Statistical analysis of genomic protein family and domain controlled annotations for functional investigation of classified gene lists

Marco Masseroli*1,2, Elisa Bellistri2, Andrea Franceschini2 and Francesco Pinciroli2

Address: 1Dipartimento di Elettronica e Informazione, Politecnico di Milano, piazza Leonardo da Vinci 32, 20133 Milano, Italy and 2BioMedical Informatics Laboratory, Dipartimento di Bioingegneria, Politecnico di Milano, piazza Leonardo da Vinci 32, 20133 Milano, Italy

Email: Marco Masseroli* - masseroli@elet.polimi.it; Elisa Bellistri - elisa.bellistri@fastwebnet.it; Andrea Franceschini - atariw@gmail.com; Francesco Pinciroli - francesco.pinciroli@polimi.it

* Corresponding author

Abstract

Background: The increasing protein family and domain based annotations constitute important information to understand protein functions and gain insight into relations among their codifying genes. To allow analyzing of gene proteomic annotations, we implemented novel modules within GFINDer, a Web system we previously developed that dynamically aggregates functional and phenotypic annotations of user-uploaded gene lists and allows performing their statistical analysis and mining.

Results: Exploiting protein information in Pfam and InterPro databanks, we developed and added in GFINDer original modules specifically devoted to the exploration and analysis of functional signatures of gene protein products. They allow annotating numerous user-classified nucleotide sequence identifiers with controlled information on related protein families, domains and functional sites, classifying them according to such protein annotation categories, and statistically analyzing the obtained classifications. In particular, when uploaded nucleotide sequence identifiers are subdivided in classes, the Statistics Protein Families&Domains module allows estimating relevance of Pfam or InterPro controlled annotations for the uploaded genes by highlighting protein signatures significantly more represented within user-defined classes of genes. In addition, the Logistic Regression module allows identifying protein functional signatures that better explain the considered gene classification.

Conclusion: Novel GFINDer modules provide genomic protein family and domain analyses supporting better functional interpretation of gene classes, for instance defined through statistical and clustering analyses of gene expression results from microarray experiments. They can hence help understanding fundamental biological processes and complex cellular mechanisms influenced by protein domain composition, and contribute to unveil new biomedical knowledge about the codifying genes.
Background
A great quantity of biomolecular information about genes and proteins is increasingly accumulating in form of textual annotations within heterogeneous and widely distributed databanks [1], which are often publicly accessible through the Internet. Among such information, gene function controlled annotations are the most interesting because their analysis can highlight new biomedical knowledge, including the identification of functional relationships among genes or the involvement of specific genes in complex patho-physiological processes.

The gene Ontology (GO) [2] is currently considered the most important source of functional information and numerous studies are based on GO biological processes or molecular function controlled annotations. However, in many cases the Gene Ontology provides only high level annotations of processes or functions that can be associated with many disorders and in which numerous genes can be involved. Moreover, many genes and proteins still have very few or no GO annotations, and several GO annotations have been assigned only according to computational inferences but not yet experimentally confirmed or revised by experts. Thus, to investigate gene function at a genomic level we think it is important to consider all different types of functional information available. Among them protein family and domain annotations constitute one of the most useful information to understand protein functions and gain insight into relations among their coding genes [3]. Comprehension of domain structure of proteins within genomes is also fundamental to better understand the evolutionary forces and emerging functions shaping genomes [3]. In fact, protein families and domains respectively represent groups of evolutionarily related proteins and their components that fold independently from the remaining protein chain. The increasing number of proteins for which domain-based annotations are available hence represents an important background for computational genome-wise analyses [3].

In the last years, several databanks have been creating to collect and provide proteomic information in organized form, including protein family and domain controlled annotations. Among them, the Pfam [4] and InterPro [5] databanks are considered the most reliable and comprehensive, respectively. Pfam is a collection of protein families and domains that contains information about multiple alignments of protein domains and conserved protein regions. InterPro is an integrated documentation resource for protein families, domains and functional sites. It combines a number of databases, which use different methodologies and a varying degree of biological information on well-characterized proteins to derive protein signatures. Pfam, InterPro and other databanks supply a great amount of valuable information. However, for the interpretation of large experimental datasets, such as those from high-throughput technologies, it is paramount the support of automated tools able to automatically retrieve the information of interest from these databanks and use it for exhaustive analysis and mining.

