UNIT 1.11

Using The Arabidopsis Information Resource (TAIR) to Find Information About Arabidopsis Genes

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ABSTRACT

The *Arabidopsis* Information Resource (TAIR; *http://arabidopsis.org*) is a comprehensive Web resource of *Arabidopsis* biology for plant scientists. TAIR curates and integrates information about genes, proteins, gene function, orthologs gene expression, mutant phenotypes, biological materials such as clones and seed stocks, genetic markers, genetic and physical maps, genome organization, images of mutant plants, protein sub-cellular localizations, publications, and the research community. The various data types are extensively interconnected and can be accessed through a variety of Web-based search and display tools. This unit primarily focuses on some basic methods for searching, browsing, visualizing, and analyzing information about *Arabidopsis* genes and genome. Additionally, we describe how members of the community can share data via JBrowse and the Generic Online Annotation Submission Tool (GOAT), in order to make their published research more accessible and visible.

Keywords: Arabidopsis • databases • bioinformatics • data mining • genomics

INTRODUCTION

The *Arabidopsis* Information Resource (TAIR; *http://arabidopsis.org*) is a comprehensive Web resource for the biology of *Arabidopsis thaliana* (Huala et al., 2001; Garcia-Hernandez et al., 2002; Rhee et al., 2003; Weems et al., 2004; Swarbreck et al., 2008, Lamesch, et al., 2012, Berardini et al., 2015). The TAIR database contains information about genes, proteins, gene expression, mutant phenotypes, germplasms, clones, genetic markers, genetic and physical maps, genome organization, publications, and the research community.

TAIR is a curated database; data are processed by Ph.D.-level plant biologists who ensure their accuracy. Curation adds value to the large-scale genomic data by incorporating information from diverse sources and making accurate associations between related data. Data from manual literature curation, such as protein localization, biochemical function, gene expression, and phenotypes, are added to the corpus of knowledge presented for each locus in the genome. TAIR aims to produce a 'gold standard' functionally annotated plant genome that plant biologists can use as a reference for understanding gene function in crop species and other plants of importance to humans (Berardini, et al., 2016).

The database content and other information relevant to plant scientists can be accessed through dynamic Web interfaces and static hypertext (HTML) pages. Users can perform simple searches of much of the database using names or keywords. Advanced search forms for different data types are used for more complex or specialized queries. Genomic data can be accessed through text-based queries, via the graphical genome browsers (see *BASIC PROTOCOL 2* and *BASIC PROTOCOL 3*), and sequence similarity tools such as BLAST. Data from TAIR can also be obtained in bulk from selected query tools (see *BASIC PROTOCOL 5*) and downloaded from the web site. TAIR provides an extensive set of links from the database and web site to other sources of *Arabidopsis* genomic data around the world.

The data and services of TAIR are organized into eight categories, which appear on the • navigation toolbar on all TAIR pages. Text-based query tools for performing simple and complex searches of specific types of data in TAIR, such as genes (see BASIC *PROTOCOL* 2), DNA, proteins, polymorphisms (including alleles), people, laboratories, and germplasms are found in the Search section. The Browse section allows the user to browse the Gene Symbol Registry, Arabidopsis transposon families, Arabidopsis gene families, as well as Gene and Plant Ontology terms (see BASIC PROTOCOL 3) and recently added literature (see BASIC PROTOCOL 9) and other data types. Within the **Tools** section are TAIR's graphical genome browsers (SeqViewer, JBrowse and GBrowse; see BASIC PROTOCOL 3), MapViewer for aligning physical and genetic maps, sequence similarity software (NCBI BLAST), Motif Analysis and Patmatch (see BASIC *PROTOCOL* 6), the TAIR Synteny viewer (see *BASIC PROTOCOL* 10), the literature full-text search tool Textpresso (see Commentary), an Arabidopsis chromosome map tool (see Commentary), among other data analysis and visualization tools. Under the Tools section, one will also find tools for downloading sets of sequences, protein data, Gene Ontology assignments (see BASIC PROTOCOL 5), and other curated data sets for a set of genes, as well as a GO term enrichment tool for Arabidopsis and other plant species (see BASIC PROTOCOL 4). The **Portals** section hosts pages with links to other databases and Web sites containing useful data and tools. Portals also contains comprehensive lists of community resources curated by the Bioinformatics section of the Multinational Arabidopsis Steering Committee and TAIR curators (https://conf.phoenixbioinformatics.org/display/COM/Resources). The Download directory contains several logically organized directories containing large data sets related to genes, sequences, Gene Ontology annotations and more. The Submit section contains forms and documentation for submitting data to TAIR. Users can contribute functional annotation for submitted papers using the web-based Generic Online Annotation Tool (GOAT; see BASIC PROTOCOL 8), or by providing data in preformatted spreadsheets. TAIR also maintains the Gene Symbol Registry for Arabidopsis and registered users can submit Gene Symbols via the web. In the News section are links to the Arabidopsis community newsgroup, announcements from TAIR, meetings, and job postings.

For *Arabidopsis* to be effectively used as a reference plant species, it is essential that researchers know what data are available, how to use the information they obtain and the provenance of that data. This unit includes several basic protocols for accessing the wealth of information about *Arabidopsis* genes that has been generated by the research community and made available through TAIR. The types of data and tools at TAIR are diverse and cannot all be described in a single unit. Therefore, this unit focuses on the data and tools that are related to retrieving, mining, and visualizing information about Arabidopsis genes. These protocols are based upon data and

tools available as of June 2022. As with any actively updated Web-based informatics resource, the data and tools will change over time.

BASIC PROTOCOL 1

TAIR HOMEPAGE, SITEMAP, AND NAVIGATION

The TAIR home page (*http://arabidopsis.org*) is the main entry point to the database and Web site (Fig. 1.11.1). To facilitate navigation of the TAIR Web site, a navigation toolbar is located at the top of all TAIR pages containing headings such as **Tools**, **Search**, and **Portals**. When mousing over each item in the toolbar, a drop-down menu appears with clickable submenus that lead to a variety of datasets, tools, and external links. Several additional buttons are located above the main toolbar, including items such as **Help**, **About Us**, **Subscribe**, **Register** and **Login**. The **Help** section of the Web site (*http://arabidopsis.org/help/*) provides a quick guide to new users, frequently asked questions, a glossary of terms used on the web site, tutorials, a search help function, and user guides for database searches, specific tools, and registration. Registered users can click on **Login** to register gene symbols and update personal information. The **About Us** subscriptions will see their institution name displayed in the upper right side of the main toolbar. On the bottom of the home page are quick links to connect with TAIR via social media (Facebook and Twitter) as well as our YouTube channel where users can view video tutorials.

Necessary Resources

Hardware

Computer with Internet access

Software

Up-to-date Web browser. The browser must have cookies enabled to log in and submit gene symbols. See *http://www.arabidopsis.org/help/index.jsp* for information on properly configuring one's browser.

Performing a quick search

1. Go to the TAIR home page (*http://www.arabidopsis.org*). Type the search term into the text box in the upper right corner of the page and choose a category from the drop-down menu (see Fig. 1.11.1 item a). Click the Search button.

