Plant MPSS databases: signature-based transcriptional resources for analyses of mRNA and small RNA

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ABSTRACT

MPSS (massively parallel signature sequencing) is a sequencing-based technology that uses a unique method to quantify gene expression level, generating millions of short sequence tags per library. We have created a series of databases for four species (Arabidopsis, rice, grape and Magnaporthe grisea, the rice blast fungus). Our MPSS databases measure the expression level of most genes under defined conditions and provide information about potentially novel transcripts (antisense transcripts, alternative splice isoforms and regulatory intergenic transcripts). A modified version of MPSS has been used to perform deep profiling of small RNAs from Arabidopsis, and we have recently adapted our database to display these data. Interpretation of the small RNA MPSS data is facilitated by the inclusion of extensive repeat data in our genome viewer. All the data and the tools introduced in this article are available at http://mpss.udel.edu.

INTRODUCTION

DNA sequencing technologies have improved dramatically in the last decade and numerous whole-genome sequences are now available. These resources include two plant genomes, Arabidopsis and rice, as well as a number of plant pathogen genomes (1–4). Gene predictions and genome annotations are available for these genomes, built using prediction software with integrated experimental data from expressed sequence tags (ESTs) and full-length cDNA sequences (1). However, the experimental data lack the depth required to saturate the identification of mRNA transcripts, and this justifies the development of more advanced technologies. MPSS (massively parallel signature sequencing) sequences 17–20 nt (a ‘signature’) adjacent to the 3' most DpnII site from millions of molecules in a sample (5,6). This depth provides a quantitative assessment of transcript abundance, while greatly increasing the likelihood of discovering novel transcripts. Gene expression differences can be determined using comparisons across multiple samples, as with DNA microarrays or other gene expression platforms (7).

The recent discovery and analysis of small RNAs (20–25 nt) is exciting and has demonstrated the biological importance of these non-coding molecules. Small RNAs have been isolated from diverse eukaryotic organisms and are typically classified into two major types: small interfering RNAs (siRNAs) and microRNAs (miRNAs) (8,9). Both types of molecules are processed from double-stranded RNA by RNase III enzymes called DICERs, although, their biogenesis and functions differ (10–12). siRNAs can target and degrade complementary mRNA molecules (13) and can trigger transcriptional silencing via histone modifications and/or DNA methylation (14,15). miRNA molecules originate from distinct genomic loci predicted to form ‘hairpin’ structures (11) and can induce degradation of homologous target mRNAs or can prevent mRNA translation. The short length of these small RNAs is more than sufficient to specifically match nearly any given RNA encoded in a genome. The very deep sampling capabilities of MPSS have demonstrated tremendous diversity among Arabidopsis small RNAs (16).

In this report, we describe our databases that facilitate the use of MPSS data. Our databases contain data derived from polyadenylated RNA or size selected small RNAs; we refer to these MPSS datasets as ‘mRNA’ and ‘small RNA’, respectively. We have developed four mRNA MPSS databases
from Arabidopsis, rice, grape and the rice blast fungus (*Magnaporthe grisea*); currently, only our Arabidopsis database has small RNA MPSS data. These databases are built on a common set of web interfaces and are equipped with various graphical and analytical tools that allow the user to retrieve and analyze the data. This article focuses on the essential tools for the first-time user and describes some of the many features that we have added or improved during the past year.

**DATABASE CONTENTS**

Our databases require two sets of data, one of which comprises genomic information such as chromosomes, genes and potential signatures, the other of which is MPSS expression data derived from different tissues or treatments of the target organism, including signature sequences and abundance values for each signature (17). We obtain the genomic information from outside sources (see below) and build specific tables for each organism. The MPSS data are generated with collaborators in different projects. The database tables are built with Oracle9i and transferred to MySQL for the public web server. The web interface, mainly written in PHP, extracts the data requested by the user and displays the query results in a graphical and analytical output (Figures 1 and 2).

