A Paradigmatic Regression Algorithm for Gene Selection Problems

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Abstract

Motivation: Gene selection has become a common task in most gene expression studies. The objective of such research is often to identify the smallest possible set of genes that can still achieve good predictive performance. The problem of assigning tumours to a known class is a particularly important example that has received considerable attention in the last ten years. Many of the classification methods proposed recently require some form of dimension-reduction of the problem. These methods provide a single model as an output and, in most cases, rely on the likelihood function in order to achieve variable selection.

Results: We propose a prediction-based objective function that can be tailored to the requirements of practitioners and can be used to assess and interpret a given problem. The direct optimization of such a function can be very difficult because the problem is potentially discontinuous and nonconvex. We therefore propose a general procedure for variable selection that resembles importance sampling to explore the feature space. Our proposal compares favorably with competing alternatives when applied to two cancer data sets in that smaller models are obtained for better or at least comparable classification errors. Furthermore by providing a set of selected models instead of a single one, we construct a network of possible models for a target prediction accuracy level.

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1 INTRODUCTION

Gene selection has become a common task in most gene expression studies. The goal of this research is often to identify the smallest possible set of genes that can still achieve good predictive performance (Díaz-Uriarte and De Andres, 2006). The problem of assigning tumours to a known class is an example that is of particular importance and has received considerable attention in the last ten years. Conventional class prediction methods of leukemia or other cancers are in general based on microscopical examination of stained tissue specimens. However, such methods require highly trained specialists and are subjective (Tibshirani et al., 2002).

To avoid these drawbacks, many automatic classification methods have been proposed recently. These methods have the advantage of being objective and have improved the correct classification rate in various cases. Among the different methodologies brought forward in this context we can find those proposed by Tibshirani et al. (2002), Dudoit et al. (2002), Zhu and Hastie (2004), Zou and Hastie (2005). See also Díaz-Uriarte and De Andres (2006) and the references therein for other approaches.

Nonetheless, many of these methods do not necessarily respond to the needs of practitioners and researchers when they approach the gene selection process. First of all, many of them have to rely on some form of size reduction and often require a subjective input to determine the dimension of the problem. Also, many of these methods often provide a single model as an output whereas genes interact inside biological systems and can be interchangeable in explaining a specific response. The idea of interchangeability of genes in explaining responses appears for instance in Kristensen et al. (2012). These authors use the PARADIGM algorithm of Vaske et al. (2010) to combine mRNA expression and DNA copy number in order to construct clusters of patients that provide the best predictive value. The resulting clusters can be seen as being characterized by different significantly expressed genes.

Another issue of most existing gene selection methods is their reliance on the likelihood function, or a penalized version of it, as a means to develop a selection criterion. However, the likelihood function may not necessarily be the quantity that users are interested in as they may want to target some other kind of loss function such as, for example, the classification error. Of course, maximizing the likelihood function is not typically the same as minimizing a particular loss function. Moreover, adapting these methods to handle missing or contaminated data is not straightforward. This has limited the applicability and reliability of these methods in many practical cases.

To eliminate the limitations of the gene selection procedures described above, this paper proposes an objective function for out-of-sample predictions that can be tailored to the requirements of practitioners and researchers. This is achieved by enabling them to select a criterion according to which they would like to assess and/or interpret a
given problem. However, the optimization of such a criterion function is typically not an easy task since the function can be discontinuous, non-convex and would require computationally intensive techniques. To tackle this issue, we propose a solution using a different approach based on a procedure that resembles importance sampling. This new approach provides a general and flexible framework for gene selection as well as for other model selection problems.

The advantages of this proposal are multiple:

- **Flexibility**: It allows the users to specify a criterion that can be tailored to the specific problem setting. It is able to handle different kinds of responses, problems of missing and contaminated data, multicollinearity, etc.

- **Prediction Power**: The result of the procedure is a set of models with high predictive power with respect to the specified criterion. It is especially suitable in selecting genes and models to achieve accurate predictions.

- **Dimension-reduction**: It can provide an assessment of the dimension of the problem because it greatly reduces the number of necessary covariates and eases the interpretation without requiring any preliminary size reduction.

- **Network-building**: With the reduced model size, it preserves the capacity to build gene-networks to provide a more general view of the potential paradigmatic structures of the genetic information.

This last aspect is of great interest for gene selection since this list can provide insight into the complex mechanisms behind different biological phenomena. Different cases, some of which can be found in Section 4, indicate that this method appears to outperform other methods in terms of criteria minimization while, at the same time, selects models of considerably smaller dimension which allow improved interpretation of the results. The set of selected models can naturally be viewed as a network of possible structures of genetic information. We call this a paradigmatic network. In Section 4 we give an example of a graphical representation of such networks based on the analysis of one of two cancer data sets which are discussed therein.

In this paper we first describe and formalize the proposed approach within the model selection statistical framework in Section 2. In Section 3 we illustrate the techniques and algorithms used to address the criterion minimization problem highlighted in Section 2. The performance of our approach is then illustrated on two data sets concerning leukemia classification (Golub et al., 1999) and breast cancer classification (Chin et al., 2006) in Section 4. We conclude the paper in Section 5 by summarizing the benefits of the new approach and providing an outlook on other potential applications that can benefit from this methodology.
2 Approach

To introduce the proposed method, let us first define some notation which will be used throughout this paper:

1. Let \( J_f = \{ 1, 2, ..., p \} \) be the set of indices for \( p \) potential covariates included in the \( n \times p \) matrix \( X \). We allow \( X \) to include a vector of 1s.

2. Let \( J = \mathcal{P}(J_f) \setminus \emptyset, |J| = 2^p - 1 \), be the power set including all possible models that can be constructed with the \( p \) covariates excluding the empty set.

3. Let \( j \in J \) be a model belonging to the above mentioned power set.

4. Let \( \beta_j \in \mathbb{R}^p \) be the parameter vector for model \( j \), i.e.

\[
\beta_j = \left\{ \begin{array}{ll}
\beta_k & \text{if } k \in j \\
0 & \text{if } k \notin j
\end{array} \right.
\]

where \( \beta_j, \beta \) are respectively the \( k \)th element of \( \beta_j \) and \( \beta \).

Keeping this notation in mind, for a given model \( j \in J \) we have

\[
Y = g(X, \beta_j) + \varepsilon,
\]

where \( g(\cdot, \cdot) \) is a link function known up to the parameter vector \( \beta_j \in \mathbb{R}^p \) and \( \varepsilon \) is a mean zero random error. Models of the form (1) are very general and include all parametric models and a large class of semiparametric models when \( g(\cdot, \cdot) \) is not completely known or the distribution of \( \varepsilon \) is not specified. A few examples of model (1) are given in Appendix A.