The quick search performs a name search for most of the objects in the database (e.g., Genes, Clones, ESTs or BAC ends, People/Labs, Polymorphisms/Alleles, Germplasms, Ecotypes, Keywords, Genetic Markers, Proteins and Vectors). By default, this is a "contains" search (a search for aba1 retrieves both ABA1 and ATRABA1A). It is also important to be aware that this search is not limited to the name field. For example, if the gene category is chosen, the gene description and keywords fields will be searched as well as the name. This is done to avoid

missing any potentially relevant results, but may produce a large number of results. Note that searching for Metabolic Pathways sends the query term to the Plant Metabolic Network Database (http://pmn.plantcyc.org/.)

- 2. A list of all matching records is displayed for the data type chosen. Click on each record to access full details for that object, or download the current page of results using the download button at the top of the page. For gene search results, the additional option "download all" provides a way to download the entire result set at once, and "get all sequences" provides an option to download sequences for all the genes in the result set.
- 3. Alternatively, to search for any data type in TAIR by name, choose "Exact name search" from the drop-down menu to the right of the box where the search term was typed in step 1. The query will return a summary (TAIR Search Result) page listing **all** data types with matching records and the number of records for each data type. Click on any item in the list to display a summary of all the records retrieved for that data type. In this example, clicking on Proteins displays a list of the two ABA1 proteins encoded by different splice forms of the *ABA1* gene.
- 4. In the event that a general query returns too many results, try an Advanced Search for the specific data type (see *BASIC PROTOCOL 2* for an example of an advanced search for Genes). The advanced search parameters can be used to narrow down an overly broad query.

BASIC PROTOCOL 2

FINDING COMPREHENSIVE INFORMATION ABOUT ARABIDOPSIS GENES

The locus detail pages represent the most comprehensive starting point for a researcher interested in finding out what is known about a gene. The physical location of an annotated gene on the genome is called a locus in TAIR. The locus serves as a useful concept for grouping genes with other objects having the same genomic location. For convenience, genetically defined genes (i.e., those identified by linkage studies but which are not yet associated with a genomic sequence) are also included as loci that have a genetic, but no physical location. Each locus is associated with at least one gene model, which can be thought of as a version of a gene. Several gene models (labeled as splice variants in TAIR) can be associated to a gene locus based on the existence of predicted or verified alternative transcripts. Every sequenced locus is assigned a unique identifier, the Arabidopsis Genome Initiative (AGI) locus identifier. This has the format AT (for Arabidopsis thaliana) X (where X is either a number from 1-5 corresponding to one of the 5 nuclear chromosomes or C for chloroplast or M for mitochondrion) NNNNN (a 5 digit number). The locus detail page collects information such as gene symbols and full names, experimentally determined or predicted function, gene expression data, mutant phenotypes, associated germplasms, polymorphisms, clones, and publications. Because data in TAIR are highly integrated, it is possible to access the locus detail page from detail pages of almost every other type of object in the database. This protocol illustrates a commonly used way of finding genes using the Advanced Gene Search form.

Necessary Resources

See Basic Protocol 1

Searching for information about a specific gene or set of genes

1. Go to the TAIR home page (*http://www.arabidopsis.org*). In the top navigation bar click on the Search header (see Fig. 1.11.1) and select the Genes link to go to the TAIR Gene Search page

(http://www.arabidopsis.org/servlets/Search?action=new_search&type=gene).

2. To search by name, choose "Gene name" as the option from the Search Name drop-down menu (the options include "Gene name," "description," "phenotype," "GenBank accession," "GenBank gi", "Locus TAIR object ID" or "Gene TAIR object ID"). Using the drop-down menu to the right of this, set the search to an exact match or an inexact match (the options are "contains," "starts with," "ends with," or "exactly") and type the name in the text box on the right-hand side of the same line. For example, to find a set of related genes sharing a gene symbol, such as ARF for Auxin Response Factor family members (Hagen and Guilfoyle, 2002), type in ARF as the name term and choose the "starts with" option to the left of this. Click the "submit query" button.

Gene names include systematic names assigned based on chromosomal location (so called 'AGI locus identifiers' such as AT1G01010) or gene symbols. For more information about Arabidopsis gene nomenclature, see the Arabidopsis Gene Nomenclature Guidelines (http://www.arabidopsis.org/portals/nomenclature/guidelines.jsp).

3. All of the loci that match the query term will be displayed in a list of results (on a page titled TAIR Gene Search Results). Click on the locus name to view the locus detail page. A sample locus detail page obtained by using the search name ABA1, and then selecting the AT5G67030 locus from the TAIR Gene Search results page, is shown in Figure 1.11.2.

The default search only retrieves genes that are active in the database. Checking the "include obsoleted genes" check box will retrieve both active and obsoleted genes, along with the history of their status in the database. Genes may become obsolete if they are merged with other genes—or if improved genome annotation methods find inadequate evidence for their existence. TAIR retains information about obsolete genes in order to maintain a record of their histories and associations.

Using the detail pages to find information about a locus

- 4. On a locus detail page (Fig. 1.11.2) related data are grouped together into different sections. The following annotations (the red lettered items on the left side in Fig. 1.11.2) summarize the typical information displayed on a locus detail page. Definitions of each data type can be obtained by clicking on the adjacent question mark image to display a pop-up definition window.
 - a. Representative gene model and summary information (Fig. 1.11.2A, a items). Unless there is specific experimental evidence to support one transcript over another, the default representative gene model for a protein coding gene is

the gene model with the longest coding sequence (CDS); for other gene types, the representative model is set as default to the .1 model. If there are multiple CDS of the same length the lowest numbered gene model is the representative model.

Data in this section includes Gene Model Type, Other Names and Summary. Example gene model types are protein coding, pseudogene, non-coding RNA, among others. Other names include gene symbols and full names curated from the literature or provided by researchers via the Gene Symbol Registry. The Description field is a short summary of the gene's function either manually composed by a curator or computationally generated. The latter is only shown if the locus has not yet been curated manually. Descriptions from Araport 11 were computationally generated (Cheng, et al., 2017).

- b. Other Gene Models/Map Image (Fig. 1.11.2A, b items). Links to other gene models (termed splice variants in TAIR) are displayed below the representative gene model information. Clicking the gene model name will open a new window displaying the gene model detail page. View this page to see gene model specific data such as gene features in a tabular format and annotations that are specific to individual gene models. The Map detail image is a graphical display of the exon-intron boundaries of all the gene models of a locus. Clicking on the image directs the user to JBrowse (see *BASIC PROTOCOL 3*)
- c. Gene function, biological role, and localization (Fig. 1.11.2A, c item).

The **Annotations** section contains all of the controlled vocabulary terms that have been assigned to describe the molecular function, biological role, subcellular localization, and expression of the gene product. The annotations are grouped according to the type of vocabulary and summarized on the locus page. Click on the **Annotation Detail** link (located at the bottom right of the Annotations section) to display the full annotation details, which include the type of evidence supporting the annotation and the corresponding reference that is the source of the data supporting the annotation.

d. Sequences (Fig. 1.11.2A, d item).

Links to genomic sequence, full-length CDS, full-length cDNA, and protein sequence are located in the **Sequence** section. Clicking on the sequence name will display a new window containing the sequence, which can be uploaded directly into TAIR'S BLAST tool. TAIR BLAST includes specialized Arabidopsis sequence data sets such as intergenic regions, upstream and downstream sequences, and UTRs (http://www.arabidopsis.org/help/helppages/BLAST help.jsp#datasets). BLAST can also be accessed from the TAIR homepage under the **Tools** section.

e. Gene expression (Fig. 1.11.2A, e item).

