. (2001). A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inference of gene changes. *Bioinformatics* **17**, 509-519.
- Bar-Joseph, Z., Gerber, G., Simon, I., Gifford, D., and Jaakkola, T. (2003). Comparing the continuous representation of time-series expression profiles to identify differentially expressed genes. *Proceedings of the National Academy of Sciences* **100**, 10146-10151.

Baras, J. S. and Finesso, L. (1992). Consistent estimation of the order of hidden Markov chains. *Stochastic Theory and Adaptive Control: Proceedings of a Workshop held in Lawrence, Kansas, September 26-28, 1991*. Berlin: Springer-Verlag.

Bickel, P. J., Ritov, Y., and Ryden, T. (1998). Asymptotic normality of the maximum-likelihood estimator for general hidden Markov models. *Annals of Statistics* **26**, 1614-1635.

Biernacki, C., Celeux, G., and Govaert, G. (1998). Assessing a mixture model for clustering with the integrated classification likelihood. *Technical Report No. 3521*. Rhone-Aples: INRIA.

Black, M. A. and Doerge, R. W. (2001). Calculation of the minimum number of replicates spots required for detection of significant gene expression fold change in microarray experiments. In *Proceeding of the Conference on Applied Statistics in Agriculture* 144-158.

Bolstad, B., Irizarry, R., Astrand, M., and Speed, T. (2002). *A comparison of normalization methods for high density oligonucleotide array data based on variance and bias*. Technical report, UC Berkeley.

Borjesson, D. L., Kobayashi, S. D., Whitney, A. R., Voyich, J. M., Argue, C. M., and DeLeo, F. R. (2005). Insights into pathogen immune evasion mechanisms: anaplasma

phagocytophilum fails to induce an apoptosis differentiation program in human neutrophils. *The Journal of Immunology* **174**, 6364-6372.

Calvano, S. E., Xiao, W., Richards, D. R., Felciano, R. M., Baker, H. V., Cho, R. J., Chen, R. O., Brownstein, B. H., Cobb, J. P., Tschoeke, S. K., Miller-Graziano, C., Moldawer, L. L., Mindrinos, M. N., Davis, R. W., Tompkins, R. G., and Lowry, S. F. (2005). A network-based analysis of systemic inflammation in humans. *Nature* **437**, 1032-1037.

Cappe, O., Moulines, E., and Ryden, T. (2005). *Inference in Hidden Markov Models*. USA: Springer.

Chen, J. and Kalbfleisch, J. D. (1996). Penalized minimum-distance estimates in finite mixture models. *The Canadian Journal of Statistics* **24**, 167-175.

Chen, Y., Dougherty, E. R., and Bittner, M. L. (1997). Ratio-based decisions and the quantitative analysis of cDNA microarray images. *Journal of Biomedical Optics* **2**, 364-374.

Chen, H., Chen, J., and Kalbfleisch, J. D. (2001). A modified likelihood ratio test for homogeneity in finite mixture models. *Journal of the Royal Statistical Society, Series B* **63**, 19-29.

Chen, J. and Khalili, A. (2005). *Order selection in finite mixture models*. Technical report. Available at <http://www.math.uwaterloo.ca/jhchen/publ.html>.

Craig, B. A, Black, M. A., and Doerge, R. W. (2003). Gene expression data: the technology and statistical analysis. *Journal of Agricultural, Biological and Environmental Statistics* **8**, 1-28.

Craven, P. and Wahba, G. (1979). Smoothing noisy data with spline functions: estimating the correct degree of smoothing by the methods of generalized cross-validation, *Numerische Mathematika* **31**, 377-403.

Csiszar, I. and Shields, P. C. (2000). The consistency of the BIC Markov order estimator. *Annals of Statistics* **28**, 1601-1619.

Cwik, J. and Koronacki, J. (1997). A combined adaptive-mixtures estimator of multivariate probability densities. *Computational Statistics and Data Analysis* **26**, 199-218.

Dempster, A. P., Laird, N. M., and Rubin, D. B. (1977). Maximum likelihood from incomplete data via the EM algorithm (with discussion). *Journal of the Royal Statistical Society, Series B* **39**, 1-38.

Dennis, B. and Patil, G. P. (1984). The gamma distribution and weighted multimodal gamma distributions as models of population abundance. *Mathematical Biosciences* **68**, 187-212.

Dudoit, S., Fridlyand, J., and Speed, T. P. (2002). Comparison of discrimination methods for the classification of tumors using gene expression data. *Journal of the American Statistical Association* **97**, 77-87.

Dudoit, S., Yang, Y. H., Callow, M. J., and Speed, T. P. (2000). *Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments*. Technical Report 578, Statistics Department, University of California at Berkeley.

Durbin, B. P., Hardin, J. S., Hawkins, D. M., and Rocke, D. M. (2002). A variance-stabilizing transformation for gene-expression microarray data. *Bioinformatics* **18**, S105-S110.

Eisen, M., Spellman, P., Brown, P., and Botstein, D. (1998). Cluster analysis of genome-wide expression patterns. *Proceeding of the National Academy of Sciences* **95**, 14863-14868.

Fan, J. and Li, R. (2001). Variable selection via non-concave penalized likelihood and its oracle properties. *Journal of the Acoustical Society of America* **96**, 1348-1360.

Fan, J. and Li, R. (2002). Variable selection for Cox's proportional hazards model and frailty model. *Annual of Statistics* **30**, 74-99.

Feng, Z. D. and McCulloch, C. E. (1996). Using bootstrap likelihood ratio in finite mixture models. *Journal of the Royal Statistical Society, Series B* **58**, 609-617.

Hansen, B. (1992). The likelihood ratio test under nonstandard conditions: testing the Markov switching model of GNP. *Journal of Applied Econometrics* 7,127-157, *Erratum*, (1996). 11, 195-198.

Hamilton, J. (1996). Specification testing in Markov switching time series models. *Journal of Econometrics* 70, 127-157.

Heckman, J. J., Robb, R. and Walker, J. R. (1990). Testing the mixture of exponentials hypothesis and estimating the mixing distribution by the method of moments. *Journal of American Statistical Association* 85, 582-589.

Hughes, J. P. and Guttorp, P. (1994). A class of stochastic models for relating synoptic atmospheric patterns to regional hydrologic phenomena. *Water Resources Research* 30,1535-1546.

Ideker, T., Thorsson, V., Siegel, A. F., and Hood, L. E. (2000). Testing for differentially-expressed genes by maximum-likelihood analysis of microarray data. *Journal of Computational Biology* 7, 805-817.

James, L. F., Priebe, C. E., and Marchette, D. J. (2001). Consistent estimation of mixture complexity. *Annals of Statistics* 29, 1281-1296.

Jiang, H. Y., Wek, S. A., McGrath, B. C., Scheuner, D., Kaufman, R., Cavener, D. R., and Wek, R. C. (2003). Phosphorylation of the alpha subunit of eukaryotic initiation factor 2 is required for activation of NFkB in response to diverse cellular stresses. *Molecular and Cellular Biology* **23**, 5651-5663.

Kendzioriski, C. M., Newton, M. A., Lan, H., and Gould, M. N. (2003). On parametric empirical Bayes methods for comparing multiple groups using replicated gene expression profiles. *Statistics in Medicine* **22**, 3899-3914.

Kerr, M. K., Martin, M., and Churchill, G. A. (2000). Analysis of variance for gene expression microarray data. *Journal of Computational Biology* **7**, 819-837.

Lee, M. T., Kuo, F. C., Whitmore, G. A., and Sklar, J. (2000). Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridization. *Proceedings of the National Academy of Science* **97**, 9834-9839.

Leroux, B.G. (1992). Consistent estimation of a mixing distribution. *Annals of Statistics* **20**, 1350-1360.

Leroux, B. G. and Puterman, M. L. (1992). Maximum-penalized likelihood estimation for independent and Markov-dependent mixture models. *Biometrics* **48**, 545-558.

Li, C. and Wong, W. H. (2001). Model-based analysis of oligonucleotide arrays: expression

index computation and outlier detection. *Proceedings of the National Academy of Science* **98**, 31-36.

Liang, G., Audas, T. E., Li, Y. Cockram, G. P., Dean, J. D., Martyn, A. C., Kokame, K., and Lu, R. (2006). Luman/CREB3 induces transcription of the endoplasmic reticulum (ER) stress response protein herp through an ER stress response element. *Molecular and Cellular Biology* **26**, 7999-8010.

Lo, K. and Gottardo, K. (2007). Flexible empirical Bayes models for differential gene expression. *Bioinformatics* **23**, 328-335.

Lockhart, D. J., Dong, H. L., Dyne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittman, M., Wang, C. W., Kobayashi, M., Horton, H., and Brown, E. L. (1996). Expression monitoring by hybridization to high density oligonucleotide arrays. *Nature Biotechnology* **14**, 1675-1680.

Luan, Y. and Li, H. (2004). Model-based methods for identifying periodically expressed genes based on time course microarray gene expression data. *Bioinformatics* **20**, 332-339.

MacDonald, I.L. and Zucchini, W. (1997). *Hidden Markov Models and Other Models for Discrete-Valued Time Series*. London: Chapman & Hall.

MacKay, R. J. (2002). Estimating the order of a hidden Markov model. *The Canadian Journal of Statistics* **30**, 573-589.

McLachlan, G., and Peel, D. (2000). *Finite Mixture Models*. New York, Chichester, Weinheim, Brisbane, Singapore, Toronto: John Wiley & Sons, Inc.

Nadon, R. and Shoemaker, J. (2002), Statistical issues with microarrays: processing and analysis. *Trends in Genetics* **18**, 265-271.

Newton, M. A., Kendzioriski, C. M., Richmond, C. S., Blattner, F. R., and Tsui, K. W. (2001). On differential variability of expression ratios: improving statistical inference about gene expression changes from microarray data. *Journal of Computational Biology* **8**, 37-52.

Newton, M. A., Noueiry, A., Sarkar, D., and Ahlquist, P. (2004). Detecting differential gene expression with a semi-parametric hierarchical mixture method. *Biostatistics* **5**, 155-176.

Nguyen, D., Bulak, A., Naisyin, W., and Carroll, R. (2002). DNA microarray experiments: biological and technological aspects. *Biometrics* **58**, 701-717.

Park, T., Yi, S. G., Lee, S., Lee, S. Y., Yoo, D. H., Ahn, J. I., and Lee, Y. S. (2003). Statistical tests for identifying differentially expressed genes in time-course microarray experiments. *Bioinformatics* **19**, 694-703.

Poskitt, D. S. and Zhang, J. (2005). Estimating components in finite mixtures and hidden Markov models. *Australian and New Zealand Journal of Statistics* **47**, 269-286.

Rabiner, L. R. (1989). A Tutorial on Hidden Markov Models and Selected Applications in Speech Recognition. *Proceedings of the IEEE* **77**, 257-286.

Richardson, S. and Green, P. J. (1997). On Bayesian analysis of mixtures with an unknown number of components. *Journal of the Royal Statistical Society, Series B* **59**, 731-792.

Richmond, C. S., Glasner, J. D. Mau, R., Jin, H., and Blattner, F. R. (1997). Genome-wide expression profiling in Escherichia coli K-12. *Nucleic Acids Research* **27**, 3821-3835.

Rocke, D. M. and Durbin, B. (2001). A model for measurement error for gene expression arrays. *Journal of Computational Biology* **8**, 557-570.

Roeder, K. and Wasserman, L. (1997). Practical density estimation using mixtures of normals. *Journal of the American Statistical Association* **92**, 894-902.

Ryden, T. (1995). Estimating the order of hidden Markov models. *Statistics* **26**, 345-354.

Schadt, E. E., Li, C., Su, C., and Wang, W. H. (2000). Analyzing high-density oligonucleotide gene expression array data. *Journal of Cellular Biochemistry* **80**, 192-202.

Schena, M., Shalon, D., Davis, R. W., and Brown, P. O., (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467-470.

Schliep, A., Schonhuth, A., and Steinhoff, C. (2003). Using hidden Markov models to analyze gene expression time course data. *Bioinformatics* **19**, i255-i263.

Schliep, A., Steinhoff, C., and Schonhuth, A. (2004). Robust inference of groups in gene expression time-course using mixtures of HMMs. *Bioinformatics* **20**, i283-i289.

Schliep, A., Costa, I. G., Steinhoff, C., and Schonhuth, A. (2005). Analyzing gene expression time-courses. *IEEE/ACM Transactions on Computational Biology and Bioinformatics* **2**, 179-193.

Snustad, D. P. and Simmons, M. J. (2003), *Principles of Genetics* (3rd edition). John Wiley & Son, Inc.

Solka, J. L., Wegman, E. J., Priebe, C. E., Poston, W. L., and Rogers, W. (1998). Mixture structure analysis using the Akaike criterion and the bootstrap. *Statistics and Computing* **8**, 177-188.

Stone, M. (1974). Cross-validatory choice and assessment of statistical predictions (With discussion). *Journal of the Royal Statistical Society, Series B* **36**, 111-147.

Tai, Y. C. and Speed, T. (2006). A multivariate empirical Bayes statistic for replicated microarray time course data. *Annals of Statistics* **34**, 2387-2412.

Theilhaber, J., Bushnell, S., Jackson, A., and Fuchs, R. (2001). Bayesian estimation of fold-changes in the analysis of gene expression: the pfold algorithm. *Journal of Computational Biology* **8**, 585-614.

Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society, Series B* **58**, 267-288.

Tsodikov, A., Szabo, A., and Jones, D. (2002). Adjustments and tests for differential expression with microarray data. *Bioinformatics* **18**, 251-260.

Yang, Y. H., Buckley, M. J., Dudoit, S., and Speed, T. P. (2000). *Comparison of methods for image analysis on cDNA microarray data*. Technical Report 584, Statistics Department, University of California at Berkeley.

Yuan, M. and Kendziorski, C. (2006). Hidden Markov models for microarray time course data in multiple biological conditions. *Journal of the American Statistical Association* **101**, 1323-1332.

Wang, P. and Puterman, M. L. (1999). Markov Poisson regression models for discrete time series. *Journal of Applied Statistics* **26**, 855-869.

Appendix A. Response Gene Tables.

Table A.1. Response gene set for GDS1428 control group. Total 1644 response genes.

[1]	AA045174	AA114166	AA521267	ABAT	ABCA1	ABCC1
[7]	ABCC3	ABCD4	ABCG1	ABHD2	ABI1	ABR
[13]	ACAA1	ACOT9	ACSL1	ACSL3	ACTN1	ACTR2
[19]	ACTR3	AD7C-NTP	ADA	ADAM10	ADAM17	ADAM9
[25]	ADAMDEC1	ADD3	ADM	ADNP	ADORA2A	AF086790
[31]	AF090895	AF119911	AF164622	AF226044	AF320070	AFTPH
[37]	AGA	AGER	AGPAT7	AGTPBP1	AHCTF1	AHCYL1
[43]	AI140364	AI432196	AI695595	AIF1	AJ275371	AK000185
[49]	AK000834	AK000918	AK022211	AL050043	AL137378	AL157484
[55]	ALG13	ALOX5	ALOX5AP	ALPL	AMPD2	AMPD3
[61]	ANKHD1	ANPEP	ANXA11	ANXA4	ANXA7	AP1G1
[67]	AP1S2	AP2A2	AP2S1	AP3B2	APBB1IP	APLP2
[73]	APOBEC3A	APOL2	AQP9	ARAF	ARCN1	ARF1
[79]	ARFGAP3	ARFIP1	ARFRP1	ARHGAP15	ARHGAP26	ARHGDI
[85]	ARHGDI	ARHGEF16	ARID1A	ARID4B	ARID5A	ARIH1
[91]	ARL1	ARL4A	ARPC3	ARPC4	ASCC3	ASL
[97]	ASNS	ATF1	ATF6	ATG3	ATG4B	ATP11A
[103]	ATP13A3	ATP1B1	ATP2B1	ATP2B4	ATP2C2	ATP6V1A
[109]	ATP6V1C1	ATP8B4	ATPBD1C	ATXN1	ATXN7	AU144792
[115]	AU148274	AU148611	AV700891	AYTL2	AZIN1	B3GNT1
[121]	B4GALT1	BAIAP2	BANP	BASP1	BAT2D1	BAZ1A
[127]	BC000265	BC005884	BCAM	BCAP31	BCAS2	BCAT1
[133]	BCAT2	BCL10	BCL2A1	BCL3	BCLAF1	BCOR
[139]	BEST1	BF114906	BHLHB2	BICD2	BID	BIN2
[145]	BIN3	BIRC2	BLZF1	BMP2K	BMPR2	BNIP2
[151]	BNIP3	BNIP3L	BRAP	BRWD1	BSG	BST1
[157]	BTD	BTG2	BTN3A2	BZW1	C10orf6	C10orf76
[163]	C10orf97	C13orf18	C13orf24	C14orf2	C14orf32	C15orf29
[169]	C16orf68	C16orf72	C17orf75	C19orf2	C19orf22	C19orf56
[175]	C1orf108	C1orf121	C1orf38	C1orf63	C1orf80	C1orf9
[181]	C1RL	C20orf111	C20orf19	C20orf23	C20orf67	C3AR1
[187]	C5AR1	C6orf106	C6orf111	C6orf211	C6orf32	CA12
[193]	CA2	CA4	CABIN1	CABP1	CALM1	CAMKK2
[199]	CAND1	CANT1	CANX	CAPN2	CAPN7	CAPZA2
[205]	CARS2	CASP4	CASP8	CAST	CBFA2T2	CBR4
[211]	CBWD1	CBX1	CBX4	CCDC109B	CCDC69	CCDC76
[217]	CCDC93	CCND3	CCNG2	CCNH	CCNJL	CCNL1
[223]	CCNT2	CCPG1	CCRL2	CCT2	CD164	CD300A

[229]	CD33	CD37	CD44	CD46	CD47	CD53
[235]	CD55	CD58	CD6	CD79B	CD82	CD93
[241]	CD96	CD97	CDA	CDC2L6	CDC42	CDC42EP3
[247]	CDC73	CDK7	CDKN2D	CDV3	CEACAM3	CEACAM4
[253]	CEBPD	CECR5	CENTA2	CENTB1	CENTB2	CENTD2
[259]	CENTD3	CEP170	CEP350	CEP63	CFLAR	CFP
[265]	CHD1	CHERP	CHFR	CHKA	CHMP1B	CHMP2A
[271]	CHMP2B	CHMP5	CHP	CHST11	CHST7	CIRBP
[277]	CITED2	CKAP4	CKS2	CLEC1A	CLEC2D	CLEC4A
[283]	CLEC5A	CLIC1	CLIP1	CLK1	CLTA	CLU
[289]	CMTM6	CNOT2	CNOT3	COL1A1	COL4A3BP	COL9A3
[295]	COPE	COQ2	CORO1C	COX11	COX4I1	CPD
[301]	CPVL	CR1	CREB1	CREM	CRISPLD2	CRK
[307]	CRSP2	CRY1	CSF2RB	CSF3R	CSNK1A1	CSNK1D
[313]	CSNK1G2	CST3	CST7	CTBP2	CTBS	CTDP1
[319]	CTNND1	CTSL1	CTSS	CUEDC1	CUL2	CUL3
[325]	CUTL1	CXCL1	CXCL2	CYC1	CYFIP2	CYLD
[331]	CYP1B1	CYP4F3	DAB2	DBN1	DDIT4	DDX17
[337]	DDX18	DDX19A	DDX3X	DDX5	DECR1	DENND2D
[343]	DERL2	DFNA5	DHCR7	DHRS7	DHX34	DHX40
[349]	DHX8	DICER1	DIMT1L	DKFZP564J102	DLG1	DLGAP4
[355]	DMTF1	DNAJA2	DNAJB1	DNAJB12	DNAJB6	DNAJB9
[361]	DNAJC10	DNAJC3	DNASE1L1	DNM2	DNTTIP2	DOCK2
[367]	DOCK4	DOK3	DOM3Z	DPEP2	DPM1	DUSP1
[373]	DUSP2	DUSP3	DUSP4	DUSP6	DVL3	DYNC1L11
[379]	DYNLT1	DYRK1A	DYRK2	DYSF	E2F3	EAPP
[385]	ECE1	ECGF1	ECOP	ECT2	EFCBP2	EFEMP2
[391]	EFHD2	EGLN1	EGR3	EHD1	EIF1	EIF2AK3
[397]	EIF4A1	EIF4A3	EIF4EBP1	EIF4H	EIF5	ELF1
[403]	ELF4	ELL	ELL2	ELMO3	ELOVL5	EMD
[409]	EML3	EMP1	EMR2	EMR3	EP300	EPB41L3
[415]	EPOR	EPRS	ERBB2IP	ERCC1	EREG	ERGIC2
[421]	ERLIN1	ESR2	ESRRA	ETF1	ETS2	ETV6
[427]	EVI2B	EVI5	EXOC7	EXOSC4	EXTL3	F11R
[433]	F25965	F5	F8A1	FAIM3	FAM129A	FAM12A
[439]	FAM45A	FAM49A	FAM49B	FAM53C	FAM55C	FANCF
[445]	FARSA	FAS	FASTKD3	FBS1	FBXL11	FBXL4
[451]	FBXO11	FBXO34	FBXW7	FCAR	FCER1G	FCGR1A
[457]	FCGR1B	FCGR2A	FCGR2C	FCGR3B	FCHO1	FCN1
[463]	FDFT1	FGFR1OP	FGFR2	FGL2	FGR	FKBP1A
[469]	FKBP5	FKBP8	FLII	FLJ10357	FLJ11151	FLJ11506
[475]	FLJ12529	FLJ13611	FLJ20273	FLJ22662	FLJ23861	FLNA
[481]	FLOT1	FLOT2	FMNL1	FMO5	FMR1	FNBP1
[487]	FNDC3A	FOS	FOSB	FOSL1	FOSL2	FPGT

[493]	FPR1	FRAT1	FRAT2	FRMD4B	FTH1	FTHP1
[499]	FTO	FUCA1	FUS	FUT6	FUT7	FXR1
[505]	FXVD2	FYB	G0S2	G3BP2	GAB2	GABARAP
[511]	GABARAPL1	GABARAPL2	GABARAPL3	GABPB2	GADD45B	GAPDH
[517]	GARNL1	GARNL4	GBP2	GCA	GCH1	GCSH
[523]	GFPT1	GHITM	GIMAP4	GIMAP6	GINS2	GIT2
[529]	GLIPR1	GLRX	GLT8D1	GLUL	GMDS	GMFG
[535]	GMIP	GMPR2	GNAI3	GNAQ	GNAS	GNB1
[541]	GNB2	GNB2L1	GNPDA1	GOLGA8A	GOLGA8B	GP1BB
[547]	GPNMB	GPR109B	GPR15	GPR157	GPR171	GPR65
[553]	GPR77	GRAMD1C	GRB2	GRIN2D	GRK6	GSK3B
[559]	GSPT1	GTF2B	GTF2H2	GTF2I	GTPBP4	GZMM
[565]	H1F0	H2AFY	H2AFZ	H3F3A	H3F3B	HAL
[571]	HAT1	HBA1	HBP1	HBS1L	hCG_2015956	HCLS1
[577]	HEBP2	HECTD3	HEXIM1	HFE	HHEX	HIF1A
[583]	HIPK1	HIST1H1C	HIST1H2AC	HIST1H2BC	HIST1H2BD	HIST1H4J
[589]	HIST2H2AA3	HIVEP1	HIVEP2	HK3	HLA-DPA1	HLA-E
[595]	HMGCS1	HMOX2	HN1	HNRPA3	HNRPC	HNRPD
[601]	HNRPDL	HNRPH2	HNRPH3	HNRPK	HNRPR	HPCAL1
[607]	HPGD	HPS5	HR44	HRBL	HSD17B11	HSD17B4
[613]	HSDL2	HSF1	HSPA4	HSPA5	HSPA6	HSPA9
[619]	IBRDC3	IBSP	ICAM3	ID4	IDH2	IDI1
[625]	IDS	IER2	IER3	IER5	IFI16	IFIT1
[631]	IFIT5	IFITM1	IFITM2	IFITM3	IFNGR2	IFT20
[637]	IGBP1	IGF1R	IGFBP4	IGSF6	IK	IKBKAP
[643]	IKZF1	IL10RA	IL10RB	IL11RA	IL17RA	IL1R1
[649]	IL1R2	IL1RAP	IL1RL2	IL23A	IL32	IL4R
[655]	IL6R	IL8	IL8RA	IL8RB	IL9R	IMPA2
[661]	IMPACT	IMPDH1	ING1	ING3	INPP5A	INSIG1
[667]	IQGAP1	IQGAP2	IQSEC1	IRF1	IRF3	IRS2
[673]	ISCA1	ISG20	ITGA5	ITGAM	ITGAX	ITPK1
[679]	IVNS1ABP	JAK1	JAK2	JARID2	JMJD1A	JMJD2B
[685]	JMJD3	JMJD6	JOSD1	JUN	JUNB	JUND
[691]	KBTBD2	KBTBD4	KCNJ15	KCNK7	KCNQ1	KCTD12
[697]	KCTD13	KIAA0174	KIAA0182	KIAA0232	KIAA0240	KIAA0241
[703]	KIAA0247	KIAA0329	KIAA0372	KIAA0404	KIAA0406	KIAA0409
[709]	KIAA0509	KIAA0513	KIAA0701	KIAA0999	KIAA1026	KIAA1033
[715]	KIAA1279	KIAA1539	KIDINS220	KIFC3	KLC1	KLF10
[721]	KLF2	KLF4	KLF6	KLF9	KLHL2	KPNA1
[727]	KPNA4	KRAS	KRT23	KYNU	L08961	L35253
[733]	LAMB3	LAMP2	LAPTM5	LARP5	LASP1	LCK
[739]	LCN2	LCP1	LDHA	LDLR	LENG4	LGALS3
[745]	LGALS8	LGALS9	LHFPL2	LIF	LILRA1	LILRA2
[751]	LILRA6	LILRB1	LILRB2	LILRB3	LILRB4	LIMD2

