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The Filamentous Fungal Gene Expression Database (FFGED)

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Abstract

Filamentous fungal gene expression assays provide essential information for understanding systemic cellular regulation. To aid research on fungal gene expression, we constructed a novel, comprehensive, free database, the Filamentous Fungal Gene Expression Database (FFGED), available at <http://bioinfo.townsend.yale.edu>. FFGED features user-friendly management of gene expression data, which are assorted into experimental metadata, experimental design, raw data, normalized details, and analysis results. Data may be submitted in the process of an experiment, and any user can submit multiple experiments, thus classifying the FFGED as an “active experiment” database. Most importantly, FFGED functions as a collective and collaborative platform, by connecting each experiment with similar related experiments made public by other users, maximizing data sharing among different users, and correlating diverse gene expression levels under multiple experimental designs within different experiments. A clear and efficient web interface is provided with enhancement by AJAX (Asynchronous JavaScript and XML) and through a collection of tools to effectively facilitate data submission, sharing, retrieval and visualization.

Keywords

public experiments; public results; data submission; related experiments; experimental design; AJAX; microarray

1. Introduction

Due to their close relationship with Animalia and their experimental tractability, filamentous fungi are frequently used as model organisms for understanding diverse aspects of basic cellular regulation, including cell cycle progression, gene expression, circadian timing, light sensing, recombination, secretion, multicellular development (Davis 1966; Ballance 1986; Dunlap *et*

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Author Contributions

ZZ designed, programmed, and implemented this database and drafted the manuscript. JPT contributed core design principles, supervised the development, and revised the manuscript. Both authors have read and approved the final manuscript.

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al. 2007), and evolution (Bruns *et al.* 1991; Kohn 2005). For instance, the multicellular filamentous fungus *Neurospora crassa* (Galagan *et al.* 2003; Wu *et al.* 2007) has been studied in research laboratories for decades (Beadle and Tatum 1941; Davis and Perkins 2002; Dong *et al.* 2008), primarily owing to its interesting and diverse biology, ease of culture, facile genetics, and rapid growth rate (Borkovich *et al.* 2004; Merrow and Roenneberg 2007). A number of other fungi, such as *Aspergillus nidulans* (Galagan *et al.* 2005a), *A. fumigatus* (Schoberle and May 2007), *Fusarium graminearum* (Cuomo *et al.* 2007), *Magnaporthe grisea* (Soderlund *et al.* 2006), have become important fungal models for animal and plant pathogenesis (Gow *et al.* 2002; Tunlid and Talbot 2002). Increasingly, gene expression data for filamentous fungi provide a rich resource for the identification of candidate genes and the characterization of systemic biological responses to growth conditions and environmental stresses (Soderlund *et al.* 2006; Breakspear and Momany 2007). However, few resources have been made available for storage and access to diverse yet interrelated filamentous fungal gene expression studies.

A number of databases have been constructed to provide storage and management of gene expression data, several (Demeter *et al.* 2007; Hong *et al.* 2008; Barrett *et al.* 2009; Parkinson *et al.* 2009). The Stanford Microarray Database (SMD) (Demeter *et al.* 2007) serves as a research tool that is free of charge for Stanford researchers and their collaborators. The Saccharomyces Genome Database (SGD) (Hong *et al.* 2008) is a central repository for the budding yeast *Saccharomyces cerevisiae*, allowing the retrieval of microarray expression data for Saccharomyces (Ball *et al.* 2001).

Among them, the Gene Expression Omnibus (GEO) database (Barrett *et al.* 2009) at the US National Center for Biotechnology Information (NCBI) and the ArrayExpress repository (Parkinson *et al.* 2009) at the European Bioinformatics Institute (EBI) are major databases for public gene expression data. However, these databases simply collect user-submitted results and lack a clear classification of gene expression data. Gene expression metadata include a variety of information from wet-lab experiment to in-silico procedure, which may be classified into different sections. Considering the burgeoning collection of gene expression data, a necessary next step is to make full use of classified expression data to automatically correlate relevant experiments and discover some correlations with different experimental conditions. However, such an operation is not well-supported by these generalized unclassified databases. Moreover, none of these databases provide specific resources to facilitate well-annotated deposition or facile comparison of filamentous fungal gene expression studies.