Information about the expression of the gene can be found in the **RNA Data** section and the lower part of the **Annotations** section. In the RNA Data section, array elements from one-channel and/or two-channel experiments that map to the locus are listed. Array element names are linked to detail pages. Note that TAIR stopped integrating and updating microarray data in 2005, see Commentary for more current datasets and tools. Lists of full-length cDNAs and expressed sequence tags (ESTs) can be found in the **Associated Transcripts** subsection within the RNA Data section. Click on the number next to the type name to see a list of all the clone records. The clone records are linked to GenBank, where information about the cDNA libraries (and therefore expression) can be found. Finally, information about gene expression, curated from the literature, is shown in the **Annotations** band along with the Plant Ontology associations (see Fig. 1.11.2A, section "c": "expressed during", "expressed in").

f. BAR eFPbrowser image

Gene expression data are displayed using the Application Programming Interface (API) to show the electronic pictographic representation of gene expression from the BioAnalytic Resource (BAR, http://bar.utoronto.ca/). Users can toggle between different views representing different experimental datasets using the 'Data Source' drop down menu. Users can also click on the link to the BAR website to make use of the features of this resource.

g. Protein data (Fig. 1.11.2A, fitem).

Structural and physical characteristics of the protein encoded by the reference gene model, including molecular weight, conserved domains, and isoelectric point, are displayed in this section. Click on the AGI name in the protein section to open a new window displaying more detailed information and the amino acid sequence itself.

h. Plant homologs (Fig. 1.11.2A, g item).

The **Plant Homologs** data section displays information about proteins that are evolutionarily related to the locus. The homologs are phylogenetic predictions made by the PANTHER project (Mi et al. 2017). Clicking on the PhyloGenes tree view glyph will open the corresponding gene tree in PhyloGenes (Zhang et al., 2019) in a new window. PhyloGenes presents gene function data alongside phylogenetic trees to aid in gene function prediction or comparing functions in different species (see BASIC PROTOCOL 8). The Arabidopsis paralogs display includes links to the individual locus pages for each paralog. Users can also click on a button to retrieve paralog sequences, or download a list of AGI locus IDS to use in subsequent analyses. Plant homologs from the PANTHER families are listed according to their taxonomic distribution and the entire list can be downloaded and saved as a tab delimited file. The 'Search Gene Families' section uses the selected locus ID as a query to search for orthologs in external resources such as Ensembl Plants (Bolser et al., 2016), PLAZA (Proost et al., 2015) and Phytozome (Goodstein, et al., 2012), among others.

i. Map locations (Fig. 1.11.2A, h item).

The **Map Locations** section displays the chromosome and coordinates of the locus for the maps on which it is found. The gene can be viewed in a whole-genome context by clicking on one of the three map options (Map Viewer, Sequence Viewer, GBrowse and JBrowse) in the Map Links section (See BASIC PROTOCOL 3).

j. Markers, Alleles and Polymorphisms (Fig. 1.11.2A, i item).

Genetic markers that map within the locus are displayed along with the type (e.g., visible, RFLP, SSLP etc...). All of the polymorphisms that map within the locus are shown in the Polymorphisms section, along with the type of variation. This section includes natural variations found in different ecotypes and induced mutations (e.g., T-DNA insertions) that have been mapped by sequence identity and alleles that have been curated from the literature. To find detailed information about a polymorphism, click on the name of the polymorphism.

k. Germplasm information (Fig. 1.11.2A, j item).

The Germplasm section provides information on all germplasms available for a locus, including phenotype descriptions and images of plants (if available). If a germplasm is a stock, that Stock ID and a link to ABRC will be shown. Links to the three main stock centers are provided at the bottom on the Germplasm section to facilitate searching for the stocks at those resources.

l. Clones (Fig. 1.11.2B, k item).

Clones linked to a locus may include vectors, BACS, clone ends (ESTs) that contain sequences from the locus of interest. If the clone is an available stock, that information will be displayed along with a link to order from ABRC.

m. External links (Fig. 1.11.2B, l item).

There are other web sites that provide either alternate views or different information about a locus (see Commentary). In order to provide access to as much information about a locus as possible, TAIR provides links to the corresponding locus pages in other databases and Web sites. Types of external links include other Arabidopsis genome annotation databases, gene expression databases, and functional genomics sites, as well as links to tools for further analysis. For example, all sequenced loci are linked to other Arabidopsis annotation databases including Thalemine at BAR, NCBI, and Gramene. Links are grouped by data types such as: Genomics, Expression/Localization, or Interactions. TAIR also provides links to UniProt and NCBI Reference genome from the protein detail pages.

n. Community Comments (Fig. 1.11.2B, m item).

Comments may contain additional data contributed by registered TAIR users, and are included in the display for nearly all of the TAIR detail pages. This function can be used to report new data, as well as errors or omissions related to the displayed object (see http://www.arabidopsis.org/help/helppages/addcomment.jsp).

o. Publications (Fig. 1.11.2B, n item).

Papers and conference abstracts are shown at the bottom of the detail page in the section marked Publications. Publications include published literature imported from PubMed, Agricola, and BIOSIS, along with abstracts from the International Conference on Arabidopsis Research. Only the most recent 15 papers are listed on the detail page; to retrieve the complete list, click on the View Complete List link. Clicking on the title of the publication opens a new link to the detailed record where one can read the abstract, link to the PubMed citation, associated loci and annotations, and find authors among TAIR's community. Users are encouraged to contact the TAIR curators to report missing or incorrectly associated papers.

p. Update History (Fig 1.11.2B, o item).

TAIR maintains a history of changes to the genome annotation status a locus for the purposes of tracking. Recorded changes include merges, splits or insertions.

Saving the results of a search to a file

1. Return to the list of results obtained by the query submitted in step 2 (page titled TAIR Gene Search Results). Check the box to the far left of the results summary. Each page of results must be saved separately. Only those results that are selected will be saved. Use the Check All function to save all of the results displayed on the page.

Before downloading a large set of results, use the browser to go back to the Advanced Search page, make sure the number of records per page of results is set to the maximum (usually 200 records/page), and resubmit the query.

- 2. After selecting all of the desired results on a page, click on the Download Checked button (or Download All if you wish to export all results) in the upper right corner of the TAIR Gene Search Results page. The checked results will then be displayed in the browser window as tab-delimited text file. Use the Save As function under the File menu in the browser toolbar to save the results in a file on the local computer. This process must be repeated for each page of results.
- 3. In order to retrieve sequences for the selected results, click on the Get Checked Sequences button (or Get All Sequences if you wish to retrieve sequences for all results) on top of the TAIR Gene Search Results page. This will bring you to the

Sequence Bulk Download and Analysis page from where you can retrieve different types of sequences for your list of genes. For more information about that tool, see *BASIC PROTOCOL 5*.

The download feature is found on all of the search results pages. Each set of results includes different information in the downloadable file. See the help documents for the specific search to view a listing and description of the downloaded fields. The files contain tab-delimited text that can be opened using a text editor or spreadsheet software such as Microsoft Excel. The download sequence option is only available on the Gene Search Results page.