**ARABIDOPSIS mRNA MPSS DATABASE**

The sequence of Arabidopsis is the most complete plant genomic sequence, and The Institute for Genomic Research (TIGR) has for several years provided a comprehensive annotation (1). Our primary database is built on this annotation.
(TIGR v5.0) with 17 MPSS libraries, representing treated and untreated tissues and flower mutants. The database currently contains 43 810 569 signatures from poly(A) RNA (297 313 distinct signature sequences).

Nearly all sections and tools for the Arabidopsis site can be accessed through an entry page (http://mpss.udel.edu/at). The ‘Simple Query’ (Basic Queries) is useful for the users who are working on a specific gene, specific BAC clone or specific gene family; gene IDs can be used to retrieve all the associated MPSS data (Figure 1B). Briefly, the numbered boxes indicate TIGR-annotated exons and the triangles with colors (not gray) represent the location and strand of the expressed MPSS signatures. Different colors of the triangles indicate different ‘classes’ which are determined based on the position of each signature relative to gene structure (17). The expression level of each signature in each library is displayed in table format at the bottom of the Gene Analysis page.

We have added numerous new features to this page since we first introduced our web interface in 2004 (17). TIGR’s v5.0 annotation indicates untranslated regions (UTRs), which we have displayed with pink or light-blue shading (Figure 1B), and splice variants for which we now provide a separate page that displays each variant. The navigation bars at the top of the image linked to adjacent genes and intergenic regions (Figure 1), BLASTP results and a sequence extraction function are also added features that make this website a more comprehensive genome analysis tool. The ability to view intergenic regions was added because many MPSS signatures represent previously unannotated or non-coding transcripts mapped within intergenic regions, particularly the small RNAs described below.

The ‘Query by Chromosome Position’ tool (linked from the main page) is useful for the users who are interested in certain location of a chromosome. Alternatively, users can click on the image map and view specific regions of interest on one of the chromosomes (Figure 2A). This viewer scales to allow the user to pinpoint precisely the target region for display in the primary chromosome viewer (Figure 2B and C). As a combined visual representation of the genomic annotation information and the MPSS expression data, the primary chromosome viewer displays the annotated genes with identifiers on both strands of DNA, along with expressed signatures. The user can view all the annotated genes as well as the potential novel transcripts expressed in the selected region.

**RICE mRNA MPSS DATABASE**

The rice database includes the most comprehensive set of libraries among our databases, and it can be accessed at http://mpss.udel.edu/rice. With our collaborators, we have generated more than 20 MPSS libraries derived from diverse tissues and abiotically stressed (cold, drought and salt) tissues. These libraries include different growth conditions (light and dark), different developmental stages and several biological replicates. Some of these libraries can be used as control libraries for the abiotic stressed libraries. Numerous additional rice mRNA libraries are underway (see Future Directions).

Similar to the Arabidopsis mRNA MPSS database, the rice MPSS website is built on genomic annotation data from TIGR (currently version 3.1) (4). The web interface includes nearly all of the tools available in Arabidopsis database, with some minor modifications specific to rice. Owing to historical changes in gene identifiers, we added a link to ‘TIGR version converter’ to the site. Since this database uses ‘Os’ gene identifiers from TIGR’s annotation, users with different or older TIGR gene IDs may translate their gene IDs, as these ‘Os’ identifiers are likely to be more permanent.

**GRAPE mRNA MPSS DATABASE**

Unlike Arabidopsis and rice, the genomic sequence of grape is not complete. Therefore, this website (http://mpss.udel.edu/grape) is built around an extensive set of Cabernet Sauvignon ESTs created at UC Davis and other institutions (18). The structure of our grape MPSS database is different from that of Arabidopsis and rice because of a lack of data about physical position, introns and intergenic regions. The web interface was modified accordingly, with an added BLAST query function to allow the user query with novel nucleotide sequences and compare these with grape EST collections. The result displays related EST contigs and associated MPSS signatures. This tool enables unique types of queries using the grape MPSS data, such as the identification and transcript abundance of genes expressed in grape berries or the identification of potential alternative splicing and/or antisense transcripts. The dataset also contains substantial numbers of high abundance MPSS signatures not yet corresponding to any identified EST; these data will be increasingly useful for the annotation of previously unidentified genes as grape genomic sequence becomes available.