To simplify data submission and management and to facilitate discovery of correlations among diverse gene expression experiments in filamentous fungi, we constructed a novel, comprehensive, free database, the Filamentous Fungal Gene Expression Database (FFGED; <http://bioinfo.townsend.yale.edu>), to serve as a collective and collaborative platform for filamentous fungi. Unlike existing related databases, FFGED focuses on comparisons of diverse experimental designs and correlations of different experimental results for filamentous fungi. The aim of FFGED is not only to provide users with friendly web interfaces for facilitating data submission and management, but also to rapidly share gene expression data within the scientific community at large, to automatically identify related gene expression experiments to assist users in connecting their experiments with similar experiments, and to effectively ease virtual comparisons of each gene's expression under multiple experimental designs within different experiments across diverse fungi.

2. Materials and Methods

2.1. Database architecture

To develop FFGED, we used MySQL (a free and popular relational database management system, Version 5.1.30; <http://www.mysql.org>) on a Mac OS X Server. To provide efficient access to the database and to simplify data submission and management, we provide user-friendly graphical user interfaces (GUI) implemented with HTML, JSP (Java Server Pages), JavaBean, Servlet, and AJAX (Asynchronous JavaScript and XML, a collection of web development technologies for creating highly interactive web applications) with two web service applications, Apache and Resin (Figure 1). Apache serves static content (like HTML) and Resin serves dynamic content (like JSP, JavaBean, and Servlet). AJAX is used to transfer data between server and browser, based on JQuery (an open source, free, and lightweight framework for interaction between JavaScript and HTML; <http://www.jquery.com>).

2.2. Annotation

To provide gene annotations to result files from raw or statistical analyses of microarray data, we retrieved annotations for *N. crassa* from three databases: PEDANT (Protein Extraction, Description and Analysis Tool; <http://pedant.gsf.de>) (Riley et al. 2005), the *Neurospora crassa* e-Compendium (<http://bioinformatics.leeds.ac.uk/~gen6ar/newgenelist/genes/>), and the Broad Institute of MIT and Harvard (<http://www.broadinstitute.org/annotation/genome/neurospora/>). Annotation data for *Aspergillus fumigatus*, *A. nidulans*, *Coccidioides immitis*, *Coprinus cinereus*, *Fusarium graminearum*, *F. verticillioides*, *Histoplasma capsulatum*, and *Magnaporthe grisea* were obtained from the Broad Institute. Additionally, pathway information for several species was extracted from KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa and Goto 2000).

2.3. Normalization

To ease data analysis, FFGED features an online normalization tool, providing normalization for two-color arrays as in (Townsend *et al.* 2003). For evaluation of hybridization quality a helpful illustrative plot of the normalization regression, can be constructed and exported as an image in SVG or PNG formats.

2.4. Identification of related experiments

To link related experiments together, FFGED calculates the cosine similarity coefficient for two experiments. The cosine similarity coefficient is widely used in information retrieval and data mining (Salton and McGill 1983; Baeza-Yates and Ribeiro-Neto 1999; Dhillon and Modha 2001). It is the cosine of the angle (θ) between two vectors of n dimensions when each experiment is represented by its experimental design, which can include multiple developmental, genetic, and/or environmental states. Therefore, given two vectors of experiments $x(x_1, x_2, \dots, x_n)$ and $y(y_1, y_2, \dots, y_n)$, the cosine similarity coefficient between x and y is obtained by

$$\cos(\theta) = \frac{\sum_{i=1}^n x_i \times y_i}{\sqrt{\sum_{i=1}^n x_i^2 \times \sum_{i=1}^n y_i^2}}, \quad (1)$$

$$\text{where } x_i = \begin{cases} 1, & \text{if experimental state } i \text{ is in } x \\ 0, & \text{otherwise} \end{cases} \quad \text{and } y_i = \begin{cases} 1, & \text{if experimental state } i \text{ is in } y \\ 0, & \text{otherwise} \end{cases}.$$