We assume that for a fixed \( j \), based on a specific choice for model (1) with corresponding parameter vector \( \beta_j \) and given a new covariate vector \( X_0 \), the user can construct a prediction \( \hat{Y}(X_0, \beta_j) \). To assess the quality of this prediction we assume that we have a divergence measure available which we denote as \( D\{\hat{Y}(X_0, \beta_j), Y_0\} \). The only requirement imposed on the divergence measure is that it satisfies the property of positiveness, i.e.

\[
D(u, v) > 0 \text{ for } u \neq v
\]

\[
D(u, v) = 0 \text{ for } u = v.
\]

With this property being respected, the divergence measure can arbitrarily be specified by the user according to the interest in the problem. Examples of such divergence measures include the \( L_1 \) loss function

\[
D\{\hat{Y}(X_0, \beta_j), Y_0\} = |\hat{Y}(X_0, \beta_j) - Y_0|
\]
or an asymmetric classification error

\[ D\{\hat{Y}(X_0, \beta'), Y_0\} = I\{\hat{Y}(X_0, \beta') = 1, Y_0 = 0\}w_1 + I\{\hat{Y}(X_0, \beta') = 0, Y_0 = 1\}w_2. \]

where \( w_1, w_2 \geq 0 \). The latter is for a Bernoulli response and is typically an interesting divergence measure when asymmetric classification errors have to be considered. Indeed, in most clinical situations, the consequences of classification errors are not equivalent with respect to the direction of the misclassification. For instance, the prognosis and the treatment of Estrogen Receptor (ER) positive Breast Cancers (BC) are quite different from those of ER negative ones. Indeed, if a patient with ER negative is treated with therapies designed for patients with ER positive, the consequence is much more severe than if this were done the other way round because of the excessive toxicities and potentially severe side effects. It therefore makes sense to give different values to \( w_1 \) and \( w_2 \). By defining \( w_1 > w_2 \) we would take these risks into account, where \( w_1 \) would be the weight for a misclassification from ER negative to ER positive BC and \( w_2 \) for the opposite direction. Weight values can be modulated according to the current medical knowledge and the clinical intuition of the physicians.

Considering this divergence measure \( D(\cdot, \cdot) \), we are consequently interested in finding the best models within the general class given in (1). To do so, we would ideally aim at solving the following risk minimization problem:

\[ \hat{\beta}' \in \mathcal{B} \equiv \operatorname{arg min}_{\beta' \in J} \min_{\beta^p} \mathbb{E}_0[ D\{\hat{Y}(X_0, \beta'), Y_0\}], \tag{2} \]

where \( \mathbb{E}_0 \) denotes the expectation on the new observation \((Y_0, X_0)\). Let \( j_0 \) denote the models with the smallest cardinality among all \( \hat{\beta}' \in \mathcal{B} \). Note that there could be more than one model with the same prediction property and of the same size, hence \( j_0 \) could contain more than one model. Let us define the models corresponding to \( j_0 \) as the “true” models. Thus, our “true” models are essentially the most parsimonious models that minimize the expected prediction error.

The optimization problem in (2) is typically very difficult to solve. First of all, supposing we do not consider interaction terms, the outer minimization would require to compare a total of \( 2^p - 1 \) results, each a result of the inner minimization problem. In addition, each of the \( 2^p - 1 \) inner minimization problems is also very hard to solve, even if the risk \( \mathbb{E}_0[D\{\hat{Y}(X_0, \beta'), Y_0\}] \) were a known function of \( \beta' \). Indeed, the inner minimization problem is in general non-convex and could be combinatorial, implying that the minimizer might not be unique. For example, when \( D(\cdot, \cdot) \) is the classification error, this problem is combinatorial by nature. In practice, the computational challenge is even greater because the risk function \( \mathbb{E}_0[D\{\hat{Y}(X_0, \beta'), Y_0\}] \) is a function of \( \beta' \) without explicit form and needs to be approximated.

We propose to estimate \( \mathbb{E}_0[D\{\hat{Y}(X_0, \beta'), Y_0\}] \) via an \( m \)-fold cross-validation (typically \( m = 10 \)) repeated \( K \) times. More specifically, for a sample of size \( n \), we repeat
the following procedure \( K \) times. At the \( k \)th repetition, we randomly select \( \lfloor n/m \rfloor \) observations to form a “test” data set, subindexed \( i = 1, \ldots, \lfloor n/m \rfloor \) and superindexed \( k = 1, \ldots, K \), i.e. \((X^k_i, Y^k_i)\), then the estimated risk is

\[
\hat{E}_0 \left[ D \left\{ \hat{Y}(X_0, \beta^0), Y_0 \right\} \right] = \frac{1}{\lfloor n/m \rfloor K} \sum_{k=1}^{K} \sum_{i=1}^{\lfloor n/m \rfloor} D \left\{ \hat{Y}(X^k_i, \beta^0), Y^k_i \right\}.
\] (3)

The reason we only use \( \lfloor n/m \rfloor \) observations out of the whole data set will become clear further on. Having approximated the expectation \( E_0 \), the minimization problem in (2) becomes

\[
\argmin_{j \in J} \argmin_{\beta^j} \hat{E}_0 \left[ D \left\{ \hat{Y}(X_0, \beta^0), Y_0 \right\} \right].
\] (4)

Despite the above approximation, the minimization problem remains complicated for the reasons mentioned earlier. Thus, we further eliminate the inner minimization problem in (4) by inserting an estimator \( \hat{\beta}^j \) obtained independently from the minimization procedure. More specifically, we assume that an estimator of \( \beta^j \), say \( \hat{\beta}^{j,k} \), is available based on model (1) and “training” observations \((X^k_{\lfloor n/m \rfloor+1}, Y^k_{\lfloor n/m \rfloor+1}), \ldots, (X_n^k, Y_n^k)\) (i.e. those observations excluded from the above mentioned “test” data sets). This estimator can be any available estimator, for example, the maximum likelihood estimator (MLE), a moment based estimator, or a quantile regression based estimator, etc. We then replace the inner minimization in (4) directly with the approximate expectation evaluated at \( \hat{\beta}^{j,k} \)'s and simplify (4) to

\[
\argmin_{j \in J} \frac{1}{\lfloor n/m \rfloor K} \sum_{k=1}^{K} \sum_{i=1}^{\lfloor n/m \rfloor} D \left\{ \hat{Y}(X^k_i, \hat{\beta}^{j,k}), Y^k_i \right\}.
\]

The intuition of replacing the inner minimization in (4) with a sample average evaluated at an arbitrary estimator is due to the fact that this estimator, under a fixed “true” model and regardless of whether this estimator is a standard MLE or a minimizer of the divergence measure \( D \), is an approximation to the “true” parameter. This means that, consequently, different estimators are “close” to each other. As a consequence, \((\lfloor n/m \rfloor K)^{-1} \sum_{k=1}^{K} \sum_{i=1}^{\lfloor n/m \rfloor} D \left\{ \hat{Y}(X^k_i, \hat{\beta}^{j,k}), Y^k_i \right\}\) is a close approximation to \( \min_{\beta^j} E_0 \left[ D \left\{ \hat{Y}(X_0, \beta^j), Y_0 \right\} \right] \).