BASIC PROTOCOL 3

USING THE ARABIDOPSIS GENOME BROWSER-JBROWSE

TAIR provides three Web applications (JBrowse, SeqViewer and GBrowse) that allow users to explore the annotated *Arabidopsis* genome sequence. SeqViewer is a graphical genome browser developed by TAIR while JBrowse (Buels et al., 2016) and its precursor, GBrowse (Stein et al., 2002) were developed by the Generic Model Organism Database project (GMOD; www.gmod.org). These tools allow the user to search for and display various sequence features such as genes, polymorphisms, T-DNA insertions, and transcripts (ESTs/cDNAs), provide a mechanism for navigating around the genome, and allow individual users to customize the type of data displayed. These tools are useful for a wide variety of tasks including positional cloning, identifying mutants in a gene of interest, finding cDNA and ESTs for a gene of interest, and finding and displaying the distribution of sequence features (e.g., polymorphisms, T-DNA insertions) in a whole-genome context. While all of these tools share some functionality, each tool has its own specific set of features. JBrowse is highly customizable, contains many data types not represented in SeqViewer or GBrowse and is more frequently refreshed with new data. SeqViewer and GBrowse are legacy software that TAIR continues to maintain.

The following protocol highlights some of the TAIR specific features of our JBrowse instance. For a more exhaustive guide to JBrowse and its features see the JBrowse User Guide (https://jbrowse.org/jbrowse1.html). As of June 2022, the current JBrowse software version is 1.16.6. Gene function data in JBrowse is updated on a quarterly basis (i.e., gene names/symbols and descriptions) and community tracks are updated as needed.

Necessary Resources

See Basic Protocol 1

Exploring JBrowse

Viewing Arabidopsis genomes and genes in JBrowse

1. Go to the TAIR home page (*http://www.arabidopsis.org*). In the Tools section of the

This is the submitted version. For the final, edited version see: Reiser, L., Subramaniam, S., Zhang, P., & Berardini, TZ., (2022) Using the Arabidopsis Information Resource (TAIR) to find information about Arabidopsis genes. Current Protocols in Bioinformatics. DOI:10.1002/cpz1.574 menu bar, click on the link to JBrowse. Alternatively, go directly to the URL https://jbrowse.arabidopsis.org/index.html?data=Araport11

- 2. The JBrowse display is organized into two main panels. The left side of the browser window contains the Track Selector (Fig 11.1.3 A) and the right side displays the genome browser along with controls including Genome, Track, View and Help and search functions (Fig 11.1.3 B).
- 3. The Genome selector (Fig 11.1.3 B item a) is used to switch between different genome versions. To change the genome, click on the named version in the drop-down menu to choose from among the preloaded options. Alternatively, one can choose to upload a custom sequence file. Once selected, the display will refresh to the new genome. This will rewrite both the browser view and available tracks displays.

The default genome in TAIR is the Arabidopsis thaliana Col-0 genome originally sequenced in 1999. Since then, the genome has undergone 10 subsequent reannotations up to Araport 11(Cheng et al, 2017) and the assembly (actual sequence of nucleotides along the chromosome) has been updated to the current TAIR10 version. As new Arabidopsis genomes have been sequenced, they can be added to JBrowse such as the ColCEN genome (Naish, et al., 2021).

Altering the Genome changes the chromosome sequence, gene models, and other available tracks to those of the specified release. Note that gene models or other features present in different releases may potentially be absent, located at a different position, or otherwise altered relative to an earlier release. A description of the latest genome release can be found at http://www.arabidopsis.org/portals/genAnnotation/gene_structural_annotation/g enome_annotation.jsp), details of earlier releases can be found on the TAIR site (http://www.arabidopsis.org/download/indexauto.jsp?dir=/download files/Genes)

3. The names and position of genomic features such as genes or genetic markers can be entered in the search box (Fig. 1.11.3. B, item e). For genes, either the AGI code (e.g., AT1G05460) or gene symbol (e.g., SDE3) is a valid search query. Nucleotide ranges can also be entered to allow specific regions of interest to be displayed. The chromosome and start and end coordinate of the desired region must be entered in the following format Chr1:1504365..1514364.

If a query returns multiple hits, JBrowse will display these as distinct rows with the position of each feature shown. Clicking the desired hyperlink will open the detail display for the selected region.

4. Entering a feature name or region and clicking Go (Fig 1.11.3 B item e) will update the overview and details display. The overview map shows the position of the region displayed in the detail view relative to the rest of the chromosome. The size of this region is shown in the Scroll/Zoom drop-down. The default display includes the following tracks: reference genome, protein coding loci, and T-DNA seq data.

To highlight an area of interest in the genome, click on the highlighter icon to the left of the Go (search) button (Fig 1.11.3.X B item f). When active, the button will be yellow. With the mouse in the nucleotide numbering track just below the

controls, click on the start point of the region all the way to the end point. This highlighting provides a convenient way of keeping the feature of interest in view when expanding the display, a larger region (Fig 11.1.4 A).

- 11 The zoom feature (Fig 1.11. 3.B item d) can be used to adjust the viewing dimensions in order to display a larger-scale view of the genome all the way down to the nucleotide sequence level. As with the highlighter, click any start point of the nucleotide numbering track (a vertical red line will appear) and then drag the mouse to the desired end point. When the mouse is released the genome view will be redrawn to the new start and end points.
- 6. To move along the chromosome, click on the grey arrows (Fig 1.11.3 item b) to shift the display to the left or right. Moving the cursor over a feature and left clicking the feature glyph will bring up a pop-up window that displays additional information about that feature. For genes, this includes known symbols or common names (Fig 1.11.4 A item a). Right clicking on a feature will open a pop-up window with additional data display options. For example, clicking on a protein coding gene model will give options to view the gene info in JBrowse, view the TAIR locus page for the corresponding locus or view the sequence using SeqLighter (see below.)

Viewing pre-loaded community generated data tracks

The following section describes features and data types available when the Araport 11 Col-0 reference genome is selected

- 7. The Tracks menu (Fig 1.11.3 A and Fig 11.1.4 B) allows you to customize the display of preloaded tracks within the JBrowse genome view. TAIR JBrowse includes the following major track categories: Arabidopsis Genome Assemblies, Community Data and Whole Genome Alignments. Within each track category there are subsections that can be expanded to display different types of data (Fig. 1.11.3 A). Clicking on the down arrow in the section header will expand to display the full list of available tracks in each category.
- 8. To add or remove tracks from the detail display simply check or uncheck the required tracks. The track order can be adjusted by clicking the track title in the details panel and dragging the track up or down to a new position.
- 9. Configure individual tracks by clicking the down arrow next to the track title. This allows the user to choose the shape and color of the glyphs, put a limit on the number of features displayed in any one region, and set preferences if a text label is displayed.

TAIR welcomes community data submissions. Community members who wish to provide data to TAIR for JBrowse should send an email to curator@arabidopsis.org and provide the data in the specified format (typically GFF or BED formats). Please specify if the data is for pre-publication review so that it can be displayed on a private server for peer review.

Adding tracks from CoGE or local sources

10. In addition to the preloaded tracks, JBrowse enables you to upload your own

annotation to visualize within the context of the *Arabidopsis* genome. To upload your own data to JBrowse go to the "Tracks" option in the Genome panel (Fig 1.11.3 B item g). Clicking on the Tracks option will open a pop-up window where you can select the custom data.

- 11. Users can upload any combination of data files and URLs. To upload a local file either drop in the file or use the browser to locate and select the file on your computer. To upload from a remote site, enter the complete URL address for the file. Choose the option to either open immediately or to add to tracks for future viewing. Once the file is uploaded, JBrowse will use the data to suggest the type of track to display. Note that custom tracks are only visible to the user in the current JBrowse session.
- 12 Users can also use the CoGE plug-in (Fig 1.11.4 B item a) to search and display publicly available tracks from CoGE (REF: https://genomevolution.org/CoGe/; Lyons and Freeling, 2008). Clicking on the Epic CoGE link will display a pop up showing all of the available community tracks. Check all the tracks you wish to include.