In order to allow comprehensive evaluation of gene annotations sparsely available in numerous different databanks accessible through the Internet, we previously developed GFINDer [6]. It is a publicly accessible Web server [7] that dynamically aggregates and keeps updated functional and phenotypic annotations of user-uploaded gene lists and allows performing their statistical analysis and mining. For this purpose, in GFINDer independent and interconnected modules use several controlled vocabularies describing gene related biomolecular processes and functions.

With the aim of effectively take advantage of the valuable protein information present in Pfam and InterPro databanks, in GFINDer we implemented new specific modules dedicated to exploring and analyzing functional signatures of gene protein products. They allow annotating user-classified nucleotide sequence identifiers with controlled information on codified protein families, domains, and functional sites; classifying them according to such protein annotation categories; and statistically analyzing the classifications defined. The novel GFINDer modules support a genomic approach in the biological interpretation of high-throughput experimental results and hence in the understanding of fundamental biological processes and complex cellular patho-physiological mechanisms.

Results
Structuring and hierarchical tree reconstruction of protein family and domain controlled annotations
Pfam databank version 19.0 contained 8,183 protein family domain entries and InterPro databank release 12.1 included 12,967 entries (9,065 protein families, 3,587 protein domains and 315 functional sites including post translational modifications, repeats, active and binding sites). We structured all these entries in a GFINDer database and found that 3,495 InterPro entries (2,587 protein families, 877 protein domains and 31 functional sites) were grouped in 927 hierarchical trees of parent/child relations (610 of protein families, 304 of protein domains and 13 of functional sites). We reconstructed such parent/child hierarchical trees within the GFINDer database and noticed that protein family trees had a maximum of 6 levels, with an average of 431 entries per level; protein domain trees had a maximum of 5 levels, with an average of 175 entries per level; and functional site trees had a maximum of 2 levels, with an average of 14 entries per level. Thus, only a total of 26.95% of the protein family
(28.54%), domain (24.45%) and functional sites (9.84%) controlled annotations available in InterPro are hierarchically organized in many unrelated and very large, but not deep, trees of parent/child hierarchical relations.

**Statistical analysis of protein family and domain annotations**

We developed and added within the GFINDer Web system new original modules specifically devoted to the exploration and analysis of functional signatures of gene protein products. They first enable the annotation of numerous user-classified nucleotide sequence identifiers with controlled information on related protein families, domains, and functional sites available in Pfam and InterPro databases. Then, they allow classifying the annotated nucleotide sequence identifiers according to their protein annotation categories, and statistically analyzing the obtained classifications. In particular, the **Exploration Protein Families & Domains** module (Figure 1) allows to easily and graphically understand either how many and which protein families, domains, and functional sites are associated with each considered gene, or how many of the user-selected genes refer to each protein family, domain, or functional site. When the user-uploaded genes are subdivided in classes (e.g. from clustering analysis of microarray results), the **Statistics Protein Families & Domains** module (Figures 2 and 3) allows evaluating the importance of each Pfam or InterPro controlled annotation for the uploaded genes by highlighting protein annotations significantly more represented within the user-defined classes of genes. The annotated genes are grouped accordingly to their class and annotation categories, and their distribution among the considered categories is statistically evaluated. For this aim, different statistical tests and type of corrections for multiple tests have been implemented in GFINDer (see the Methods section). After selecting a specific gene class, for each protein family or domain annotation category in that class the module provides the observed number of input genes, their expected number, and the significance p-value for that category with its histogram (Figures 2 and 3). In addition, external links to Pfam or InterPro protein family or domain descriptions related to the considered genes are given.