[757]	LIMK2	LIN7A	LITAF	LMNB1	LMO2	LOC137886
[763]	LOC151579	LOC283537	LOC440248	LOC440926	LOC552891	LPIN2
[769]	LPPR2	LRMP	LRP10	LRRC17	LRRC6	LRRFIPI
[775]	LSM14A	LST1	LTA4H	LTB4R	LTBR	LY75
[781]	LY96	LYN	LYPD3	MAEA	MAF	MAFF
[787]	MAGOH	MAK	MAN1B1	MANSC1	MAP1LC3B	MAP2K1IP1
[793]	MAP2K3	MAP2K7	MAP3K1	MAP3K3	MAP3K7IP2	MAP3K8
[799]	MAP4K4	MAPBPIP	MAPK1	MAPK14	MAPK6	MAPKAP1
[805]	MAPKAPK2	MARCH6	MARCH7	MARCKS	MARCKSL1	MARK3
[811]	MARS	MAX	MBD4	MBP	MCART1	MCL1
[817]	MED12	MEF2D	MET	METTL3	MGC14376	MGC31957
[823]	MGC4093	MGC5139	MGEA5	MICALL2	MIR16	MKLN1
[829]	MKRN1	MKRN2	MLF1IP	MLF2	MLSTD1	MNDA
[835]	MNT	MOAP1	MOBK1B	MOG	MON1B	MORC3
[841]	MORF4L2	MOSC1	MPP1	MPPE1	MRC1	MRC2
[847]	MRLC2	MRPL13	MRPS14	MRTO4	MS4A6A	MT1X
[853]	MT2A	MTHFS	MTMR14	MTMR6	MTX1	MVP
[859]	MX2	MXD1	MYBPC1	MYBPC3	MYD88	MYH9
[865]	MYLIP	MYLPF	MYO1F	MYST1	MYST4	N-PAC
[871]	N4BP1	NAB1	NACAP1	NADK	NAPA	NARF
[877]	NBEAL2	NBN	NBPF11	NBPF15	NBR1	NCF1
[883]	NCF2	NCF4	NCK1	NCLN	NCOA1	NCOA2
[889]	NCOA4	NDEL1	NDFIP1	NDRG1	NDRG3	NDUFB6
[895]	NEDD9	NEU1	NF1	NFATC3	NFE2	NFE2L2
[901]	NFIL3	NFKB1	NFKB2	NFKBIB	NFKBIE	NFYA
[907]	NFYC	NIPBL	NLRP3	NM_000051	NM_000064	NM_000177
[913]	NM_000201	NM_000269	NM_000311	NM_000321	NM_000389	NM_000416
[919]	NM_000430	NM_000551	NM_000576	NM_000594	NM_000600	NM_000610
[925]	NM_000636	NM_000902	NM_001007245	NM_001008540	NM_001009607	NM_001025076
[931]	NM_001186	NM_001455	NM_001550	NM_001620	NM_001660	NM_001706
[937]	NM_001964	NM_001968	NM_002157	NM_002444	NM_002658	NM_002719
[943]	NM_003054	NM_003059	NM_003150	NM_003244	NM_003254	NM_003370
[949]	NM_003379	NM_003418	NM_003588	NM_003955	NM_004226	NM_004241
[955]	NM_004313	NM_004380	NM_004444	NM_004652	NM_005242	NM_005345
[961]	NM_005428	NM_005445	NM_005543	NM_005565	NM_005746	NM_006292
[967]	NM_006305	NM_006561	NM_006665	NM_006766	NM_007287	NM_007318
[973]	NM_014076	NM_014314	NM_014664	NM_014778	NM_014821	NM_014856
[979]	NM_014863	NM_015208	NM_016119	NM_016384	NM_018468	NM_018579
[985]	NM_020237	NM_020529	NM_021039	NM_021212	NM_022354	NM_022718
[991]	NM_024524	NM_024974	NM_030756	NMI	NOC3L	NOL12
[997]	NOLA2	NOLA3	NOTCH2	NOTCH3	NP	NPM1
[1003]	NPTXR	NR1D2	NR2C2	NR2F2	NR3C1	NR4A2
[1009]	NR4A3	NRBF2	NRGN	NRIP3	NSFL1C	NSMAF

[1015]	NSUN5C	NSUN7	NT5C2	NUAK2	NUCB1	NUMB
[1021]	NUP98	NXF1	NXT1	OAT	OAZ1	ODC1
[1027]	OGT	OLR1	ORC4L	ORC5L	OSBP	OSBPL11
[1033]	OSBPL2	OSBPL8	OSM	OSTM1	OTUB1	OXSRI
[1039]	P2RY13	PADI4	PAFAH1B1	PAICS	PAK1	PAK2
[1045]	PAM	PANX1	PAPOLA	PARP8	PAWR	PBX2
[1051]	PBXIP1	PCAF	PCDHB12	PCMT1	PDE2A	PDE4B
[1057]	PDE4DIP	PDE6D	PDE8B	PDLIM5	PDLIM7	PDSS1
[1063]	PDXK	PECAM1	PELI1	PER1	PEX3	PFKFB3
[1069]	PGK1	PGS1	PHACTR1	PHC2	PHF20L1	PHF21A
[1075]	PHF3	PHKA2	PHLDA1	PI3	PIAS1	PICALM
[1081]	PID1	PIGA	PIGB	PILRA	PIP5K3	PISD
[1087]	PITPNA	PJA2	PKN2	PKP4	PLA2G12A	PLA2G4C
[1093]	PLA2G7	PLAGL1	PLAGL2	PLAU	PLAUR	PLCB2
[1099]	PLCL2	PLEK	PLEKHB2	PLEKHG3	PLEKHM1	PLK3
[1105]	PLP2	PLXNC1	PMAIP1	PNN	POLR2J	POLR3G
[1111]	POR	PPAP2B	PPARD	PPFIA1	PPIE	PPIF
[1117]	PPIG	PPM1A	PPM1B	PPM1F	PPP1CA	PPP1CB
[1123]	PPP1R10	PPP1R13B	PPP1R14B	PPP1R15A	PPP2CA	PPP2R2A
[1129]	PPP3CA	PPP3R1	PPP4C	PPP4R1	PRDM2	PREI3
[1135]	PRKACA	PRKACB	PRKAR1A	PRKCB1	PRKCSH	PRKD2
[1141]	PRKD3	PRKRA	PRLH	PRMT2	PRPF18	PRR13
[1147]	PRR14	PSAP	PSCD4	PSD4	PSMA7	PSMB3
[1153]	PSMB4	PSMB9	PSME3	PSME4	PSMF1	PTAFR
[1159]	PTBP1	PTEN	PTGER4	PTGS2	PTHLH	PTK2B
[1165]	PTP4A1	PTPN12	PTPRC	PTPRE	PTX3	PYCARD
[1171]	QDPR	QKI	QSCN6	RAB11FIP1	RAB11FIP2	RAB1A
[1177]	RAB21	RAB22A	RAB27A	RAB2A	RAB31	RAB3D
[1183]	RAB3GAP1	RAB5A	RAB5C	RAB6IP1	RAB7A	RAB8B
[1189]	RABGEF1	RABL4	RAC1	RAC2	RAD17	RALB
[1195]	RALGDS	RANBP2	RANBP3	RANBP5	RANBP6	RAP2C
[1201]	RAPGEF2	RARA	RASA2	RASGRP2	RASSF2	RASSF3
[1207]	RASSF4	RB1CC1	RBBP6	RBM13	RBM22	RBM26
[1213]	RBM39	RBMS1	RBPJ	RC3H2	RCBTB2	RCN2
[1219]	RCN3	RCOR1	RCOR3	RECQL	REL	RELB
[1225]	REPS2	RFC1	RFC3	RGL2	RGS12	RGS14
[1231]	RHO	RHOF	RHOG	RHOH	RHOQ	RHOT1
[1237]	RIN3	RIOK3	RMND5A	RNASET2	RNF11	RNF141
[1243]	RNF167	RNF170	RNF24	RNGTT	ROCK1	RORA
[1249]	RPA1	RRAGD	RRN3	RRS1	RTN4	RXRA
[1255]	RYBP	S100A11	S100A12	S100A4	S100A8	S100A9
[1261]	S100P	SACS	SAP30BP	SAR1A	SAT1	SAV1
[1267]	SC4MOL	SCARF1	SCML1	SCO2	SDC2	SDCBP
[1273]	SDHC	SEC14L1	SEC22B	SEC31A	SEC61A2	SECTM1

[1279]	SEH1L	SEL1L	SELL	SELPLG	SEMA3C	SENP3
[1285]	SENP6	SEPX1	SERBP1	SERINC1	SERINC3	SERP1
[1291]	SERPINA1	SERPINB1	SERPINB2	SERPINB8	SERTAD2	SETD1B
[1297]	SETD2	SETDB1	SETX	SFPQ	SFRS2	SFRS2IP
[1303]	SFRS3	SFRS5	SFRS6	SFRS7	SGK3	SH2B1
[1309]	SH2B2	SH3BGRL	SH3BGRL3	SH3BP2	SH3BP5	SH3GL1
[1315]	SHOC2	SIGLEC5	SIGLEC9	SIPA1	SIRPB1	SIRT7
[1321]	SKI	SKIL	SLA	SLAMF8	SLC11A1	SLC11A2
[1327]	SLC12A6	SLC12A9	SLC13A4	SLC15A3	SLC16A3	SLC16A6
[1333]	SLC19A1	SLC19A2	SLC1A3	SLC25A13	SLC25A37	SLC25A44
[1339]	SLC29A1	SLC2A14	SLC2A3	SLC31A1	SLC31A2	SLC35A1
[1345]	SLC35A2	SLC4A7	SLC6A6	SLC6A8	SLC7A11	SLC7A5
[1351]	SLC9A1	SLC9A8	SLCO4C1	SLK	SLMO2	SMCHD1
[1357]	SMPDL3B	SNED1	SNIP1	SNN	SNRPD1	SNX10
[1363]	SNX13	SNX17	SNX2	SOAT1	SOD2	SON
[1369]	SORBS3	SORL1	SOS2	SP100	SP110	SP3
[1375]	SPAG9	SPATA2L	SPG21	SPI1	SQSTM1	SRGN
[1381]	SRPK2	SRRM2	SS18	SSBP1	SSFA2	SSR1
[1387]	ST3GAL2	STAG2	STAM2	STAT3	STAT5B	STAT6
[1393]	STC1	STIP1	STK10	STK17A	STK38L	STK4
[1399]	STMN1	STRAP	STX10	STX16	STX3	STX4
[1405]	STX6	STX7	STXBP3	SUB1	SUCLG2	SUMO1
[1411]	SUPT4H1	SUPT6H	SVIL	SYF2	SYK	SYNCRIP
[1417]	SYNJ2	TACC1	TACC3	TAF1A	TAF7	TAGLN2
[1423]	TALDO1	TANK	TAOK3	TAPBP	TBC1D1	TBC1D15
[1429]	TBC1D17	TBC1D22A	TBK1	TBKBP1	TBL1XR1	TBXAS1
[1435]	TCF3	TCP1	TEGT	TESK2	TFCP2	TFDP1
[1441]	TFE3	TFEB	TFEC	TFG	TFRC	TGFBR3
[1447]	TGM2	THAP1	THBD	THBS1	THOC2	THOC5
[1453]	THRAP1	THRAP2	THRAP5	TIA1	TIMM17A	TIPARP
[1459]	TLE3	TLR1	TM2D3	TMED2	TMEM1	TMEM127
[1465]	TMEM140	TMEM176B	TMEM4	TMEM41B	TMEM50A	TMF1
[1471]	TncRNA	TNFAIP2	TNFAIP3	TNFAIP6	TNFRSF10B	TNFRSF10C
[1477]	TNFRSF14	TNFRSF1B	TNFRSF25	TNFRSF9	TNFSF14	TNIP1
[1483]	TNRC5	TNRC6B	TNXB	TOM1	TOP1	TOR1AIP1
[1489]	TOX4	TP53BP2	TP53I11	TPD52L2	TPM4	TPP1
[1495]	TRAF1	TRAF5	TREM1	TREML2	TRIB1	TRIM23
[1501]	TRIM27	TRIM36	TRIM38	TRIM8	TRIOBP	TRIP12
[1507]	TSC22D2	TSEN34	TTC13	TTC19	TTF1	TTF2
[1513]	TUBA1A	TUBA1B	TUBA1C	TUBA3D	TUBA4A	TUBB
[1519]	TUBB2A	TUBB2C	TUBGCP3	TUG1	TWF2	TXN2
[1525]	TXNDC13	TXNRD1	U00956	U34919	UAP1	UBAP1
[1531]	UBAP2L	UBE2B	UBE2D1	UBE2D3	UBE2G1	UBE2H
[1537]	UBE2J1	UBE2L3	UBE2O	UBE3A	UBL3	UBN1

[1543]	UBR2	UBTD1	UBTF	UBXD2	ULK1	UNC119
[1549]	UNC50	UPF1	UPF3A	UQCRC2	UROS	USH2A
[1555]	USP10	USP15	USP3	USP32	USP33	USP36
[1561]	USP8	UTX	VAMP2	VAMP3	VCL	VCPIP1
[1567]	VDR	VEGFA	VIL2	VIM	VPS13C	VPS24
[1573]	VPS26A	VPS37B	VRK1	W88821	WAC	WDR1
[1579]	WDR26	WDR47	WDR8	WIPF1	WTAP	XBP1
[1585]	XM_094581	XM_370635	XM_374529	XM_378250	XPO6	XPO7
[1591]	XR_000228	XRCC4	XRCC5	YIPF3	YIPF4	YKT6
[1597]	YPEL5	YTHDC2	YTHDF3	YWHAB	YWHAE	YWHAZ
[1603]	ZBTB1	ZBTB43	ZC3H11A	ZC3H12A	ZCCHC6	ZDHHHC18
[1609]	ZEB1	ZFAND3	ZFAND6	ZFP36	ZFP36L2	ZFX
[1615]	ZFYVE26	ZHX2	ZMIZ1	ZMYM1	ZMYM2	ZNF12
[1621]	ZNF148	ZNF165	ZNF180	ZNF224	ZNF227	ZNF24
[1627]	ZNF250	ZNF267	ZNF292	ZNF350	ZNF394	ZNF467
[1633]	ZNF508	ZNF518	ZNF573	ZNF588	ZNF668	ZNF7
[1639]	ZNF710	ZNF750	ZNF804A	ZUBR1	ZYX	ZZEF1

Table A.2 Response gene set for GDS1428 treatment group. Total 1902 response genes.

[1] 3.8-1	AA017721	AA203487	AA355179	AA365670	AA521267
[7] ABAT	ABCA1	ABCB9	ABCC1	ABCC3	ABCD1
[13] ABCF2	ABCG1	ABHD14B	ABHD2	ABHD3	ABHD5
[19] ABI1	ACACB	ACBD3	ACIN1	ACSL1	ACSL3
[25] ACSL5	ACTN1	ACTR1A	ACTR3	ADA	ADAM10
[31] ADAM19	ADAM22	ADAM9	ADAMDEC1	ADAMTS8	ADD1
[37] ADH1B	ADMR	ADORA2A	ADRB2	ADRBK1	AF103530
[43] AF291676	AF320070	AGK	AGPAT7	AGTPBP1	AI278204
[49] AI472320	AI523613	AI683552	AI695595	AIF1	AK025360
[55] AK026682	AK1	AK3L1	AK3L2	AKAP8L	AL044078
[61] AL049435	AL109696	AL137624	AL390145	ALG13	ALLC
[67] ALOX5	ALOX5AP	ALPL	AMD1	AMPD2	AMPD3
[73] AMPH	ANGPT1	ANKHD1	ANKRD15	ANP32E	ANPEP
[79] ANXA11	ANXA4	ANXA5	ANXA7	AOAH	AOC2
[85] AP1G1	AP1S1	AP3M2	APBA3	APC	APLP2
[91] APOBEC3A	APOBEC3B	APPBP2	AQP3	ARCNI	ARF6
[97] ARFGEF1	ARFGEF2	ARFIP1	ARFRP1	ARHGAP1	ARHGAP15
[103] ARHGAP19	ARHGAP26	ARHGEF18	ARHGEF3	ARHGEF4	ARID4B
[109] ARID5A	ARIH1	ARL3	ARL6IP2	ARL8B	ARMC9
[115] ARPC2	ARPC3	ARPP-19	ARS2	ARTS-1	ASPH
[121] ASXL1	ATBF1	ATF1	ATG7	ATHL1	ATOX1
[127] ATP13A3	ATP2A3	ATP2B1	ATP2B4	ATP2C2	ATP5F1
[133] ATP6V1F	ATP8B4	ATPBD1C	ATXN1	ATXN3	ATXN3L
[139] AU144792	AU147851	AUH	AW301235	AW836210	AYTL2
[145] AZGP1	AZIN1	B4GALT1	B4GALT2	B4GALT4	BAG2
[151] BAT1	BAT2	BAT2D1	BAZ1A	BAZ2B	BBS4
[157] BC002791	BC003528	BC006164	BCAS2	BCAT1	BCL2A1
[163] BCL3	BCLAF1	BE327172	BF114906	BID	BIN2
[169] BIN3	BIRC2	BLVRB	BLZF1	BMP2	BMP6
[175] BMX	BNIP3	BRCA1	BRCC3	BRD8	BTG2
[181] BTG3	BTN3A1	BZRAP1	BZW1	C10orf95	C11orf30
[187] C12orf5	C13orf15	C13orf18	C13orf24	C14orf159	C14orf32
[193] C15orf29	C15orf39	C16orf68	C16orf72	C16orf80	C17orf68
[199] C19orf10	C19orf22	C1D	C1orf107	C1orf183	C1orf38
[205] C1orf63	C1QTNF1	C1R	C1RL	C20orf121	C20orf32
[211] C20orf67	C21orf33	C2orf25	C3AR1	C4BPB	C4orf16
[217] C4orf20	C5AR1	C5orf15	C6orf106	C6orf108	C6orf111
[223] C6orf211	C6orf32	C6orf62	C7orf42	C7orf44	C9orf46
[229] CA2	CA4	CABIN1	CACNA1I	CACNA2D3	CADM3
[235] CALU	CAMK2G	CAMKK2	CAMTA2	CAND2	CANT1
[241] CANX	CAP1	CAPG	CAPN7	CAPRIN1	CARS2
[247] CASP8	CAST	CAT	CBFA2T3	CBWD1	CBX1

[253]	CBX4	CCDC49	CCDC76	CCDC93	CCL18	CCL19
[259]	CCL2	CCL3	CCL4	CCNA1	CCND3	CCNH
[265]	CCNL1	CCNT2	CCPG1	CCRL2	CCT6A	CD22
[271]	CD226	CD2BP2	CD302	CD37	CD44	CD46
[277]	CD48	CD55	CD58	CD59	CD68	CD69
[283]	CD82	CD93	CD97	CDA	CDC2L2	CDC2L6
[289]	CDC42	CDC42EP2	CDC42EP3	CDKN2D	CDV3	CEBPD
[295]	CENTB2	CENTD2	CENTD3	CEP164	CEP170	CEP63
[301]	CETN2	CFLAR	CFP	CHD1	CHD4	CHD9
[307]	CHI3L1	CHIC2	CHMP1B	CHMP2A	CHMP2B	CHMP5
[313]	CHRNA10	CHST11	CHST7	CHST8	CIB1	CIR
[319]	CIRBP	CITED2	CIZ1	CKAP4	CLASP2	CLCN3
[325]	CLCN4	CLCN6	CLCN7	CLDND1	CLEC1B	CLEC5A
[331]	CLIC1	CLIC4	CLINT1	CLIP1	CLK1	CLK2
[337]	CLN5	CLU	CNN2	CNOT2	CNOT3	CNOT8
[343]	COL13A1	COL1A1	COL6A3	COMMD4	COPE	COX5B
[349]	CPD	CPM	CPVL	CR1	CREB1	CREB5
[355]	CREBL2	CREG1	CREM	CRHR1	CRIP2	CRIP2
[361]	CRISPLD2	CRK	CRKL	CROP	CRSP2	CRY1
[367]	CSAD	CSF3R	CSNK1A1	CSNK1D	CSNK2A1	CST3
[373]	CSTB	CTBS	CTGLF1	CTSB	CTSE	CTSL1
[379]	CTSL2	CTSS	CTSW	CUGBP1	CUTL1	CXCL5
[385]	CXXC1	CYB5R4	CYFIP2	CYHR1	CYLD	CYP17A1
[391]	CYP19A1	CYP4F2	CYP4F3	D85181	DAPP1	DCTN3
[397]	DDIT3	DDIT4	DDX18	DDX21	DDX23	DDX39
[403]	DDX5	DDX52	DENND1A	DENND2D	DENND3	DERL2
[409]	DFNA5	DHCR7	DHRS12	DHRS7	DHRS9	DHTKD1
[415]	DHX34	DHX8	DIAPH2	DICER1	DIS3	DLAT
[421]	DLC1	DLG3	DMC1	DMXL1	DMXL2	DNAJA1
[427]	DNAJA2	DNAJB12	DNAJB14	DNAJB6	DNAL4	DNASE1L1
[433]	DNPEP	DNTTIP2	DOCK4	DOK3	DPEP3	DPM2
[439]	DRAM	DSCR1L1	DSCR3	DSE	DTX2	DUSP1
[445]	DUSP10	DUSP2	DUSP3	DUSP6	DYNLT3	DYSF
[451]	E2F3	E2F8	EBI3	ECE1	ECGF1	EDD1
[457]	EDEM3	EDG4	EFHC2	EFHD2	EGLN1	EGR3
[463]	EHD1	EIF1	EIF1B	EIF2AK3	EIF2S1	EIF2S2
[469]	EIF3S1	EIF3S10	EIF4A1	EIF4A3	EIF4G3	EIF5
[475]	EIF5A	ELF1	ELF2	ELF3	ELF4	ELK3
[481]	ELL	ELL2	ELL3	ELN	ELOVL5	EMD
[487]	ENC1	ENO3	ENOPH1	ENPP4	ENSA	EP300
[493]	EPB41L3	EPB41L5	EPM2AIP1	EPOR	EPS15L1	ERBB2IP
[499]	EREG	ERLIN1	ESF1	ESR2	ESRRA	ETF1
[505]	ETNK1	ETS1	ETS2	EVA1	EVI2A	EWSR1
[511]	EXOSC4	EXTL3	F5	F8A1	FABP5	FAM120A

[517]	FAM128B	FAM129A	FAM3C	FAM49A	FAM49B	FAM63A
[523]	FAM65A	FAS	FBXL15	FBXL5	FBXO11	FBXO31
[529]	FBXO34	FBXO42	FBXW7	FCAR	FCGR2A	FCGR3B
[535]	FCGRT	FCHSD2	FCN1	FDPS	FEM1B	FGFR1
[541]	FGFR1OP	FGL2	FGR	FIS1	FKBP15	FKBP1A
[547]	FKBP8	FKSG30	FLCN	FLI1	FLJ10154	FLJ10213
[553]	FLJ10357	FLJ11151	FLJ11506	FLJ12529	FLJ12716	FLJ12886
[559]	FLJ14213	FLJ20186	FLJ20254	FLJ20273	FLJ20433	FLJ21908
[565]	FLJ22662	FLJ23861	FLOT1	FMR1	FNBP4	FNDC3A
[571]	FNDC3B	FOS	FOSB	FOSL1	FOSL2	FOXJ3
[577]	FOXK2	FPR1	FRAT1	FRAT2	FRMD4B	FSHB
[583]	FSTL3	FTH1	FUT4	FUT7	FXYD5	FYB
[589]	FYN	G0S2	G3BP2	GABARAPL1	GABARAPL3	GABPB2
[595]	GAD1	GADD45B	GAL	GALC	GALNACT-2	GALNT2
[601]	GALNT3	GALNT7	GAPDH	GAPVD1	GARNL1	GARNL4
[607]	GARS	GAS7	GATAD1	GCA	GCH1	GCLM
[613]	GDPD3	GFPT1	GGA3	GGT1	GGT3	GHITM
[619]	GIMAP4	GIMAP6	GIN51	GIT2	GK	GLA
[625]	GLIPR1	GLUL	GMFB	GMFG	GNAI3	GNAQ
[631]	GNB1	GNB2	GNB5	GNL1	GNPAT	GNPDA1
[637]	GNS	GOLGA5	GOLGA8B	GPLD1	GPR171	GPR175
[643]	GPR176	GPR177	GPR18	GPR65	GPR77	GREM1
[649]	GRINA	GRK5	GRK6	GRPEL1	GSH1	GSPT1
[655]	GSR	GTF2H1	GTF2H2	GTPBP1	GTPBP4	GUCA1B
[661]	GUK1	GYG1	GZMA	H2AFY	H2AFZ	H3F3B
[667]	HAL	HARS2	HAT1	HBEGF	HBXIP	HCFC2
[673]	HCK	HCLS1	HDAC2	HDLBP	HERC4	HERPUD1
[679]	HEXA	HHEX	HIF1A	HIP1	HIST1H1C	HIST1H2AC
[685]	HIST1H2AJ	HIST1H2BC	HIST1H3G	HIST1H3H	HIST2H2AA3	HIST2H2BE
[691]	HIVEP1	HIVEP2	HK3	HLA-E	HLA-F	HLA-G
[697]	HMG20B	HMHA1	HNF4A	HNRPA3	HNRPC	HNRPD
[703]	HNRPD	HNRPH3	HNRPM	HNRPU	HOXA1	HP1BP3
[709]	HPCAL1	HPN	HPS5	HRH1	HRK	HS2ST1
[715]	HSD17B14	HSD17B4	HSDL2	HSMPP8	HSP90AA1	HSP90AB1
[721]	HSPA4	HSPA6	HSPA9	HTR2A	HTR2B	HTR6
[727]	HTRA2	IARS2	IBRDC3	ICAM4	ICK	IDH3G
[733]	IDI1	IDS	IER2	IER5	IFI16	IFIH1
[739]	IFIT2	IFITM1	IFITM2	IFITM3	IFNAR1	IFNG
[745]	IFNGR1	IFNGR2	IFT57	IGF1R	IGFBP5	IGFBP7
[751]	IL10RB	IL11RA	IL12B	IL18RAP	IL1A	IL1F9
[757]	IL1R1	IL1R2	IL1RAP	IL23A	IL24	IL4R
[763]	IL5RA	IL6R	IL6ST	IL8RA	IL8RB	ILF3
[769]	ING1	INHBA	INHBC	INPP4A	INPP5A	INSIG1
[775]	INTS3	INTS8	IPO4	IPO7	IQGAP1	IQGAP2