Other specialized tracks

- 13 In addition to adding data tracks from the available preloaded track menu and custom datasets, JBrowse can be configured to include some specialized track functions. These options are available from the 'Track' link.
 - a. 'Add combination track' allows you to create a new track that displays merged data from two tracks. Click add combination track and then drag the two tracks you wish to display into the new combination track. This creates a new track (Fig 1.11.5 A) that merges the features of both tracks.
 - b. Add sequence search track allows you to include a DNA or amino acid search function. Click add sequence search track, choose the parameters (e.g., DNA or AA, case sensitive or not, etc...) enter the query term and hit search. This feature is very useful for identifying sequence features within a whole genome context.

Obtaining decorated sequence files

JBrowse includes a module called SeqLighter that allows you to extract a protein coding region and highlight specific features of interest.

- 14 In the genome display right click on the sequence glyph of interest in the 'protein coding gene models' track and choose the 'View Sequence' option.
- 15 Choose the add flanking region to add 500 to 4000bp upstream and downstream sequences.
- 16. The SeqLighter function will display the corresponding sequence in default CODATA format (Fig 1.11.5.B). Use the option selectors to choose which features to annotate such as introns and exons and UTRs. The default format can be changed to FASTA, PRIDE, or RAW formats and the image can be saved as JPEG, SVG and PNG files.

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Reiser, L., Subramaniam, S., Zhang, P., & Berardini, TZ., (2022) Using the Arabidopsis Information Resource (TAIR) to find information about Arabidopsis genes. Current Protocols in Bioinformatics. DOI:10.1002/cpz1.574

BASIC PROTOCOL 4

USING THE GENE ONTOLOGY ANNOTATIONS FOR GENE DISCOVERY AND GENE FUNCTION ANALYSIS

Annotations (associations of controlled vocabularies or keywords to data objects) provide a richer, more complex picture of a gene that is also more computationally accessible for the purpose of querying, classification, and making correlations among seemingly unrelated data. TAIR makes extensive use of controlled vocabularies for describing data in the database. The controlled vocabularies (ontologies) that are used by TAIR are also used by other model organism databases, thereby facilitating cross-species comparisons. All of the ontologies used by TAIR are included in the Open Biological Ontologies Project (*http://www.obofoundry.org/*) where they are freely accessible.

TAIR is member of the Gene Ontology (GO) Consortium (http://www.geneontology.org) and participates by developing and refining the ontologies and annotating Arabidopsis gene products (The Gene Ontology Consortium, 2010). The GO controlled vocabularies describe three aspects of gene products: molecular function, biological process, and subcellular location. TAIR also imports manual and computational annotations for Arabidopsis made by other groups including UniProt, BioGrid, JCVI (formerly TIGR) and others (Wortman et al., 2003; Berardini et al., 2004). These annotations are contributed independently by each organization to the GO database, where they are accessible through the AmiGO query tool for making cross-species queries (http://amigo.geneontology.org/amigo). The other main ontology used at TAIR is the Plant Ontologies developed by the Plant Ontology Consortium (POC; http://www.plantontology.org). The POC has used the GO model to develop controlled vocabularies for plant structures and developmental stages (Jaiswal, et al, 2005). In TAIR, both of these ontologies are used to annotate many additional types of data such as high throughput proteomics data, low throughput gene expression data, phenotypes, and publications. TAIR also collects and displays annotations contributed by members of the community who can use a simple web tool (Generic Online Annotation Tool, GOAT) to provide GO and PO annotations for genes based on published works (see BASIC PROTOCOL 7).

Necessary Resources

See Basic Protocol 1

Files

WRKYFamily.txt (available at https://www.arabidopsis.org/download_files/Help_Documents/WRKYFamily.txt)

Using the Keyword Browser to find candidate genes

For researchers, finding candidate genes involved in a particular pathway typically involves a fishing expedition using a variety of genetic, molecular, and biochemical assays. The GO annotations can be useful in making educated guesses about what genes may act in a pathway or are members of transcriptional/signaling cascades. Because TAIR and its community contributors have focused on GO curation from the literature, Arabidopsis is the most well annotated plant genome, with a large number of experiment-based annotations (Berardini). Thus, Arabidopsis GO annotations can be particularly useful for being able to infer gene function for unknown genes in other plant species based on sequence similarity or evolutionary relatedness. Another common use of GO annotations is to identify sets of genes associated with a given function or process in Arabidopsis as a starting point to identify genes with similar functions in other species.

Go to the TAIR home page (*http://www.arabidopsis.org*), click Search in the toolbar (Fig. 1.11.1), and select Keywords from the drop-down menu that appears. The page shown in Figure 1.11.6A is returned (TAIR Keyword Search and Browse; can be directly accessed at *http://www.arabidopsis.org/servlets/Search?action=new_search&type=keyword*). Enter term (keyword) "root development" in the text box and choose "contains" (an inexact search) from the drop-down menu to the left of the text box. From the group of check boxes for restricting the search, choose GO Biological Process as the keyword type and click the Submit Query button.

Many of the terms in GO exist as complex phrases. TAIR searches take the entire entered term or phrase as a complete phrase rather than a set of words. Consequently, an "exact match" search will often not retrieve any entries. Therefore, the authors recommend using the "contains" option for keyword searches.

2. On the Keyword Search Results page (Fig. 1.11.6A), each controlled vocabulary term is displayed along with a count of all data objects (e.g., loci, publications, annotations) annotated to that term. Click "loci" to display the genes annotated to "root development." The results are displayed as a Gene Search Result page (see *BASIC PROTOCOL 2*) where all of the genes associated to the term 'root development" or its children, are displayed. Click on the locus name to view the locus details or save the list as a text file (see *BASIC PROTOCOL 2*)

Finding genes annotated to related functions

3. On the Keyword Search Results page, find the listing for "root development," and click on the "treeview" link. This will open a window displaying the term in a hierarchical tree view (Fig. 1.11.6 B).

In the Gene Ontology, terms have a parent-child relationship to one another. Parent terms are less specific than their child terms. A child term may be a part of the parent (as thylakoid is part of chloroplast) or a type of the parent (as chloroplast is a type of plastid). In contrast to simple hierarchies, a child term may have more than one parent. The ontologies are intended to be as biologically accurate as possible. Terms and their relationships are defined by what is known about the biology of the process, function, or cellular component. By examining the structure of the ontology to find related terms, related gene products can also be found via their annotations to the terms.

- 4. Click on the plus sign next to the parent term ("root development") to expand the node and display all of the child terms.
- 5. To display genes annotated to each of the parent and child terms, select the "loci" radio button from the top of the tree view page (Fig. 1.11.6 B), then click the Display button. The display will be redrawn to show a count of the number of loci annotated to each term and the number of loci annotated to the children of each term. Click on the link to list loci annotated to the term "regulation of root development" to find all loci that are annotated to this term.

Retrieving GO annotations for sets of genes

GO Annotations can also be used to rapidly classify sets of genes such as gene families or coclustered genes revealed by analysis of high throughput expression data.