3. Results

3.1. Data submission and management

In FFGED, any data can be submitted in the process of an experiment or after its completion. Entry of an experiment is divided into five sections: experimental Metadata (e.g., experimental name), experimental Design (e.g., cyanine-3 and cyanine-5 treatments), Raw data (e.g., GenePix Results), Normalized details (e.g., normalization methods), and analysis Results (e.g., gene expression levels). This division is hereafter denoted as MDRNR (see Figure 2B). MDRNR is also compatible with MIAME (Minimum Information about Microarray Experiment; <http://www.mged.org/Workgroups/MIAME/miame.html>) (Brazma et al. 2001), further facilitating data exchange with other institutions. By providing resources for archiving raw data through processed results at each of these stages, FFGED facilitates use as an “active experiment” database, increasing the immediate utility to the user and decreasing the burden of post-experimental submission and annotation.

Unlike existing related databases (Killion *et al.* 2003; Demeter *et al.* 2007; Hong *et al.* 2008; Barrett *et al.* 2009; Parkinson *et al.* 2009), FFGED not only functions as a storage database but also as an analysis tool that facilitates progression and processing of data through the MDRNR classifications. Several online programs, for example, normalization (Townsend *et al.* 2003), generation of an input file for BAGEL (Bayesian Analysis of Gene Expression Levels; (Townsend and Hartl 2002), annotation of results, and figure plotting (implemented with JfreeChart, an open-source framework for creation of professional charts; <http://www.jfree.org>), have been integrated into FFGED. These tools greatly ease data analysis and visualization. In addition, user-friendly web interfaces with clear progression monitoring of experiment submission are provided for diverse competencies of submitters, lowering technological entrance barriers (Ball *et al.* 2004).

3.2. Public experiments

Any user can submit multiple experiments to FFGED. Each experiment can be set to be private or public. Public experiments are freely accessible by users. MDRNR classifies gene expression data into five different sections, with “experimental design” as the core section that shapes the entries for other sections. Although experiments may differ greatly, similar experimental states submitted in experimental design indicate that experiments may be compared very productively. Therefore, FFGED employs experimental states to link each experiment with similar experiments made public by other users.

For each public experiment in FFGED, detailed information is shown in a tab format according to MDRNR (Figure 2), and related experiments are also displayed according to their similarity coefficients (see Materials and Methods). As a result, related experiments are identified, which can lead to synthetic knowledge discovery. Furthermore, users with similar experiments are connected to each other, enhancing communication and collaboration within the scientific community.

3.3. Public results

Public results are gene expression levels under a variety of experimental states, extracted from analysis results within public experiments. Such public results provide a forum for the study of the expression of individual genes and their correlations under different experimental states. For each gene, FFGED quantifies expression levels under diverse experimental variables within different public experiments (Figure 3), enabling comparative gene expression analysis.

To share this information with the scientific community at large and to provide content for other institutions, we provide an XML retrieval mode via Internet for structured data exchange. In addition, FFGED offers a dynamic interface to embed up-to-the-minute experimental results for the expression of a relevant gene into any web site. For instance, the *N. crassa* database at the Broad Institute has implemented this tool, embedding experimental results for each gene's expression into its gene-specific web page (See the FAQ page at <http://bioinfo.townsend.yale.edu/faq.jsp#sharing>).

4. Discussion and Future Development

Our newly developed database, FFGED, is a collective and collaborative platform that provides a useful tool for filamentous fungal gene expression studies. The AJAX-based database has several unique advantages over other currently available databases; the database provides users with friendly and highly interactive web interfaces, lowers technological barriers and emphasizes ease of data submission, retrieval, visualization, and sharing. Furthermore, unlike existing generalized databases, FFGED provides a specific platform for filamentous fungal gene expression studies, and most importantly, facilitates direct comparisons of diverse experimental designs and correlations of different experimental results for filamentous fungi. Additionally, in FFGED, gene expression data can be shared rapidly within the scientific community, related gene expression experiments can be identified easily, and gene expression systems can be compared virtually under multiple experimental designs across diverse fungi.