[781]	IQSEC1	IRAK1	IRAK3	IRF5	IRGC	IRS2
[787]	ISCA1	ISG20	ISG20L2	ISGF3G	ITCH	ITGA2B
[793]	ITGA5	ITGA6	ITGAX	ITGB3	ITGB8	ITM2B
[799]	ITPK1	ITPR1	IVNS1ABP	JAG1	JAKMIP2	JARID1B
[805]	JARID2	JMJD2B	JMJD3	JMJD6	JOSD1	JUN
[811]	JUNB	JUND	KCNAB3	KCNE1	KCNJ15	KCNJ5
[817]	KCNK1	KCNK7	KCNMB1	KCNQ1	KCTD13	KCTD2
[823]	KCTD20	KDELR3	KEAP1	KHDRBS1	KIAA0143	KIAA0174
[829]	KIAA0241	KIAA0247	KIAA0286	KIAA0329	KIAA0404	KIAA0467
[835]	KIAA0692	KIAA0701	KIAA0746	KIAA0892	KIAA0913	KIAA0922
[841]	KIAA0984	KIAA0999	KIAA1026	KIAA1033	KIAA1324	KIAA1539
[847]	KIAA1655	KIAA1815	KIAA1840	KIDINS220	KIF1B	KLC1
[853]	KLF2	KLF4	KLF6	KLHL2	KLHL21	KLHL24
[859]	KPNA1	KPNA2	KPNA4	KPTN	KRCC1	KRT32
[865]	KRT6B	Kua-UEV	KYNU	L35253	LAMA2	LAMA4
[871]	LAMB3	LAPTM5	LARP5	LCK	LDLR	LGALS3
[877]	LGALS8	LIF	LILRA1	LILRA5	LILRB2	LILRB3
[883]	LIMK2	LIMS1	LMNB1	LMO2	LOC130074	LOC137886
[889]	LOC151579	LOC152719	LOC339457	LOC388335	LOC388458	LOC439992
[895]	LOC440354	LOC51136	LOC54103	LOC552891	LPPR2	LRCH4
[901]	LRMP	LRRC8D	LRRFIP1	LSM5	LST1	LY6G6D
[907]	LY75	LY96	LYPD3	LYRM4	M6PR	M6PRBP1
[913]	MACF1	MAEA	MAFF	MAFG	MAG	MAGEA8
[919]	MAGEB2	MAGOH	MAK	MAN1A1	MAN2B2	MANSC1
[925]	MAP2K3	MAP3K3	MAP3K4	MAP3K5	MAP3K7IP2	MAP4K4
[931]	MAPK1	MAPK13	MAPK14	MAPK6	MAPK8IP2	MAPKAPK2
[937]	MARCH2	MARCH6	MARCH7	MARCKS	MARK2	MARK3
[943]	MARS	MAST2	MATR3	MBP	MBTD1	MCAM
[949]	MCAT	MCFD2	MCL1	MCM4	MCOLN1	MCTP1
[955]	MCTP2	MDM2	MECP2	MEF2A	MEF2D	METTL3
[961]	MGAM	MGC13098	MGC14376	MGLL	MICA	MIER2
[967]	MKKS	MLF1IP	MLSTD1	MLX	MMD	MMP1
[973]	MMP14	MMS19L	MOBK1B	MORC3	MORF4L2	MOXD1
[979]	MPPE1	MPZL1	MRPL12	MRPL34	MS4A6A	MSC
[985]	MSRA	MSRB2	MT1E	MT1JP	MT1X	MT3
[991]	MTA1	MTERFD1	MTF1	MTHFS	MTM1	MTMR10
[997]	MTMR2	MTMR6	MVK	MVP	MX2	MXD1
[1003]	MXD3	MXI1	MYCBP	MYD88	MYH7	MYH9
[1009]	MYO9B	N4BP1	NAB1	NACAP1	NAGK	NANS
[1015]	NAPA	NARF	NBEAL2	NBN	NBR1	NCF4
[1021]	NCK1	NCLN	NCOA1	NCOR2	NCR2	NDEL1
[1027]	NDFIP1	NDRG1	NDUFB6	NDUFB8	NDUFV2	NEDD4L
[1033]	NEDD9	NENF	NF1	NFATC3	NFE2	NFE2L1
[1039]	NFE2L2	NFIL3	NFKB1	NFKB2	NFKBIE	NFYA

[1045]	NGLY1	NHP2L1	NINJ1	NIPBL	NISCH	NLRP3
[1051]	NM_000064	NM_000110	NM_000125	NM_000129	NM_000201	NM_000311
[1057]	NM_000321	NM_000358	NM_000389	NM_000553	NM_000610	NM_000636
[1063]	NM_000885	NM_000902	NM_001007245	NM_001012478	NM_001250	NM_001455
[1069]	NM_001550	NM_001622	NM_001660	NM_001787	NM_001964	NM_001968
[1075]	NM_001993	NM_002015	NM_002185	NM_002228	NM_002562	NM_002658
[1081]	NM_002746	NM_003017	NM_003059	NM_003072	NM_003103	NM_003137
[1087]	NM_003150	NM_003244	NM_003254	NM_003266	NM_003370	NM_003379
[1093]	NM_003605	NM_003810	NM_003885	NM_004226	NM_004233	NM_004241
[1099]	NM_004313	NM_004380	NM_004652	NM_004682	NM_004846	NM_005242
[1105]	NM_005259	NM_005428	NM_005445	NM_005565	NM_005746	NM_005821
[1111]	NM_005965	NM_006021	NM_006292	NM_006305	NM_006561	NM_006665
[1117]	NM_006766	NM_007287	NM_007318	NM_007319	NM_012115	NM_013421
[1123]	NM_014076	NM_014129	NM_014242	NM_014314	NM_014645	NM_014664
[1129]	NM_014796	NM_014856	NM_015208	NM_015372	NM_015987	NM_016119
[1135]	NM_016415	NM_017795	NM_017874	NM_017920	NM_018468	NM_018605
[1141]	NM_019061	NM_020037	NM_020149	NM_020213	NM_020415	NM_020661
[1147]	NM_021730	NM_021941	NM_022837	NM_024524	NM_024614	NM_024716
[1153]	NM_024777	NM_024853	NM_024984	NM_030756	NM_030897	NM_033111
[1159]	NM_145237	NM_152516	NME5	NMI	NOC3L	NOL1
[1165]	NOL14	NONO	NOTCH2	NOTCH2NL	NOV	NP
[1171]	NPC1	NPHP4	NPM1	NQO2	NR1H2	NR3C1
[1177]	NR4A2	NR4A3	NR6A1	NRAS	NRBF2	NRGN
[1183]	NRIP3	NSFL1C	NSMAF	NSUN5C	NT5C2	NUAK2
[1189]	NUFIP1	NUMB	NUP210	NUP62	NUP98	NXF1
[1195]	NXT1	OAT	OAZ1	OCLM	ODC1	ODZ2
[1201]	OGT	OLFM4	OLR1	ORM1	OSBPL1A	OSBPL2
[1207]	OSBPL8	OSGEP	OSTM1	OXR1	P2RX4	P2RY13
[1213]	PADI4	PAFAH1B1	PAK1	PAK2	PAM	PAPOLA
[1219]	PARP8	PATZ1	PAX8	PAXIP1	PBX2	PCAF
[1225]	PCF11	PCMT1	PCOLCE2	PCTK2	PCYT1A	PDCD4
[1231]	PDE4B	PDE4DIP	PDE6B	PDE6D	PDE8B	PDK3
[1237]	PDLIM1	PDLIM2	PDLIM3	PDLIM5	PDLIM7	PDPK1
[1243]	PDXK	PER1	PER2	PF4	PFAAP5	PFKFB3
[1249]	PGCP	PGD	PGK1	PGLS	PHACTR1	PHACTR2
[1255]	PHC1	PHC2	PHF20	PHF20L1	PHF3	PHKA2
[1261]	PHLDA1	PHLPP	PI3	PIAS1	PICALM	PID1
[1267]	PIGA	PIGB	PIGV	PIK3CD	PIK3CG	PILRA
[1273]	PIP5K2A	PIR	PITPNA	PKIG	PKM2	PKN2
[1279]	PLA1A	PLA2G4C	PLA2G6	PLA2G7	PLAGL2	PLAU
[1285]	PLAUR	PLCB2	PLCL2	PLD1	PLEC1	PLEK
[1291]	PLEKHB2	PLEKHF2	PLEKHG3	PLEKHM1	PLK2	PLK3
[1297]	PLOD1	PLSCR4	PMAIP1	PMF1	PMVK	PNN

[1303]	POLG2	POLM	POLR3E	POMZP3	POPDC2	POU6F2
[1309]	PPAP2B	PPBP	PPFIA1	PPIAL4	PPIF	PPP1CB
[1315]	PPP1R10	PPP1R12A	PPP1R14B	PPP1R15A	PPP2CB	PPP2R3C
[1321]	PPP3CA	PPP4R1	PPP4R2	PQBP1	PRDM14	PRDM2
[1327]	PREI3	PREPL	PRKAA1	PRKAR1A	PRKAR2B	PRKCB1
[1333]	PRKCH	PRKCI	PRKD2	PRKD3	PRKDC	PRPF18
[1339]	PRPF38B	PRPF4	PRPF6	PRPSAP1	PRR13	PRR7
[1345]	PRSS3	PSD4	PSMA3	PSMA6	PSMB4	PSMC5
[1351]	PSMD12	PSMD13	PSMD3	PSMD4	PSMF1	PSORS1C2
[1357]	PTAFR	PTEN	PTGS2	PTP4A1	PTPN12	PTPN18
[1363]	PTPRE	PTPRN	PTRH2	PTS	PTTG1IP	PTX3
[1369]	PUM2	PYCR1	QKI	QSCN6	RAB11A	RAB13
[1375]	RAB1A	RAB21	RAB27A	RAB31	RAB3D	RAB5C
[1381]	RAB6IP1	RAB8B	RABGAP1L	RABGEF1	RABGGTA	RABL2A
[1387]	RAC2	RAD21	RAD23B	RAD51	RAGE	RALB
[1393]	RALGDS	RANBP2	RANBP3	RANBP5	RAPGEF2	RARA
[1399]	RASGRF1	RASGRP2	RB1CC1	RBM16	RBM22	RBM26
[1405]	RBM28	RBM38	RBM5	RBM7	RBM9	RBMS1
[1411]	RBPJ	RC3H2	RCBTB2	RCN2	RCN3	RCOR1
[1417]	REC8	REL	REPS2	RERE	RFK	RGL1
[1423]	RGL2	RGPD5	RGS12	RGS14	RGS7	RHOF
[1429]	RHOQ	RHOT1	RIF1	RIN2	RIOK3	RIPK1
[1435]	RLN2	RNASET2	RNF10	RNF111	RNF138	RNF141
[1441]	RNF167	RNF170	RNGTT	RNPEP	RP4-724E16.2	RPGRIP1
[1447]	RPL10	RPS6KA5	RPS6KB2	RPS9	RRM1	RRN3
[1453]	RRP12	RSRC2	RTN2	RTN4	RXRA	RXRG
[1459]	RYBP	S100A11	S100A12	S100A6	S100A9	S100P
[1465]	SAFB	SAMD9	SAMSN1	SAP18	SAP30	SAP30BP
[1471]	SAP30L	SAR1A	SAT1	SBNO2	SCARA3	SCARF1
[1477]	SCN1B	SCO2	SCRN3	SDC2	SDCCAG3	SDHD
[1483]	SEC22B	SEC23B	SEC23IP	SECISBP2	SECTM1	SEL1L
[1489]	SELL	SELPLG	SEMA6B	SEN3P	SEN5P	SEN6P
[1495]	SEP15	SEPX1	SERBP1	SERINC3	SERP1	SERPINB1
[1501]	SERPINB2	SERPINB7	SERPINB8	SERPINB9	SERPINI2	SERTAD2
[1507]	SETX	SF1	SF3B4	SFN	SFPQ	SFRS15
[1513]	SFRS2	SFRS2IP	SFRS3	SFRS5	SFRS7	SFT2D2
[1519]	SGK3	SH2B2	SH2D3A	SH2D3C	SH3BP5	SH3GLB1
[1525]	SHCBP1	SIAH1	SIGLEC7	SIRPA	SIRPG	SIRT7
[1531]	SKAP2	SKI	SKIL	SLA	SLAMF7	SLBP
[1537]	SLC11A1	SLC11A2	SLC12A9	SLC14A2	SLC15A3	SLC19A1
[1543]	SLC19A2	SLC1A2	SLC1A3	SLC24A1	SLC25A13	SLC25A32
[1549]	SLC25A37	SLC25A44	SLC26A2	SLC2A6	SLC31A2	SLC35A2
[1555]	SLC35E3	SLC39A6	SLC39A8	SLC3A2	SLC43A3	SLC4A2
[1561]	SLC5A3	SLC6A6	SLC7A11	SLC7A5	SLC7A7	SLC9A8

[1567]	SLCO4A1	SLCO4C1	SLK	SLMO2	SLPI	SMARCD3
[1573]	SMARCE1	SMCHD1	SMG7	SMPD2	SNAP23	SNIP1
[1579]	SNN	SNRPA1	SNRPB	SNTB2	SNX1	SNX10
[1585]	SNX17	SNX2	SNX3	SOAT1	SON	SOS2
[1591]	SP100	SP110	SPAG1	SPAG5	SPAG7	SPAG9
[1597]	SPATA2L	SPATA5L1	SPATA6	SPCS1	SPG20	SPHK1
[1603]	SPI1	SPINLW1	SPINT1	SPINT2	SPOCK2	SPTB
[1609]	SPTBN1	SPTLC1	SQSTM1	SRC	SRF	SRGAP3
[1615]	SRGN	SRP72	SRPK2	SS18	SSFA2	SSR1
[1621]	SSSCA1	ST13	ST3GAL1	ST3GAL2	ST8SIA4	STAG1
[1627]	STAG2	STAM2	STARD8	STAT3	STAT5B	STATH
[1633]	STK10	STMN1	STRAP	STS	STX16	STX3
[1639]	STX4	STX7	STXBP2	SUB1	SUCLG1	SUMO1
[1645]	SUPT6H	SUPV3L1	SVIL	SYCP2	SYNCRIP	SYNE2
[1651]	SYNJ1	SYNJ2	TACC3	TAF1C	TAF7	TAF9
[1657]	TAGLN2	TAL1	TANK	TAPBPL	TARDBP	TBC1D1
[1663]	TBC1D2	TBC1D22A	TBC1D2B	TBC1D3	TBK1	TBXAS1
[1669]	TCFL5	TDG	TECT1	TES	TFDP1	TFEC
[1675]	TFR2	TGFB3	TGIF2	THBD	THBS1	THOC5
[1681]	THRAP1	THRAP2	THRAP5	TIA1	TICAM1	TIMM17A
[1687]	TIPARP	TKT	TLE3	TLN1	TLR1	TLR5
[1693]	TM2D3	TM9SF2	TM9SF3	TMCO1	TMCO3	TMED10
[1699]	TMED2	TMED7	TMEM110	TMEM165	TMEM24	TMEM33
[1705]	TMEM4	TMEM41B	TMEM53	TMEM8	TMEPAI	TNFAIP3
[1711]	TNFAIP6	TNFRSF10C	TNFRSF1A	TNFRSF1B	TNFRSF8	TNFRSF9
[1717]	TNFSF14	TNIP1	TNNI2	TNRC5	TOB1	TOM1
[1723]	TOMM20	TOP1	TOPORS	TOR1A	TOR1AIP1	TOR1B
[1729]	TPD52L2	TPM4	TPP1	TPR	TRA@	TRA2A
[1735]	TRAC	TRADD	TRAF1	TRAF3	TRAF3IP2	TRAF3IP3
[1741]	TRAPPC3	TREM1	TREML2	TRIB1	TRIB3	TRIM13
[1747]	TRIM16	TRIM27	TRIM34	TRIM36	TRIP10	TRIP12
[1753]	TRPC2	TSC22D2	TSEN34	TSNAX	TSPAN2	TSPAN3
[1759]	TTC1	TTC19	TTC35	TTF1	TTN	TUBA1A
[1765]	TUBA1B	TUBA1C	TUBA3D	TUBA4A	TXNDC13	TXNRD1
[1771]	U34919	U56725	UAP1	UBAP1	UBAP2L	UBE2B
[1777]	UBE2D1	UBE2D3	UBE2E1	UBE2G1	UBE2H	UBE2J1
[1783]	UBE2S	UBE4B	UBL3	UBN1	UCHL5	UCKL1
[1789]	UCP2	UGP2	UIMC1	UPB1	UPF3A	UPP1
[1795]	UQCRC2	USP10	USP15	USP33	USP4	UTP18
[1801]	UTP6	VAC14	VAMP2	VCAN	VCL	VDP
[1807]	VDR	VEGFA	VIL2	VILL	VIM	VNN2
[1813]	VPS24	VPS37B	VPS41	W88821	WDR1	WDR26
[1819]	WDR42A	WDR47	WDR74	WHDC1	WIPF1	WSB1
[1825]	WSB2	WTAP	WWC1	XM_094581	XM_208778	XM_370838

[1831]	XM_372632	XM_374529	XM_378250	XM_496132	XM_496217	XM_497663
[1837]	XM_498825	XM_498877	XM_499165	XPO1	XPO6	YAF2
[1843]	YIPF4	YIPF6	YPEL5	YRDC	YTHDC1	YWHAZ
[1849]	YY1	ZBED1	ZBTB1	ZBTB17	ZBTB3	ZBTB43
[1855]	ZC3H12A	ZC3H7A	ZCCHC10	ZCCHC14	ZCCHC6	ZDHHC17
[1861]	ZEB1	ZEB2	ZFAND5	ZFP36	ZFP36L2	ZFX
[1867]	ZFYVE26	ZH2C2	ZHX2	ZMAT3	ZMYM1	ZMYND10
[1873]	ZNF124	ZNF155	ZNF165	ZNF177	ZNF202	ZNF221
[1879]	ZNF225	ZNF238	ZNF24	ZNF250	ZNF254	ZNF259
[1885]	ZNF267	ZNF277P	ZNF331	ZNF350	ZNF394	ZNF500
[1891]	ZNF508	ZNF552	ZNF586	ZNF589	ZNF652	ZNF668
[1897]	ZNF675	ZNF692	ZNF783	ZNF84	ZYX	ZZEF1

Table A.3 The common response gene set for GDS1428 treatment group and control group. Total 894 genes.

[1]	AA521267	ABAT	ABCA1	ABCC1	ABCC3
[6]	ABCG1	ABHD2	ABI1	ACSL1	ACSL3
[11]	ACTN1	ACTR3	ADA	ADAM10	ADAM9
[16]	ADAMDEC1	ADORA2A	AF320070	AGPAT7	AGTPBP1
[21]	AI695595	AIF1	ALG13	ALOX5	ALOX5AP
[26]	ALPL	AMPD2	AMPD3	ANKHD1	ANPEP
[31]	ANXA11	ANXA4	ANXA7	AP1G1	APLP2
[36]	APOBEC3A	ARCN1	ARFIP1	ARFRP1	ARHGAP15
[41]	ARHGAP26	ARID4B	ARID5A	ARIH1	ARPC3
[46]	ATF1	ATP13A3	ATP2B1	ATP2B4	ATP2C2
[51]	ATP8B4	ATPBD1C	ATXN1	AU144792	AYTL2
[56]	AZIN1	B4GALT1	BAT2D1	BAZ1A	BCAS2
[61]	BCAT1	BCL2A1	BCL3	BCLAF1	BF114906
[66]	BID	BIN2	BIN3	BIRC2	BLZF1
[71]	BNIP3	BTG2	BZW1	C13orf18	C13orf24
[76]	C14orf32	C15orf29	C16orf68	C16orf72	C19orf22
[81]	C1orf38	C1orf63	C1RL	C20orf67	C3AR1
[86]	C5AR1	C6orf106	C6orf111	C6orf211	C6orf32
[91]	CA2	CA4	CABIN1	CAMKK2	CANT1
[96]	CANX	CAPN7	CARS2	CASP8	CAST
[101]	CBWD1	CBX1	CBX4	CCDC76	CCDC93
[106]	CCND3	CCNH	CCNL1	CCNT2	CCPG1
[111]	CCRL2	CD37	CD44	CD46	CD55
[116]	CD58	CD82	CD93	CD97	CDA
[121]	CDC2L6	CDC42	CDC42EP3	CDKN2D	CDV3
[126]	CEBPD	CENTB2	CENTD2	CENTD3	CEP170
[131]	CEP63	CFLAR	CFP	CHD1	CHMP1B
[136]	CHMP2A	CHMP2B	CHMP5	CHST11	CHST7
[141]	CIRBP	CITED2	CKAP4	CLEC5A	CLIC1
[146]	CLIP1	CLK1	CLU	CNOT2	CNOT3
[151]	COL1A1	COPE	CPD	CPVL	CR1
[156]	CREB1	CREM	CRISPLD2	CRK	CRSP2
[161]	CRY1	CSF3R	CSNK1A1	CSNK1D	CST3
[166]	CTBS	CTSL1	CTSS	CUTL1	CYFIP2
[171]	CYLD	CYP4F3	DDIT4	DDX18	DDX5
[176]	DENND2D	DERL2	DFNA5	DHCR7	DHRS7
[181]	DHX34	DHX8	DICER1	DNAJA2	DNAJB12
[186]	DNAJB6	DNASE1L1	DNTTIP2	DOCK4	DOK3
[191]	DUSP1	DUSP2	DUSP3	DUSP6	DYSF
[196]	E2F3	ECE1	ECGF1	EFHD2	EGLN1

[201]	EGR3	EHD1	EIF1	EIF2AK3	EIF4A1
[206]	EIF4A3	EIF5	ELF1	ELF4	ELL
[211]	ELL2	ELOVL5	EMD	EP300	EPB41L3
[216]	EPOR	ERBB2IP	EREG	ERLIN1	ESR2
[221]	ESRRA	ETF1	ETS2	EXOSC4	EXTL3
[226]	F5	F8A1	FAM129A	FAM49A	FAM49B
[231]	FAS	FBXO11	FBXO34	FBXW7	FCAR
[236]	FCGR2A	FCGR3B	FCN1	FGFR1OP	FGL2
[241]	FGR	FKBP1A	FKBP8	FLJ10357	FLJ11151
[246]	FLJ11506	FLJ12529	FLJ20273	FLJ22662	FLJ23861
[251]	FLOT1	FMR1	FNDC3A	FOS	FOSB
[256]	FOSL1	FOSL2	FPR1	FRAT1	FRAT2
[261]	FRMD4B	FTH1	FUT7	FYB	G0S2
[266]	G3BP2	GABARAPL1	GABARAPL3	GABPB2	GADD45B
[271]	GAPDH	GARNL1	GARNL4	GCA	GCH1
[276]	GFPT1	GHITM	GIMAP4	GIMAP6	GIT2
[281]	GLIPR1	GLUL	GMFG	GNAI3	GNAQ
[286]	GNB1	GNB2	GNPDA1	GOLGA8B	GPR171
[291]	GPR65	GPR77	GRK6	GSPT1	GTF2H2
[296]	GTPBP4	H2AFY	H2AFZ	H3F3B	HAL
[301]	HAT1	HCLS1	HHEX	HIF1A	HIST1H1C
[306]	HIST1H2AC	HIST1H2BC	HIST2H2AA3	HIVEP1	HIVEP2
[311]	HK3	HLA-E	HNRPA3	HNRPC	HNRPD
[316]	HNRPDL	HNRPH3	HPCAL1	HPS5	HSD17B4
[321]	HSDL2	HSPA4	HSPA6	HSPA9	IBRDC3
[326]	IDI1	IDS	IER2	IER5	IFI16
[331]	IFITM1	IFITM2	IFITM3	IFNGR2	IGF1R
[336]	IL10RB	IL11RA	IL1R1	IL1R2	IL1RAP
[341]	IL23A	IL4R	IL6R	IL8RA	IL8RB
[346]	ING1	INPP5A	INSIG1	IQGAP1	IQGAP2
[351]	IQSEC1	IRS2	ISCA1	ISG20	ITGA5
[356]	ITGAX	ITPK1	IVNS1ABP	JARID2	JMJD2B
[361]	JMJD3	JMJD6	JOSD1	JUN	JUNB
[366]	JUND	KCNJ15	KCNK7	KCNQ1	KCTD13
[371]	KIAA0174	KIAA0241	KIAA0247	KIAA0329	KIAA0404
[376]	KIAA0701	KIAA0999	KIAA1026	KIAA1033	KIAA1539
[381]	KIDINS220	KLC1	KLF2	KLF4	KLF6
[386]	KLHL2	KPNA1	KPNA4	KYNU	L35253
[391]	LAMB3	LAPTM5	LARP5	LCK	LDLR
[396]	LGALS3	LGALS8	LIF	LILRA1	LILRB2
[401]	LILRB3	LIMK2	LMNB1	LMO2	LOC137886
[406]	LOC151579	LOC552891	LPPR2	LRMP	LRRFIP1
[411]	LST1	LY75	LY96	LYPD3	MAEA
[416]	MAFF	MAGOH	MAK	MANSC1	MAP2K3