- Go to the TAIR home page (*http://www.arabidopsis.org*), click Search in the toolbar (Fig. 1.11.1), and select Gene Ontology Annotations from the drop-down menu that appears. Alternatively, go to the URL *http://www.arabidopsis.org/tools/bulk/go/index.jsp.*
- 7. Upload a list of AGI locus identifiers using the sample data file WRKYFamily.txt. This file contains a list of 74 loci all belonging to the WRKY transcription factor family (Eulgem et al., 2000; http://www.arabidopsis.org/browse/genefamily/WRKY-Som.jsp). Select the Text radio button under "Select output type"; to view results locally in a table format. Click on the "Get all GO Annotations" button. The output file contains a list of all the specified loci and their annotations to all three aspects of the GO ontology.

The annotations include the evidence code and reference for the data supporting the annotation. The file can be saved onto a local computer as a tab-delimited text file. If the HTML option is chosen, the results are hyperlinked to TAIR detail pages for loci, keywords, and publications. The Web output also has links to the corresponding keyword entry in the GO database, where one can find annotations to genes from other organisms.

Classifying sets of genes into functional categories

8. Alternatively, instead of getting a list of all annotations, the genes can be grouped into broader categories based on their annotations. After uploading the gene list (step 7 above), choose "HTML output" and click the Functional Categorization button.

For each aspect of the GO ontologies, a subset of terms have been selected to represent major broad categories, called GO Slim categories. If a gene is annotated to a child term of one of the GO Slim terms, it is included in the broader category. The GO Slim is less specific, but presents a simpler classification. The results include gene annotations that are both experimentally supported and computationally predicted. To find sets of annotated genes based on evidence codes, use the Evidence sub option in the Search by Associated Keyword section on the Gene Search page

(http://www.arabidopsis.org/servlets/Search?action=new_search&type=gene). GO Slim assignments are also included in the detailed GO annotation output

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(from step 7). See http://arabidopsis.org/help/helppages/go_slim_help.jsp for a list of all GO Slim terms and their definitions.

9. The database will return a functional categorization list showing all categories represented in the genes from the input file, along with the frequency of distribution of the genes within the set (Fig. 1.11.7A). To view a list of genes in each category, click on the number in the "Gene count" column.

Only the categories represented by the genes in the list are included; the absence of any of the GO Slim categories means that there are no genes in the list that fall into that particular group. The default option displays the list grouped by keyword type and then by categories sorted by the number of annotations in each category. The table can be re-sorted to list by gene count. Frequency refers to the number of occurrences of a gene-keyword pair in the list. Multiple annotations to the same term are essentially compressed in this view, in contrast to the Get all GO annotations option. Genes that are annotated to multiple terms that fall into different categories will be included in each of the GO Slim bins. Therefore, the total number of annotations to each aspect of the GO ontologies may be greater than the total number of genes in the query list.

Displaying the functional classification as a chart

- 10. The distribution of functional categories can be displayed graphically as either an annotation pie chart or gene bar chart. To display as a pie chart, above the Functional Category column (Fig. 1.11.7A), select 'Annotation Pie Chart' and click on the 'Draw' button." This will create a new page showing three separate pie charts, one for each aspect of the Gene Ontology (Fig. 1.11.7 B). Depending on how the results are sorted, the sections can be displayed from most to least frequent category, or by related categories. The percentage of the total is shown in the color key for each graph.
- 11. To save the graph images, hold down the Ctrl key while clicking on the image, or right click the mouse if using a PC, and save the image to the clipboard or to a file. The images are in Graphic Interchange Format (GIF), which can be opened using a variety of graphics software.

Downloading the entire set of Arabidopsis GO annotations

In some cases, it may be useful to download the set of Arabidopsis GO annotations for the entire genome. For example, a common use of TAIR's curated annotations is as a reference for annotation of other species using sequence similarity or homology based methods. In such cases it may be useful to import the Arabidopsis annotations into an analysis tool.

- 12. On the home page (Fig 1.11.1) go to the Downloads section of the main toolbar, choose Downloads and then GO and PO Annotations. Alternatively go directly to http://www.arabidopsis.org/download/index-auto.jsp?dir=%2Fdownload_files%2FGO_and_PO_Annotations%2FGene_Ontol ogy Annotations.
- Navigate to the file named gene_association.tair.gz. This compressed file contains all of the GO annotations for Arabidopsis genes annotated by TAIR, community members, UniProt, the GO Consortium, IntAct, TIGR, and others, in

the standard GAF2.2 format (http://geneontology.org/docs/go-annotation-file-gaf-format-2.2/). The file is updated on a quarterly basis.

14. Another option is to use the ATH_GO_SLIM.txt file. This text document is a tab-delimited file that contains all the annotations to the narrow (granular) GO term as well as a column that maps the annotations to the corresponding GO Slim category. Users should consult the README file (http://www.arabidopsis.org/download_files/GO_and_PO_Annotations/Gene_On tology_Annotations/ATH_GO.README.txt) for information on each of the data fields.

GO Term Enrichment/ Statistical over-underrepresentation test.

In addition to GO functional categorization, for any given set of genes users may also wish to determine if there are terms that are over or underrepresented in that set as a means to generate hypotheses about gene function or biological events. TAIR uses a web service, provided by PANTHER DB to facilitate GO Term statistical enrichment tests for Arabidopsis and other plants represented in the PANTHER database

(http://www.arabidopsis.org/tools/go_term_enrichment.jsp; Mi, et al., 2013). Users can enter a list of locus identifiers, choose the appropriate species, and select the GO aspect (biological process, cellular component or molecular function). PANTHER's tool accesses a comprehensive list of GO annotations from the GO Consortium as well as a recent version of the ontology itself, both of which are updated monthly. Because annotations are constantly being updated as new information is obtained, the monthly updating schedule ensures that analyses done using the PANTHER tool rely on the most current annotation data.

- 15. Go to the TAIR home page (*http://www.arabidopsis.org*), click Tools in the upper menu bar (Fig. 1.11.1), and select GO Term Enrichment from the drop-down menu that appears. Alternatively, go to the URL *http://www.arabidopsis.org/tools/go term enrichment.jsp*
- 16. Enter in a list of gene identifiers such as AGI Locus IDs (e.g., AT5G61160), UniProt IDs (e.g., Q9FNP9) or NCBI Entrez GeneIDs (e.g., Gene: 836237), separated by newlines or commas.
- 17. Choose the appropriate plant species from the drop down menu.

The web service implemented at TAIR can be used to analyze Arabidopsis as well as any of the other plant species included in the PANTHER database.

- 18. Select the ontology aspect that you wish to analyze. The options are 'biological process', 'molecular function', and 'cellular component.'
- 19. Click Submit, to send the data to PANTHER.

Evaluating the results

The web service sends the data to PANTHER and the results are returned in a new window on the PANTHER website (Figure 1.11.8).

20. The analysis summary box (Figure 1.11.8 A) displays the analysis type (PANTHER can do several types of gene list analysis), annotation version and

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annotation dataset. This information is important to record and report in your publications, as the same analysis performed with different software versions and different annotation releases may yield different results.