**Logistic regression of protein family and domain annotations**

In GFINDer Web system we also implemented a new **Logistic Regression** module. It exploits controlled protein family and domain information provided by Pfam and InterPro databanks to allow executing logistic regression analysis of functional signature annotations that characterize protein products of user-uploaded classified gene lists. Such analysis can allow identifying which protein functional characteristics better explain the binary classification of a set of genes, for instance obtained through statistical and clustering analysis of gene expression results from microarray experiments. It can also allow estimating the probability that a gene, which codifies proteins with certain functional characteristics, is functionally similar to a set of genes whose protein products have those functional characteristics. This can help in revealing or better understanding the functions of new or less studied genes.

Figure 4 shows an example of logistic regression analysis results obtained for a set of genes whose protein product domains and relation to neurobiology (121 genes), or cardiovascular system (55 genes), are known [8-10]. Figure 5 shows the distribution of such considered genes according to their values of calculated logistic regression and estimated probability of being related to neurobiology.
Validation of implemented protein family and domain analysis

In order to test effectiveness of our approach and assess capabilities of implemented GFINDer Protein Families & Domains modules, we used them to evaluate two distinct sets of genes. The first consisted of 142 genes codifying tyrosine kinase proteins (90 genes) or tyrosine phosphatase proteins (52 genes) [11-13]. The second included 179 genes related to apoptosis (92 genes) or growth factors (87 genes) [14-16]. We used the GFINDer Exploration and Statistics Protein Families & Domains modules to evaluate the relevant presence of genes associated with specific protein families or domains within the tyrosine kinase genes (TK) versus the tyrosine phosphatase genes (TP), or within the apoptosis genes (APO) versus the growth factor genes (GF) considered.

First, with the Exploration module we observed the distribution of protein families or domains within the two considered TK and TP classes of genes (Figure 1). Then, using

Table 1

| Type | Tree Level | Category Name | P-value_{test-type} | Log(1/P) |
|------|------------|---------------|---------------------|----------|
| D 25 1 | Protein kinase [0.04, 0.77, 0.1, 0.42] | $p = 0.00301$ |          |
| D 29 0 | Protein kinase [0.04, 0.77, 0.1, 0.42] | $p = 0.01599$ |          |
| D 29 2 | Protein kinase [0.04, 0.77, 0.1, 0.42] | $p = 0.00010$ |          |
| D 260 0 | Src kinases [0.21, 0.14, 0.8, 0.16] | $p = 0.00016$ |          |
| D 29 0 | Src kinases [0.21, 0.14, 0.8, 0.16] | $p = 0.01599$ |          |
| D 29 2 | Src kinases [0.21, 0.14, 0.8, 0.16] | $p = 0.00010$ |          |
| D 12 1 | Protein receptors, signal transduction [0.13, 0.4, 0.8, 0.1] | $p = 0.00010$ |          |
| D 62 1 | Src-like src family of kinases [0.13, 0.4, 0.8, 0.1] | $p = 0.00010$ |          |
| D 264 1 | Growth factor receptors [0.13, 0.77, 0.4, 0.42] | $p = 0.02305$ |          |
| D 264 2 | Growth factor receptors [0.13, 0.77, 0.4, 0.42] | $p = 0.02923$ |          |
| D 21 1 | Protein-protein interaction [0.0, 0.2, 0.4, 0.02] | $p = 0.00010$ |          |
| D 21 1 | Protein-protein interaction [0.0, 0.2, 0.4, 0.02] | $p = 0.00010$ |          |
| D 5 1 | Protein-protein interaction [0.0, 0.2, 0.4, 0.02] | $p = 0.00010$ |          |
| D 5 0 | Protein-protein interaction [0.0, 0.2, 0.4, 0.02] | $p = 0.00010$ |          |

Figure 2

GFINDer Statistics Protein Families & Domains module: protein family analysis. InterPro protein families most significantly over- (red) and under-represented (green) in the considered tyrosine kinase versus tyrosine phosphatase gene classes; Type: type of InterPro entry (F: protein family); Tree: tree label in the defined protein family parent/child hierarchy, if any; Level: level in the related tree of the defined protein family parent/child hierarchy, if any (higher levels correspond to more specific protein families); $P_{value_{test-type}}$: p value defining relevance of a given protein family (Category Name) for a considered class of genes, and initial of used statistical test name (h: hypergeometric distribution test).