We now have an optimization problem in (2) which requires a comparison of \( 2^p - 1 \) values and is much easier to solve. To further reduce the number of comparisons, the following section describes some procedures and algorithms allowing to solve this problem in a more efficient manner.
3 Heuristic Procedure

To solve the optimization problem in (2), we propose an approach designed to have the following three features:

1. Identify a set of models that carry large predictive power instead of a single “best” model;
2. Find this set of models within a reasonable time, without having to explore all possible models;
3. This set achieves sparsity, i.e. most of the parameters in $\beta$ will be fixed at zero in each of the models in the set.

Note that the last feature above reflects the belief that most of the covariates are irrelevant for the problem under consideration and should be excluded. Indeed, our method is designed to work effectively if such a sparsity assumption holds, putting it on the same level of almost all variable selection procedures in the literature. Moreover, we require the method to have the first feature in order to increase flexibility in terms of interpretation. Indeed, in many domains such as gene selection, for example, the aim may not be to find a single model but a set of variables (genes) that can be inserted in a paradigmatic structure to better understand the contribution of each of them via their interactions.

Given this goal, assume that we have at our disposal an estimate of the measure of interest $D(\cdot, \cdot)$ for all possible $2^p - 1$ models. In this case, our interest would be to select a set of “best” models by simply keeping the set of models that have a low discrepancy measure $D(\cdot, \cdot)$. It is of course unrealistic to obtain a discrepancy measure for all models in most practical cases because this would require a considerable amount of time for computation. Therefore, in order to achieve the second feature, instead of examining all possible models, we can randomly sample covariates from $J$. The random sampling needs to be carefully devised because in practice, for example in gene selection problems, the number of covariates $p$ can easily reach thousands or tens of thousands (see examples in Section 4, where $p = 7, 129$ and $p = 22, 215$ respectively). In such situations, $2^p - 1$ is an extremely large number and the probability of randomly sampling a “good” set of variables from the $2^p - 1$ variables is very small. Using the sparsity property of the problem, we propose to start with the set of variables $M_0$ (typically an empty set) and increase the model complexity stepwise. Throughout this procedure, we ensure that at step $k$, the more promising covariates based on the evaluation at step $k-1$ are given higher probabilities of being randomly drawn. The last idea is in the spirit of “importance sampling” in the sense that covariates with more importance based on the previous step are “encouraged” to be selected in the current step. Note
that by construction we achieve sparsity if we stop the stepwise search at models of size $d_{\text{max}} \ll p$.

More formally, let us first define the set of all possible models of size $d$ as 

$$S_d = \{ (i_1, \ldots, i_d) \mid i_1, \ldots, i_d \in J; \ i_1 < \ldots < i_d \}.$$ 

We then define the set of promising models, $S^*_d$, as the ones with an estimated out-of-sample divergence measure $D(\cdot, \cdot)$ below a certain estimated $\alpha$-quantile. The value of $\alpha$ is user-defined depending on the problem at hand, and is typically a small value such as $\alpha = 1\%$. The formal definition of this set would then be

$$S^*_d = \{ \mathbf{j} \mid \mathbf{j} \in S_d; \hat{D}_j \leq \hat{q}_d(\alpha) \},$$

where

$$\hat{D}_j = \frac{1}{[n/m]K} \sum_{k=1}^{K} \sum_{i=1}^{[n/m]} D(\hat{\mathbf{Y}}(X_{i,k}, \hat{\beta}_{j,k}), Y_{i,k}), \quad (5)$$

and $\hat{q}_d(\alpha)$ is the $\alpha$-quantile of the $\hat{D}_j (j \in S_d)$ values issued from the $B$ randomly selected models. Finally, we define the set of indices of covariates that are in $S^*_d$ as

$$I^*_d = \{ i \mid i \in \mathbf{j}, \mathbf{j} \in S^*_d \}$$

whose complement we define as $I^c_d$ (i.e. all those covariates that are not included in $I^*_d$).

With this approach in mind and using the above notations, to start the procedure we assume that we have $p$ variables from which to select.

A. **Initial Step:** We start by adding the number of variables $d = 1$ to our initial variable set $M_0$ with the goal of finally obtaining the set $I^*_1$.

1. Construct the $p$ possible one variable models by augmenting $M_0$ with each of the $p$ available variables.
2. Compute $\hat{D}$ for every model obtained in Step A.1.
3. From Steps A.1 and A.2, construct the set $I^*_1$ using (3). Go to Step B and let $d = 2$.

B. **General Step:** We define here the general procedure to construct $I^*_d$ for $2 \leq d \leq d_{\text{max}}$.

1. Augment $M_0$ with $d$ variables as follows:
   
   (i) Randomly select a set, either set $I^*_{d-1}$ with probability $\pi$ or its complement $I^c_{d-1}$ with probability $1 - \pi$.
   
   (ii) Select one variable uniformly at random and without replacement from the set chosen in Step (i) and add this variable to $M_0$. 

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(iii) Repeat Steps (i) and (ii) until $d$ variables are added to $\mathcal{M}_0$.

2. Construct a model of dimension $d$ using the $d$ variables selected in Step B.1. Repeat Step B.1 $B$ times to construct $B$ such models.

3. From Steps B.1 and B.2, construct the set $\mathcal{I}_d^*$ according to (3). If $d < d_{\text{max}}$, go to Step B and let $d = d + 1$, otherwise exit algorithm.

3.1 Discussion

Some ideas that our approach and our algorithm use can be found in the literature. We relegate a short discussion of these to Appendix D. Here we will discuss instead some practical issues arising when implementing our algorithm.

3.1.1 Choice of algorithm inputs:

The parameters $d_{\text{max}}$, $B$, $\alpha$ and $\pi$ of the above algorithm are to be fixed by the user. As mentioned earlier $d_{\text{max}}$ represents a reasonable upper bound for the model dimension which is constrained to $d_{\text{max}} \leq l$, where $l$ depends on the limitations of the estimation method and is commonly the sample size $n$. As for the parameter $B$, a larger value is always preferable to better explore the covariate space. However, a larger $B$ implies heavier computations, hence a rule of thumb that could be used is to choose this parameter such that $p \leq B \leq (\frac{p}{2})$. As mentioned earlier, the parameter $\alpha$ should define a small quantile, typically 1%. Finally, $\pi$ determines to what extent the user assigns importance to the variables selected at the previous step. Given that $d_{\text{max}} \ll p$ and $\alpha$ is small, we will typically have that $|I_{d-1}^*| < |I_{d-1}^c|$. In this setting, a choice of $\pi = .5$ for example would deliver a higher probability for the variables in $I_{d-1}^*$ to be included in $I_{d}^*$. All other parameters being equal, increasing the value of $\pi$ would decrease the probability of choosing a variable in $I_{d-1}^c$ and vice versa. Moreover, we discuss in Appendix B how the proposed algorithm can be adjusted to situations where $p$ is either small or very large.

As a final note, it is also possible for the initial model $\mathcal{M}_0$ to already contain a set of $p_0$ covariates which the user considers to be essential for the final output. In this case, the procedure described above would remain exactly the same since the procedure would simply select from the $p$ covariates which are not in the user-defined set and the final model dimension would simply be $p_0 + d$.