[421]	MAP3K3	MAP3K7IP2	MAP4K4	MAPK1	MAPK14
[426]	MAPK6	MAPKAPK2	MARCH6	MARCH7	MARCKS
[431]	MARK3	MARS	MBP	MCL1	MEF2D
[436]	METTL3	MGC14376	MLF1IP	MLSTD1	MOBK1B
[441]	MORC3	MORF4L2	MPPE1	MS4A6A	MT1X
[446]	MTHFS	MTMR6	MVP	MX2	MXD1
[451]	MYD88	MYH9	N4BP1	NAB1	NACAP1
[456]	NAPA	NARF	NBEAL2	NBN	NBR1
[461]	NCF4	NCK1	NCLN	NCOA1	NDEL1
[466]	NDFIP1	NDRG1	NDUFB6	NEDD9	NF1
[471]	NFATC3	NFE2	NFE2L2	NFIL3	NFKB1
[476]	NFKB2	NFKBIE	NFYA	NIPBL	NLRP3
[481]	NM_000064	NM_000201	NM_000311	NM_000321	NM_000389
[486]	NM_000610	NM_000636	NM_000902	NM_001007245	NM_001455
[491]	NM_001550	NM_001660	NM_001964	NM_001968	NM_002658
[496]	NM_003059	NM_003150	NM_003244	NM_003254	NM_003370
[501]	NM_003379	NM_004226	NM_004241	NM_004313	NM_004380
[506]	NM_004652	NM_005242	NM_005428	NM_005445	NM_005565
[511]	NM_005746	NM_006292	NM_006305	NM_006561	NM_006665
[516]	NM_006766	NM_007287	NM_007318	NM_014076	NM_014314
[521]	NM_014664	NM_014856	NM_015208	NM_016119	NM_018468
[526]	NM_024524	NM_030756	NMI	NOC3L	NOTCH2
[531]	NP	NPM1	NR3C1	NR4A2	NR4A3
[536]	NRBF2	NRGN	NRIP3	NSFL1C	NSMAF
[541]	NSUN5C	NT5C2	NUAK2	NUMB	NUP98
[546]	NXF1	NXT1	OAT	OAZ1	ODC1
[551]	OGT	OLR1	OSBPL2	OSBPL8	OSTM1
[556]	OXSRI	P2RY13	PADI4	PAFAH1B1	PAK1
[561]	PAK2	PAM	PAPOLA	PARP8	PBX2
[566]	PCAF	PCMT1	PDE4B	PDE4DIP	PDE6D
[571]	PDE8B	PDLIM5	PDLIM7	PDXK	PER1
[576]	PFKFB3	PGK1	PHACTR1	PHC2	PHF20L1
[581]	PHF3	PHKA2	PHLDA1	PI3	PIAS1
[586]	PICALM	PID1	PIGA	PIGB	PILRA
[591]	PITPNA	PKN2	PLA2G4C	PLA2G7	PLAGL2
[596]	PLAU	PLAUR	PLCB2	PLCL2	PLEK
[601]	PLEKHB2	PLEKHG3	PLEKHM1	PLK3	PMAIP1
[606]	PNN	PPAP2B	PPFIA1	PPIF	PPP1CB
[611]	PPP1R10	PPP1R14B	PPP1R15A	PPP3CA	PPP4R1
[616]	PRDM2	PREI3	PRKAR1A	PRKCB1	PRKD2
[621]	PRKD3	PRPF18	PRR13	PSD4	PSMB4
[626]	PSMF1	PTAFR	PTEN	PTGS2	PTP4A1
[631]	PTPN12	PTPRE	PTX3	QKI	QSCN6
[636]	RAB1A	RAB21	RAB27A	RAB31	RAB3D

[641]	RAB5C	RAB6IP1	RAB8B	RABGEF1	RAC2
[646]	RALB	RALGDS	RANBP2	RANBP3	RANBP5
[651]	RAPGEF2	RARA	RASGRP2	RB1CC1	RBM22
[656]	RBM26	RBMS1	RBPJ	RC3H2	RCBTB2
[661]	RCN2	RCN3	RCOR1	REL	REPS2
[666]	RGL2	RGS12	RGS14	RHOF	RHOQ
[671]	RHOT1	RIOK3	RNASET2	RNF141	RNF167
[676]	RNF170	RNGTT	RRN3	RTN4	RXRA
[681]	RYBP	S100A11	S100A12	S100A9	S100P
[686]	SAP30BP	SAR1A	SAT1	SCARF1	SCO2
[691]	SDC2	SEC22B	SECTM1	SEL1L	SELL
[696]	SELPLG	SENP3	SENP6	SEPX1	SERBP1
[701]	SERINC3	SERP1	SERPINB1	SERPINB2	SERPINB8
[706]	SERTAD2	SETX	SFPQ	SFRS2	SFRS2IP
[711]	SFRS3	SFRS5	SFRS7	SGK3	SH2B2
[716]	SH3BP5	SIRT7	SKI	SKIL	SLA
[721]	SLC11A1	SLC11A2	SLC12A9	SLC15A3	SLC19A1
[726]	SLC19A2	SLC1A3	SLC25A13	SLC25A37	SLC25A44
[731]	SLC31A2	SLC35A2	SLC6A6	SLC7A11	SLC7A5
[736]	SLC9A8	SLCO4C1	SLK	SLMO2	SMCHD1
[741]	SNIP1	SNN	SNX10	SNX17	SNX2
[746]	SOAT1	SON	SOS2	SP100	SP110
[751]	SPAG9	SPATA2L	SPI1	SQSTM1	SRGN
[756]	SRPK2	SS18	SSFA2	SSR1	ST3GAL2
[761]	STAG2	STAM2	STAT3	STAT5B	STK10
[766]	STMN1	STRAP	STX16	STX3	STX4
[771]	STX7	SUB1	SUMO1	SUPT6H	SVIL
[776]	SYNCRIP	SYNJ2	TACC3	TAF7	TAGLN2
[781]	TANK	TBC1D1	TBC1D22A	TBK1	TBXAS1
[786]	TFDP1	TFEC	TGFBR3	THBD	THBS1
[791]	THOC5	THRAP1	THRAP2	THRAP5	TIA1
[796]	TIMM17A	TIPARP	TLE3	TLR1	TM2D3
[801]	TMED2	TMEM4	TMEM41B	TNFAIP3	TNFAIP6
[806]	TNFRSF10C	TNFRSF1B	TNFRSF9	TNFSF14	TNIP1
[811]	TNRC5	TOM1	TOP1	TOR1AIP1	TPD52L2
[816]	TPM4	TPP1	TRAF1	TREM1	TREML2
[821]	TRIB1	TRIM27	TRIM36	TRIP12	TSC22D2
[826]	TSEN34	TTC19	TTF1	TUBA1A	TUBA1B
[831]	TUBA1C	TUBA3D	TUBA4A	TXNDC13	TXNRD1
[836]	U34919	UAP1	UBAP1	UBAP2L	UBE2B
[841]	UBE2D1	UBE2D3	UBE2G1	UBE2H	UBE2J1
[846]	UBL3	UBN1	UPF3A	UQCRC2	USP10
[851]	USP15	USP33	VAMP2	VCL	VDR
[856]	VEGFA	VIL2	VIM	VPS24	VPS37B

[861]	W88821	WDR1	WDR26	WDR47	WIPF1
[866]	WTAP	XM_094581	XM_374529	XM_378250	XPO6
[871]	YIPF4	YPEL5	YWHAZ	ZBTB1	ZBTB43
[876]	ZC3H12A	ZCCHC6	ZEB1	ZFP36	ZFP36L2
[881]	ZFX	ZFYVE26	ZHX2	ZMYM1	ZNF165
[886]	ZNF24	ZNF250	ZNF267	ZNF350	ZNF394
[891]	ZNF508	ZNF668	ZYX	ZZEF1	

Table A.4. The response gene set only for GDS1428 control group. Total 750 genes.

[1]	AA045174	AA114166	ABCD4	ABR	ACAA1	ACOT9
[7]	ACTR2	AD7C-NTP	ADAM17	ADD3	ADM	ADNP
[13]	AF086790	AF090895	AF119911	AF164622	AF226044	AFTPH
[19]	AGA	AGER	AHCTF1	AHCYL1	AI140364	AI432196
[25]	AJ275371	AK000185	AK000834	AK000918	AK022211	AL050043
[31]	AL137378	AL157484	AP1S2	AP2A2	AP2S1	AP3B2
[37]	APBB1IP	APOL2	AQP9	ARAF	ARF1	ARFGAP3
[43]	ARHGDIA	ARHGDIB	ARHGEF16	ARID1A	ARL1	ARL4A
[49]	ARPC4	ASCC3	ASL	ASNS	ATF6	ATG3
[55]	ATG4B	ATP11A	ATP1B1	ATP6V1A	ATP6V1C1	ATXN7
[61]	AU148274	AU148611	AV700891	B3GNT1	BAIAP2	BANP
[67]	BASP1	BC000265	BC005884	BCAM	BCAP31	BCAT2
[73]	BCL10	BCOR	BEST1	BHLHB2	BICD2	BMP2K
[79]	BMPR2	BNIP2	BNIP3L	BRAP	BRWD1	BSG
[85]	BST1	BTD	BTN3A2	C10orf6	C10orf76	C10orf97
[91]	C14orf2	C17orf75	C19orf2	C19orf56	C1orf108	C1orf121
[97]	C1orf80	C1orf9	C20orf111	C20orf19	C20orf23	CA12
[103]	CABP1	CALM1	CAND1	CAPN2	CAPZA2	CASP4
[109]	CBFA2T2	CBR4	CCDC109B	CCDC69	CCNG2	CCNJL
[115]	CCT2	CD164	CD300A	CD33	CD47	CD53
[121]	CD6	CD79B	CD96	CDC73	CDK7	CEACAM3
[127]	CEACAM4	CECR5	CENTA2	CENTB1	CEP350	CHERP
[133]	CHFR	CHKA	CHP	CKS2	CLEC1A	CLEC2D
[139]	CLEC4A	CLTA	CMTM6	COL4A3BP	COL9A3	COQ2
[145]	CORO1C	COX11	COX4I1	CSF2RB	CSNK1G2	CST7
[151]	CTBP2	CTDP1	CTNND1	CUEDC1	CUL2	CUL3
[157]	CXCL1	CXCL2	CYC1	CYP1B1	DAB2	DBN1
[163]	DDX17	DDX19A	DDX3X	DECR1	DHX40	DIMT1L
[169]	DKFZP564J102	DLG1	DLGAP4	DMTF1	DNAJB1	DNAJB9
[175]	DNAJC10	DNAJC3	DNM2	DOCK2	DOM3Z	DPEP2
[181]	DPM1	DUSP4	DVL3	DYNC1LI1	DYNLT1	DYRK1A
[187]	DYRK2	EAPP	ECOP	ECT2	EFCBP2	EFEMP2
[193]	EIF4EBP1	EIF4H	ELMO3	EML3	EMP1	EMR2
[199]	EMR3	EPRS	ERCC1	ERGIC2	ETV6	EVI2B
[205]	EVI5	EXOC7	F11R	F25965	FAIM3	FAM12A
[211]	FAM45A	FAM53C	FAM55C	FANCF	FARSA	FASTKD3
[217]	FBS1	FBXL11	FBXL4	FCER1G	FCGR1A	FCGR1B
[223]	FCGR2C	FCHO1	FDFT1	FGFR2	FKBP5	FLII
[229]	FLJ13611	FLNA	FLOT2	FMNL1	FMO5	FNBP1
[235]	FPGT	FTHP1	FTO	FUCA1	FUS	FUT6
[241]	FXR1	FXRD2	GAB2	GABARAP	GABARAPL2	GBP2
[247]	GCSH	GINS2	GLRX	GLT8D1	GMDS	GMIP

[253]	GMPR2	GNAS	GNB2L1	GOLGA8A	GP1BB	GPNMB
[259]	GPR109B	GPR15	GPR157	GRAMD1C	GRB2	GRIN2D
[265]	GSK3B	GTF2B	GTF2I	GZMM	H1F0	H3F3A
[271]	HBA1	HBP1	HBS1L	hCG_2015956	HEBP2	HECTD3
[277]	HEXIM1	HFE	HIPK1	HIST1H2BD	HIST1H4J	HLA-DPA1
[283]	HMGCS1	HMOX2	HN1	HNRPH2	HNRPK	HNRPR
[289]	HPGD	HR44	HRBL	HSD17B11	HSF1	HSPA5
[295]	IBSP	ICAM3	ID4	IDH2	IER3	IFIT1
[301]	IFIT5	IFT20	IGBP1	IGFBP4	IGSF6	IK
[307]	IKBKAP	IKZF1	IL10RA	IL17RA	IL1RL2	IL32
[313]	IL8	IL9R	IMPA2	IMPACT	IMPDH1	ING3
[319]	IRF1	IRF3	ITGAM	JAK1	JAK2	JMJD1A
[325]	KBTBD2	KBTBD4	KCTD12	KIAA0182	KIAA0232	KIAA0240
[331]	KIAA0372	KIAA0406	KIAA0409	KIAA0509	KIAA0513	KIAA1279
[337]	KIFC3	KLF10	KLF9	KRAS	KRT23	L08961
[343]	LAMP2	LASP1	LCN2	LCP1	LDHA	LENG4
[349]	LGALS9	LHFPL2	LILRA2	LILRA6	LILRB1	LILRB4
[355]	LIMD2	LIN7A	LITAF	LOC283537	LOC440248	LOC440926
[361]	LPIN2	LRP10	LRRC17	LRRC6	LSM14A	LTA4H
[367]	LTB4R	LTBR	LYN	MAF	MAN1B1	MAP1LC3B
[373]	MAP2K1IP1	MAP2K7	MAP3K1	MAP3K8	MAPBPIP	MAPKAP1
[379]	MARCKSL1	MAX	MBD4	MCART1	MED12	MET
[385]	MGC31957	MGC4093	MGC5139	MGEA5	MICALL2	MIR16
[391]	MKLN1	MKRN1	MKRN2	MLF2	MNDA	MNT
[397]	MOAP1	MOG	MON1B	MOSC1	MPP1	MRC1
[403]	MRC2	MRLC2	MRPL13	MRPS14	MRTO4	MT2A
[409]	MTMR14	MTX1	MYBPC1	MYBPC3	MYLIP	MYLPF
[415]	MYO1F	MYST1	MYST4	N-PAC	NADK	NBPF11
[421]	NBPF15	NCF1	NCF2	NCOA2	NCOA4	NDRG3
[427]	NEU1	NFKBIB	NFYC	NM_000051	NM_000177	NM_000269
[433]	NM_000416	NM_000430	NM_000551	NM_000576	NM_000594	NM_000600
[439]	NM_001008540	NM_001009607	NM_001025076	NM_001186	NM_001620	NM_001706
[445]	NM_002157	NM_002444	NM_002719	NM_003054	NM_003418	NM_003588
[451]	NM_003955	NM_004444	NM_005345	NM_005543	NM_014778	NM_014821
[457]	NM_014863	NM_016384	NM_018579	NM_020237	NM_020529	NM_021039
[463]	NM_021212	NM_022354	NM_022718	NM_024974	NOL12	NOLA2
[469]	NOLA3	NOTCH3	NPTXR	NR1D2	NR2C2	NR2F2
[475]	NSUN7	NUCB1	ORC4L	ORC5L	OSBP	OSBPL11
[481]	OSM	OTUB1	PAICS	PANX1	PAWR	PBXIP1
[487]	PCDHB12	PDE2A	PDSS1	PECAM1	PELI1	PEX3

[493]	PGS1	PHF21A	PIP5K3	PISD	PJA2	PKP4
[499]	PLA2G12A	PLAGL1	PLP2	PLXNC1	POLR2J	POLR3G
[505]	POR	PPARD	PPIE	PPIG	PPM1A	PPM1B
[511]	PPM1F	PPP1CA	PPP1R13B	PPP2CA	PPP2R2A	PPP3R1
[517]	PPP4C	PRKACA	PRKACB	PRKCSH	PRKRA	PRLH
[523]	PRMT2	PRR14	PSAP	PSCD4	PSMA7	PSMB3
[529]	PSMB9	PSME3	PSME4	PTBP1	PTGER4	PTHLH
[535]	PTK2B	PTPRC	PYCARD	QDPR	RAB11FIP1	RAB11FIP2
[541]	RAB22A	RAB2A	RAB3GAP1	RAB5A	RAB7A	RABL4
[547]	RAC1	RAD17	RANBP6	RAP2C	RASA2	RASSF2
[553]	RASSF3	RASSF4	RBBP6	RBM13	RBM39	RCOR3
[559]	RECQL	RELB	RFC1	RFC3	RHO	RHOG
[565]	RHOH	RIN3	RMND5A	RNF11	RNF24	ROCK1
[571]	RORA	RPA1	RRAGD	RRS1	S100A4	S100A8
[577]	SACS	SAV1	SC4MOL	SCML1	SDCBP	SDHC
[583]	SEC14L1	SEC31A	SEC61A2	SEH1L	SEMA3C	SERINC1
[589]	SERPINA1	SETD1B	SETD2	SETDB1	SFRS6	SH2B1
[595]	SH3BGRL	SH3BGRL3	SH3BP2	SH3GL1	SHOC2	SIGLEC5
[601]	SIGLEC9	SIPA1	SIRPB1	SLAMF8	SLC12A6	SLC13A4
[607]	SLC16A3	SLC16A6	SLC29A1	SLC2A14	SLC2A3	SLC31A1
[613]	SLC35A1	SLC4A7	SLC6A8	SLC9A1	SMPDL3B	SNED1
[619]	SNRPD1	SNX13	SOD2	SORBS3	SORL1	SP3
[625]	SPG21	SRRM2	SSBP1	STAT6	STC1	STIP1
[631]	STK17A	STK38L	STK4	STX10	STX6	STXBP3
[637]	SUCLG2	SUPT4H1	SYF2	SYK	TACC1	TAF1A
[643]	TALDO1	TAOK3	TAPBP	TBC1D15	TBC1D17	TBKBP1
[649]	TBL1XR1	TCF3	TCP1	TEGT	TESK2	TFCP2
[655]	TFE3	TFEB	TFG	TFRC	TGM2	THAP1
[661]	THOC2	TMEM1	TMEM127	TMEM140	TMEM176B	TMEM50A
[667]	TMF1	TncRNA	TNFAIP2	TNFRSF10B	TNFRSF14	TNFRSF25
[673]	TNRC6B	TNXB	TOX4	TP53BP2	TP53I11	TRAF5
[679]	TRIM23	TRIM38	TRIM8	TRIOBP	TTC13	TTF2
[685]	TUBB	TUBB2A	TUBB2C	TUBGCP3	TUG1	TWF2
[691]	TXN2	U00956	UBE2L3	UBE2O	UBE3A	UBR2
[697]	UBTD1	UBTF	UBXD2	ULK1	UNC119	UNC50
[703]	UPF1	UROS	USH2A	USP3	USP32	USP36
[709]	USP8	UTX	VAMP3	VCPIP1	VPS13C	VPS26A
[715]	VRK1	WAC	WDR8	XBP1	XM_370635	XPO7
[721]	XR_000228	XRCC4	XRCC5	YIPF3	YKT6	YTHDC2
[727]	YTHDF3	YWHAB	YWHAE	ZC3H11A	ZDHC18	ZFAND3
[733]	ZFAND6	ZMIZ1	ZMYM2	ZNF12	ZNF148	ZNF180
[739]	ZNF224	ZNF227	ZNF292	ZNF467	ZNF518	ZNF573
[745]	ZNF588	ZNF7	ZNF710	ZNF750	ZNF804A	ZUBR1

Table A.5. The response gene set only for GDS1428 treatment group. Total 1008 genes.

[1]	3.8-1	AA017721	AA203487	AA355179	AA365670	ABCB9
[7]	ABCD1	ABCF2	ABHD14B	ABHD3	ABHD5	ACACB
[13]	ACBD3	ACIN1	ACSL5	ACTR1A	ADAM19	ADAM22
[19]	ADAMTS8	ADD1	ADH1B	ADMR	ADRB2	ADRBK1
[25]	AF103530	AF291676	AGK	AI278204	AI472320	AI523613
[31]	AI683552	AK025360	AK026682	AK1	AK3L1	AK3L2
[37]	AKAP8L	AL044078	AL049435	AL109696	AL137624	AL390145
[43]	ALLC	AMD1	AMPH	ANGPT1	ANKRD15	ANP32E
[49]	ANXA5	AOAH	AOC2	AP1S1	AP3M2	APBA3
[55]	APC	APOBEC3B	APBP2	AQP3	ARF6	ARFGEF1
[61]	ARFGEF2	ARHGAP1	ARHGAP19	ARHGEF18	ARHGEF3	ARHGEF4
[67]	ARL3	ARL6IP2	ARL8B	ARMC9	ARPC2	ARPP-19
[73]	ARS2	ARTS-1	ASPH	ASXL1	ATBF1	ATG7
[79]	ATHL1	ATOX1	ATP2A3	ATP5F1	ATP6V1F	ATXN3
[85]	ATXN3L	AU147851	AUH	AW301235	AW836210	AZGP1
[91]	B4GALT2	B4GALT4	BAG2	BAT1	BAT2	BAZ2B
[97]	BBS4	BC002791	BC003528	BC006164	BE327172	BLVRB
[103]	BMP2	BMP6	BMX	BRCA1	BRCC3	BRD8
[109]	BTG3	BTN3A1	BZRAP1	C10orf95	C11orf30	C12orf5
[115]	C13orf15	C14orf159	C15orf39	C16orf80	C17orf68	C19orf10
[121]	C1D	C1orf107	C1orf183	C1QTNF1	C1R	C20orf121
[127]	C20orf32	C21orf33	C2orf25	C4BPB	C4orf16	C4orf20
[133]	C5orf15	C6orf108	C6orf62	C7orf42	C7orf44	C9orf46
[139]	CACNA1I	CACNA2D3	CADM3	CALU	CAMK2G	CAMTA2
[145]	CAND2	CAP1	CAPG	CAPRIN1	CAT	CBFA2T3
[151]	CCDC49	CCL18	CCL19	CCL2	CCL3	CCL4
[157]	CCNA1	CCT6A	CD22	CD226	CD2BP2	CD302
[163]	CD48	CD59	CD68	CD69	CDC2L2	CDC42EP2
[169]	CEP164	CETN2	CHD4	CHD9	CHI3L1	CHIC2
[175]	CHRNA10	CHST8	CIB1	CIR	CIZ1	CLASP2
[181]	CLCN3	CLCN4	CLCN6	CLCN7	CLDND1	CLEC1B
[187]	CLIC4	CLINT1	CLK2	CLN5	CNN2	CNOT8
[193]	COL13A1	COL6A3	COMMD4	COX5B	CPM	CREB5
[199]	CREBL2	CREG1	CRHR1	CRIP2	CRIP2	CRKL
[205]	CROP	CSAD	CSNK2A1	CSTB	CTGLF1	CTSB
[211]	CTSE	CTSL2	CTSW	CUGBP1	CXCL5	CXXC1
[217]	CYB5R4	CYHR1	CYP17A1	CYP19A1	CYP4F2	D85181
[223]	DAPP1	DCTN3	DDIT3	DDX21	DDX23	DDX39
[229]	DDX52	DENND1A	DENND3	DHRS12	DHRS9	DHTKD1
[235]	DIAPH2	DIS3	DLAT	DLC1	DLG3	DMC1
[241]	DMXL1	DMXL2	DNAJA1	DNAJB14	DNAL4	DNPEP

[247]	DPEP3	DPM2	DRAM	DSCR1L1	DSCR3	DSE
[253]	DTX2	DUSP10	DYNLT3	E2F8	EBI3	EDD1
[259]	EDEM3	EDG4	EFHC2	EIF1B	EIF2S1	EIF2S2
[265]	EIF3S1	EIF3S10	EIF4G3	EIF5A	ELF2	ELF3
[271]	ELK3	ELL3	ELN	ENC1	ENO3	ENOPH1
[277]	ENPP4	ENSA	EPB41L5	EPM2AIP1	EPS15L1	ESF1
[283]	ETNK1	ETS1	EVA1	EVI2A	EWSR1	FABP5
[289]	FAM120A	FAM128B	FAM3C	FAM63A	FAM65A	FBXL15
[295]	FBXL5	FBXO31	FBXO42	FCGRT	FCHSD2	FDPS
[301]	FEM1B	FGFR1	FIS1	FKBP15	FKSG30	FLCN
[307]	FLI1	FLJ10154	FLJ10213	FLJ12716	FLJ12886	FLJ14213
[313]	FLJ20186	FLJ20254	FLJ20433	FLJ21908	FNBP4	FNDC3B
[319]	FOXJ3	FOXK2	FSHB	FSTL3	FUT4	FXYS5
[325]	FYN	GAD1	GAL	GALC	GALNACT-2	GALNT2
[331]	GALNT3	GALNT7	GAPVD1	GARS	GAS7	GATAD1
[337]	GCLM	GDPD3	GGA3	GGT1	GGT3	GIN51
[343]	GK	GLA	GMFB	GNB5	GNL1	GNPAT
[349]	GNS	GOLGA5	GPLD1	GPR175	GPR176	GPR177
[355]	GPR18	GREM1	GRINA	GRK5	GRPEL1	GSH1
[361]	GSR	GTF2H1	GTPBP1	GUCA1B	GUK1	GYG1
[367]	GZMA	HARS2	HBEGF	HBXIP	HCFC2	HCK
[373]	HDAC2	HDLBP	HERC4	HERPUD1	HEXA	HIP1
[379]	HIST1H2AJ	HIST1H3G	HIST1H3H	HIST2H2BE	HLA-F	HLA-G
[385]	HMG20B	HMHA1	HNF4A	HNRPM	HNRPU	HOXA1
[391]	HP1BP3	HPN	HRH1	HRK	HS2ST1	HSD17B14
[397]	HSMPP8	HSP90AA1	HSP90AB1	HTR2A	HTR2B	HTR6
[403]	HTRA2	IARS2	ICAM4	ICK	IDH3G	IFIH1
[409]	IFIT2	IFNAR1	IFNG	IFNGR1	IFT57	IGFBP5
[415]	IGFBP7	IL12B	IL18RAP	IL1A	IL1F9	IL24
[421]	IL5RA	IL6ST	ILF3	INHBA	INHBC	INPP4A
[427]	INTS3	INTS8	IPO4	IPO7	IRAK1	IRAK3
[433]	IRF5	IRGC	ISG20L2	ISGF3G	ITCH	ITGA2B
[439]	ITGA6	ITGB3	ITGB8	ITM2B	ITPR1	JAG1
[445]	JAKMIP2	JARID1B	KCNAB3	KCNE1	KCNJ5	KCNK1
[451]	KCNMB1	KCTD2	KCTD20	KDEL3	KEAP1	KHDRBS1
[457]	KIAA0143	KIAA0286	KIAA0467	KIAA0692	KIAA0746	KIAA0892
[463]	KIAA0913	KIAA0922	KIAA0984	KIAA1324	KIAA1655	KIAA1815
[469]	KIAA1840	KIF1B	KLHL21	KLHL24	KPNA2	KPTN
[475]	KRCC1	KRT32	KRT6B	Kua-UEV	LAMA2	LAMA4
[481]	LILRA5	LIMS1	LOC130074	LOC152719	LOC339457	LOC388335
[487]	LOC388458	LOC439992	LOC440354	LOC51136	LOC54103	LRCH4
[493]	LRRC8D	LSM5	LY6G6D	LYRM4	M6PR	M6PRBP1
[499]	MACF1	MAFG	MAG	MAGEA8	MAGEB2	MAN1A1
[505]	MAN2B2	MAP3K4	MAP3K5	MAPK13	MAPK8IP2	MARCH2