Figure 3

GFINDer Statistics Protein Families & Domains module: protein domain analysis. InterPro protein domains most significantly over- (red) and under-represented (green) in the considered tyrosine kinase versus tyrosine phosphatase gene classes; Type: type of InterPro entry (D: protein domain); Tree: tree label in the defined protein domain parent/child hierarchy, if any; Level: level in the related tree of the defined protein domain parent/child hierarchy, if any (higher levels correspond to more specific protein domains); $P_{value_{test-type}}$: p value defining relevance of a given protein domain (Category Name) for a considered class of genes, and initial of used statistical test name (h: hypergeometric distribution test).
the Statistics module we evaluated the protein families most represented in the TK versus TP class. We concentrated only on genes with protein family annotations and on protein family categories associated with at least three of the considered genes. As shown in Figure 2, statistical analysis correctly selected protein families related to the appropriate class of considered genes. In fact, the significant protein families selected included: "Receptor tyrosine kinase, class V" (\( p = 0.00932 \)) and "Receptor tyrosine kinase, class II" (\( p = 0.02122 \)) categories for the TK class; "Protein-tyrosine phosphatase, non-receptor 1/2" (\( p = 0.00375 \)) for the TP class.

![Figure 4](image-url) **GFINDer Logistic Regression module: selected protein domains.** InterPro protein domains (Protein Category Name) with a relevant logistic regression coefficient and codified by the considered neurobiology or cardiovascular system related genes; Tree: tree label in the defined protein domain parent/child hierarchy, if any; Level: level in the related tree of the defined protein domain parent/child hierarchy, if any (higher levels correspond to more specific protein domains).

![Figure 5](image-url) **Calculated logistic regression curve for the considered neurobiology (NEURO) or cardiovascular system (CARDIO) related genes.** Each point area indicates the number of considered genes with a calculated regression value (logit\( (p) \)) and estimated probability \( (p) \), of belonging to the NEURO class, corresponding to the point center.
Finally, we analyzed the protein domains most represented in the TK versus TP class. Focusing only on genes with protein domain annotations and on protein domain categories associated with at least five of the considered genes, GFINDer statistical analysis properly highlighted as most relevant in each gene class protein domains logically pertaining to that class (Figure 3). In fact, the protein domains selected as most significant after FDR multiple test correction included: "Protein kinase" \((p < 0.00001)\), "Protein kinase-like" \((p < 0.00001)\), "Tyrosine protein kinase" \((p < 0.00001)\), "Src homology-3" \((p = 0.000016)\), and "SH2 motif" \((p = 0.01459)\) protein domains for the TK gene class; "Tyrosine specific protein phosphatase and dual specificity protein phosphatase" \((p < 0.00001)\), "Tyrosine specific protein phosphatase" \((p < 0.00001)\), and "Protein tyrosine phosphatase, catalytic region" \((p < 0.00001)\) for the TP class.

Similarly, we used the Statistics module to evaluate the protein domains most represented in the APO versus GF class. Also in this case, GFINDer statistical analysis properly selected as most relevant in each gene class protein domains correctly pertaining to that class (Figure 6). In fact, they included "DEATH-like" \((p < 0.00001)\), "TNFR/CD27/30/40/95 cysteine-rich region" \((p = 0.00066)\), "Caspase Recruitment" \((p = 0.00085)\), and "Apoptosis regulator Bcl-2, BH" \((p = 0.00296)\) for the APO class; "Transforming growth factor beta" \((p < 0.00001)\), "Transforming growth factor beta (TGFb), N-terminal" \((p < 0.00001)\), and "Growth factor, cystine knot" \((p = 0.00002)\) for the GF class.

Obtained results demonstrate validity of the approach for the analysis of protein families, domains and related genes that we developed, implemented and made available within the GFINDer Web system.