3.1.2 Algorithm output:

Once the algorithm is implemented, the user obtains an out-of-sample discrepancy measure for all evaluated models. The final goal is then to find a subset of models
of dimension $d^*$ that in some way minimize the considered discrepancy. A possible solution would be to select the set of models $S_{d^*}^*$ such that $d^* = \min_{d \in \{1, \ldots, d_{\text{max}}\}} q_d(\alpha)$. However, the quantity $q_d(\alpha)$ is unknown and replaced by its estimator $\hat{q}_d(\alpha)$. Due to this, a solution that might be more appropriate would be to consider a testing procedure to obtain $d^*$ taking into account the variability of $\hat{q}_d(\alpha)$. For example, we could find the dimension $d^*$ such that we cannot reject the hypothesis that $\hat{q}_{d^*}(\alpha) = \hat{q}_{d^*+1}(\alpha)$. Thus we sequentially test whether $\hat{q}_{j+1}$ is smaller than $\hat{q}_j$ for $j = 1, \ldots, d_{\text{max}}$. As long as the difference is significant we increment $j$ by one unit, otherwise the minimum is reached and $d^* = j$. A more detailed discussion on the type of tests that can be used for this purpose is presented in Appendix C.

4 Case Studies

In this section we provide an example of how the methodology proposed in this paper selects and groups genes to explain, describe and predict specific outcomes. We focus on the data-set (hereinafter leukemia) which collects information on Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) and is frequently used as an example for gene selection procedures. Indeed, Golub et al. (1999) were among the first to use this data to propose a gene selection procedure which was then followed up by other proposals that used the same data to compare their performance. We will use this data-set to underline the features and advantages of the proposed method. A second data-set concerning the research on breast cancer (presented in Chin et al. (2006)) is analysed in Appendix F to show the outputs of the proposed method from another example.

The analysis of these data-sets focuses both on the advantages of the proposed methodology and the biological interpretation of the outcomes. One of the goals of our method is to help decipher the complexity of biological systems. We will take on an overly simplified view of the cellular processes in which we will assume that one biomarker maps to only one gene that in turn has only one function. Although this assumption is not realistic, it allows us to give a straightforward interpretation of the selected models or “networks” which can therefore provide an approximate first insight into the relationships between variables and biomarkers (as well as between the biomarkers themselves). We clarify that we do not claim any causal nature in the conclusions we present in these analyses but we believe that the selected covariates can eventually be strongly linked to other covariates that may have a more obvious and direct interpretation for the problem at hand. Finally, the data-set has binary outcomes (as does the data-set in Appendix F), hence we will make use of the Classification Error (CE) as a measure of prediction performance and we will not assign weights to a given prediction error. This means that misclassification errors are given the same weight, in the sense that a false positive prediction (e.g. predicted “presence” when the truth
is “absence”) is considered as undesirable as a false negative prediction. However, our method can consider also divergence measures based on unequal weights as highlighted in Section 2.

4.1 Acute Leukemia

4.1.1 Statistical analysis

Golub et al. (1999) were among the first to propose an automatic selection method for cancer classification and demonstrated the advantages of using such a method. One of the main applications of their method was on the leukemia data-set in which information regarding 72 patients is included, namely their type of leukemia (25 patients with AML and 47 patients with ALL) and 7,129 gene expressions used as explanatory variables to distinguish between two types of leukemia. As explained in Golub et al. (1999) this distinction is critical for successful treatment which substantially differs between classes. In fact, although remissions can be achieved using any of these therapies, cure rates are markedly increased and unwarranted toxicities are avoided when targeting the specific type of leukemia with the right therapy.

In order to understand how our proposed methodology performs compared to existing ones, we split the leukemia data into the same training set (38 patients) and test set (34 patients) as in the original work by Golub et al. (1999). We employ our method on the training set to understand the dimension of the model and to select the most relevant genes. Setting $\alpha = 0.01$, the corresponding observed quantile of the 10-fold cross-validation CE ($\hat{D}$) is shown in Figure 1. It can be seen that the error immediately decreases to almost zero when using two covariates instead of one, after which it monotonically increases, suggesting that the optimal model dimension is two.

In Figure 1 we also plotted the performance of the other selection methods used on this training data which are represented by labelled dots reporting the acronyms of these methods that are listed in Table 1. The approach proposed in this work compares favourably to all other methods in terms of prediction power. Indeed, all the other methods lie under the curve to the right of its minimum indicating that, compared to our method, they select models of considerably higher dimensions without achieving the same degree of performance in terms of CE. Therefore, for this particular case, our method outperforms the other methods. The sparsity and tenfold CV error are further illustrated in Table 1, where we also present the average prediction error on the test data. Considering the latter, it can be seen how the performance of the different methods are similar but the proposed method is able to achieve the same performance by selecting models of a considerably lower dimension. As a final note to the table, the last line reports the performance of model averaging. Indeed if the interest lies in predicting, as described earlier, the algorithm of Section 3 provides a set of models.
Figure 1: Number of covariates vs. $\hat{D}$ on leukemia cancer classification training set. The names are abbreviations for other selection method referred in Table 1.
whose CE is below a given quantile $\alpha$. The predictions of these models can be used in the spirit of model averaging where a general prediction can be obtained by taking the average of predictions of the selected set of models. The proposed methodology can therefore be potentially seen as a bridge between model selection and model averaging.

| Method                      | Tenfold CV error | Test error | Number of genes |
|-----------------------------|------------------|------------|-----------------|
| Golub                       | 3/38             | 4/34       | 50              |
| Support vector machine      | 2/38             | 1/34       | 31              |
| (with recursive feature elimination) |                |            |                 |
| Penalised logistic regression | 2/38        | 1/34       | 26              |
| (with recursive feature elimination) |                |            |                 |
| Nearest shrunken centroids  | 2/38             | 2/34       | 21              |
| Elastic net                 | 3/38             | 0/34       | 45              |
| Proposed                    |                  |            |                 |
| Model a                     | 0/38             | 2/34       | 2               |
| Model b                     | 0/38             | 2/34       | 2               |
| Model c                     | 0/38             | 2/34       | 2               |
| [... ]                      |                  |            |                 |
| Model averaging             |                  | 2/34       | 2               |

Table 1: Summary of Leukemia classification results. The table is taken from Zou and Hastie (2005) except for the Proposed part. We obtained a total of 107 models of size 2 (109 different biomarker) using a probability $\alpha = 0.01$, $B = 20000$ bootstrap replicates, a selection probability $\pi = 0.5$ with $D$ the tenfold-CV repeated 10 times. Model a to c are three examples out of the 107 models. All 107 models have a tenfold-CV error of 0. The best test error is 2 and the worst is 12. For model averaging all models are equally weighted.