**MAGNAPORTHE mRNA MPSS DATABASE**

The Magnaporthe MPSS site (http://mpss.udel.edu/mg) was developed using the whole-genome shotgun sequence assembly from the Broad Institute (version 2.3, October, 2003) (2). An important difference from our other MPSS sites is that this genome is segmented, with many gaps and unordered contigs. Therefore, the Magnaporthe MPSS web interface uses the same contig and super-contig numbers, and it is equipped with the tools described above to analyze genome-matching MPSS signatures derived from mycelium and appressorium libraries.

**ARABIDOPSIS SMALL RNA MPSS DATABASE**

The most fundamental advance in our website is the addition of small RNA MPSS data. The method that led to the development of this dataset is described elsewhere (16), but the display and interpretation of these data required extensive additions to the mRNA MPSS website. And while the dataset and analysis tools use similar approaches to other plant small RNA databases, our more extensive data has required substantially new analytical tools (19). In addition, the small RNA signatures do not start with a DpnII site (GATC), so they may be from the Broad Institute (version 2.3, October, 2003) (2). An important difference from our other MPSS sites is that this genome is segmented, with many gaps and unordered contigs. Therefore, the Magnaporthe MPSS web interface uses the same contig and super-contig numbers, and it is equipped with the tools described above to analyze genome-matching MPSS signatures derived from mycelium and appressorium libraries.
and 2D). In this viewer, small RNAs appear as black triangles, pointing toward the DNA strands and representing each genomic match of an endogenous small RNA sequenced by MPSS. Because repetitive sequences are known to be sources of siRNAs, we used low-stringency analyses with RepeatMasker (http://repeatmasker.org) (20), inverted and tandem (21) to identify and display these genomic regions. The low stringency identifies many transposon- or retrotransposon-related sequences not annotated by TIGR, and these predictions are often supported by the experimentally derived small RNA data. We indicate different repeat classes in pastel-shaded background colors (e.g., light pink, yellow, blue, etc.) (Figures 1A and 2D). For individual genes or intergenic regions, we provide links to the FindMiRNA site that can provide supporting data for novel miRNAs (22).

FUTURE DIRECTIONS

Although we anticipate additional small RNA MPSS data for Arabidopsis, rice and Magnaporthe, and we are therefore adding repeat data to our rice and Magnaporthe websites, most of the new growth in our database is likely to be rice mRNA MPSS data. In collaboration with the laboratory of Dr Guoliang Wang (Ohio State University), we currently have ~40 rice mRNA libraries in preparation. These libraries include analyses of indica rice and F1 hybrids with Nipponbare, as well as resistant and susceptible responses to Magnaporthe or Xanthomonas oryzae pv oryzae treatments. We are currently working on novel tools to dissect allele-specific expression level polymorphisms in the japonica and indica rice subspecies, because the MPSS signatures will vary based on SNPs or indels. The inclusion of Magnaporthe-infected rice will necessitate direct connections between our rice and Magnaporthe databases. These rice libraries will contain a small proportion of Magnaporthe-derived signatures, facilitating the identification and measurement of host and pathogen transcription during infection.

We also anticipate the release of a library analysis tool (‘LIBAN’) to facilitate and automate the characterization of entire libraries, queries based on library comparisons, and the production of lists of signatures or genes with defined characteristics or expression patterns. This tool uses a Java-based web interface to customize and generate database queries without requiring bioinformatics skills. It represents a significant advance over our existing ‘advanced tools’ page which will be superseded by LIBAN.

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