**Discussion**

Information about protein families and domains is paramount to understanding gene functional characteristics. As the most reliable source of such information we selected Pfam, the curated database specifically concerning protein families and domains. In addition, we also considered InterPro database, since it is the most comprehensive source of annotations on the subject. In fact, it contains data from many member databases such as UniProt, PROSITE, PRINTS, ProDom, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D, PANTHER, and also Pfam. Furthermore, InterPro provides parent/child relations among many of its entries. Such relations indicate hierarchical levels of detail between a parent entry and a
more specific child entry. The GFINDER Protein Families & Domains modules we developed allow exploring and analyzing the available protein family and domain annotations by exploiting such relations. In the Exploration module, the user can select the level of detail at which exploring the protein family and domain annotations associated with a considered set of genes, or explore all levels at the same time (Figure 1). In the Statistics module, consecutive statistical tests are executed on each categorical annotation independently on its level of detail. Then, analysis results are shown listing each tested categorical annotation with its hierarchical level and the obtained p-value (Figures 2, 3 and 6). This simultaneously provides a comprehensive view of the statistical significance of all considered annotations and clearly highlights the evaluated protein characteristics with lowest p-value within each of the considered user-defined classes of genes, specifying also their granularity level. Validation results showed that this is correctly performed also when the same protein family or domain is associated with genes in different classes, as it happens for domains contained both in tyrosine kinase (TK) and tyrosine phosphatase (TP) proteins (Figure 1). In this case, although obtained p-values do not reach statistical significance, lower p-values properly indicate more relevant protein domains for each gene class (e.g. "Immunoglobulin subtype" ($p = 0.21715$) and "Immunoglobulin-like" ($p = 0.234$) for the TK class, or "Fibronectin, type III-like fold" ($p = 0.16395$) and "Fibronectin, type III" ($p = 0.20151$) for the TP class).

In the Logistic Regression module, the user can select the level of detail of the protein annotations to be included in the regression, or focus only on those independent annotations that are not linked by parent/child relations. Although logistic regression requires several samples (number of considered genes associated with each evaluated annotation) to provide reliable results [17], application results obtained with the new GFINDER Logistic Regression module are promising. They show that this module can support better interpretation of gene classes identified through gene expression experiments by finding the functional characteristics that better explain the considered gene classification. Furthermore, it can aid in better characterizing genes whose functional role is not yet completely understood, or even unknown, by estimating the probability of a gene to be functionally similar to a set of genes with certain functional characteristics. Thus, the implemented logistic regression analysis could contribute to unveil new biomedical knowledge about the considered genes.

Even if several tools are available for the analysis of gene annotations according to the Gene Ontology, at present to our knowledge only DAVID [18] and FatiGO+ [19] support analysis of protein family and domain controlled vocabularies, although in a more basic way than GFINDER does. Therefore, the novel implemented modules for evaluating protein family and domain annotations considerably enrich GFINDER, which is freely available on-line for non-profit use [7], and make it a unique valuable tool for genomic functional and phenotypic analysis of gene sets.

**Conclusion**

The novel GFINDER modules for the evaluation of protein family and domain annotations of sets of genes can help in better interpreting high-throughput gene lists and in unveiling new functional knowledge about the considered genes, as our validation demonstrated. Thus, they can support a genomic approach in the understanding of fundamental biological processes and complex cellular mechanisms influenced by protein structural and functional characteristics related to domain composition of the codified proteins.

**Methods**

**Structuring and hierarchical tree reconstruction of protein family and domain annotations**

By using standard text-parsing procedures, we extracted protein accession numbers, Pfam IDs and protein family domain categories from the uniprot sprot.dat text file, which contains the whole protein information in the UniProt databank [20] and is freely available from the ExpASy FTP site [21]. Then, we structured the extracted controlled annotations in a GFINDER database table that we related to a previously created gene2accession correspondence table. This table contains nucleotide sequence and gene IDs, and the associated accession numbers of their protein products, which are freely available from the FTP site [22] of the Entrez Gene databank [23].

From the InterPro FTP site [24] we freely downloaded the MySQL dump of the entire InterPro databank [5]. Then, in GFINDER multi-database system we imported those InterPro tables containing controlled annotations of protein families, domains and functional sites and their hierarchical relations, and related them to the gene2accession correspondence table. Furthermore, we reconstructed the trees of parent/child hierarchical relations defined among the InterPro entries and structured them within the GFINDER/InterPro database in order to use them more efficiently during user protein annotation analysis.