Once this procedure is completed, we can create a gene network to facilitate interpretation. This is a direct benefit of our method which does not deliver a single model after the selection process but provides a series of models that can be linked to each other and interpreted jointly. Indeed, the existence of a single model that links the covariates to the explained variable is probably not realistic in many settings, especially for gene classification. For this reason, the frequency with which each gene is included within the selected models and with which these genes are coupled with other genes provides the building block to create an easy-to-interpret gene network with powerful explanatory and predictive capacities. A graphical representation of this gene network can be found in Appendix E together with a table where the biomarkers are listed according to their position in the model. These positions represent families of biomarkers (or genes) whose members are interchangeable. By the latter we mean that, given the presence of biomarkers from other families, specific biomarkers can be replaced by another biomarker from within the same family without losing predictive power. This is the idea behind finding a paradigmatic network for gene selection purposes. In
the following paragraph we provide a summary biological interpretation of the three main biomarkers (i.e. the most frequent in the selected models) which we call “hubs” from which the networks start.

4.1.2 Biological interpretation

The three hubs that were identified are the following:

1. Cystatin C: a secreted cysteine protease inhibitor abundantly expressed in body fluids (see Xu et al., 2015);
2. Zyxin: a zinc-binding phosphoprotein that concentrates at focal adhesions and along the actin cytoskeleton;
3. Complement factor D: a rate-limiting enzyme in the alternative pathway of complement activation (see White et al., 1992).

In the current state of knowledge about acute leukemia, these three hubs appear to make sense from a biological viewpoint. Cystatin C is directly linked to many pathologic processes through various mechanisms and recent studies indicate that the roles of Cystatin C in neuronal cell apoptosis induction include decreasing B-cell leukemia-2 (BCL-2) whose deregulation is known to be implicated in resistant AML (see Sakamoto et al., 2015). Zyxin is a protein that interacts with Vasodilator-stimulated phosphoprotein (VASP) with both being involved in cellular adhesion and motility. VASP interacts with ABL (breakpoint cluster region-abelson) and is a substrate of the BcrAbl oncoprotein which drives oncogenesis in patients with chronic myeloid leukemia (CML) due to a constitutive activation of tyrosine kinase activity (see Bernusso et al., 2015). Further results suggest that the phosphorylation and dephosphorylation cycle of VASP by the Abi-1-bridged mechanism regulates association of VASP with focal adhesions, which may regulate adhesion of Bcr-Abl-transformed leukaemic cells (see Masahiro et al., 2012). Finally, Complement factor D, together with several other components of both the classical and alternative complement cascade, is primarily expressed through both adipocytes and monocytes-macrophages in human subjects (see White et al., 1992; Gabrielsson et al., 2003). A recent review in Ratajczak (2014) has stressed the role of the complement cascade as a trigger for hematopoietic stem cells from bone marrow into blood.

The interpretation of the network can be carried out through plots or tables such as those presented in Appendix E where the biomarkers can be grouped together into clusters having the same biological traits, e.g. transcription/translation factor activity, DNA repair and catabolism, apoptotic activity. This grouping allows a more straightforward interpretation of the links between the different families thereby providing a more general overview of how the elements of the identified network interact.
5 Conclusions

This paper has proposed a new model selection method with various advantages compared to existing approaches. Firstly, it allows the user to specify the criterion according to which they would like to assess the quality of a model. In this setting, it gives an estimate of the dimension of the problem, allowing the user to understand how many gene expressions are needed in a model to well describe and predict the response of interest. Building on this, it provides a paradigmatic structure of the selected models where the selected covariates are considered as elements in an interconnected biological network. The approach can handle more variables than observations without going through dimension-reduction techniques such as pre-screening or penalization.

The problem definition of this method and the algorithmic structure used to solve it deliver further advantages such as the ability to cope with noisy inputs, missing data, multicollinearity and the capacity to deal with outliers within the response and the explanatory variables (robustness).

Some issues which must be taken into account concerning the proposed method are (i) its computational demand and (ii) its need for an external validation. As far as the first aspect goes, this can be considered indeed negligible compared to the time often required to collect the data it should analyse and can be greatly reduced according to the needs and requirements of the user. Concerning the second aspect, external validation is a crucial point which is often overlooked and is required for any model selection procedure. In this sense, the proposed method does not differ from any other existing approach in terms of additional requirements.

Having proposed a method with considerable advantages for gene selection using statistical ideas in model selection and machine learning, we expect future research concerning the statistical properties of this approach aiming at understanding its asymptotic behaviour and developing inference tools, which will be highly challenging and rewarding.

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A  Example for Model (1)

A few examples of model (1) are as follows:

- \( Y \) can be a continuous variable representing, for example, the concentration of a certain substance in the body. In this case, a common model is \( Y = X^T \beta^p + \epsilon \), which corresponds to \( g(X, \beta^p) = X^T \beta^p \).

- \( Y \) can also be a count variable, such as when it represents the number of tumours in a patient. A common Poisson model is
  \[
  P(Y = y | X) = \exp(-e^{X^T \beta^p}) e^{X^T \beta^p y / y!},
  \]
  which can be equivalently written as \( Y = e^{X^T \beta^p} + \epsilon \), where \( \epsilon = \exp(-e^{X^T \beta^p}) e^{X^T \beta^p Y / Y!} - e^{X^T \beta^p} \) has mean zero and \( g(X, \beta^p) = e^{X^T \beta^p} \).

- \( Y \) can be a Bernoulli random variable (\( Y \in \{0,1\} \)), when it records the presence/absence of a tumour. A common logistic model is
  \[
  P(Y = 1 | X) = 1 - 1/(e^{X^T \beta^p} + 1)
  \]
  and can be equivalently written as \( Y = 1 - 1/(e^{X^T \beta^p} + 1) + \epsilon \), where \( \epsilon = Y - 1 + 1/(e^{X^T \beta^p} + 1) \) also has mean zero. Thus \( g(X, \beta^p) = 1 - 1/(e^{X^T \beta^p} + 1) \).

B  Adapting the Algorithm to \( p \)

In this subsection we provide two variants of the algorithm proposed in Section 3 in order to adapt it to situations where \( p \) is either small or large.

B.1  Adapting the Algorithm to Very Large \( p \)

In situations where \( p \) is extremely large and the initial step of the algorithm is not computationally feasible, this step can, for example, be replaced by the following modified initial step:

\[ A'. \]  Large \( p \) Modified Initial Step: We start by augmenting our initial variable set \( M_0 \) with \( d = 1 \) variable in order to construct the set \( I^*_1 \).