[511]	MARK2	MAST2	MATR3	MBTD1	MCAM	MCAT
[517]	MCFD2	MCM4	MCOLN1	MCTP1	MCTP2	MDM2
[523]	MECP2	MEF2A	MGAM	MGC13098	MGLL	MICA
[529]	MIER2	MKKS	MLX	MMD	MMP1	MMP14
[535]	MMS19L	MOXD1	MPZL1	MRPL12	MRPL34	MSC
[541]	MSRA	MSRB2	MT1E	MT1JP	MT3	MTA1
[547]	MTERFD1	MTF1	MTM1	MTMR10	MTMR2	MVK
[553]	MXD3	MXI1	MYCBP	MYH7	MYO9B	NAGK
[559]	NANS	NCOR2	NCR2	NDUFB8	NDUFV2	NEDD4L
[565]	NENF	NFE2L1	NGLY1	NHP2L1	NINJ1	NISCH
[571]	NM_000110	NM_000125	NM_000129	NM_000358	NM_000553	NM_000885
[577]	NM_001012478	NM_001250	NM_001622	NM_001787	NM_001993	NM_002015
[583]	NM_002185	NM_002228	NM_002562	NM_002746	NM_003017	NM_003072
[589]	NM_003103	NM_003137	NM_003266	NM_003605	NM_003810	NM_003885
[595]	NM_004233	NM_004682	NM_004846	NM_005259	NM_005821	NM_005965
[601]	NM_006021	NM_007319	NM_012115	NM_013421	NM_014129	NM_014242
[607]	NM_014645	NM_014796	NM_015372	NM_015987	NM_016415	NM_017795
[613]	NM_017874	NM_017920	NM_018605	NM_019061	NM_020037	NM_020149
[619]	NM_020213	NM_020415	NM_020661	NM_021730	NM_021941	NM_022837
[625]	NM_024614	NM_024716	NM_024777	NM_024853	NM_024984	NM_030897
[631]	NM_033111	NM_145237	NM_152516	NME5	NOL1	NOL14
[637]	NONO	NOTCH2NL	NOV	NPC1	NPHP4	NQO2
[643]	NR1H2	NR6A1	NRAS	NUFIP1	NUP210	NUP62
[649]	OCLM	ODZ2	OLFM4	ORM1	OSBPL1A	OSGEP
[655]	P2RX4	PATZ1	PAX8	PAXIP1	PCF11	PCOLCE2
[661]	PCTK2	PCYT1A	PDCD4	PDE6B	PDK3	PDLIM1
[667]	PDLIM2	PDLIM3	PDPK1	PER2	PF4	PFAAP5
[673]	PGCP	PGD	PGLS	PHACTR2	PHC1	PHF20
[679]	PHLPP	PIGV	PIK3CD	PIK3CG	PIP5K2A	PIR
[685]	PKIG	PKM2	PLA1A	PLA2G6	PLD1	PLEC1
[691]	PLEKHF2	PLK2	PLOD1	PLSCR4	PMF1	PMVK
[697]	POLG2	POLM	POLR3E	POMZP3	POPDC2	POU6F2
[703]	PPBP	PPIAL4	PPP1R12A	PPP2CB	PPP2R3C	PPP4R2
[709]	PQBP1	PRDM14	PREPL	PRKAA1	PRKAR2B	PRKCH
[715]	PRKCI	PRKDC	PRPF38B	PRPF4	PRPF6	PRPSAP1

[721]	PRR7	PRSS3	PSMA3	PSMA6	PSMC5	PSMD12
[727]	PSMD13	PSMD3	PSMD4	PSORS1C2	PTPN18	PTPRN
[733]	PTRH2	PTS	PTTG1IP	PUM2	PYCR1	RAB11A
[739]	RAB13	RABGAP1L	RABGGTA	RABL2A	RAD21	RAD23B
[745]	RAD51	RAGE	RASGRF1	RBM16	RBM28	RBM38
[751]	RBM5	RBM7	RBM9	REC8	RERE	RFK
[757]	RGL1	RGPD5	RGS7	RIF1	RIN2	RIPK1
[763]	RLN2	RNF10	RNF111	RNF138	RNPEP	RP4-724E16.2
[769]	RPGRIP1	RPL10	RPS6KA5	RPS6KB2	RPS9	RRM1
[775]	RRP12	RSRC2	RTN2	RXRG	S100A6	SAFB
[781]	SAMD9	SAMSN1	SAP18	SAP30	SAP30L	SBNO2
[787]	SCARA3	SCN1B	SCRN3	SDCCAG3	SDHD	SEC23B
[793]	SEC23IP	SECISBP2	SEMA6B	SENP5	SEP15	SERPINB7
[799]	SERPINB9	SERPINI2	SF1	SF3B4	SFN	SFRS15
[805]	SFT2D2	SH2D3A	SH2D3C	SH3GLB1	SHCBP1	SIAH1
[811]	SIGLEC7	SIRPA	SIRPG	SKAP2	SLAMF7	SLBP
[817]	SLC14A2	SLC1A2	SLC24A1	SLC25A32	SLC26A2	SLC2A6
[823]	SLC35E3	SLC39A6	SLC39A8	SLC3A2	SLC43A3	SLC4A2
[829]	SLC5A3	SLC7A7	SLCO4A1	SLPI	SMARCD3	SMARCE1
[835]	SMG7	SMPD2	SNAP23	SNRPA1	SNRPB	SNTB2
[841]	SNX1	SNX3	SPAG1	SPAG5	SPAG7	SPATA5L1
[847]	SPATA6	SPCS1	SPG20	SPHK1	SPINLW1	SPINT1
[853]	SPINT2	SPOCK2	SPTB	SPTBN1	SPTLC1	SRC
[859]	SRF	SRGAP3	SRP72	SSSCA1	ST13	ST3GAL1
[865]	ST8SIA4	STAG1	STARD8	STATH	STS	STXBP2
[871]	SUCLG1	SUPV3L1	SYCP2	SYNE2	SYNJ1	TAF1C
[877]	TAF9	TAL1	TAPBPL	TARDBP	TBC1D2	TBC1D2B
[883]	TBC1D3	TCFL5	TDG	TECT1	TES	TFR2
[889]	TGIF2	TICAM1	TKT	TLN1	TLR5	TM9SF2
[895]	TM9SF3	TMCO1	TMCO3	TMED10	TMED7	TMEM110
[901]	TMEM165	TMEM24	TMEM33	TMEM53	TMEM8	TMEPAI
[907]	TNFRSF1A	TNFRSF8	TNNI2	TOB1	TOMM20	TOPORS
[913]	TOR1A	TOR1B	TPR	TRA@	TRA2A	TRAC
[919]	TRADD	TRAF3	TRAF3IP2	TRAF3IP3	TRAPPC3	TRIB3
[925]	TRIM13	TRIM16	TRIM34	TRIP10	TRPC2	TSNAX
[931]	TSPAN2	TSPAN3	TTC1	TTC35	TTN	U56725
[937]	UBE2E1	UBE2S	UBE4B	UCHL5	UCKL1	UCP2
[943]	UGP2	UIMC1	UPB1	UPP1	USP4	UTP18
[949]	UTP6	VAC14	VCAN	VDP	VILL	VNN2
[955]	VPS41	WDR42A	WDR74	WHDC1	WSB1	WSB2
[961]	WWC1	XM_208778	XM_370838	XM_372632	XM_496132	XM_496217
[967]	XM_497663	XM_498825	XM_498877	XM_499165	XPO1	YAF2
[973]	YIPF6	YRDC	YTHDC1	YY1	ZBED1	ZBTB17

[979]	ZBTB3	ZC3H7A	ZCCHC10	ZCCHC14	ZDHHC17	ZEB2
[985]	ZFAND5	ZH2C2	ZMAT3	ZMYND10	ZNF124	ZNF155
[991]	ZNF177	ZNF202	ZNF221	ZNF225	ZNF238	ZNF254
[997]	ZNF259	ZNF277P	ZNF331	ZNF500	ZNF552	ZNF586
[1003]	ZNF589	ZNF652	ZNF675	ZNF692	ZNF783	ZNF84

Table A.6. The common set of differentially regulated gene set (gene set A) and the response gene set under treatment (gene set B). Total 232 genes.

[1]	ABAT	ABHD2	ABHD5	ADA	ADD1	ADORA2A
[7]	AGTPBP1	AIF1	AMD1	AMPD2	AMPD3	ANXA5
[13]	ANXA7	ARCN1	ARIH1	ARTS-1	ATF1	ATP2B4
[19]	BCL3	BID	BIN2	BRD8	BTG2	BTG3
[25]	BTN3A1	C14orf159	C20orf121	CA4	CAMK2G	CAMKK2
[31]	CANX	CAPN7	CCL19	CCL3	CCL4	CCRL2
[37]	CD44	CD48	CD59	CD69	CDA	CFLAR
[43]	CHST7	CIB1	CIRBP	CLCN7	CLIC4	COX5B
[49]	CR1	CREB1	CREB5	CREM	CSAD	CSF3R
[55]	CSTB	DICER1	DNAJB6	DUSP10	DUSP6	EGR3
[61]	ELOVL5	EREG	ETS2	F5	FABP5	FBXO11
[67]	FCGR2A	FGL2	FGR	FLI1	FPR1	FRAT1
[73]	G0S2	G3BP2	GADD45B	GALNT3	GCH1	GCLM
[79]	GGT1	GLIPR1	GMFB	GTF2H2	HAL	HHEX
[85]	HNRPDL	IDS	IER5	IL12B	IL1F9	IL1R1
[91]	IL1R2	IL8RA	IL8RB	INHBA	INPP4A	INSIG1
[97]	IQGAP1	ISGF3G	ITM2B	ITPK1	ITPR1	JAG1
[103]	KIAA0143	KIF1B	LILRA1	LILRB2	LILRB3	LIMS1
[109]	LOC54103	LRMP	LST1	M6PR	MAN1A1	MAP3K3
[115]	MAP4K4	MAPK6	MATR3	MCL1	MCM4	MGLL
[121]	MPPE1	MPZL1	NARF	NCOA1	NDUFB6	NFATC3
[127]	NFKB1	NMI	NR4A2	NR4A3	NRIP3	NSFL1C
[133]	NSMAF	OLR1	ORM1	OSBPL2	OSTM1	P2RX4
[139]	PAK1	PAM	PBX2	PDE4B	PDE4DIP	PDPK1
[145]	PDXK	PFKFB3	PGCP	PGLS	PHLDA1	PI3
[151]	PICALM	PIGA	PLAGL2	PLAU	PLCL2	PLEK
[157]	PLEKHB2	PLSCR4	PMAIP1	PPAP2B	PPIF	PPP1R15A
[163]	PSMA6	PSMB4	PSMD12	PSMD4	PTGS2	PTX3
[169]	RAB5C	RAD21	RAD23B	RALGDS	RBMS1	REL
[175]	RERE	RGS14	RNASET2	RNGTT	RNPEP	RPS6KA5
[181]	RPS9	RXRA	S100A11	S100A6	SEC23B	SEPX1
[187]	SERPINB2	SERPINB9	SFPQ	SFRS7	SH3BP5	SIAH1
[193]	SLBP	SLC11A2	SLC12A9	SLC19A1	SLC25A13	SLC43A3
[199]	SLC7A7	SLK	SLPI	SNN	SP110	SPG20
[205]	SPHK1	SPINLW1	SQSTM1	SRPK2	SYNJ2	TACC3
[211]	TBC1D2	TFEC	TKT	TLR1	TNFAIP3	TNFAIP6
[217]	TNFRSF10C	TNFRSF1A	TNFRSF14	TNRC5	TPM4	TRA@
[223]	TRAF1	UBE2G1	UBN1	VAMP2	VNN2	WTAP
[229]	ZFP36L2	ZFX	ZFYVE26	ZYX		

Table A.7. Those genes only in differentially regulated gene set (gene set A) but not in the response gene set under treatment (gene set B). Total 578 genes.

[1]	101F6	2TRPA1	ABCA7	ACAT2	ACINUS
[6]	ACTN4	ADAM17	ADAM8	ADCY3	ADD3
[11]	ADRA1A	ADSL	AHCY	AK2	AK3
[16]	AKT1	ALAS1	ANP32B	APG4B	AQP9
[21]	ARFGAP3	ARHGAP11A	ARHGAP4	ARHGDIB	ARHGEF6
[26]	ARHQ	ARHT1	ARPC4	ASK	ATF5
[31]	ATP1B1	ATP5J	ATP6V1A1	ATPIF1	AUP1
[36]	B3GNT4	B4GALT5	BAGE	BCAP31	BCL10
[41]	BCL11A	BCOR	BFAR	BICD2	BIGM103
[46]	BM045	BMP2K	BRAF	BRD1	BTBD14A
[51]	BTF	BTG1	BTK	BTN2A1	BTN3A2
[56]	BTN3A3	C11orf10	C13orf10	C14orf109	C14orf147
[61]	C19orf7	C1orf16	C1orf24	C1QBP	C20orf104
[66]	C21orf66	C21orf91	C22orf19	C3	C9orf10
[71]	Cab45	CABC1	CASC3	CASP2	CASP4
[76]	CBX7	CCL20	CCNG2	CCR1	CCR3
[81]	CCR9	CD74	CD9	CDC16	CDC34
[86]	CDC5L	CDC6	CECR5	CES1	CGI-72
[91]	CHES1	ChGn	CHS1	CHST2	CHST6
[96]	cig5	CKLF	CLC	CLECSF12	CLECSF6
[101]	CLECSF9	CLN2	CMAH	COL15A1	COL18A1
[106]	COPEB	COPS3	CORO1A	CPR8	CRSP3
[111]	CSNK1G2	CSPG2	CTBP1	CTBP2	CTNNA1
[116]	CUGBP2	CXCL1	CXCL2	CXCL3	DAMS
[121]	DARS	DCL-1	DDEF1	DGCR2	DIAPH1
[126]	DKFZP434C171	DKFZP566A1524	DKFZp586i1420	DKFZP586L151	DKFZP586M1523
[131]	DKFZp761P1010	DLEU2	DLGAP4	DMN	DNAH7
[136]	DNAH9	DNAJB9	DNAJC8	DOK1	DPEP2
[141]	DSIPI	E1B-AP5	EEF1A1	EGLN2	EHD4
[146]	EIF3S6IP	EIF4E	EIF4EL3	EMR3	ERAL1
[151]	ETFA	EZH1	EZI	F2RL1	FACL3
[156]	FACL6	FAD104	FBS1	FBXO9	FCER1G
[161]	FCGR3A	FDX1	FETUB	FGF7	FHOD1
[166]	FLII	FLJ10055	FLJ10707	FLJ10726	FLJ10858
[171]	FLJ10996	FLJ11036	FLJ11088	FLJ11142	FLJ11259
[176]	FLJ12150	FLJ13195	FLJ13386	FLJ20038	FLJ20189
[181]	FLJ20274	FLJ20287	FLJ20373	FLJ20449	FLJ20502
[186]	FLJ20530	FLJ20559	FLJ20811	FLJ20986	FLJ20989
[191]	FLJ21047	FLJ21308	FLJ21588	FLJ22169	FLJ22649
[196]	FLJ22843	FLJ22938	FLJ23056	FLJ23142	FLJ23231

[201]	FLJ90005	FLRT2	FMNL	FNBP1	FOXO1A
[206]	FOXO3A	FPRL1	FZD6	G2	G3BP
[211]	GAGE4	GATA2	GBE1	GBP1	GCLC
[216]	GGTLA4	GL004	GLRX	GLRX2	GMCL
[221]	GMIP	GMPR2	GOLPH3	GPNMB	GPR58
[226]	GPR86	GPS2	GPX3	GRP58	H1FX
[231]	H2AFX	H2AV	HA-1	HCGVIII-1	HDAC1
[236]	HEBP2	HFE	HGF	HIPK1	HIST1H2BK
[241]	HMGCL	HMGCS1	HMGN1	hnRNPA3:	HOXB7
[246]	HPIP	HRH4	HRIHFB2122	HS3ST2	HSPA1B
[251]	ICAM1	ICAM3	IER3	IFI30	IFNA10
[256]	IFRD1	IKBKG	IL13RA1	IL1B	IL1RN
[261]	IL22RA1	IL6	IL7R	IL8	IMPA2
[266]	INPP5D	INPPL1	IRF1	IRF3	IRS1
[271]	ITGAL	ITGAM	ITGB1	ITGB2	JK
[276]	KCNB1	KCNJ14	KIAA0053	KIAA0103	KIAA0140
[281]	KIAA0146	KIAA0191	KIAA0222	KIAA0290	KIAA0352
[286]	KIAA0415	KIAA0433	KIAA0471	KIAA0561	KIAA0592
[291]	KIAA0625	KIAA0650	KIAA0779	KIAA0802	KIAA0847
[296]	KIAA0853	KIAA0876	KIAA0930	KIAA0980	KIAA1039
[301]	KIAA1049	KIAA1109	KIAA1185	KIAA1240	KIAA1466
[306]	KIAA1473	KIF14	KMO	KPNB2	LAMP1
[311]	LAMP2	LBR	LCT	LEPR	LILRA2
[316]	LIM	LIN7C	LOC115207	LOC116150	LOC54499
[321]	LOC56267	LOC90410	LPIN2	LTA4H	LTBR
[326]	LUC7L2	LYPLA2	LYZ	MADH4	MAF
[331]	MAN2A2	MAP3K2	MAP3K8	MAX	MBD4
[336]	MBTPS1	ME1	ME2	MEIS2	MEL
[341]	MGAT2	MGC10986	MGC12518	MGC17528	MGC3121
[346]	MGC31957	MGC8902	MGEA5	MGRN1	MIR
[351]	MIR16	MKL1	MKRN1	MLF2	MLL4
[356]	MME	MMP25	MNDA	MRCL3	MRLC2
[361]	MRPL44	MT1G	NACA	NCF1	NCKAP1
[366]	NDUFA2	NEK3	NFKBIA	NFYC	NICAL
[371]	NINJ2	NPAT	NSEP1	NUP214	Nup37
[376]	NUPL1	OGFR	oncogene	OPHN1	OR1D2
[381]	OR1E1	OSM	OTUB1	PC326	PCOLN3
[386]	PCTP	PDE4D	PDX1	PECAM1	PELI1
[391]	PEX3	PEX6	PFC	PFDN4	PFN1
[396]	PHF11	PHLDA2	PIASY	PILB	PINK1
[401]	PKP2	PLA2G4A	PLEKHE1	PLS3	PLXNC1
[406]	POLR2J	PPM1F	PPP1CA	PPP1R9A	PPP2R5C
[411]	PPP6C	PREB	PRKAB1	PRKAR2A	PRKCL2
[416]	PRO0097	PRO0971	PRO2037	PRO2198	proteoglycan

[421]	PRPF4B	PSG1	PSMB10	PSMB8	PSME2
[426]	PSME4	PTD008	PTGER3	PTGER4	PTK2
[431]	PTK9L	PTN	PTP4A2	PTPN13	PTPN2
[436]	PYGL	RAB20	RAB33B	RAD1	RAD51L3
[441]	RALBP1	RALY	RASA1	RBSK	RGS2
[446]	RHAG	RHOBTB3	RI58	RIMS2	RNASE6
[451]	RNASE6PL	RNPC1	ROCK2	RPEL1	RPL13
[456]	RPL18A	RPL27A	RPL3	RPL30	RPL34
[461]	RPL39	RPL5	RPS11	RPS14	RPS17
[466]	RPS18	RPS27A	RPS6KA1	RPS6KA3	RUVBL1
[471]	S100A4	SALL1	SAT	SCAMP1	SCAP
[476]	SCARB2	SCD4	SCN11A	SEC14L1	SEC22L1
[481]	SEC24A	SERPINE2	SFRS6	SH3GL3	SIAT4A
[486]	SIPA1	SLC1A4	SLC21A11	SLC30A9	SLC4A4
[491]	SLC4A7	SLC6A14	SMARCA1	SMARCA5	SMT3H2
[496]	SMURF2	SNAPC1	SNARK	SNRPG	SOD2
[501]	SORL1	SPG4	SPINK1	SPTA1	SPTLC2
[506]	SQRDL	SSNA1	SSR2	STIM1	STK17A
[511]	STX10	STX4A	SULT1A1	SV2C	SYK
[516]	TAF4	TAPBP-R	TBX6	TCEB2	TCF20
[521]	TFAM	TFEB	TFRC	THG-1	TIMP1
[526]	TLK1	TLR2	TLR4	TLR6	TMSNB
[531]	TNF	TNFRSF10B	TNFRSF6	TNFSF15	TNKS
[536]	TPMT	TPRA40	TPT1	TPTE	TRAF6
[541]	TRIM8	TRIP-Br2	TRPC4AP	TSC22	TSPAN-3
[546]	TUBA1	TUBB2	TUSC2	TXNIP	TXNL2
[551]	UBE1	UBE2I	UBE2L3	UBE2N	UBE3A
[556]	UGCG	ULK1	UNC84B	UQCRB	VAMP3
[561]	VCP	VEGF	VNN3	VPS28	WAC
[566]	WAS	WBP2	WBSCR5	XBP1	ZBTB7
[571]	ZFP106	ZNF216	ZNF239	ZNF281	ZNF313
[576]	ZNF337	ZNF36	ZNF435		

Table A.8. Those genes not in differentially regulated gene set (gene set A) but only in the response gene set under treatment (gene set B). Total 1670 genes.

[1]	3.8-1	AA017721	AA203487	AA355179	AA365670	AA521267
[7]	ABCA1	ABCB9	ABCC1	ABCC3	ABCD1	ABCF2
[13]	ABCG1	ABHD14B	ABHD3	ABI1	ACACB	ACBD3
[19]	ACIN1	ACSL1	ACSL3	ACSL5	ACTN1	ACTR1A
[25]	ACTR3	ADAM10	ADAM19	ADAM22	ADAM9	ADAMDEC1
[31]	ADAMTS8	ADH1B	ADMR	ADRB2	ADRBK1	AF103530
[37]	AF291676	AF320070	AGK	AGPAT7	AI278204	AI472320
[43]	AI523613	AI683552	AI695595	AK025360	AK026682	AK1
[49]	AK3L1	AK3L2	AKAP8L	AL044078	AL049435	AL109696
[55]	AL137624	AL390145	ALG13	ALLC	ALOX5	ALOX5AP
[61]	ALPL	AMPH	ANGPT1	ANKHD1	ANKRD15	ANP32E
[67]	ANPEP	ANXA11	ANXA4	AOAH	AOC2	APIG1
[73]	AP1S1	AP3M2	APBA3	APC	APLP2	APOBEC3A
[79]	APOBEC3B	APBP2	AQP3	ARF6	ARFGEF1	ARFGEF2
[85]	ARFIP1	ARFRP1	ARHGAP1	ARHGAP15	ARHGAP19	ARHGAP26
[91]	ARHGEF18	ARHGEF3	ARHGEF4	ARID4B	ARID5A	ARL3
[97]	ARL6IP2	ARL8B	ARMC9	ARPC2	ARPC3	ARPP-19
[103]	ARS2	ASPH	ASXL1	ATBF1	ATG7	ATHL1
[109]	ATOX1	ATP13A3	ATP2A3	ATP2B1	ATP2C2	ATP5F1
[115]	ATP6V1F	ATP8B4	ATPBD1C	ATXN1	ATXN3	ATXN3L
[121]	AU144792	AU147851	AUH	AW301235	AW836210	AYTL2
[127]	AZGP1	AZIN1	B4GALT1	B4GALT2	B4GALT4	BAG2
[133]	BAT1	BAT2	BAT2D1	BAZ1A	BAZ2B	BBS4
[139]	BC002791	BC003528	BC006164	BCAS2	BCAT1	BCL2A1
[145]	BCLAF1	BE327172	BF114906	BIN3	BIRC2	BLVRB
[151]	BLZF1	BMP2	BMP6	BMX	BNIP3	BRCA1
[157]	BRCC3	BZRAP1	BZW1	C10orf95	C11orf30	C12orf5
[163]	C13orf15	C13orf18	C13orf24	C14orf32	C15orf29	C15orf39
[169]	C16orf68	C16orf72	C16orf80	C17orf68	C19orf10	C19orf22
[175]	C1D	C1orf107	C1orf183	C1orf38	C1orf63	C1QTNF1
[181]	C1R	C1RL	C20orf32	C20orf67	C21orf33	C2orf25
[187]	C3AR1	C4BPB	C4orf16	C4orf20	C5AR1	C5orf15
[193]	C6orf106	C6orf108	C6orf111	C6orf211	C6orf32	C6orf62
[199]	C7orf42	C7orf44	C9orf46	CA2	CABIN1	CACNA1I
[205]	CACNA2D3	CADM3	CALU	CAMTA2	CAND2	CANT1
[211]	CAP1	CAPG	CAPRIN1	CARS2	CASP8	CAST
[217]	CAT	CBFA2T3	CBWD1	CBX1	CBX4	CCDC49
[223]	CCDC76	CCDC93	CCL18	CCL2	CCNA1	CCND3
[229]	CCNH	CCNL1	CCNT2	CCPG1	CCT6A	CD22
[235]	CD226	CD2BP2	CD302	CD37	CD46	CD55
[241]	CD58	CD68	CD82	CD93	CD97	CDC2L2