Regarding gene expression data storage and management, FFGED provides a flexible framework, MDRNR, which clearly classifies gene expression data into five different sections and can be used with diverse designs and platforms (Burgoon 2006). This classification scheme accordingly makes FFGED completely different from other databases. Compared with extant unclassified databases, FFGED also promises to provide correlations among different experiments that may be easily retrieved and interpreted. Moreover, MDRNR separates wet-bench work conducted by biologists (experimental metadata and experimental design) from dry-bench work done by bioinformaticians (raw data, normalized details and analysis results).

In contrast with some databases which are not free of charge for all users (e.g., SMD), FFGED features free private submission and free public access to public data for any user, with the aim of increasing collaboration by maximizing data sharing and facilitating knowledge discovery from collective data. This feature of knowledge discovery through collective public data is a key element for data-intensive bioinformatics studies (Stajich and Lapp 2006; Zhang *et al.* 2009).

FFGED has served as an in-house database for gene expression data since October 2007, and has been regularly updated and improved. Potential expansions of this database would be the addition of other fungal genome browsers (<http://fungal.genome.duke.edu>), facilitating genome annotations and comparative sequence analysis. In addition, providing specific formats for submission of other types of gene expression data, such as MPSS (Massively Parallel Signature Sequencing, Reinartz *et al.* 2002; Torres *et al.* 2008) and DGE (Digital Gene Expression) profiling (Linsen *et al.* 2009), into the expression level tables would facilitate further gene expression comparisons across multiple samples, as with DNA microarrays and other gene expression platforms (Nakano *et al.* 2006). Moreover, integrating more online tools for gene expression analysis, for example, tools for detecting co-expressed genes within and among experiments, would greatly facilitate expression analysis. Lastly, expanding comparative expression analysis across fungi would be essential for studying their vast and diverse biology and morphology (Galagan *et al.* 2005b).

In summary, we have constructed the Filamentous Fungal Gene Expression Database and it features user-friendly web interfaces, free access, clear classification of expression data (MDRNR), novel identification of similar related experiments and correlation of diverse gene expression levels within multiple experiments. It will facilitate in-depth comparative expression studies on diverse filamentous fungi.

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Figure 1. Database architecture of FFGED, including three tiers, web browser, application server and data management system. These tiers facilitate ease of use, efficient data processing, and reliable archiving of data.

A

Experiment ID	Experiment Name	Description	Submitter	Public Date	Date Submitted
2	Neurospora crassa early sexual development and MTP	Neurospora crassa mat A FGSC#2489 Three developmental stages and two different primers used for reverse transcription: mycelium oligo(dT) M1 protoperithecia oligo(dT) PP1 perithecia oligo(dT) PT1 mycelium oligo(dT)+ Multi-Targeted Primer [MTP] (M2) protoperithecia oligo(dT)+ MTP (PP2) perithecia oligo(dT)+ MTP (PT2)	Travis Clark	2008-11-11	2007-08-30
3	Cocci Array Experiment	Compares different stages of growth of Coccidioides	Jeffrey Townsend	2008-04-10	2007-08-30
4	Nc mating type, Tol, and homo vs heterokaryon	Neurospora crassa mat a wt, mat A wt, mat A Tol KO, mat A Tol KO, mat a/A Tol KO heterokaryon. All possible comparisons with dye swaps (5 nodes, 16 hybridizations)	Travis Clark	2008-10-09	2007-08-31
5	Nc Mat A Tol, wt, HET preliminary	This experiment was the start of the mating type, Tol, and HET comparison. Nc mat A wt (FGSC#2489), mat A Tol deletion, and mat A/a Heterokaryon were compared in all possible combinations with dye swap. Arrays had the final scan incorrectly at 10um instead of 5um. These hybs were redone in the full experiment with both mating types. Data accessioned here for addition or future analysis.	Travis Clark	2008-04-10	2007-09-06
7	Neurospora crassa mating type, Tol, and homo vs heterokaryon	Neurospora crassa mating type, Tol, and homo vs heterokaryon	Zhang Zhang	2009-03-01	2007-09-15
10	Conidial Germination	Conidial germination profiling from time 0 to 16 hours in liquid medium.	Takao Kasuga	2008-04-10	2007-09-19
13	Cycle 1 (circadian response)	Time point 13 is really 0 hrs. The ordering of time points is backwards. The reason will be clear from the design. We were controlling total growth time.	Jonathan Arnold	2008-08-29	2007-10-10
14	Magnaporthe 1	Fungal gene expression in 5 different conditions (stress, WT and in planta).	Nicole Donofrio	2007-10-26	2007-10-26
16	Genomic Hybridization / Saccharomyces Sensu Stricto	Test of BAGEL output of genomic hybridization data for species of Saccharomyces Sensu Stricto Complex	Regina Wilpiszeski	2008-01-11	2008-01-11
19	Vegetative Growth	N. crassa grown on solid Birds medium	Takao Kasuga	2008-07-18	2008-03-14