1. Augment \( M_0 \) with \( d = 1 \) variable selected uniformly at random in \( J_f \).
2. Construct \( B \) models of dimension 1 by repeating Step \( A'.1 \) \( B \) times.
3. From Steps \( A'.1 \) and \( A'.2 \), construct the set \( I^*_1 \) using (3). Go to Step B and let \( d = 2 \).
B.2 Adapting the algorithm to small $p$

On the other hand, when $p$ is of reasonable size it may be possible to compute and evaluate all the $\binom{p}{d'}$ models of dimension $2 \leq d' \leq d_{\text{max}}$. In such cases, it may be feasible to also modify the initial step of the proposed algorithm to a different modified initial step. A possible modification is the following:

\begin{enumerate}
\item We augment our initial variable set $\mathcal{M}_0$ with $d$ ($1 \leq d \leq d'$) variables in order to construct the sets $\mathcal{I}_1^*, \ldots, \mathcal{I}_{d'}^*$.
\begin{enumerate}
\item Construct the $p$ possible models obtained by augmenting $\mathcal{M}_0$ with each of the $p$ available variables.
\item Compute $\hat{D}(\cdot, \cdot)$ for every model obtained in Step (i).
\item From Steps (i) and (ii), construct the set $\mathcal{I}_1^*$ using (3). Go to Step A''.2 and let $d = 2$.
\end{enumerate}
\item We augment our initial model $\mathcal{M}_0$ set by $d$ variables in order to construct the set $\mathcal{I}_d^*$.
\begin{enumerate}
\item Construct the $\binom{p}{d}$ possible models and augment $\mathcal{M}_0$ with all variables of these constructed models.
\item Compute $\hat{D}$ for every model obtained in Step (i).
\item From Steps (i) and (ii), construct the set $\mathcal{I}_d^*$ using (3) and let $d = d + 1$. Go to Step A''.2 (if $d < d'$) or Step B.1 (if $d \geq d'$), with model dimension starting value $d$.
\end{enumerate}
\end{enumerate}

C Determining the Models Dimension via Testing

The type of test and its corresponding rejection level are determined by the user based on the nature of the divergence measure. For example, if we take the $L_1$ loss function as a divergence, one could opt for the Mann-Whitney test or if the loss function is a classification error (as in the applications in Section 4), one could choose the binomial test or other tests for proportions. The rejection level will depend, among others, on the number of tests that need to be run, typically less than $d_{\text{max}} - 1$, and need to be adjusted using, for example, the Bonferroni correction. Finally, once the set $\mathcal{S}_{d'}^*$ is obtained, the user may still want to “filter” the resulting models. Indeed, the number of models in the solution $\mathcal{S}_{d'}^*$ may be large and the corresponding divergence estimates may vary considerably from model to model. Since these divergence measures are estimators, we again propose a multiple testing procedure to reduce the number of models in $\mathcal{S}_{d'}^*$. 

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Before doing so, we eliminate redundant models, thereby making sure that every model is included only once. Then, we start the testing procedure with an empty set \( S^0 = \emptyset \) to which we add the model (or one of the models) that has the minimum divergence measure estimate, denoted \( \hat{D}_{j_{\text{min}}} \), where \( j_{\text{min}} \in S^* \) denotes this model. Then for every model \( j \in S^* \setminus j_{\text{min}} \), we test whether \( \hat{D}_j \) is greater than \( \hat{D}_{j_{\text{min}}} \). We add the model to \( S^0 \) if the difference is not significant and stop adding models as soon as the test deems that the divergence of the next model is indeed larger. By doing so we finally obtain \( S^0 \subseteq S^* \), which is the set containing the models (and hence covariates) which can be interpreted in a paradigmatic network.

D RELATED LITERATURE

Some of the ideas put forth in this work have also been considered in the literature. An extensive survey of the related works goes beyond the scope of this paper. Here we briefly describe some of the connections to three main ideas that have been explored previously.

The first one is recognizing that practitioners might aim to minimize some criterion that differs from likelihood-type losses. An interesting paper illustrating this point is Juang et al. (1997) in the context of speech recognition. For their classification problem, these authors propose to minimize a “smoothed” version of the decision rule used for classification. The advantage of this procedure is that it yields better misclassification errors than using pure likelihood based criteria which intrinsically fit a distribution to the data. In the approach presented in this work we also deliver an approximate solution but, as opposed to approximating the problem and solving the latter in an exact manner as in Juang et al. (1997), we define the exact problem and try to approximately minimize the misclassification error through our algorithm.

Second, there is a large literature that uses stochastic search procedures to explore the space of candidate models. Influential work in this direction include George and McCulloch (1993) and George and McCulloch (1997) who postulate hierarchical Bayesian models. In their set up, subsets of promising predictors form models with higher posterior probabilities. An interesting application of this framework for disease classification using gene expression data is the work of Yang and Song (2010). Cantoni et al. (2007) also consider a random exploration of the space of possible models, but avoiding the Bayesian formulation of George and McCulloch (1993). Their approach defines a probability distribution for the various candidate models based on a cross-validated prediction error criterion and then uses a Markov Chain Monte-Carlo method to generate a sample from this probability distribution. An important feature of the stochastic search implied by our algorithm is that it is a greedy method, while the aforementioned methods are not. The typical forward/backward greedy algorithms proposed in the literature are not
random, while existing stochastic procedures are not greedy. Thus, the combination of greedy approach and random search approach seems to be new. See for instance Zhang (2011) for some theory on greedy algorithms in sparse scenarios.

Third, other authors have also considered providing a set of interesting models as opposed to a single “best” model. The stochastic search procedures mentioned in the above paragraph can naturally be used to obtain a group of interesting models. For example, Cantoni et al. (2007) consider a set of best indistinguishable models in terms of prediction. Random forests can be used to select variables and account for the stability of the chosen model as in Díaz-Uriarte and De Andres (2006). These methods can also be used to construct a set of interesting models.

E MORE RESULTS ON ACUTE LEUKEMIA

Figure 2 graphically represents the network created by the proposed method for the leukemia data-set where the size of a disk represents the frequency with which a particular biomarker is included in the selected models, and the line connecting the disks indicates the biomarkers that are included in the same model. Since the model dimension in this case is two, each biomarker is connected with only one other biomarker and, as can be observed, the proposed method identifies three main “hubs” for the networks (green disks) generating three networks.

Table 2 reports the main biomarker hubs and related biomarker networks for the leukemia data set analysed in Section 4.1.

F BREAST CANCER

The second data-set we analyze is the breast cancer data presented in Chin et al. (2006) for which we only provide a summary biological interpretation of the results of the proposed method, having already discussed the working of our approach in Section 4.1. The main goal behind analyzing this data is to identify the estrogen receptor expression on tumor cells which is a crucial step for the correct management of breast cancer. Figure 3 shows the paradigmatic network identified by our method for the breast cancer data for which the selected model dimension is three (i.e. only three biomarkers are needed in a model to well classify the breast cancer). Table 3 provides the details of the networks based on the three main hubs and is to be interpreted as described in Section 4.1.