[247]	CDC2L6	CDC42	CDC42EP2	CDC42EP3	CDKN2D	CDV3
[253]	CEBPD	CENTB2	CENTD2	CENTD3	CEP164	CEP170
[259]	CEP63	CETN2	CFP	CHD1	CHD4	CHD9
[265]	CHI3L1	CHIC2	CHMP1B	CHMP2A	CHMP2B	CHMP5
[271]	CHRNA10	CHST11	CHST8	CIR	CITED2	CIZ1
[277]	CKAP4	CLASP2	CLCN3	CLCN4	CLCN6	CLDND1
[283]	CLEC1B	CLEC5A	CLIC1	CLINT1	CLIP1	CLK1
[289]	CLK2	CLN5	CLU	CNN2	CNOT2	CNOT3
[295]	CNOT8	COL13A1	COL1A1	COL6A3	COMMD4	COPE
[301]	CPD	CPM	CPVL	CREBL2	CREG1	CRHR1
[307]	CRIP2	CRIP2	CRISPLD2	CRK	CRKL	CROP
[313]	CRSP2	CRY1	CSNK1A1	CSNK1D	CSNK2A1	CST3
[319]	CTBS	CTGLF1	CTSB	CTSE	CTSL1	CTSL2
[325]	CTSS	CTSW	CUGBP1	CUTL1	CXCL5	CXXC1
[331]	CYB5R4	CYFIP2	CYHR1	CYLD	CYP17A1	CYP19A1
[337]	CYP4F2	CYP4F3	D85181	DAPP1	DCTN3	DDIT3
[343]	DDIT4	DDX18	DDX21	DDX23	DDX39	DDX5
[349]	DDX52	DENND1A	DENND2D	DENND3	DERL2	DFNA5
[355]	DHCR7	DHRS12	DHRS7	DHRS9	DHTKD1	DHX34
[361]	DHX8	DIAPH2	DIS3	DLAT	DLC1	DLG3
[367]	DMC1	DMXL1	DMXL2	DNAJA1	DNAJA2	DNAJB12
[373]	DNAJB14	DNAL4	DNASE1L1	DNPEP	DNTTIP2	DOCK4
[379]	DOK3	DPEP3	DPM2	DRAM	DSCR1L1	DSCR3
[385]	DSE	DTX2	DUSP1	DUSP2	DUSP3	DYNLT3
[391]	DYSF	E2F3	E2F8	EBI3	ECE1	ECGF1
[397]	EDD1	EDEM3	EDG4	EFHC2	EFHD2	EGLN1
[403]	EHD1	EIF1	EIF1B	EIF2AK3	EIF2S1	EIF2S2
[409]	EIF3S1	EIF3S10	EIF4A1	EIF4A3	EIF4G3	EIF5
[415]	EIF5A	ELF1	ELF2	ELF3	ELF4	ELK3
[421]	ELL	ELL2	ELL3	ELN	EMD	ENC1
[427]	ENO3	ENOPH1	ENPP4	ENSA	EP300	EPB41L3
[433]	EPB41L5	EPM2AIP1	EPOR	EPS15L1	ERBB2IP	ERLIN1
[439]	ESF1	ESR2	ESRRA	ETF1	ETNK1	ETS1
[445]	EVA1	EVI2A	EWSR1	EXOSC4	EXTL3	F8A1
[451]	FAM120A	FAM128B	FAM129A	FAM3C	FAM49A	FAM49B
[457]	FAM63A	FAM65A	FAS	FBXL15	FBXL5	FBXO31
[463]	FBXO34	FBXO42	FBXW7	FCAR	FCGR3B	FCGRT
[469]	FCHSD2	FCN1	FDPS	FEM1B	FGFR1	FGFR1OP
[475]	FIS1	FKBP15	FKBP1A	FKBP8	FKSG30	FLCN
[481]	FLJ10154	FLJ10213	FLJ10357	FLJ11151	FLJ11506	FLJ12529
[487]	FLJ12716	FLJ12886	FLJ14213	FLJ20186	FLJ20254	FLJ20273
[493]	FLJ20433	FLJ21908	FLJ22662	FLJ23861	FLOT1	FMR1
[499]	FNBP4	FNDC3A	FNDC3B	FOS	FOSB	FOSL1
[505]	FOSL2	FOXJ3	FOXK2	FRAT2	FRMD4B	FSHB

[511]	FSTL3	FTH1	FUT4	FUT7	FXYD5	FYB
[517]	FYN	GABARAPL1	GABARAPL3	GABPB2	GAD1	GAL
[523]	GALC	GALNACT-2	GALNT2	GALNT7	GAPDH	GAPVD1
[529]	GARNL1	GARNL4	GARS	GAS7	GATAD1	GCA
[535]	GDPD3	GFPT1	GGA3	GGT3	GHITM	GIMAP4
[541]	GIMAP6	GIN51	GIT2	GK	GLA	GLUL
[547]	GMFG	GNAI3	GNAQ	GNB1	GNB2	GNB5
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[559]	GPLD1	GPR171	GPR175	GPR176	GPR177	GPR18
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[571]	GRPEL1	GSH1	GSPT1	GSR	GTF2H1	GTPBP1
[577]	GTPBP4	GUCA1B	GUK1	GYG1	GZMA	H2AFY
[583]	H2AFZ	H3F3B	HARS2	HAT1	HBEGF	HBXIP
[589]	HCFC2	HCK	HCLS1	HDAC2	HDLBP	HERC4
[595]	HERPUD1	HEXA	HIF1A	HIP1	HIST1H1C	HIST1H2AC
[601]	HIST1H2AJ	HIST1H2BC	HIST1H3G	HIST1H3H	HIST2H2AA3	HIST2H2BE
[607]	HIVEP1	HIVEP2	HK3	HLA-E	HLA-F	HLA-G
[613]	HMG20B	HMHA1	HNF4A	HNRPA3	HNRPC	HNRPD
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[625]	HPN	HPS5	HRH1	HRK	HS2ST1	HSD17B14
[631]	HSD17B4	HSDL2	HSMPP8	HSP90AA1	HSP90AB1	HSPA4
[637]	HSPA6	HSPA9	HTR2A	HTR2B	HTR6	HTRA2
[643]	IARS2	IBRDC3	ICAM4	ICK	IDH3G	IDI1
[649]	IER2	IFI16	IFIH1	IFIT2	IFITM1	IFITM2
[655]	IFITM3	IFNAR1	IFNG	IFNGR1	IFNGR2	IFT57
[661]	IGF1R	IGFBP5	IGFBP7	IL10RB	IL11RA	IL18RAP
[667]	IL1A	IL1RAP	IL23A	IL24	IL4R	IL5RA
[673]	IL6R	IL6ST	ILF3	ING1	INHBC	INPP5A
[679]	INTS3	INTS8	IPO4	IPO7	IQGAP2	IQSEC1
[685]	IRAK1	IRAK3	IRF5	IRGC	IRS2	ISCA1
[691]	ISG20	ISG20L2	ITCH	ITGA2B	ITGA5	ITGA6
[697]	ITGAX	ITGB3	ITGB8	IVNS1ABP	JAKMIP2	JARID1B
[703]	JARID2	JMJD2B	JMJD3	JMJD6	JOSD1	JUN
[709]	JUNB	JUND	KCNAB3	KCNE1	KCNJ15	KCNJ5
[715]	KCNK1	KCNK7	KCNMB1	KCNQ1	KCTD13	KCTD2
[721]	KCTD20	KDELR3	KEAP1	KHDRBS1	KIAA0174	KIAA0241
[727]	KIAA0247	KIAA0286	KIAA0329	KIAA0404	KIAA0467	KIAA0692
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[739]	KIAA0999	KIAA1026	KIAA1033	KIAA1324	KIAA1539	KIAA1655
[745]	KIAA1815	KIAA1840	KIDINS220	KLC1	KLF2	KLF4
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[763]	KYNU	L35253	LAMA2	LAMA4	LAMB3	LAPTM5
[769]	LARP5	LCK	LDLR	LGALS3	LGALS8	LIF

[775]	LILRA5	LIMK2	LMNB1	LMO2	LOC130074	LOC137886
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[799]	LYRM4	M6PRBP1	MACF1	MAEA	MAFF	MAFG
[805]	MAG	MAGEA8	MAGEB2	MAGOH	MAK	MAN2B2
[811]	MANSC1	MAP2K3	MAP3K4	MAP3K5	MAP3K7IP2	MAPK1
[817]	MAPK13	MAPK14	MAPK8IP2	MAPKAPK2	MARCH2	MARCH6
[823]	MARCH7	MARCKS	MARK2	MARK3	MARS	MAST2
[829]	MBP	MBTD1	MCAM	MCAT	MCFD2	MCOLN1
[835]	MCTP1	MCTP2	MDM2	MECP2	MEF2A	MEF2D
[841]	METTL3	MGAM	MGC13098	MGC14376	MICA	MIER2
[847]	MKKS	MLF1IP	MLSTD1	MLX	MMD	MMP1
[853]	MMP14	MMS19L	MOBK1B	MORC3	MORF4L2	MOXD1
[859]	MRPL12	MRPL34	MS4A6A	MSC	MSRA	MSRB2
[865]	MT1E	MT1JP	MT1X	MT3	MTA1	MTERFD1
[871]	MTF1	MTHFS	MTM1	MTMR10	MTMR2	MTMR6
[877]	MVK	MVP	MX2	MXD1	MXD3	MXI1
[883]	MYCBP	MYD88	MYH7	MYH9	MYO9B	N4BP1
[889]	NAB1	NACAP1	NAGK	NANS	NAPA	NBEAL2
[895]	NBN	NBR1	NCF4	NCK1	NCLN	NCOR2
[901]	NCR2	NDEL1	NDFIP1	NDRG1	NDUFB8	NDUFV2
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[1063]	OAT	OAZ1	OCLM	ODC1	ODZ2	OGT
[1069]	OLFM4	OSBPL1A	OSBPL8	OSGEP	OXSRI	P2RY13
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[1099]	PER1	PER2	PF4	PFAAP5	PGD	PGK1
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[1111]	PHF3	PHKA2	PHLPP	PIAS1	PID1	PIGB
[1117]	PIGV	PIK3CD	PIK3CG	PILRA	PIP5K2A	PIR
[1123]	PITPNA	PKIG	PKM2	PKN2	PLA1A	PLA2G4C
[1129]	PLA2G6	PLA2G7	PLAUR	PLCB2	PLD1	PLEC1
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[1141]	PMF1	PMVK	PNN	POLG2	POLM	POLR3E
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[1153]	PPP1CB	PPP1R10	PPP1R12A	PPP1R14B	PPP2CB	PPP2R3C
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[1165]	PREI3	PREPL	PRKAA1	PRKAR1A	PRKAR2B	PRKCB1
[1171]	PRKCH	PRKCI	PRKD2	PRKD3	PRKDC	PRPF18
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[1183]	PRSS3	PSD4	PSMA3	PSMC5	PSMD13	PSMD3
[1189]	PSMF1	PSORS1C2	PTAFR	PTEN	PTP4A1	PTPN12
[1195]	PTPN18	PTPRE	PTPRN	PTRH2	PTS	PTTG1IP
[1201]	PUM2	PYCR1	QKI	QSCN6	RAB11A	RAB13
[1207]	RAB1A	RAB21	RAB27A	RAB31	RAB3D	RAB6IP1
[1213]	RAB8B	RABGAP1L	RABGEF1	RABGGTA	RABL2A	RAC2
[1219]	RAD51	RAGE	RALB	RANBP2	RANBP3	RANBP5
[1225]	RAPGEF2	RARA	RASGRF1	RASGRP2	RB1CC1	RBM16
[1231]	RBM22	RBM26	RBM28	RBM38	RBM5	RBM7
[1237]	RBM9	RBPJ	RC3H2	RCBTB2	RCN2	RCN3
[1243]	RCOR1	REC8	REPS2	RFK	RGL1	RGL2
[1249]	RGPD5	RGS12	RGS7	RHOF	RHOQ	RHOT1
[1255]	RIF1	RIN2	RIOK3	RIPK1	RLN2	RNF10
[1261]	RNF111	RNF138	RNF141	RNF167	RNF170	RP4-724E16.2
[1267]	RPGRIP1	RPL10	RPS6KB2	RRM1	RRN3	RRP12
[1273]	RSRC2	RTN2	RTN4	RXRG	RYBP	S100A12
[1279]	S100A9	S100P	SAFB	SAMD9	SAMSN1	SAP18
[1285]	SAP30	SAP30BP	SAP30L	SAR1A	SAT1	SBNO2
[1291]	SCARA3	SCARF1	SCN1B	SCO2	SCRN3	SDC2

[1297]	SDCCAG3	SDHD	SEC22B	SEC23IP	SECISBP2	SECTM1
[1303]	SEL1L	SELL	SELPLG	SEMA6B	SENP3	SENP5
[1309]	SENP6	SEP15	SERBP1	SERINC3	SERP1	SERPINB1
[1315]	SERPINB7	SERPINB8	SERPINI2	SERTAD2	SETX	SF1
[1321]	SF3B4	SFN	SFRS15	SFRS2	SFRS2IP	SFRS3
[1327]	SFRS5	SFT2D2	SGK3	SH2B2	SH2D3A	SH2D3C
[1333]	SH3GLB1	SHCBP1	SIGLEC7	SIRPA	SIRPG	SIRT7
[1339]	SKAP2	SKI	SKIL	SLA	SLAMF7	SLC11A1
[1345]	SLC14A2	SLC15A3	SLC19A2	SLC1A2	SLC1A3	SLC24A1
[1351]	SLC25A32	SLC25A37	SLC25A44	SLC26A2	SLC2A6	SLC31A2
[1357]	SLC35A2	SLC35E3	SLC39A6	SLC39A8	SLC3A2	SLC4A2
[1363]	SLC5A3	SLC6A6	SLC7A11	SLC7A5	SLC9A8	SLCO4A1
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[1387]	SON	SOS2	SP100	SPAG1	SPAG5	SPAG7
[1393]	SPAG9	SPATA2L	SPATA5L1	SPATA6	SPCS1	SPI1
[1399]	SPINT1	SPINT2	SPOCK2	SPTB	SPTBN1	SPTLC1
[1405]	SRC	SRF	SRGAP3	SRGN	SRP72	SS18
[1411]	SSFA2	SSR1	SSSCA1	ST13	ST3GAL1	ST3GAL2
[1417]	ST8SIA4	STAG1	STAG2	STAM2	STARD8	STAT3
[1423]	STAT5B	STATH	STK10	STMN1	STRAP	STS
[1429]	STX16	STX3	STX4	STX7	STXBP2	SUB1
[1435]	SUCLG1	SUMO1	SUPT6H	SUPV3L1	SVIL	SYCP2
[1441]	SYNCRIP	SYNE2	SYNJ1	TAF1C	TAF7	TAF9
[1447]	TAGLN2	TAL1	TANK	TAPBPL	TARDBP	TBC1D1
[1453]	TBC1D22A	TBC1D2B	TBC1D3	TBK1	TBXAS1	TCFL5
[1459]	TDG	TECT1	TES	TFDP1	TFR2	TGFBR3
[1465]	TGIF2	THBD	THBS1	THOC5	THRAP1	THRAP2
[1471]	THRAP5	TIA1	TICAM1	TIMM17A	TIPARP	TLE3
[1477]	TLN1	TLR5	TM2D3	TM9SF2	TM9SF3	TMCO1
[1483]	TMCO3	TMED10	TMED2	TMED7	TMEM110	TMEM165
[1489]	TMEM24	TMEM33	TMEM4	TMEM41B	TMEM53	TMEM8
[1495]	TMEPA1	TNFRSF1B	TNFRSF8	TNFRSF9	TNIP1	TNNI2
[1501]	TOB1	TOM1	TOMM20	TOP1	TOPORS	TOR1A
[1507]	TOR1AIP1	TOR1B	TPD52L2	TPP1	TPR	TRA2A
[1513]	TRAC	TRADD	TRAF3	TRAF3IP2	TRAF3IP3	TRAPPC3
[1519]	TREM1	TREML2	TRIB1	TRIB3	TRIM13	TRIM16
[1525]	TRIM27	TRIM34	TRIM36	TRIP10	TRIP12	TRPC2
[1531]	TSC22D2	TSEN34	TSNAX	TSPAN2	TSPAN3	TTC1
[1537]	TTC19	TTC35	TTF1	TTN	TUBA1A	TUBA1B
[1543]	TUBA1C	TUBA3D	TUBA4A	TXNDC13	TXNRD1	U34919
[1549]	U56725	UAP1	UBAP1	UBAP2L	UBE2B	UBE2D1
[1555]	UBE2D3	UBE2E1	UBE2H	UBE2J1	UBE2S	UBE4B

[1561]	UBL3	UCL5	UCKL1	UCP2	UGP2	UIMC1
[1567]	UPB1	UPF3A	UPP1	UQCRC2	USP10	USP15
[1573]	USP33	USP4	UTP18	UTP6	VAC14	VCAN
[1579]	VCL	VDP	VDR	VEGFA	VIL2	VILL
[1585]	VIM	VPS24	VPS37B	VPS41	W88821	WDR1
[1591]	WDR26	WDR42A	WDR47	WDR74	WHDC1	WIPF1
[1597]	WSB1	WSB2	WWC1	XM_094581	XM_208778	XM_370838
[1603]	XM_372632	XM_374529	XM_378250	XM_496132	XM_496217	XM_497663
[1609]	XM_498825	XM_498877	XM_499165	XPO1	XPO6	YAF2
[1615]	YIPF4	YIPF6	YPEL5	YRDC	YTHDC1	YWHAZ
[1621]	YY1	ZBED1	ZBTB1	ZBTB17	ZBTB3	ZBTB43
[1627]	ZC3H12A	ZC3H7A	ZCCHC10	ZCCHC14	ZCCHC6	ZDHHHC17
[1633]	ZEB1	ZEB2	ZFAND5	ZFP36	ZH2C2	ZHX2
[1639]	ZMAT3	ZMYM1	ZMYND10	ZNF124	ZNF155	ZNF165
[1645]	ZNF177	ZNF202	ZNF221	ZNF225	ZNF238	ZNF24
[1651]	ZNF250	ZNF254	ZNF259	ZNF267	ZNF277P	ZNF331
[1657]	ZNF350	ZNF394	ZNF500	ZNF508	ZNF552	ZNF586
[1663]	ZNF589	ZNF652	ZNF668	ZNF675	ZNF692	ZNF783
[1669]	ZNF84	ZZEF1				

Table A.9. Those genes in both differentially regulated gene set (gene set A) and in the response gene set under treatment (gene set C). Total 80 genes.

[1]	ABHD5	ADD1	AMD1	ANXA5	ARTS-1	BRD8	BTG3	BTN3A1
[9]	C14orf159	C20orf121	CAMK2G	CCL19	CCL3	CCL4	CD48	CD59
[17]	CD69	CIB1	CLCN7	CLIC4	COX5B	CREB5	CSAD	CSTB
[25]	DUSP10	FABP5	FLI1	GALNT3	GCLM	GGT1	GMFB	IL12B
[33]	IL1F9	INHBA	INPP4A	ISGF3G	ITM2B	ITPR1	JAG1	KIAA0143
[41]	KIF1B	LIMS1	LOC54103	M6PR	MAN1A1	MATR3	MCM4	MGLL
[49]	MPZL1	ORM1	P2RX4	PDPK1	PGCP	PGLS	PLSCR4	PSMA6
[57]	PSMD12	PSMD4	RAD21	RAD23B	RERE	RNPEP	RPS6KA5	RPS9
[65]	S100A6	SEC23B	SERPINB9	SIAH1	SLBP	SLC43A3	SLC7A7	SLPI
[73]	SPG20	SPHK1	SPINLW1	TBC1D2	TKT	TNFRSF1A	TRA@	VNN2

Table A.10. Those genes only in differentially regulated gene set (gene set A) but not in the response gene set under treatment (gene set C). Total 730 genes.

[1]	101F6	2TRPA1	ABAT	ABCA7	ABHD2	ACAT2
[7]	ACINUS	ACTN4	ADA	ADAM17	ADAM8	ADCY3
[13]	ADD3	ADORA2A	ADRA1A	ADSL	AGTPBP1	AHCY
[19]	AIF1	AK2	AK3	AKT1	ALAS1	AMPD2
[25]	AMPD3	ANP32B	ANXA7	APG4B	AQP9	ARCN1
[31]	ARFGAP3	ARHGAP11A	ARHGAP4	ARHGDIB	ARHGEF6	ARHQ
[37]	ARHT1	ARIH1	ARPC4	ASK	ATF1	ATF5
[43]	ATP1B1	ATP2B4	ATP5J	ATP6V1A1	ATPIF1	AUP1
[49]	B3GNT4	B4GALT5	BAGE	BCAP31	BCL10	BCL11A
[55]	BCL3	BCOR	BFAR	BICD2	BID	BIGM103
[61]	BIN2	BM045	BMP2K	BRAF	BRD1	BTBD14A
[67]	BTF	BTG1	BTG2	BTK	BTN2A1	BTN3A2
[73]	BTN3A3	C1orf10	C13orf10	C14orf109	C14orf147	C19orf7
[79]	C1orf16	C1orf24	C1QBP	C20orf104	C21orf66	C21orf91
[85]	C22orf19	C3	C9orf10	CA4	Cab45	CABC1
[91]	CAMKK2	CANX	CAPN7	CASC3	CASP2	CASP4
[97]	CBX7	CCL20	CCNG2	CCR1	CCR3	CCR9
[103]	CCRL2	CD44	CD74	CD9	CDA	CDC16
[109]	CDC34	CDC5L	CDC6	CECR5	CES1	CFLAR
[115]	CGI-72	CHES1	ChGn	CHS1	CHST2	CHST6
[121]	CHST7	cig5	CIRBP	CKLF	CLC	CLECSF12
[127]	CLECSF6	CLECSF9	CLN2	CMAH	COL15A1	COL18A1
[133]	COPEB	COPS3	CORO1A	CPR8	CR1	CREB1
[139]	CREM	CRSP3	CSF3R	CSNK1G2	CSPG2	CTBP1
[145]	CTBP2	CTNNA1	CUGBP2	CXCL1	CXCL2	CXCL3
[151]	DAMS	DARS	DCL-1	DDEF1	DGCR2	DIAPH1
[157]	DICER1	DKFZP434C171	DKFZP566A1524	DKFZp586I1420	DKFZP586L151	DKFZP586M1523
[163]	DKFZp761P1010	DLEU2	DLGAP4	DMN	DNAH7	DNAH9
[169]	DNAJB6	DNAJB9	DNAJC8	DOK1	DPEP2	DSIP1
[175]	DUSP6	E1B-AP5	EEF1A1	EGLN2	EGR3	EHD4
[181]	EIF3S6IP	EIF4E	EIF4EL3	ELOVL5	EMR3	ERAL1
[187]	EREG	ETFA	ETS2	EZH1	EZI	F2RL1
[193]	F5	FACL3	FACL6	FAD104	FBS1	FBXO11
[199]	FBXO9	FCER1G	FCGR2A	FCGR3A	FDX1	FETUB
[205]	FGF7	FGL2	FGR	FHOD1	FLII	FLJ10055
[211]	FLJ10707	FLJ10726	FLJ10858	FLJ10996	FLJ11036	FLJ11088
[217]	FLJ11142	FLJ11259	FLJ12150	FLJ13195	FLJ13386	FLJ20038
[223]	FLJ20189	FLJ20274	FLJ20287	FLJ20373	FLJ20449	FLJ20502
[229]	FLJ20530	FLJ20559	FLJ20811	FLJ20986	FLJ20989	FLJ21047
[235]	FLJ21308	FLJ21588	FLJ22169	FLJ22649	FLJ22843	FLJ22938

[241]	FLJ23056	FLJ23142	FLJ23231	FLJ90005	FLRT2	FMNL
[247]	FNBP1	FOXO1A	FOXO3A	FPR1	FPRL1	FRAT1
[253]	FZD6	G0S2	G2	G3BP	G3BP2	GADD45B
[259]	GAGE4	GATA2	GBE1	GBP1	GCH1	GCLC
[265]	GGTLA4	GL004	GLIPR1	GLRX	GLRX2	GMCL
[271]	GMIP	GMPR2	GOLPH3	GPNMB	GPR58	GPR86
[277]	GPS2	GPX3	GRP58	GTF2H2	H1FX	H2AFX
[283]	H2AV	HA-1	HAL	HCGVIII-1	HDAC1	HEBP2
[289]	HFE	HGF	HHEX	HIPK1	HIST1H2BK	HMGCL
[295]	HMGCS1	HMGNI	hnRNPA3:	HNRPDL	HOXB7	HPIP
[301]	HRH4	HRIHFB2122	HS3ST2	HSPA1B	ICAM1	ICAM3
[307]	IDS	IER3	IER5	IFI30	IFNA10	IFRD1
[313]	IKBKG	IL13RA1	IL1B	IL1R1	IL1R2	IL1RN
[319]	IL22RA1	IL6	IL7R	IL8	IL8RA	IL8RB
[325]	IMPA2	INPP5D	INPPL1	INSIG1	IQGAP1	IRF1
[331]	IRF3	IRS1	ITGAL	ITGAM	ITGB1	ITGB2
[337]	ITPK1	JK	KCNB1	KCNJ14	KIAA0053	KIAA0103
[343]	KIAA0140	KIAA0146	KIAA0191	KIAA0222	KIAA0290	KIAA0352
[349]	KIAA0415	KIAA0433	KIAA0471	KIAA0561	KIAA0592	KIAA0625
[355]	KIAA0650	KIAA0779	KIAA0802	KIAA0847	KIAA0853	KIAA0876
[361]	KIAA0930	KIAA0980	KIAA1039	KIAA1049	KIAA1109	KIAA1185
[367]	KIAA1240	KIAA1466	KIAA1473	KIF14	KMO	KPNB2
[373]	LAMP1	LAMP2	LBR	LCT	LEPR	LILRA1
[379]	LILRA2	LILRB2	LILRB3	LIM	LIN7C	LOC115207
[385]	LOC116150	LOC54499	LOC56267	LOC90410	LPIN2	LRMP
[391]	LST1	LTA4H	LTBR	LUC7L2	LYPLA2	LYZ
[397]	MADH4	MAF	MAN2A2	MAP3K2	MAP3K3	MAP3K8
[403]	MAP4K4	MAPK6	MAX	MBD4	MBTPS1	MCL1
[409]	ME1	ME2	MEIS2	MEL	MGAT2	MGC10986
[415]	MGC12518	MGC17528	MGC3121	MGC31957	MGC8902	MGEA5
[421]	MGRN1	MIR	MIR16	MKL1	MKRN1	MLF2
[427]	MLL4	MME	MMP25	MNDA	MPPE1	MRCL3
[433]	MRLC2	MRPL44	MT1G	NACA	NARF	NCF1
[439]	NCKAP1	NCOA1	NDUFA2	NDUFB6	NEK3	NFATC3
[445]	NFKB1	NFKBIA	NFYC	NICAL	NINJ2	NMI
[451]	NPAT	NR4A2	NR4A3	NRIP3	NSEP1	NSFL1C
[457]	NSMAF	NUP214	Nup37	NUPL1	OGFR	OLR1
[463]	oncogene	OPHN1	OR1D2	OR1E1	OSBPL2	OSM
[469]	OSTM1	OTUB1	PAK1	PAM	PBX2	PC326
[475]	PCOLN3	PCTP	PDE4B	PDE4D	PDE4DIP	PDX1
[481]	PDXK	PECAM1	PEL1	PEX3	PEX6	PFC
[487]	PFDN4	PFKFB3	PFN1	PHF11	PHLDA1	PHLDA2
[493]	PI3	PIASY	PICALM	PIGA	PILB	PINK1
[499]	PKP2	PLA2G4A	PLAGL2	PLAU	PLCL2	PLEK

[505]	PLEKHB2	PLEKHE1	PLS3	PLXNC1	PMAIP1	POLR2J
[511]	PPAP2B	PIIF	PPM1F	PPP1CA	PPP1R15A	PPP1R9A
[517]	PPP2R5C	PPP6C	PREB	PRKAB1	PRKAR2A	PRKCL2
[523]	PRO0097	PRO0971	PRO2037	PRO2198	proteoglycan	PRPF4B
[529]	PSG1	PSMB10	PSMB4	PSMB8	PSME2	PSME4
[535]	PTD008	PTGER3	PTGER4	PTGS2	PTK2	PTK9L
[541]	PTN	PTP4A2	PTPN13	PTPN2	PTX3	PYGL
[547]	RAB20	RAB33B	RAB5C	RAD1	RAD51L3	RALBP1
[553]	RALGDS	RALY	RASA1	RBMS1	RBSK	REL
[559]	RGS14	RGS2	RHAG	RHOBTB3	RI58	RIMS2
[565]	RNASE6	RNASE6PL	RNASET2	RNGTT	RNPC1	ROCK2
[571]	RPEL1	RPL13	RPL18A	RPL27A	RPL3	RPL30
[577]	RPL34	RPL39	RPL5	RPS11	RPS14	RPS17
[583]	RPS18	RPS27A	RPS6KA1	RPS6KA3	RUVBL1	RXRA
[589]	S100A11	S100A4	SALL1	SAT	SCAMP1	SCAP
[595]	SCARB2	SCD4	SCN11A	SEC14L1	SEC22L1	SEC24A
[601]	SEPX1	SERPINB2	SERPINE2	SFPQ	SFRS6	SFRS7
[607]	SH3BP5	SH3GL3	SIAT4A	SIPA1	SLC11A2	SLC12A9
[613]	SLC19A1	SLC1A4	SLC21A11	SLC25A13	SLC30A9	SLC4A4
[619]	SLC4A7	SLC6A14	SLK	SMARCA1	SMARCA5	SMT3H2
[625]	SMURF2	SNAPC1	SNARK	SNN	SNRPG	SOD2
[631]	SORL1	SP110	SPG4	SPINK1	SPTA1	SPTLC2
[637]	SQRDL	SQSTM1	SRPK2	SSNA1	SSR2	STIM1
[643]	STK17A	STX10	STX4A	SULT1A1	SV2C	SYK
[649]	SYNJ2	TACC3	TAF4	TAPBP-R	TBX6	TCEB2
[655]	TCF20	TFAM	TFEB	TFEC	TFRC	THG-1
[661]	TIMP1	TLK1	TLR1	TLR2	TLR4	TLR6
[667]	TMSNB	TNF	TNFAIP3	TNFAIP6	TNFRSF10B	TNFRSF10C
[673]	TNFRSF6	TNFSF14	TNFSF15	TNKS	TNRC5	TPM4
[679]	TPMT	TPRA40	TPT1	TPTE	TRAF1	TRAF6
[685]	TRIM8	TRIP-Br2	TRPC4AP	TSC22	TSPAN-3	TUBA1
[691]	TUBB2	TUSC2	TXNIP	TXNL2	UBE1	UBE2G1
[697]	UBE2I	UBE2L3	UBE2N	UBE3A	UBN1	UGCG
[703]	ULK1	UNC84B	UQCRB	VAMP2	VAMP3	VCP
[709]	VEGF	VNN3	VPS28	WAC	WAS	WBP2
[715]	WBSR5	WTAP	XBP1	ZBTB7	ZFP106	ZFP36L2
[721]	ZFX	ZFYVE26	ZNF216	ZNF239	ZNF281	ZNF313
[727]	ZNF337	ZNF36	ZNF435	ZYX		

Table A.11. Those genes not in differentially regulated gene set (gene set A) but in the response gene set under treatment (gene set C). Total 928 genes.