**Implemented GFINDER system for protein family and domain investigation**

GFINDER Web system is built as three-tier architecture based on a multi-database structure. In the first tier, the data tier, a MySQL DBMS manages all considered biomolecular annotations stored in different relational databases. In this tier, we added a specifically designed relational database where we stored and hierarchically
structured all annotations retrieved about protein families and domains. To associate a protein family or domain with their codifying genes we considered the protein codes associated with a gene, as provided by the Entrez Gene database. Using Java programming language, we implemented procedures able to automatically import and keep updated, in GFINDER data tier, protein family and domain information and related gene annotations when new releases of them become available in UniProt, InterPro and Entrez Gene databanks. Particular procedures automatically reconstruct protein family and domain hierarchies according to the hierarchical information provided by InterPro, and structure them in the specific GFINDER database.

In GFINDER processing tier we used Microsoft ActiveX Data Object technology and Standard Query Language to interact with the MySQL DBMS server on the data tier, and created new management and analysis routines in Javascript and Active Server Page scripts. In the analysis routines categorical and logistic regression statistical approaches were implemented to evaluate protein family and domain category terms. They employ different tests (described in the Statistical analysis section below) to assess statistical significance of the over- and under-representation of categorical protein family and domain annotations in a group of user-classified genes.

In GFINDER user tier, which is composed of any client computer connected to the Web server on the processing tier through an Internet/intranet communication network, we employed Hyper Text Markup Language and Javascript to implement a Web graphic interface for the new developed Protein Families & Domains modules.

**Statistical analysis**

Categorical statistical techniques were implemented in GFINDER to analyze protein family and domain controlled annotations. Because a gene may or may not be associated with a certain protein annotation category, the number of genes and their frequency, distribution, and probability of occurrence is calculated for each protein family or domain corresponding with a certain protein annotation category. In GFINDER we implemented the hypergeometric test (more time-consuming), the binomial test (which is an asymptotic limit of the first for high number of genes), and the exact Fisher test (based on a two-way table crossing gene classes and protein family or domain categories) [6,25,26]. As usual in all significance tests, small p-values relate to relevant protein family or domain categories for a certain class of genes. However, depending on the number of considered genes and their associated protein families or domains, the number of performed statistical tests can be high. This can greatly increase the Type-I error associated with the tests, i.e. the probability of obtaining a significant p-value by chance when the null hypothesis is true (or the false-positive value, as it is known in the medical field). This requires corrections on the calculated p-values in order to obtain proper significances.

In GFINDER several correction methods for multiple tests are available. The simplest and strictest is the Bonferroni method that can be applied if the performed tests are independent [27]. It consists of changing the threshold \( \alpha \) of each single test, from which every corresponding p-value is considered significant, in such a way that the Type-I error of the whole set of tests is maintained. The correction is the following: \( \alpha_{\text{corrected}} = \frac{\alpha}{N} \), where \( N \) is the number of performed tests. From a practical point of view this is equivalent to keep the usual threshold \( \alpha \) for the performed tests and apply a correction to the observed p-values such that: \( p_{\text{corrected}} = \frac{N \cdot p}{\text{rank}(p)} \). However, the Bonferroni method greatly reduces the power of detecting a specific hypothesis when the number of tests increases. False Discovery Rate (FDR) and Family-Wise Error-rate (FWE), an extension of Bonferroni method, are milder corrections and they are even suitable when independence among tests does not hold [28]. The former briefly consists in ordering the \( N \) p-values such that the maximum has rank \( N \) and the minimum has rank 1. Then, the correction to be applied is: \( p_{\text{corrected}} = \frac{p}{\text{rank}(p)} \). The latter instead, in the implementation proposed by Benjamini and Hochberg [28], uses the following p-value correction: \( p_{\text{corrected}} = \frac{p}{(N - \text{rank}(p) + 1)} \). All three methods above illustrated have been included in GFINDER. Among them the FDR, which is the mildest of the three and practically consists of defining the maximum acceptable number of obtained false-positive tests, is considered the most suitable correction method to be applied to genomic data.