This figure is a clear example of the advantages of the proposed method since, it not only selects a set of low-dimensional models with a high predictive power, but also
Figure 2: Network representation of biomarkers selected from leukemia data-set. Colors represent the position of covariates within the model: green for first position (hub) and orange for second. The width of the connecting lines is proportional to the frequency with which two biomarkers appear in the same model. The size of the disk is proportional to the frequency with which a biomarker is present within the selected set of models.
Figure 3: Network representation of biomarkers selected from breast cancer data-set. Colors represent the position of covariates within the model: green for first position (hub), orange for second and purple for third. The width of the connecting lines is proportional to the frequency with which two biomarkers appear in the same model. The size of the circles is proportional to the frequency with which a biomarker is present within the selected set of models. (Note: biomarker “209602_s_at” is merged with biomarker “209604_s_at”).
## Table 2: Biomarker network organisation - leukemia data set - Lymphoblastic / Myeloblastic leukemia.

| Affy ID | Gene ID | Gene Function | Biological Process |
|---------|---------|---------------|--------------------|
| NETWORK 1 | | | |
| Position 1 | M27891_at | ENSG00000101439 | Cystatin C | AA |
| | Position 2 | D80006_at | ENSG00000114978 | MOB kinase activator 1A | AA |
| | | M20779_at | ENSG00000163559 | Collagen, type VI, alpha 3 | AA |
| | | U70336_at | ENSG00000108773 | K(lysine) acetyltransferase 2A | TF |
| | | U90547_at | ENSG00000182852 | High mobility group nucleosomal binding domain 4 | TF |
| | | X68699_at | ENSG00000182944 | Ewing Sarcoma region 1; RNA binding protein | TF |
| | | M74889_at | ENSG00000134872 | Adenomatous polyposis coli, DP2, DP3, PPP1R46 | TF |
| | | U31166_at | ENSG00000139372 | Thymine-DNA glycoylase | TF |
| | | Z69381_at | ENSG0000017470 | ATPase, Ca++ transporting, ubiquitously | IPT |
| | | U49248_at | ENSG00000233839 | ATP-binding cassette, sub-family C (CFTR/MRP), member 2 | IPT |
| | | X801105_at | ENSG00000102579 | Coronin, actin binding protein, IA | IPT |
| | | H28123-HT2813_at | ENSG00000092841 | Myosin, Light Chain, Alkali, Smooth Muscle (Gh-U02629) | ACC |
| | | M94145_at | ENSG000000142493 | Capping protein (actin filament), gelsolin-like | ACC |
| | | L31075_at | ENSG0000140575 | IQ motif containing GTPase activating protein 1 | ACC |
| | | L67833_at | ENSG00000392910 | Proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) | APC |
| | | 303588_at | ENSG00000102178 | Ubiquitin-like 4A | APC |
| | | D89280_at | ENSG0000085265 | FCN1, Ficolin-1 | IR |
| | | X03934_at | ENSG00000167296 | CD3d molecule, delta (CD3-TCR complex) | IR |
| NETWORK 2 | | | |
| Position 1 | X90735_at | ENSG00000159840 | Zyxin | ACC |
| | Position 2 | X04526_at | ENSG00000185688 | Guanine nucleotide binding protein (G protein), beta polypeptide 1 | ST |
| | | D79077_at | ENSG000001292495 | Tyrosine 3-monooxygenase/trihydroxyanisole 2-monooxygenase activation protein, etc | ST |
| | | U36645_at | ENSG00000102034 | E74-like factor 4 (ets-domain transcription factor) | TF |
| | | U98867_at | ENSG00000186144 | Polynucleare (RNA) III (DNA directed) polypeptide C (62kD) | TF |
| | | U291177_at | ENSG00000127616 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4 | TF |
| | | Y8029_at | ENSG00000177892 | Retinoid acid receptor, beta | TF |
| | | D17382_at | ENSG00000113967 | DEAD (Asp-Glu-Ala-Asp) Box Helicase 6 | TF |
| | | H28123-HT2813_at | ENSG00000142934 | Ras-Related Protein RagB | TF |
| | | M83233_at | ENSG00000140262 | Transcription factor 12 | TF |
| | | U94855_at | ENSG00000175390 | Eukaryotic translation initiation factor 3, subunit F | TF |
| | | L67775_at | ENSG00000136045 | FWP1 homolog | TF |
| | | D63586_at | ENSG00000116266 | Synactin binding protein 3 | IR |
| | | M33568_at | ENSG00000110655 | CD81 molecule | IR |
| | | H1G102-H1G104_at | ENSG00000171540 | Macmarcks | IR |
| | | M92287_at | ENSG00000112576 | Cyclin D3 | IR |
| | | M84383_rna1_at | ENSG00000113575 | Protein Phosphatase 2 (formerly 2A), catalytic subunit, alpha isozyme | IR |
| | | U83998_at | ENSG00000169372 | CASP2 and RIPK1 domain containing adaptor with death domain | IR |
| | | S0417_rna1_at | ENSG00000169372 | CD36 - Thrombospondin receptor | IR |

Table 2: Biomarker network organisation - leukemia data set - Lymphoblastic / Myeloblastic leukemia. TF = Transcription/translational factor activity, DNA repair and catabolism - AA = apoptotic activity - IR = immunity, inflammatory response (blood coagulation, antigen presentation and complement activation) - IPT = intracellular protein trafficking, transmembrane transport - ACC = actin activity, cytoskeleton organisation - APC = protein catabolism - ST = intracellular signal transduction - CG = cell growth, proliferation and division. Source: www.ensembl.org; www.uniprot.org

provides the basis for a more general biological interpretation which takes into account interactions between different biomarkers as opposed to one single model. The three main hubs identified through the proposed algorithm are:
1. GATA binding protein 3 (GATA3): a transcription factor regulating the differentiation of breast luminal epithelial cells;

2. IL6 Signal Transducer (IL6 ST): a pro-inflammatory cytokine signal transducer;

3. TBC1 domain family, member 9 (TBC1D9): a GTPase-activating protein for Rab family protein involved in the expression of the ER in breast tumors.