B

Experimental Metadata	Experimental Design	Raw Data	Normalized Details	Analysis Results
Name of Experiment	Conidial Germination			
Type				
Channel Data	Dual-Channel			
Description	Conidial germination profiling from time 0 to 16 hours in liquid medium.			
Citations	T. Kasuga, J. P. Townsend, C. G. Tian, L. B. Gilbert, G. Mannhaupt, J. W. Taylor and N. L. Glass (2005). Long-oligomer microarray profiling in Neurospora crassa reveals the transcriptional program underlying biochemical and physiological events of conidial germination. Nucleic Acids Research 33:6469-6485			
Publication Link				
Abstract	To test the inferences of spotted microarray technology against a biochemically well-studied process, we performed transcriptional profiling of 15 conidial germination in the filamentous fungus, Neurospora crassa. We first constructed a 70 base oligomer microarray that assays 3366 predicted genes. To estimate the relative gene expression levels and changes in gene expression during conidial 20 germination, we analyzed a circuit design of competitive hybridizations throughout a time course using a Bayesian analysis of gene expression level. Remarkable consistency of mRNA profiles with previously published northern data was observed. Genes 25 were hierarchically clustered into groups with respect to their expression profiles over the time course of conidial germination. A functional classification database was employed to characterize the global picture of gene expression. Consensus motif searches 30 identified a putative regulatory component associated with genes involved in ribosomal biogenesis. Our transcriptional profiling data correlate well with biochemical and physiological processes associated with conidial germination and will facilitate functional 35 predictions of novel genes in N. crassa and other filamentous ascomycete species. Furthermore, our dataset on conidial germination allowed comparisons to transcriptional mechanisms associated with germination processes of diverse propagules, such as 40 as teliospores of the phytopathogenic fungus Ustilago maydis and spores of the social amoeba Dictyostelium discoideum.			
Keywords				
Public Experiment	2008-04-10			

Related Experiments:

- Neurospora crassa mating type, Tol, and homo vs heterokaryon (EXPTID: 7)

Figure 2.

Screenshots from public experiments, shown in a tab format. All information in public experiments can be accessed by any user. (A) Some public experiments submitted by users. (B) Detailed information for a given experiment, including experimental metadata, experimental design, raw data, normalized details, and analysis results (only “experimental metadata” shown), and listing related experiments at the bottom.

Figure 3. Screenshots from public results, taking *Neurospora crassa* Gene NCU09997 as an example. All information in public results can be accessed by any user. (A) Results for expression of gene NCU09997 are included in three public experiments. (B) Gallery show of expression information for gene NCU09997. The gallery show for expression of a gene, for instance, can be embedded into any web site or exchanged by an XML retrieval mode. Expression information displayed in this manner is embedded into gene descriptions for *Neurospora* at the Broad Institute website (e.g. <http://www.broadinstitute.org/annotation/genome/neurospora/GeneLocus.html?>

sp=SNCU09997). The 3D figure for gene expression level can be freely downloaded in PNG and SVG formats.