**Logistic regression analysis**

Logistic regression [29] is a variation of ordinary regression \( y = b_0 + b_1 x_1 + b_2 x_2 + ... + b_n x_n \) that predicts the probability \( p \) of the occurrence of an outcome event \( y \) as a function of certain predictor variables \( x_i \). It fits a special s-shaped curve by using the non linear function:

\[
p = \left( \frac{e^y}{1 + e^y} \right)
\]

which produces p-values between 0 (as \( y \) approaches minus infinity) and 1 (as \( y \) approaches plus infinity). In our implementation, we considered the following corresponding non linear equation, called logit(p):

\[
\text{logit}(p) = \ln \left( \frac{p}{1 - p} \right) = y = b_0 + b_1 x_1 + b_2 x_2 + ... + b_n x_n
\]
where $p$ is the proportion of considered classified genes in the two evaluated classes; $x_i$ are the proportions of considered genes in the two evaluated classes that present the $i$ characteristic; $b_i$ (with $1 \leq i \leq n$) are the regression coefficients for the $i$ characteristic; and $b_0$ is the intercept, or constant term, of the regression. The absolute value of each $b_i$ coefficient indicates the importance of the corresponding $i$ characteristic in contributing to the considered gene classification.

In order to solve the non linear equation, we used a straightforward Active Server Page and Javascript implementation of a standard iterative method [30] to minimize the log likelihood function. Such function is defined as the sum of the logarithms of the predicted probabilities of belonging to the first of the two evaluated classes for those considered genes indeed belonging to that class, and the logarithms of the predicted probabilities of belonging to the second of the two evaluated classes for those considered genes indeed belonging to that second class. Minimization is by Newton’s method, with an elimination algorithm to invert and solve the simultaneous equations. No special convergence-acceleration techniques were used. The null model was used as starting guess for the iterations, i.e. all $b_i$ coefficients are zero and the $b_0$ intercept is the logarithm of the ratio of the number of considered genes belonging to the first of the two evaluated classes to the number of considered genes belonging to the second class.

In addition to providing value, standard error and $p$-value of $b_i$ coefficients, our logistic regression implementation also produces an odds ratio associated with each predictor variable $x_i$ and its 95% confidence interval. The odds of an event is defined as the probability of the event occurring divided by the probability of the event not occurring. The odds ratio for a predictor variable $x_i$ denotes the relative amount by which the odds of belonging to the first of the two evaluated classes, for those considered genes that present the $i$ characteristic, increases (odds ratio greater than 1.0) or decreases (odds ratio less than 1.0) when the value of the predictor variable $x_i$ is increased by 1.0 unit.

Authors’ contributions
MM was responsible for the overall conception and project coordination, was involved in design, development and testing of GFINDer software modules, and wrote this manuscript. EB developed GFINDer software module devoted to logistic regression, and was involved in their design and testing. AF developed the routines devoted to structuring and updating protein family and domain annotations within GFINDer database. FP provided supervision and funding of the project.

Acknowledgements
The authors thank Andrea Maffeizoli for his contribution in writing this manuscript.

This article has been published as part of BMC Bioinformatics Volume 8, Supplement 1, 2007: Italian Society of Bioinformatics (BITS): Annual Meeting 2006. The full contents of the supplement are available online at http://www.biomedcentral.com/1471-2105/8?issue=S1.