GATA3 is known to regulate the differentiation of epithelial cells in mammary glands (see Kouros-Mehr et al., 2006) and is required for luminal epithelial cell differentiation. Its expression is progressively lost during luminal breast cancer progression as cancer cells acquire a stem cell-like phenotype (see Chou et al., 2010). IL6 ST has been linked to breast cancer epithelial-mesenchymal transition and cancer stem cell traits (see Chung et al., 2014), cancer-promoting microenvironment (see Bohrer et al., 2014) and resistance (see Christer et al., 2013). Moreover, this result supports the assertion by Taniguchi and Karin (2014) that IL6 ST and related cytokines are the critical lynchpins between inflammation and cancer. Finally, concerning the third biomarker, a recent publication by Andres and Wittliff (2012) has shown that the expression of the ER on the surface of breast tumor cells is highly correlated with the coordinate expression of different genes among which we can find TBC1D9 and GATA3. These two genes are not only considered as relevant genes according to the proposed method but as actual hubs of the “best” models which define the structure of the identified network. Instead of selecting a single model with many biomarkers whose interactions may be difficult to interpret, the proposed method selects a set of models with few biomarkers that allow them to be individually easy to interpret without losing the possibility of interpreting them within the larger network. This is what this paper intends with the expression “paradigmatic network” since by taking this approach it is possible to identify a set of biomarker families within which each biomarker is interchangeable with the others.
| Affy ID   | Gene ID         | Gene Function                                                                 | Biological Process |
|-----------|-----------------|-------------------------------------------------------------------------------|--------------------|
| 209604    | ENSG00000107485 | GATA binding protein 3                                                        | TF                 |
| 205520    | ENSG00000115808 | Striatin, calmodulin binding protein                                           | ER                 |
| 204902    | ENSG00000168397 | Autophagy related 4B, cysteine peptidase (APG4B, AUTL1), DKFZp566D1822,       | APC                |
|           |                 | KIAA0943                                                                      |                    |
| 221695    | ENSG00000172243 | C-type lectin domain family 7, member A                                       | IR                 |
| 49049     | ENSG00000178498 | Deltex 3, E3 ubiquitin ligase                                                 | APC                |
| 209602    | ENSG00000107485 | GATA binding protein 3                                                        | TF                 |
| 216604    | ENSG00000003989 | Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 | IPT                |
| 218877    | ENSG00000066651 | TRNA methyltransferase 11 homolog                                             | TF                 |
| 201316    | ENSG00000106588 | Proteosome (prosome, macropain) subunit, alpha type, 2                       | APC                |
| 208019    | ENSG00000147117 | Zinc finger protein 157                                                        | TF                 |
| 219108    | ENSG00000186654 | PRR5 (Proline rich 5 (renal))                                                 | CG                 |
| 219413    | ENSG00000171241 | SHC SH2-domain binding protein 1                                              | CG                 |
| 204508    | ENSG0000129719  | Vacuolar protein sorting 33 homolog A                                         | APC                |
| 210021    | ENSG0000152669  | Cyclin O                                                                       | CG                 |
| 208915    | ENSG00001033365 | Golgi-associated, gamma adaptin ear containing, ARF binding protein 2         | IPT                |
| 214318    | ENSG00000073910 | Furry homolog                                                                  | ACC                |
| 207586    | ENSG00000173991 | Tuf-cap (Telomatin)                                                            | ACC                |
| 221695    | ENSG00000066140 | Serine/threonine/tyrrosine kinase 1                                           | CG                 |
| 202408    | ENSG0000059804  | Solute carrier family 2 (facilitated glucose transporter), member D1          | STB                |
| 20915     | ENSG00001033365 | Golgi-associated, gamma adaptin ear containing, ARF binding protein 2         | IPT                |
| 213102    | ENSG00000141959 | Phosphofructokinase, liver                                                     | STM                |
| 208915    | ENSG00001033365 | Golgi-associated, gamma adaptin ear containing, ARF binding protein 2         | IPT                |
| 201316    | ENSG00000106588 | Proteosome (prosome, macropain) subunit, alpha type, 2                       | APC                |
| 212288    | ENSG00000187239 | Formin binding protein 1                                                       | ACC                |
| 209713    | ENSG0000116704  | Solute carrier family 35 (UDP-GlcA/UDP-GaNAc transporter), member D1          | STB                |
| 208915    | ENSG00001033365 | Golgi-associated, gamma adaptin ear containing, ARF binding protein 2         | IPT                |
| 212702    | ENSG00000185963 | Bicaudal D homolog 2                                                           | ACC                |
| 221030    | ENSG00000138639 | Rho GTPase activating protein 2                                                | ACC                |
| 212956    | ENSG0000109436  | TBC1 domain family, member 9 (with GRAM domain)                               | IPT                |
| 210221    | ENSG0000008644  | Cholinergic receptor, nicotinic, alpha 3 (neuronal)                            | ITT                |
| 214194    | ENSG00000083520 | DIS3 mitotic control homolog (Ribosomal RNA-processing protein 44)            | TF                 |
| 221695    | ENSG00000060140 | Serine/threonine/tyrrosine kinase 1                                           | CG                 |
| 216841    | ENSG00000232267 | ACTR3 pseudogene 2                                                             | PUP                |
| 221102    | ENSG000000205630| Cilia and flagella associated protein 44                                       | ACC                |
| 221030    | ENSG00000138639 | Rho GTPase activating protein 2                                                | ACC                |
| 201316    | ENSG0000106588  | Proteosome (prosome, macropain) subunit, alpha type, 2                       | APC                |
| 221695    | ENSG00000060140 | Serine/threonine/tyrrosine kinase 1                                           | CG                 |
| 209287    | ENSG00000070831 | Cell division control protein 42 homolog                                     | ACC                |
| 221901    | ENSG00000138944 | KIAA1644                                                                       | PUP                |
| 208915    | ENSG00000103365 | Golgi-associated, gamma adaptin ear containing, ARF binding protein 2         | IPT                |
| 209602    | ENSG00000107485 | GATA3                                                                          | TF                 |
| 202951    | ENSG00000112079 | Serine/threonine kinase 35                                                    | CG                 |
| 220443    | ENSG00000116035 | VAX2 (ventral anterior homeobox 2)                                            | TF                 |
| 221935    | ENSG00000088256 | Guanine nucleotide binding protein (G protein), alpha 11 (Gq class)           | ITT                |
| 207303    | ENSG00000154678 | Phosphodiesterase 1C, calmodulin-dependent 70kDa                              | ST                 |
| 205152    | ENSG00000157103 | Solute carrier family 6, member 1                                             | ST                 |
| Position 1 | Position 2 | Position 3 |
|------------|------------|------------|
| 207518     | ENSG00000153933 | Diacylglycerol kinase, epsilon 64kDa | ST |
| 206270     | ENSG00000126583 | Protein kinase C, gamma | ST |
| 208964     | ENSG00000123505 | Fatty acid desaturase 1 | FAM |
| 201102     | ENSG00000141959 | ATP-dependent 6-phosphofructokinase, liver type | STM |
| 214972     | ENSG00000198408 | Protein O-GlcNAcase (Meningioma expressed antigen 5 (hyaluronidase)) | ST |
| 210477     | ENSG00000107643 | Mitogen-activated protein kinase 8 | CG |
| 205907     | ENSG00000127083 | Osteomodulin | STM |

**NETWORK 3**

| Position 1 | Position 2 | Position 3 |
|------------|------------|------------|
| 212956     | ENSG00000119436 | TBC1 domain family, member 9 (with GRAM domain) | IPT |
| 202951     | ENSG00000112079 | Serine/threonine kinase 38 | CG |
| 221955     | ENSG00000089256 | Guanine nucleotide binding protein (G protein), alpha 11 (Gq class) | ITT |
| 207303     | ENSG00000154678 | Phosphodiesterase 1C, calmodulin-dependent 70kDa | ICT |
| 216814     | ENSG00000232267 | ACTR3 pseudogene 2 | PUP |
| 221103     | ENSG00000206530 | Cilia and flagella associated protein 44 | ACC |

**Table 3:** Biomarker network organisation - breast cancer data set - Estrogen Receptor - Breast Cancer.

*TF = Transcription/translation factor activity, DNA/RNA repair and catabolism - ER = estrogen receptor activity - APC = autophagy - protein catabolism - IR = immunity, inflammatory response (blood coagulation, antigen presentation and complement activation) - CC = cell/cell communication - ST = intracellular signal transduction, protein glycosylation - CG = cell growth and division - IPT = intracellular protein trafficking, transmembrane amino-acid transporter - ACC = actin activity, cytoskeleton organisation, cell projection - STM = sugar transport and metabolism - ITT = ion transmembrane transport, transmembrane signaling systems - PUP = pseudogene, uncharacterized protein - FAM = fatty acid metabolism. Source: [www.uniprot.org](http://www.uniprot.org); [www.ncbi.nlm.nih.gov/gene](http://www.ncbi.nlm.nih.gov/gene)