[1] 3.8-1	AA017721	AA203487	AA355179	AA365670	ABCB9
[7] ABCD1	ABCF2	ABHD14B	ABHD3	ACACB	ACBD3
[13] ACIN1	ACSL5	ACTR1A	ADAM19	ADAM22	ADAMTS8
[19] ADH1B	ADMR	ADRB2	ADRBK1	AF103530	AF291676
[25] AGK	AI278204	AI472320	AI523613	AI683552	AK025360
[31] AK026682	AK1	AK3L1	AK3L2	AKAP8L	AL044078
[37] AL049435	AL109696	AL137624	AL390145	ALLC	AMPH
[43] ANGPT1	ANKRD15	ANP32E	AOAH	AOC2	AP1S1
[49] AP3M2	APBA3	APC	APOBEC3B	APPBP2	AQP3
[55] ARF6	ARFGEF1	ARFGEF2	ARHGAP1	ARHGAP19	ARHGEF18
[61] ARHGEF3	ARHGEF4	ARL3	ARL6IP2	ARL8B	ARMC9
[67] ARPC2	ARPP-19	ARS2	ASPH	ASXL1	ATBF1
[73] ATG7	ATHL1	ATOX1	ATP2A3	ATP5F1	ATP6V1F
[79] ATXN3	ATXN3L	AU147851	AUH	AW301235	AW836210
[85] AZGP1	B4GALT2	B4GALT4	BAG2	BAT1	BAT2
[91] BAZ2B	BBS4	BC002791	BC003528	BC006164	BE327172
[97] BLVRB	BMP2	BMP6	BMX	BRCA1	BRCC3
[103] BZRAP1	C10orf95	C11orf30	C12orf5	C13orf15	C15orf39
[109] C16orf80	C17orf68	C19orf10	C1D	C1orf107	C1orf183
[115] C1QTNF1	C1R	C20orf32	C21orf33	C2orf25	C4BPB
[121] C4orf16	C4orf20	C5orf15	C6orf108	C6orf62	C7orf42
[127] C7orf44	C9orf46	CACNA1I	CACNA2D3	CADM3	CALU
[133] CAMTA2	CAND2	CAP1	CAPG	CAPRIN1	CAT
[139] CBFA2T3	CCDC49	CCL18	CCL2	CCNA1	CCT6A
[145] CD22	CD226	CD2BP2	CD302	CD68	CDC2L2
[151] CDC42EP2	CEP164	CETN2	CHD4	CHD9	CHI3L1
[157] CHIC2	CHRNA10	CHST8	CIR	CIZ1	CLASP2
[163] CLCN3	CLCN4	CLCN6	CLDND1	CLEC1B	CLINT1
[169] CLK2	CLN5	CNN2	CNOT8	COL13A1	COL6A3
[175] COMMD4	CPM	CREBL2	CREG1	CRHR1	CRIP2
[181] CRIPT	CRKL	CROP	CSNK2A1	CTGLF1	CTSB
[187] CTSE	CTSL2	CTSW	CUGBP1	CXCL5	CXXC1
[193] CYB5R4	CYHR1	CYP17A1	CYP19A1	CYP4F2	D85181
[199] DAPP1	DCTN3	DDIT3	DDX21	DDX23	DDX39
[205] DDX52	DENND1A	DENND3	DHRS12	DHRS9	DHTKD1
[211] DIAPH2	DIS3	DLAT	DLC1	DLG3	DMC1
[217] DMXL1	DMXL2	DNAJA1	DNAJB14	DNAL4	DNPEP
[223] DPEP3	DPM2	DRAM	DSCR1L1	DSCR3	DSE
[229] DTX2	DYNLT3	E2F8	EBI3	EDD1	EDEM3
[235] EDG4	EFHC2	EIF1B	EIF2S1	EIF2S2	EIF3S1
[241] EIF3S10	EIF4G3	EIF5A	ELF2	ELF3	ELK3

[247]	ELL3	ELN	ENC1	ENO3	ENOPH1	ENPP4
[253]	ENSA	EPB41L5	EPM2AIP1	EPS15L1	ESF1	ETNK1
[259]	ETS1	EVA1	EVI2A	EWSR1	FAM120A	FAM128B
[265]	FAM3C	FAM63A	FAM65A	FBXL15	FBXL5	FBXO31
[271]	FBXO42	FCGRT	FCHSD2	FDPS	FEM1B	FGFR1
[277]	FIS1	FKBP15	FKSG30	FLCN	FLJ10154	FLJ10213
[283]	FLJ12716	FLJ12886	FLJ14213	FLJ20186	FLJ20254	FLJ20433
[289]	FLJ21908	FNBP4	FNDC3B	FOXJ3	FOXK2	FSHB
[295]	FSTL3	FUT4	FXYD5	FYN	GAD1	GAL
[301]	GALC	GALNACT-2	GALNT2	GALNT7	GAPVD1	GARS
[307]	GAS7	GATAD1	GDPD3	GGA3	GGT3	GINS1
[313]	GK	GLA	GNB5	GNL1	GNPAT	GNS
[319]	GOLGA5	GPLD1	GPR175	GPR176	GPR177	GPR18
[325]	GREM1	GRINA	GRK5	GRPEL1	GSH1	GSR
[331]	GTF2H1	GTPBP1	GUCA1B	GUK1	GYG1	GZMA
[337]	HARS2	HBEGF	HBXIP	HCFC2	HCK	HDAC2
[343]	HDLBP	HERC4	HERPUD1	HEXA	HIP1	HIST1H2AJ
[349]	HIST1H3G	HIST1H3H	HIST2H2BE	HLA-F	HLA-G	HMG20B
[355]	HMHA1	HNF4A	HNRPM	HNRPU	HOXA1	HP1BP3
[361]	HPN	HRH1	HRK	HS2ST1	HSD17B14	HSMPP8
[367]	HSP90AA1	HSP90AB1	HTR2A	HTR2B	HTR6	HTRA2
[373]	IARS2	ICAM4	ICK	IDH3G	IFIH1	IFIT2
[379]	IFNAR1	IFNG	IFNGR1	IFT57	IGFBP5	IGFBP7
[385]	IL18RAP	IL1A	IL24	IL5RA	IL6ST	ILF3
[391]	INHBC	INTS3	INTS8	IPO4	IPO7	IRAK1
[397]	IRAK3	IRF5	IRGC	ISG20L2	ITCH	ITGA2B
[403]	ITGA6	ITGB3	ITGB8	JAKMIP2	JARID1B	KCNAB3
[409]	KCNE1	KCNJ5	KCNK1	KCNMB1	KCTD2	KCTD20
[415]	KDEL3	KEAP1	KHDRBS1	KIAA0286	KIAA0467	KIAA0692
[421]	KIAA0746	KIAA0892	KIAA0913	KIAA0922	KIAA0984	KIAA1324
[427]	KIAA1655	KIAA1815	KIAA1840	KLHL21	KLHL24	KPNA2
[433]	KPTN	KRCC1	KRT32	KRT6B	Kua-UEV	LAMA2
[439]	LAMA4	LILRA5	LOC130074	LOC152719	LOC339457	LOC388335
[445]	LOC388458	LOC439992	LOC440354	LOC51136	LRCH4	LRRC8D
[451]	LSM5	LY6G6D	LYRM4	M6PRBP1	MACF1	MAFG
[457]	MAG	MAGEA8	MAGEB2	MAN2B2	MAP3K4	MAP3K5
[463]	MAPK13	MAPK8IP2	MARCH2	MARK2	MAST2	MBTD1
[469]	MCAM	MCAT	MCFD2	MCOLN1	MCTP1	MCTP2
[475]	MDM2	MECP2	MEF2A	MGAM	MGC13098	MICA
[481]	MIER2	MKKS	MLX	MMD	MMP1	MMP14
[487]	MMS19L	MOXD1	MRPL12	MRPL34	MSC	MSRA
[493]	MSRB2	MT1E	MT1JP	MT3	MTA1	MTERFD1
[499]	MTF1	MTM1	MTMR10	MTMR2	MVK	MXD3
[505]	MXI1	MYCBP	MYH7	MYO9B	NAGK	NANS

[511]	NCOR2	NCR2	NDUFB8	NDUFV2	NEDD4L	NENF
[517]	NFE2L1	NGLY1	NHP2L1	NINJ1	NISCH	NM_000110
[523]	NM_000125	NM_000129	NM_000358	NM_000553	NM_000885	NM_001012478
[529]	NM_001250	NM_001622	NM_001787	NM_001993	NM_002015	NM_002185
[535]	NM_002228	NM_002562	NM_002746	NM_003017	NM_003072	NM_003103
[541]	NM_003137	NM_003266	NM_003605	NM_003810	NM_003885	NM_004233
[547]	NM_004682	NM_004846	NM_005259	NM_005821	NM_005965	NM_006021
[553]	NM_007319	NM_012115	NM_013421	NM_014129	NM_014242	NM_014645
[559]	NM_014796	NM_015372	NM_015987	NM_016415	NM_017795	NM_017874
[565]	NM_017920	NM_018605	NM_019061	NM_020037	NM_020149	NM_020213
[571]	NM_020415	NM_020661	NM_021730	NM_021941	NM_022837	NM_024614
[577]	NM_024716	NM_024777	NM_024853	NM_024984	NM_030897	NM_033111
[583]	NM_145237	NM_152516	NME5	NOL1	NOL14	NONO
[589]	NOTCH2NL	NOV	NPC1	NPHP4	NQO2	NR1H2
[595]	NR6A1	NRAS	NUFIP1	NUP210	NUP62	OCLM
[601]	ODZ2	OLFM4	OSBPL1A	OSGEP	PATZ1	PAX8
[607]	PAXIP1	PCF11	PCOLCE2	PCTK2	PCYT1A	PDCD4
[613]	PDE6B	PDK3	PDLIM1	PDLIM2	PDLIM3	PER2
[619]	PF4	PFAAP5	PGD	PHACTR2	PHC1	PHF20
[625]	PHLPP	PIGV	PIK3CD	PIK3CG	PIP5K2A	PIR
[631]	PKIG	PKM2	PLA1A	PLA2G6	PLD1	PLEC1
[637]	PLEKHF2	PLK2	PLOD1	PMF1	PMVK	POLG2
[643]	POLM	POLR3E	POMZP3	POPDC2	POU6F2	PPBP
[649]	PPIAL4	PPP1R12A	PPP2CB	PPP2R3C	PPP4R2	PQBP1
[655]	PRDM14	PREPL	PRKAA1	PRKAR2B	PRKCH	PRKCI
[661]	PRKDC	PRPF38B	PRPF4	PRPF6	PRPSAP1	PRR7
[667]	PRSS3	PSMA3	PSMC5	PSMD13	PSMD3	PSORS1C2
[673]	PTPN18	PTPRN	PTRH2	PTS	PTTG1IP	PUM2
[679]	PYCR1	RAB11A	RAB13	RABGAP1L	RABGGTA	RABL2A
[685]	RAD51	RAGE	RASGRF1	RBM16	RBM28	RBM38
[691]	RBM5	RBM7	RBM9	REC8	RFK	RGL1
[697]	RGPD5	RGS7	RIF1	RIN2	RIPK1	RLN2
[703]	RNF10	RNF111	RNF138	RP4-724E16.2	RPGRIP1	RPL10
[709]	RPS6KB2	RRM1	RRP12	RSRC2	RTN2	RXRG
[715]	SAFB	SAMD9	SAMSN1	SAP18	SAP30	SAP30L
[721]	SBNO2	SCARA3	SCN1B	SCRN3	SDCCAG3	SDHD
[727]	SEC23IP	SECISBP2	SEMA6B	SENP5	SEP15	SERPINB7
[733]	SERPINI2	SF1	SF3B4	SFN	SFRS15	SFT2D2
[739]	SH2D3A	SH2D3C	SH3GLB1	SHCBP1	SIGLEC7	SIRPA
[745]	SIRPG	SKAP2	SLAMF7	SLC14A2	SLC1A2	SLC24A1
[751]	SLC25A32	SLC26A2	SLC2A6	SLC35E3	SLC39A6	SLC39A8
[757]	SLC3A2	SLC4A2	SLC5A3	SLCO4A1	SMARCD3	SMARCE1
[763]	SMG7	SMPD2	SNAP23	SNRPA1	SNRPB	SNTB2

[769]	SNX1	SNX3	SPAG1	SPAG5	SPAG7	SPATA5L1
[775]	SPATA6	SPCS1	SPINT1	SPINT2	SPOCK2	SPTB
[781]	SPTBN1	SPTLC1	SRC	SRF	SRGAP3	SRP72
[787]	SSSCA1	ST13	ST3GAL1	ST8SIA4	STAG1	STARD8
[793]	STATH	STS	STXBP2	SUCLG1	SUPV3L1	SYCP2
[799]	SYNE2	SYNJ1	TAF1C	TAF9	TAL1	TAPBPL
[805]	TARDBP	TBC1D2B	TBC1D3	TCFL5	TDG	TECT1
[811]	TES	TFR2	TGIF2	TICAM1	TLN1	TLR5
[817]	TM9SF2	TM9SF3	TMCO1	TMCO3	TMED10	TMED7
[823]	TMEM110	TMEM165	TMEM24	TMEM33	TMEM53	TMEM8
[829]	TMEPAI	TNFRSF8	TNNI2	TOB1	TOMM20	TOPORS
[835]	TOR1A	TOR1B	TPR	TRA2A	TRAC	TRADD
[841]	TRAF3	TRAF3IP2	TRAF3IP3	TRAPPC3	TRIB3	TRIM13
[847]	TRIM16	TRIM34	TRIP10	TRPC2	TSNAX	TSPAN2
[853]	TSPAN3	TTC1	TTC35	TTN	U56725	UBE2E1
[859]	UBE2S	UBE4B	UCHL5	UCKL1	UCP2	UGP2
[865]	UIMC1	UPB1	UPP1	USP4	UTP18	UTP6
[871]	VAC14	VCAN	VDP	VILL	VPS41	WDR42A
[877]	WDR74	WHDC1	WSB1	WSB2	WWC1	XM_208778
[883]	XM_370838	XM_372632	XM_496132	XM_496217	XM_497663	XM_498825
[889]	XM_498877	XM_499165	XPO1	YAF2	YIPF6	YRDC
[895]	YTHDC1	YY1	ZBED1	ZBTB17	ZBTB3	ZC3H7A
[901]	ZCCHC10	ZCCHC14	ZDHHC17	ZEB2	ZFAND5	ZH2C2
[907]	ZMAT3	ZMYND10	ZNF124	ZNF155	ZNF177	ZNF202
[913]	ZNF221	ZNF225	ZNF238	ZNF254	ZNF259	ZNF277P
[919]	ZNF331	ZNF500	ZNF552	ZNF586	ZNF589	ZNF652
[925]	ZNF675	ZNF692	ZNF783	ZNF84		

Appendix B.

Computation Codes in R version 2.3.1 or latter:

```
##### for d1428: use raw text file to form the dataset.
tT0r1<-read.delim("tT0r1.txt",header=T)[,c(1,4)]
names(tT0r1)[2]<-"tT0r1"
tT0r1[1:3,]
dim(tT0r1)
tT0r2<-read.delim("tT0r2.txt",header=T)[,c(1,4)]
names(tT0r2)[2]<-"tT0r2"
tT0r2[1:3,]
dim(tT0r2)
tT0r3<-read.delim("tT0r3.txt",header=T)[,c(1,4)]
names(tT0r3)[2]<-"tT0r3"
tT0r3[1:3,]
dim(tT0r3)

tT1o5r1<-read.delim("tT1o5r1.txt",header=T)[,c(1,4)]
names(tT1o5r1)[2]<-"tT1o5r1"
tT1o5r1[1:3,]
dim(tT1o5r1)
tT1o5r2<-read.delim("tT1o5r2.txt",header=T)[,c(1,4)]
names(tT1o5r2)[2]<-"tT1o5r2"
tT1o5r2[1:3,]
dim(tT1o5r2)
tT1o5r3<-read.delim("tT1o5r3.txt",header=T)[,c(1,4)]
names(tT1o5r3)[2]<-"tT1o5r3"
tT1o5r3[1:3,]
dim(tT1o5r3)

tT3r1<-read.delim("tT3r1.txt",header=T)[,c(1,4)]
names(tT3r1)[2]<-"tT3r1"
tT3r1[1:3,]
dim(tT3r1)
tT3r2<-read.delim("tT3r2.txt",header=T)[,c(1,4)]
names(tT3r2)[2]<-"tT3r2"
tT3r2[1:3,]
dim(tT3r2)
tT3r3<-read.delim("tT3r3.txt",header=T)[,c(1,4)]
names(tT3r3)[2]<-"tT3r3"
tT3r3[1:3,]
dim(tT3r3)

tT6r1<-read.delim("tT6r1.txt",header=T)[,c(1,4)]
names(tT6r1)[2]<-"tT6r1"
tT6r1[1:3,]
dim(tT6r1)
tT6r2<-read.delim("tT6r2.txt",header=T)[,c(1,4)]
names(tT6r2)[2]<-"tT6r2"
tT6r2[1:3,]
dim(tT6r2)

tT9r1<-read.delim("tT9r1.txt",header=T)[,c(1,4)]
```

```

names(tT9r1) [2] <- "tT9r1"
tT9r1 [1:3,]
dim(tT9r1)
tT9r2<-read.delim("tT9r2.txt",header=T) [,c(1,4)]
names(tT9r2) [2] <- "tT9r2"
tT9r2 [1:3,]
dim(tT9r2)
tT9r3<-read.delim("tT9r3.txt",header=T) [,c(1,4)]
names(tT9r3) [2] <- "tT9r3"
tT9r3 [1:3,]
dim(tT9r3)
tT9r4<-read.delim("tT9r4.txt",header=T) [,c(1,2)]
names(tT9r4) [2] <- "tT9r4"
tT9r4 [1:3,]
dim(tT9r4)
tT9r5<-read.delim("tT9r5.txt",header=T) [,c(1,2)]
names(tT9r5) [2] <- "tT9r5"
tT9r5 [1:3,]
dim(tT9r5)
tT9r6<-read.delim("tT9r6.txt",header=T) [,c(1,2)]
names(tT9r6) [2] <- "tT9r6"
tT9r6 [1:3,]
dim(tT9r6)

tT12r1<-read.delim("tT12r1.txt",header=T) [,c(1,4)]
names(tT12r1) [2] <- "tT12r1"
tT12r1 [1:3,]
dim(tT12r1)
tT12r2<-read.delim("tT12r2.txt",header=T) [,c(1,4)]
names(tT12r2) [2] <- "tT12r2"
tT12r2 [1:3,]
dim(tT12r2)

tT24r1<-read.delim("tT24r1.txt",header=T) [,c(1,4)]
names(tT24r1) [2] <- "tT24r1"
tT24r1 [1:3,]
dim(tT24r1)
tT24r2<-read.delim("tT24r2.txt",header=T) [,c(1,4)]
names(tT24r2) [2] <- "tT24r2"
tT24r2 [1:3,]
dim(tT24r2)
tT24r3<-read.delim("tT24r3.txt",header=T) [,c(1,4)]
names(tT24r3) [2] <- "tT24r3"
tT24r3 [1:3,]
dim(tT24r3)
tT24r4<-read.delim("tT24r4.txt",header=T) [,c(1,2)]
names(tT24r4) [2] <- "tT24r4"
tT24r4 [1:3,]
dim(tT24r4)
tT24r5<-read.delim("tT24r5.txt",header=T) [,c(1,2)]
names(tT24r5) [2] <- "tT24r5"
tT24r5 [1:3,]
dim(tT24r5)
tT24r6<-read.delim("tT24r6.txt",header=T) [,c(1,2)]
names(tT24r6) [2] <- "tT24r6"
tT24r6 [1:3,]
dim(tT24r6)

```

```

d1428t<-merge(tT0r1,tT0r2, by="ID_REF")
d1428t<-merge(d1428t,tT0r3, by="ID_REF")

d1428t<-merge(d1428t,tT1o5r1, by="ID_REF")
d1428t<-merge(d1428t,tT1o5r2, by="ID_REF")
d1428t<-merge(d1428t,tT1o5r3, by="ID_REF")

d1428t<-merge(d1428t,tT3r1, by="ID_REF")
d1428t<-merge(d1428t,tT3r2, by="ID_REF")
d1428t<-merge(d1428t,tT3r3, by="ID_REF")

d1428t<-merge(d1428t,tT6r1, by="ID_REF")
d1428t<-merge(d1428t,tT6r2, by="ID_REF")

d1428t<-merge(d1428t,tT9r1, by="ID_REF")
d1428t<-merge(d1428t,tT9r2, by="ID_REF")
d1428t<-merge(d1428t,tT9r3, by="ID_REF")
d1428t<-merge(d1428t,tT9r4, by="ID_REF")
d1428t<-merge(d1428t,tT9r5, by="ID_REF")
d1428t<-merge(d1428t,tT9r6, by="ID_REF")

d1428t<-merge(d1428t,tT12r1, by="ID_REF")
d1428t<-merge(d1428t,tT12r2, by="ID_REF")

d1428t<-merge(d1428t,tT24r1, by="ID_REF")
d1428t<-merge(d1428t,tT24r2, by="ID_REF")
d1428t<-merge(d1428t,tT24r3, by="ID_REF")
d1428t<-merge(d1428t,tT24r4, by="ID_REF")
d1428t<-merge(d1428t,tT24r5, by="ID_REF")
d1428t<-merge(d1428t,tT24r6, by="ID_REF")
d1428t<-d1428t[-1,]
d1428t<-merge(d1428t, d1428[,1:2],by="ID_REF")
d1428t[1:4,]
dim(d1428t) #22212, 27
#### d1428t raw dataset ready #####

##### form d1428c dataset #####
cT0r1<-read.delim("cT0r1.txt",header=T)[,c(1,4)]
names(cT0r1)[2]<-"cT0r1"
cT0r1[1:3,]
dim(cT0r1)
cT0r2<-read.delim("cT0r2.txt",header=T)[,c(1,4)]
names(cT0r2)[2]<-"cT0r2"
cT0r2[1:3,]
dim(cT0r2)

cT1o5r1<-read.delim("cT1o5r1.txt",header=T)[,c(1,4)]
names(cT1o5r1)[2]<-"cT1o5r1"
cT1o5r1[1:3,]
dim(cT1o5r1)
cT1o5r2<-read.delim("cT1o5r2.txt",header=T)[,c(1,4)]
names(cT1o5r2)[2]<-"cT1o5r2"
cT1o5r2[1:3,]
dim(cT1o5r2)
cT1o5r3<-read.delim("cT1o5r3.txt",header=T)[,c(1,4)]