- 21. ID mapping results. Uploaded IDs are mapped to the reference proteome set in PANTHER. Click on the number to review each list to see the details.
 - a. Unmapped IDs are those that could not be mapped to a corresponding UniProt reference genome protein record in the PANTHER. This list would include any non-protein coding loci.
 - b. Multiple IDs. PANTHER also provides a list of IDs where multiple IDs are mapped to the same PANTHER protein entry. Typically, this occurs when more than one gene produces the same amino acid sequence.
- 22. Term Enrichment Results (Figure 1.11.8 B). The results are displayed in a table.
 - a. Term list. The first column displays the over/underrepresented GO terms. By default, only results with a p value of less than 0.05 are displayed. The terms are presented in a hierarchical format where related terms are grouped by background color, with the most granular term at the top. Invert the sort order by clicking the arrow next to the term 'Hierarchy' in the last column header. To view it as a simple list, click 'Hierarchy'.
 - b. The second column shows the number of genes (#) in the reference genome dataset that map to the terms. This is the background frequency.
 - c. The third column shows the number of genes (#) in the sample gene set that map to the GO term. This is the sample frequency.
 - d. The fourth column displays the number of genes mapped to the term that would be **expected** based on the whole genome representation. For example, if 113/27,352 genes in the reference set mapped to cytosolic large ribosomal subunit, then the expected frequency (0.0041) to map to that term in the sample set (0.0041 X 247=1.02). Clicking on the number will retrieve a list of the genes that map to the term.
 - e. The fifth and sixth columns show the fold enrichment and a sign to show increase (+) or decrease (-). Fold change is calculated by dividing the observed by expected results.
 - f. The seventh column shows the p-value. The lower the p-value, the less likely the obtained result can be explained by random distribution.

BASIC PROTOCOL 5

USING GENE LISTS TO DOWNLOAD BULK DATASETS

TAIR provides a number of tools for obtaining data in bulk for sets of genes such as gene descriptions or sequences (http://www.arabidopsis.org/tools/bulk/index.jsp). While the gene search and locus pages can provide comprehensive information on a gene by gene basis (see *BASIC PROTOCOL 2*), it is often desirable to obtain specific data for a large number of genes. TAIR's bulk download tools can be used to take a set of AGI locus identifiers as an input and obtain gene descriptions (http://www.arabidopsis.org/tools/bulk/genes/index.jsp), GO annotations (see *BASIC PROTOCOL 4*) and PO annotations (http://www.arabidopsis.org/tools/bulk/sequences/index.jsp), protein properties (http://www.arabidopsis.org/tools/bulk/sequences/index.jsp), microarray elements (http://www.arabidopsis.org/tools/bulk/protein/index.jsp) and locus histories (http://www.arabidopsis.org/tools/bulk/microarray/index.jsp).

Necessary Resources

See Basic Protocol 1

Downloading Gene Description/Summaries

- 1. On the TAIR home page (*http://www.arabidopsis.org*) select Bulk Downloads from the Tools drop-down menu. Alternatively, go directly to the URL *http://www.arabidopsis.org/tools/bulk/index.jsp*.
- 2. Choose Gene Descriptions (http://www.arabidopsis.org/tools/bulk/genes/index.jsp).
- 3. Enter in or upload a list of AGI locus identifiers or gene model identifiers.
- 4. Choose which data set to search against to retrieve matching records. To obtain all descriptions for a locus, choose 'get descriptions for all gene models/splice forms.
- 5. Choose how you want your results returned, to the browser or in a file.

Guidelines for understanding the results

6. The results will include the locus identifier, gene model name(s), description, primary gene symbol and other gene symbols.

Each locus may be associated to one or more gene models, and each model may have distinct descriptive information that is unique for that gene product. For example, the locus AT2G42810

(http://www.arabidopsis.org/servlets/TairObject?id=33349&type=locus), encoding Protein Phosphatase5 (PP5) has a total of 5 gene models which represent different splice variants. The AT2G42810.2, or reference gene model, is an integral membrane protein whereas AT2G42810. 1 does not contain the membrane domains and is localized to the cytoplasm.

The gene description will either be a short, computationally derived description statement attributed to Araport 11, or a curated summary written by TAIR curators.

Downloading whole genome annotations in bulk

In some cases, it may be desirable to obtain data sets for the entire Arabidopsis genome such as sequences or functional annotations. TAIR provides access to curated data (e.g., PO annotations, phenotypes, gene summaries, gene aliases, etc.) after the data have been in TAIR for one year. Year old data is released on a quarterly basis and can be found in the **Download** section (see Introduction) under Public Data Releases. Subscribers can access more recent data sets from the **Download** section (Subscriber Data Releases). Sequences from TAIR10 and Araport11 are available as BLAST data files (http://www.arabidopsis.org/download/index-auto.jsp?dir=%2Fdownload_files%2FSequences).

If you are unsure of where to find a dataset, if the dataset is the most current or for custom datasets, contact the TAIR curators (curator@arabidopsis.org).

BASIC PROTOCOL 6

USING TAIR'S ANALYSIS TOOLS TO FIND SHORT SEQUENCES AND MOTIFS

Using the Motif Analysis Tool for Identifying potential cis-regulatory motifs in upstream sequences

The Motif Finder identifies six-oligomer nucleotide sequences that are statistically overrepresented in a set of input sequences when compared to the whole genome. The most common application of this tool is for identifying potential *cis*-regulatory elements in genes whose expression patterns correlate into a cluster. Consensus sequences for putative transcription factor binding sites can be used to identify additional genes having the element in the promoter using the Patmatch program

Necessary Resources

See Basic Protocol 1

Entering the search parameters

1. On the TAIR home page (*http://www.arabidopsis.org*) select Motif Analysis from the Tools drop-down menu. Alternatively, go directly to the URL *http://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp*.

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- 2. On the data entry form enter the locus identifiers of your genes of interest. This can be done manually or by uploading a list of Arabidopsis AGI identifiers from a file. In the example shown in Figure 1.11.12A, we queried for motifs in the 500 bp upstream region of 15 co-expressed genes. Note that a minimum number of 3 locus identifiers has to be entered.
- 3. Select length (500, 1000, or 3000 bp) of upstream sequence to be queried. Submit the query.

The sequence data sets are either 500-, 1000-, or 3000-base-pair sequences upstream of the translation start site of each gene in the genome. The program will search for 6-mer words that are overrepresented in the upstream regions of the set of queried genes compared to upstream sequences in the entire genome. Both forward and reverse strands are queried.

Evaluating the results

- 4. The results are displayed in a table as shown in Figure 1.11.9 B. The columns, denoted "a" through "g" in Figure 1.11.9 B, are as follows.
 - a. Oligomer.

Each over-represented six-oligomer sequence is listed in the first column of the results table.

b. Absolute number of oligos in the query set.

Number of times the oligo appears in the upstream regions (of chosen length) of the query genes. This number can be higher than the number of query sequences, as some sequences contain multiple occurrences of the motif.

c. Absolute number of oligos in the genomic set.

Number of times the oligo appears in the upstream sequences (of chosen length) of all genes in the genome.

d. Number of sequences in query set containing oligomer.

Shows the ratio of the number of queried sequences containing the oligomer over the total number of queried sequences.

e. Number of sequences (e.g., out of 34,187 in genomic set) containing oligomer.

Shows the ratio of the number of genome sequences containing the oligomer over the total number of sequences in the genome.

f. *p*-value.

This score reflects the probability of the six-oligomer sequence occurring in the selected query set by chance. The lower the score (closer to zero) the greater the likelihood the match is significant.

g. Query sequences containing this oligomer.

All of the query genes containing the oligomer are listed here. The Patmatch tool (see next section) can be used to locate other genes that contain the oligomer in the upstream sequence.