References
1. Galperin MY: The Molecular Biology Database Collection: 2006 update. Nucleic Acids Res 2006, 34(Database issue):D3-D5.
2. The Gene Ontology™ Consortium: Gene Ontology: tool for the unification of biology. Nature Genet 2000, 25(1):25-29.
3. Copley R, Doerks T, Letunic I, Bork P: Protein domain analysis in the era of complete genomes. FEBS Lett 2002, 513(1):129-34.
4. Finn RD, Mishry J, Schuster-Bockler B, Griffiths-Jones S, Hollich V, Lassmann T, Moxon S, Marshall AJ, Kiana H, Durbin R, Eddy SR, Sonnhammer EL, Bateman A: Pfam: clans, web tools and services. Nucleic Acids Res 2006:D247-D251.
5. Mulder NJ, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bradley P, Bork P, Bucher P, Cerutti L, Copley R, Courcelle E, Das U, Durbin R, Fleischmann W, Gough J, Haft D, Hart N, Hulo N, Kahn D, Kanapin A, Krestyaninova M, Lonsdale D, Lopez R, Letunic I, Madara M, Maslen J, McDowall J, Mitchell A, Nikolayska AN, Orchard S, Pagni M, Ponting CP, Quevillon E, Selengut J, Sigrist CJ, Silvestontoinen V, Studholme DJ, Vaughan R, Wu CH: InterPro, progress and status in 2005. Nucleic Acids Res 2005:D201-D205.
6. Masseroli M, Martucci D, Pinciroli F: GFINDer: Genome Function INtegrated Discoverer through dynamic annotation, statistical analysis, and mining. Nucleic Acids Res 2004-W293-W300.
7. GFINDer Web site [http://www.bioinformatics.polimi.it/GFINDer/]
8. Boguski MS, Jones AR: Neurogenomics: at the intersection of neurobiology and genome sciences. Nat Neurosci 2004, 7(5):429-433.
9. Snoudou D, Favadaki E, Arvanitis DA, Kranias E, Kontogianni-Konstantopoulos A: Array lessons from the heart: focus on the genome and transcriptome of cardiomyopathies. Physiol Genomics 2005, 21(2):131-143.
10. BD Biosciences Clontech [http://www.bdbiosciences.com/clonech]
11. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S: The protein kinase complement of the human genome. Science 2002, 298(5600):1912-1934.
12. Bardelli A, Parsons DW, Silliman N, Pisk J, Szabo S, Saha S, Markowitz S, Willson JK, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE: Mutational analysis of the tyrosine kinome in colorectal cancers. Science 2003, 302(5621):949.
13. Zhenghe W, Dong S, Willians DP, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, Van der Heijden MS, Parmigiani G, Yan H, Wang T, Riggins G, Powell SW, Willson JK, Markowitz S, Kintler KW, Vogelstein B, Velculescu VE: Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science 2004, 304(5674):1164-1166.
14. Green DR: Apoptotic pathways: paper wraps stone blunts scissors. Cell 2000, 102(1):1-4.
15. Falls DL: Neuregulins: functions, forms, and signaling strategies. Exp Cell Res 2003, 284(1):14-30.
16. SuperArray Bioscience Corporation [http://www.superarray.com]
17. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR: A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996, 49(12):1373-1379.
18. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lamb PC: DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 2003, 4(9):R60.
19. Al-Shahrour F, Minguez P, Vaquerizas JM, Conde L, Dopazo J: Bio-lemics: a suite of web tools for functional annotation and analysis of groups of genes in high-throughput experiments. Nucleic Acids Res 2005:W460-W464.
20. Wu CH, Apweiler R, Bairoch A, Natale DA, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Mazumder R, O’Donovan C, Redaschi N, Suzek B: The Universal
Protein Resource (UniProt): an expanding universe of protein information. Nucleic Acids Res 2006:D187-D191.

21. ExPASy FTP site [ftp://ftp.expasy.org/databases/uniprot/knowledgebase/]

22. Entrez Gene FTP site [ftp://ftp.ncbi.nih.gov/gene/DATA/]

23. Maglott D, Ostell J, Pruitt KD, Tatusova T: Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res 2005:D54-D58.

24. InterPro FTP site [ftp://ftp.ebi.ac.uk/pub/databases/interpro/]

25. Fisher LD, van Belle G: Biostatistics: a methodology for the health sciences New York, NY: John Wiley & Sons; 1993.

26. Casella G, Berger RL: Statistical inference 2nd edition. Belmont, CA: Duxbury Press; 2002.

27. Bonferroni CE: Teoria statistica delle classi e calcolo delle probabilità. Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze 1936, 8:13-62.

28. Benjamini Y, Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 1995, 57:289-300.

29. Hosmer DW, Lemeshow S: Applied logistic regression New York, NY: John Wiley & Sons; 1989.

30. Pezzullo JC, Sullivan KM: Logistic regression calculating page. [http://www.sph.emory.edu/~cdckms1/Logistic/logistic.html].