```

```

names(cT1o5r3) [2] <-"cT1o5r3"
cT1o5r3 [1:3,]
dim(cT1o5r3)

cT3r1<-read.delim("cT3r1.txt",header=T) [,c(1,4)]
names(cT3r1) [2] <-"cT3r1"
cT3r1 [1:3,]
dim(cT3r1)
cT3r2<-read.delim("cT3r2.txt",header=T) [,c(1,4)]
names(cT3r2) [2] <-"cT3r2"
cT3r2 [1:3,]
dim(cT3r2)
cT3r3<-read.delim("cT3r3.txt",header=T) [,c(1,4)]
names(cT3r3) [2] <-"cT3r3"
cT3r3 [1:3,]
dim(cT3r3)

cT6r1<-read.delim("cT6r1.txt",header=T) [,c(1,4)]
names(cT6r1) [2] <-"cT6r1"
cT6r1 [1:3,]
dim(cT6r1)
cT6r2<-read.delim("cT6r2.txt",header=T) [,c(1,4)]
names(cT6r2) [2] <-"cT6r2"
cT6r2 [1:3,]
dim(cT6r2)

cT9r1<-read.delim("cT9r1.txt",header=T) [,c(1,4)]
names(cT9r1) [2] <-"cT9r1"
cT9r1 [1:3,]
dim(cT9r1)
cT9r2<-read.delim("cT9r2.txt",header=T) [,c(1,4)]
names(cT9r2) [2] <-"cT9r2"
cT9r2 [1:3,]
dim(cT9r2)
cT9r3<-read.delim("cT9r3.txt",header=T) [,c(1,4)]
names(cT9r3) [2] <-"cT9r3"
cT9r3 [1:3,]
dim(cT9r3)
cT9r4<-read.delim("cT9r4.txt",header=T) [,c(1,2)]
names(cT9r4) [2] <-"cT9r4"
cT9r4 [1:3,]
dim(cT9r4)
cT9r5<-read.delim("cT9r5.txt",header=T) [,c(1,2)]
names(cT9r5) [2] <-"cT9r5"
cT9r5 [1:3,]
dim(cT9r5)
cT9r6<-read.delim("cT9r6.txt",header=T) [,c(1,2)]
names(cT9r6) [2] <-"cT9r6"
cT9r6 [1:3,]
dim(cT9r6)

cT12r1<-read.delim("cT12r1.txt",header=T) [,c(1,4)]
names(cT12r1) [2] <-"cT12r1"
cT12r1 [1:3,]
dim(cT12r1)
cT12r2<-read.delim("cT12r2.txt",header=T) [,c(1,4)]
names(cT12r2) [2] <-"cT12r2"

```

```

cT12r2[1:3,]
dim(cT12r2)

cT24r1<-read.delim("cT24r1.txt",header=T)[,c(1,4)]
names(cT24r1)[2]<-"cT24r1"
cT24r1[1:3,]
dim(cT24r1)
cT24r2<-read.delim("cT24r2.txt",header=T)[,c(1,4)]
names(cT24r2)[2]<-"cT24r2"
cT24r2[1:3,]
dim(cT24r2)
cT24r3<-read.delim("cT24r3.txt",header=T)[,c(1,4)]
names(cT24r3)[2]<-"cT24r3"
cT24r3[1:3,]
dim(cT24r3)
cT24r4<-read.delim("cT24r4.txt",header=T)[,c(1,2)]
names(cT24r4)[2]<-"cT24r4"
cT24r4[1:3,]
dim(cT24r4)
cT24r5<-read.delim("cT24r5.txt",header=T)[,c(1,2)]
names(cT24r5)[2]<-"cT24r5"
cT24r5[1:3,]
dim(cT24r5)
cT24r6<-read.delim("cT24r6.txt",header=T)[,c(1,2)]
names(cT24r6)[2]<-"cT24r6"
cT24r6[1:3,]
dim(cT24r6)

d1428c<-merge(cT0r1,cT0r2, by="ID_REF")

d1428c<-merge(d1428c,cT1o5r1, by="ID_REF")
d1428c<-merge(d1428c,cT1o5r2, by="ID_REF")
d1428c<-merge(d1428c,cT1o5r3, by="ID_REF")

d1428c<-merge(d1428c,cT3r1, by="ID_REF")
d1428c<-merge(d1428c,cT3r2, by="ID_REF")
d1428c<-merge(d1428c,cT3r3, by="ID_REF")

d1428c<-merge(d1428c,cT6r1, by="ID_REF")
d1428c<-merge(d1428c,cT6r2, by="ID_REF")

d1428c<-merge(d1428c,cT9r1, by="ID_REF")
d1428c<-merge(d1428c,cT9r2, by="ID_REF")
d1428c<-merge(d1428c,cT9r3, by="ID_REF")
d1428c<-merge(d1428c,cT9r4, by="ID_REF")
d1428c<-merge(d1428c,cT9r5, by="ID_REF")
d1428c<-merge(d1428c,cT9r6, by="ID_REF")

d1428c<-merge(d1428c,cT12r1, by="ID_REF")
d1428c<-merge(d1428c,cT12r2, by="ID_REF")

d1428c<-merge(d1428c,cT24r1, by="ID_REF")
d1428c<-merge(d1428c,cT24r2, by="ID_REF")
d1428c<-merge(d1428c,cT24r3, by="ID_REF")
d1428c<-merge(d1428c,cT24r4, by="ID_REF")
d1428c<-merge(d1428c,cT24r5, by="ID_REF")
d1428c<-merge(d1428c,cT24r6, by="ID_REF")

```



```

d1428c<-d1428c[-1,]
d1428c<-merge(d1428c, d1428[,1:2],by="ID_REF")
d1428c[1:4,]
dim(d1428c) #22214, 26
#### d1428c raw dataset ready #####

#####
check NA value and delete
x1<-numeric()
for (i in 2:dim(d1428c)[2]){
x1[(i-1)]<-length(d1428c$ID_REF[is.na(d1428c[,i])])
}
##### No NA values

##### Empirical Bayesian Estimation
#####
### get mean and std for each time point
d1428c$T0m<-apply(d1428c[,2:3],1,mean)
d1428c$T0sd<-apply(d1428c[,2:3],1,sd)
d1428c$T1o5m<-apply(d1428c[,4:6],1,mean)
d1428c$T1o5sd<-apply(d1428c[,4:6],1,sd)
d1428c$T3m<-apply(d1428c[,7:9],1,mean)
d1428c$T3sd<-apply(d1428c[,7:9],1,sd)
d1428c$T6m<-apply(d1428c[,10:11],1,mean)
d1428c$T6sd<-apply(d1428c[,10:11],1, sd)
d1428c$T9m<-apply(d1428c[,12:17],1,mean)
d1428c$T9sd<-apply(d1428c[,12:17],1, sd)
d1428c$T12m<-apply(d1428c[,18:19],1,mean)
d1428c$T12sd<-apply(d1428c[,18:19],1, sd)
d1428c$T24m<-apply(d1428c[,20:25],1,mean)
d1428c$T24sd<-apply(d1428c[,20:25],1, sd)

d1428t$T0m<-apply(d1428t[,2:4],1,mean)
d1428t$T0sd<-apply(d1428t[,2:4],1,sd)
d1428t$T1o5m<-apply(d1428t[,5:7],1,mean)
d1428t$T1o5sd<-apply(d1428t[,5:7],1,sd)
d1428t$T3m<-apply(d1428t[,8:10],1,mean)
d1428t$T3sd<-apply(d1428t[,8:10],1,sd)
d1428t$T6m<-apply(d1428t[,11:12],1,mean)
d1428t$T6sd<-apply(d1428t[,11:12],1, sd)
d1428t$T9m<-apply(d1428t[,13:18],1,mean)
d1428t$T9sd<-apply(d1428t[,13:18],1, sd)
d1428t$T12m<-apply(d1428t[,19:20],1,mean)
d1428t$T12sd<-apply(d1428t[,19:20],1, sd)
d1428t$T24m<-apply(d1428t[,21:26],1,mean)
d1428t$T24sd<-apply(d1428t[,21:26],1, sd)

##### check sd equals to zero rows, make the row number the same for
each experiment
dim(d1428c[d1428c$T0sd==0|d1428c$T1o5sd==0|d1428c$T3sd==0|d1428c$T6sd==0
|d1428c$T9sd==0|d1428c$T12sd==0|
d1428c$T24sd==0,])# 240 rows
d1428c[d1428c$T0sd==0|d1428c$T1o5sd==0|d1428c$T3sd==0|d1428c$T6sd==0|d14
28c$T9sd==0|d1428c$T12sd==0|
d1428c$T24sd==0,][,1]

```

```

dim(d1428c[d1428c$T0sd!=0 & d1428c$T1o5sd!=0 & d1428c$T3sd!=0 &
d1428c$T6sd!=0 & d1428c$T9sd!=0
& d1428c$T12sd!=0 & d1428c$T24sd!=0,]) # 21974, 40
dim(d1428c) # 22214, 40
d1428c1<-d1428c[d1428c$T0sd!=0 & d1428c$T1o5sd!=0 & d1428c$T3sd!=0 &
d1428c$T6sd!=0 & d1428c$T9sd!=0
& d1428c$T12sd!=0 & d1428c$T24sd!=0,]

dim(d1428t[d1428t$T0sd==0|d1428t$T1o5sd==0|d1428t$T3sd==0|d1428t$T6sd==0
|d1428t$T9sd==0|d1428t$T12sd==0|
d1428t$T24sd==0,])
# 203 rows
d1428t[d1428t$T0sd==0|d1428t$T1o5sd==0|d1428t$T3sd==0|d1428t$T6sd==0|d14
28t$T9sd==0|d1428t$T12sd==0|
d1428t$T24sd==0,][,1]
dim(d1428t[d1428t$T0sd!=0 & d1428t$T1o5sd!=0 & d1428t$T3sd!=0 &
d1428t$T6sd!=0 & d1428t$T9sd!=0
& d1428t$T12sd!=0 & d1428t$T24sd!=0,]) # 22009, 40
dim(d1428t) # 22212, 40
d1428t1<-d1428t[d1428t$T0sd!=0 & d1428t$T1o5sd!=0 & d1428t$T3sd!=0 &
d1428t$T6sd!=0 & d1428t$T9sd!=0
& d1428t$T12sd!=0 & d1428t$T24sd!=0,]

d1428c1<-d1428c1[d1428c1$ID_REF%in%ints,]
d1428t1<-d1428t1[d1428t1$ID_REF%in%ints,]

##### use d1428c1, d1428t1 hereafter
#####
#
# EB computation for d1428c1, d1428t1
#
#####

### hyperparameter estimation fi is the shape parameter in gamma dist.
also call it alfa,
### it is the squared stability measure.
fic28<-c(d1428c1$T0m^2/d1428c1$T0sd^2, d1428c1$T1o5m^2/d1428c1$T1o5sd^2,
d1428c1$T3m^2/d1428c1$T3sd^2,d1428c1$T6m^2/d1428c1$T6sd^2,
d1428c1$T9m^2/d1428c1$T9sd^2,d1428c1$T12m^2/d1428c1$T12sd^2,
d1428c1$T24m^2/d1428c1$T24sd^2)
fit28<-c(d1428t1$T0m^2/d1428t1$T0sd^2, d1428t1$T1o5m^2/d1428t1$T1o5sd^2,
d1428t1$T3m^2/d1428t1$T3sd^2,d1428t1$T6m^2/d1428t1$T6sd^2,
d1428t1$T9m^2/d1428t1$T9sd^2,d1428t1$T12m^2/d1428t1$T12sd^2,
d1428t1$T24m^2/d1428t1$T24sd^2)

# plot: histogram of emperical estimates of shape parameter in log
# scale:
hist(log(fic28),xlim=c(-5,10),n=50)
hist(log(fit28),xlim=c(-5,10),n=60)
hist(log(fic28),main="Histogram of Log Transformed empirical estimates
of shape parameter",
xlab="shape parameter in log scale")

alfac<-mean(log(fic28))
betac<-sd(log(fic28))
alfat<-mean(log(fit28))

```

```

betat<-sd(log(fit28))

##### for d1428c1 and d1428t1 #####
## alfac: 2.182803, betac: 1.955049.
## alfat: 2.295019, betat: 1.840149.

##### calculate CV=1/sqrt(fi)
d1428c1$lsum<-apply(log(d1428c1[,2:25]),1,sum)
d1428t1$lsum<-apply(log(d1428t1[,2:26]),1,sum)

d1428c1$lmsum<-
2*log(d1428c1$T0m)+3*log(d1428c1$T1o5m)+3*log(d1428c1$T3m)+2*log(d1428c1
$T6m)+6*log(d1428c1$T9m)+2*log(d1428c1$T12m)+6*log(d1428c1$T24m)
d1428t1$lmsum<-
3*log(d1428t1$T0m)+3*log(d1428t1$T1o5m)+3*log(d1428t1$T3m)+2*log(d1428t1
$T6m)+6*log(d1428t1$T9m)+2*log(d1428t1$T12m)+6*log(d1428t1$T24m)

B<-numeric()
for(i in 1:dim(d1428c1)[1]){
B[i]<-uniroot(function(x) 24*log(x)-24*digamma(x)+(d1428c1$lsum[i]-
d1428c1$lmsum[i])-(log(x)-
alfac+betac^2)/(x*betac^2),low=0.000001,up=1000,tol=0.0001)$root
}
d1428c1$fi<-B
d1428c1$CV<-1/sqrt(B)

B<-numeric()
for(i in 1:dim(d1428t1)[1]){
B[i]<-uniroot(function(x) 25*log(x)-25*digamma(x)+(d1428t1$lsum[i]-
d1428t1$lmsum[i])-(log(x)-
alfat+betat^2)/(x*betat^2),low=0.000001,up=1000,tol=0.0001)$root
}
d1428t1$fi<-B
d1428t1$CV<-1/sqrt(B)

##### Plots: chapter6 4 plots #####

par(mfrow=c(1,2))

plot(d1428c1$CV,main="CV Plot for GDS1428 Control",ylab="CV",
xlab="genes (probes)")
plot(d1428t1$CV,main="CV Plot for GDS1428 Treatment",ylab="CV",
xlab="genes (probes)")

plot(d1428c1$fi,main="Shape Parameter Plot for GDS1428
Control",ylab="Shape Parameter",xlab="genes (probes)")
plot(d1428t1$fi,main="Shape Parameter Plot for GDS1428
Treatment",ylab="Shape Parameter",xlab="genes (probes)")

#####
#
# HMM Main Routine for d1428c1, d1428t1
#
#

```

```
#####
#####
##### HMM for d1428c1, d1428t1 begin:
order<-numeric()
k<-5
for (g in 1:dim(d1428t1)[1]) { # d1428t1, d1428c1
#set initial parameters for each iteration
datat<-as.numeric(d1428t1[g,2:26]) # d1428t1 2:26, d1428c1 2:25
pi<-c(0.2,0.2,0.2,0.2,0.2)
alfa<-d1428t1$fi[g]      #d1428c1, d1428t1
n<-length(datat)
ck<-0.1*log(n)/sqrt(n)
# calculate initial mean
mu<-muinit(datat,k)
# calculate threshold parameter lamda
lamda<-lamdacalc(alfa)
##### below portion does not need adjustment #####
Q<-numeric(0)
for (e in 1:100){

#step1.
eta<-etaCalc(mu,alfa)
mu<-mergemu(mu,eta,lamda)
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step 2. calculate Q
#2.1 compute the penalty of eta:

penl<-numeric(0)
for (i in 1:(k-1)){
if (abs(eta[i])<lamda){
penl[i]<-lamda^2-(eta[i]-lamda)^2
}else{penl[i]<-lamda^2}
}
# penl<-16*sum(penl)
penl<-log(n)*sqrt(n)*sum(penl)

#2.2 compute penalty of log pi
pen2<-ck*sum(log(pi))

#2.3 calculate full Q value
lk<-matrix(nrow=n,ncol=k)
for (i in 1:n){
for (j in 1:k){
lk[i,j]<-
wt[i,j]*(log(dgamma(datat[i],shape=alfa,scale=(mu[j]/alfa)))+log(pi[j]))
}
}
Q[e]<-sum(lk)-pen1+pen2

#step3.update mu, and compute eta
mu<-updatemu(mu,wt,datat)
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step4. update pi
pi<-updatepi(wt,ck)
}
}
```

```

order[g]<-length(unique(round(mu,digits=0)))
} # whole iteration end
##### End of HMM for d1428c1, d1428t1
##### Most update d1428t1Multi, d1428c1Multi for GDS1428.
d1428t1Multi<-cbind(which(order>1,order),order[order>1])
dim(d1428t1Multi) ##### total 2351 genes
sort(unique(d1428t1[d1428t1Multi[,1],27]))
##### unique total 1902 gene

#total probe number and gene number:
length(unique(d1428t1[,1])) #unique probe: 21775
length(unique(d1428t1[,27])) #unique gene: 13996

d1428c1Multi<-cbind(which(order>1,order),order[order>1])
dim(d1428c1Multi) ##### total 2053 genes
sort(unique(d1428c1[d1428c1Multi[,1],26]))
##### unique total 1644 gene

#total probe number and gene number:
length(unique(d1428c1[,1])) #unique probe: 21775
length(unique(d1428c1[,26])) #unique gene: 13996

wMatrix<-function(pi=pi,mu=mu,alfa=alfa,data=data){
if (length(pi)!=length(mu)){outx<-'input data error'
outx}
wMat<-matrix(nrow=length(data),ncol=length(mu))
cel<-numeric(0)
for (i in 1:length(data)){
for (j in 1:length(mu)){
cel[j]<-pi[j]*dgamma(data[i],shape=alfa,scale=(mu[j]/alfa))
}
wMat[i,]<-cel/sum(cel)
}
return(wMat)
}

updatepi<-function(wt,ck){
pinew<-numeric(0)
for (i in 1:dim(wt)[2]){
pinew[i]<-(sum(wt[,i])+ck)/(dim(wt)[1]+dim(wt)[2]*ck)
#pinew[i]<-sum(wt[,i])/(dim(wt)[1])
}
return(pinew)
}

updatemu<-function(mu,wMat,data){
munew<-numeric(length(mu))

for (i in 1:length(mu)){
munew[i]<-sum(data*wMat[,i])/sum(wMat[,i])
}
return(munew)
}

etaCalc<-function(mu, alfa){
difmu<-mu[2:length(mu)]-mu[1:(length(mu)-1)]
eta<-difmu/(mu[1:(length(mu)-1)])
}

```

```

#eta<-difmu/(mu[1:(length(mu)-1)]*alfa)
#for (i in 1:length(eta)){
#if (eta[i]==0){eta[i]<-0.0001}
#}
return(eta)
}
mergemu<-function(mu,eta,lamda){
munew1<-numeric()
if (min(eta)>lamda){munew1<-mu}else{

mi<-which(eta<=lamda,eta)
for (i in length(mi):1){
mu[mi[i]]<-mu[(mi[i]+1)]
}

munew1<-mu
}
return(munew1)
}
munit <- function(data, Ku){
rg<-exp(( log(max(data))-log(min(data)) )/Ku)
mui<-numeric()
for (i in 1:Ku){
mui[i]<-0.5*(min(data)*rg^(i-1)+min(data)*rg^i)
}
return(mui)
}
lamdacalc<-function(alfa){
if(alfa>0.1){Sprob<-0.76
}else{
Sprob<-round(exp( log(alfa)*(-0.057242)-0.155172 ),digit=3 )}
qb<-qgamma(Sprob, shape=alfa, scale=1)
#solve for the right component's scale
rt<-uniroot(function(x) dgamma(qb, shape=alfa,scale=x)-dgamma(qb,
shape=alfa, scale=1), low=1.01, up=300, tol=0.01)
lamda1<-rt$root-1

return(lamda1)
}

Inter1428Multi<-
sort(intersect(d1428c1[d1428c1Multi[,1],26],d1428t1[d1428t1Multi[,1],27]
))
## total 894 genes in common.

##### respond only in d1428c1Multi, total gene number: 750
sort(setdiff(unique(d1428c1[d1428c1Multi[,1],26]),Inter1428Multi))

##### respond only in d1428t1Multi, total gene number: 1008
sort(setdiff(unique(d1428t1[d1428t1Multi[,1],27]),Inter1428Multi))

## generate gamma mixture dtm2 (order=2).
#### for mediate sample size:20
c2<- rmultinom(500, size = 20, prob=c(0.4,0.6))
dim(c2)
c2[,1:5] #has 500 columns

```

```

alfa1<-20
dtm2<-matrix(nrow=500,ncol=20)
for (i in 1:dim(c2)[2]){
  dtm2[i,]<-
  c(rgamma(c2[1,i],shape=alfa1,scale=15),rgamma(c2[2,i],shape=alfa1,scale=
22.35)) }

c2<- rmultinom(500, size = 300, prob=c(0.4,0.6))

alfa1<-5
dtm2<-matrix(nrow=500,ncol=300)
for (i in 1:dim(c2)[2]){
  dtm2[i,]<-
  c(rgamma(c2[1,i],shape=alfa1,scale=15),rgamma(c2[2,i],shape=alfa1,scale=
112.05)) }

c3<- rmultinom(500, size = 300, prob=c(0.2,0.3,0.5))

alfa1<-40
dtm3<-matrix(nrow=500,ncol=300)
for (i in 1:dim(c3)[2]){
  dtm3[i,]<-
  c(rgamma(c3[1,i],shape=alfa1,scale=15),rgamma(c3[2,i],shape=alfa1,scale=
23.1),
  rgamma(c3[3,i],shape=alfa1,scale=35.57)) }

c3<- rmultinom(500, size = 20, prob=c(0.2,0.3,0.5))

alfa1<-5
dtm3<-matrix(nrow=500,ncol=20)
for (i in 1:dim(c3)[2]){
  dtm3[i,]<-
  c(rgamma(c3[1,i],shape=alfa1,scale=15),rgamma(c3[2,i],shape=alfa1,scale=
27.15),
  rgamma(c3[3,i],shape=alfa1,scale=49.14)) }

#####
order<-numeric()
k<-5
alfa<-alfa1 # Reset this parameter

for (g in 1:500) {
  #set initial parameters for each iteration
  datat<-dtm3[g,]
  pi<-c(0.2,0.2,0.2,0.2,0.2)
  n<-length(datat)
  ck<-0.1*log(n)/sqrt(n)

  # calculate initial mean
  mu<-muinit(datat,k)
  # calculate threshold parameter lamda
  lamda<-lamdacalc(alfa)

  Q<-numeric(0)
  for (e in 1:100){

```

```

#step1.
eta<-etaCalc(mu,alfa)
mu<-mergemu(mu,eta,lamda)
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step 2. calculate Q
#2.1 compute the penalty of eta:

penl<-numeric(0)
for (i in 1:(k-1)){
  if (abs(eta[i])<lamda){
    penl[i]<-lamda^2-(eta[i]-lamda)^2
  }else{penl[i]<-lamda^2}
}
# penl<-16*sum(penl)
penl<-log(n)*sqrt(n)*sum(penl)

#2.2 compute penalty of log pi
pen2<-ck*sum(log(pi))

#2.3 calculate full Q value
lk<-matrix(nrow=n,ncol=k)
for (i in 1:n){
  for (j in 1:k){
    lk[i,j]<-
    wt[i,j]*(log(dgamma(datat[i],shape=alfa,scale=(mu[j]/alfa)))+log(pi[j]))
  }
}
Q[e]<-sum(lk)-penl+pen2

#step3.update mu, and compute eta
mu<-updatemu(mu,wt,datat)
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step4. update pi
pi<-updatepi(wt,ck)
}
order[g]<-length(unique(round(mu,digits=0)))
}
order
length(order[order>3])
length(order[order==3])
length(order[order<3])
#####

#### for mediate sample size:300
c1<- rmultinom(500, size = 300, prob=c(0.4,0.3,0.3))
dim(c1)

dtm<-matrix(nrow=500,ncol=300)
for (i in 1:dim(c1)[2]){
  dtm[i,<-
  c(rgamma(c1[1,i],shape=alfa1,scale=5),rgamma(c1[2,i],shape=alfa1,scale=4
0),
  rgamma(c1[3,i],shape=alfa1,scale=320)) }

#### generate gamma mixture, for small size=20

```



```

c2<- rmultinom(500, size = 20, prob=c(0.4,0.3,0.3))

dtm2<-matrix(nrow=500,ncol=20)
for (i in 1:dim(c2)[2]){
dtm2[i,]<-
c(rgamma(c2[1,i],shape=alfa1,scale=5),rgamma(c2[2,i],shape=alfa1,scale=4
0),
rgamma(c2[3,i],shape=alfa1,scale=320)) }

##### two components
c3<- rmultinom(500, size = 20, prob=c(0.6,0.4))
dtm3<-matrix(nrow=500,ncol=20)
for (i in 1:dim(c3)[2]){
dtm3[i,]<-
c(rgamma(c3[1,i],shape=alfa1,scale=5),rgamma(c3[2,i],shape=alfa1,scale=4
0)) }

#Q<-matrix(nrow=500,ncol=30)
#mu500<-matrix(nrow=500,ncol=5)

od<-numeric()
k<-5
for (g in 1:500) {
#set initial parameters for each iteration
datat<-dtm3[g,]

pi<-c(0.2,0.2,0.2,0.2,0.2)
alfa<-alfa1
n<-length(datat)
ck<-0.1*log(n)/sqrt(n)

# calculate initial mean
mu<-muinit(datat,k)
# calculate threshold parameter lamda
lamda<-lamdacalc(alfa)

for (e in 1:100){
#step1.
eta<-etaCalc(mu,alfa)
if (e > 8){ mu<-mergemu(mu,eta,lamda)}
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step 2. calculate Q
#2.1 compute the penalty of eta:

penl<-numeric(0)
for (i in 1:(k-1)){
if (abs(eta[i])<lamda){
penl[i]<-lamda^2-(eta[i]-lamda)^2
}else{penl[i]<-lamda^2}
}
# penl<-16*sum(penl)
penl<-log(n)*sqrt(n)*sum(penl)

#2.2 compute penalty of log pi
pen2<-ck*sum(log(pi))

```

```

#2.3 calculate full Q value
lk<-matrix(nrow=n,ncol=k)
for (i in 1:n){
  for (j in 1:k){
    lk[i,j]<-
    wt[i,j]*(log(dgamma(datat[i],shape=alfa,scale=(mu[j]/alfa)))+log(pi[j]))
  }
}
#Q[g,e]<- sum(lk)-pen1+pen2

#step3.update mu, and compute eta
mu<-updatemu(mu,wt,datat)
wt<-wMatrix(pi=pi,mu=mu,alfa=alfa,data=datat)

#step4. update pi
pi<-updatepi(wt,ck)
}

od[g]<-length(unique(round(mu,digits=0)))
} od

```