Using the Patmatch Tool to find short sequence patterns in DNA and protein sequences

Patmatch (Yan et al., 2005) was designed for identifying patterns in a selected TAIR dataset (e.g., genes, proteins, upstream sequences, etc.) that match regular expressions. Patmatch can be useful for finding short nucleotide patterns such as *cis*-elements, Massively Parallel Signature Sequence (MPSS), Serial Analysis of Gene Expression (SAGE) tags, or small RNA binding sites. Patmatch can also be used to search for motifs in protein sequences. Other options of this tool include the selection of a target data set, strand to be queried (in case of nucleotide search), and number of results to retrieve. If one needs to process large amounts of data or increase the number of results to be included, users can download the Patmatch1.1 program (*http://www.arabidopsis.org/download/index-*

auto.jsp?dir=%2Fdownload_files%2FSoftware%2FPatmatch/) and run it locally on a Unixbased system. The BLAST data sets used by Patmatch can also be downloaded from the TAIR site (*http://www.arabidopsis.org/download/index-auto.jsp?dir=/download_files/Sequences*).

Necessary Resources

See Basic Protocol 1

Entering the search Parameters

- 1. From the TAIR home page (www.arabidopsis.org) select Patmatch from the Tools drop down menu. Alternatively go directly to https://www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl.
- 2. Enter in a query pattern and the appropriate option for a DNA or Protein search from the drop down menu. Acceptable inputs include regular expressions that include mismatches, insertions, and deletions, and apply standard IUPAC notation to indicate ambiguous sequences Supported syntax formats are displayed on the bottom of the data entry page.
- 3. Choose a sequence dataset to search. The program uses the same target datasets as TAIR's BLAST software.

Evaluating the results

- 4. Patmatch does not generate alignments or provide scores for best hits. The results are displayed in a table format that includes the following information.
 - a. Sequence name: Name of the gene or sequence for which a hit was found.
 - b. # *of hits:* Number of times the query pattern was found in that specific sequence.
 - c. *Hit pattern:* Pattern used for the query.
 - d. *Matching positions:* Start and end position of the hit. These coordinates are always relative to the sequence (e.g., gene, upstream region, intergenic region).
 - e. *Hit sequence:* Hyperlink to the sequence for which a hit was found. The pattern match is highlighted in red letters. For nucleotide searches, coordinates shown here are always relative to the chromosome.

BASIC PROTOCOL 7

USING THE TAIR GENERIC ONLINE ANNOTATION TOOL (GOAT) TO SUBMIT FUNCTIONAL ANNOTATIONS FOR ARABIDOPSIS (OR ANY OTHER SPECIES) GENES

In order to maximize the capture of experimental information about gene function from the literature and from our expert community, TAIR has developed tools to enable researchers to curate functional annotations and make those annotations visible in TAIR. In 2021 we retired the TAIR Online Annotation Tool (TOAST, Berardini, et al, 2012) and replaced it with an easier to use Generic Online Annotation Tool (GOAT, https://goat.phoenixbioinformatics.org). Like TOAST, GOAT also enables users to submit their own GO and PO annotations, and comments. The software is more intuitive to use, allows saving in progress submissions, most importantly it allows users to annotate not only Arabidopsis genes, but genes from any species as long as they have a UniProt ID or RNA Central ID. Authentication is via the users ORCiD ID and registration at TAIR is no longer required for submission. Submitters must provide a DOI or PubMed ID. Alternatively users can download a preformatted Excel spreadsheet and email annotations to TAIR at curator@arabidopsis.org.

Necessary Resources

See Basic Protocol 1

Submitting Annotations

- 1. On the TAIR home page (*http://www.arabidopsis.org*) go to the section marked Submit and choose Online Submission for Authors and Others. Alternatively go to http://www.arabidopsis.org/doc/submit/functional_annotation/123.
- 2. Scroll to the center of the page and click the button to "Fill Online Form". Alternatively go to https://goat.phoenixbioinformatics.org/.

GOAT uses ORCiD for user authentication as a way of crediting community submission, therefore before creating a submission users should register with ORCID (https://orcid.org/). Users should include a public email address in the ORCiD profile if they wish to receive automated notifications about their submission.

- 3. Select login from the upper right menu on the home screen follow instructions to authenticate with ORCiD.
- 4. On the GOAT home page click the 'Submission" link in the upper left menu.
- 5. On the resulting data entry form (Fig 1.11.10) enter the PubMed ID or DOI for the article to annotate (Fig 1.11.10 item a).

TAIR only displays annotations from peer-reviewed, published works. Users may submit annotations for articles that have been accepted for publication that have received a temporary DOI via a preformatted spreadsheet, but TAIR does not accept annotations for unpublished work.

- 6. Enter the identifier of the first gene to annotate. Acceptable IDs for Arabidopsis genes include AGI Locus identifiers (e.g., ATNGNNNNN.), UniProt ID (e.g.) and RNA Central ID. To add additional genes, click the "Add Another Gene" button (Fig. 1.11.10 item b).
- 7. Enter the annotations. The form contains separate sections for each type of annotation (GO Molecular Function, GO Process, Expression, Protein Interaction, Comment). At least one annotation must be entered in order to be able to submit (otherwise the Submit button remains grayed out).
 - a. Enter the term in the left column. The auto-suggest function will offer a list of suggested terms. Choose one of the suggested terms, or if none is appropriate, enter a new term. A TAIR curator reviews all the contributions and will approve the annotation, update to find the best term that matches, or determine if a new ontology term needs to be added.
 - b. Enter the supporting evidence in the right column. All annotations must be backed up by evidence. Choose the evidence type from the dropdown menu that most closely fits the experimental method.
- 8. To add additional annotations, click on the 'Add Another Annotation' button and a new data entry row will appear (Fig. 1.11.10 item d). The choose the annotation type from the drop down menu. To delete an annotation, click on the red X to the right of the annotation row (Fig. 1.11.10 item c).
- 9. Enter comments. At the bottom of the form there is a section to enter comments that may include information that cannot be captured in a GO or PO annotation. This section is optional.
- 10. Submit the annotations or add another gene. Once all of the annotations for the gene entered in step 5 are done, either submit the annotations or annotate another gene described in the same paper.
 - a. To add another gene, go to the top of the form and click on the plus sign in the upper right corner (Figure 1.11.10 item b). This will append a new entry form to the bottom of the page.
 - b. To submit annotations, click on the Submit Annotations button on the lower left side of the page (Figure 1.11.10 item e).
- 11. Once the data is submitted a curator will review the submission. If there are any questions, a curator will contact the submitter. It can take a week or two before the data is visible in TAIR.

Other ways to submit data/corrections to TAIR

One of the most fundamental aspects of science is sharing data and results with the research community. The fruits of research drive new areas of discovery, and funding agencies, such as the National Science Foundation (NSF), have invested heavily in developing community resources. Web sites and databases such as TAIR make these data accessible to anyone connected to the Internet. The long-term sustainability of databases will increasingly rely upon contributions by the research community (Reiser et al., 2016; Leonelli, et al, 2017).

TAIR encourages feedback and data submission and provides several ways for researchers to contribute their expertise and data. Instructions for submitting various types of data including gene function, interaction partners, expression patterns, markers, phenotypes, and several others, are available on the Submit Overview page (*http://arabidopsis.org/submit/index.jsp*), accessible from the Submit drop-down menu in the top navigation bar. Users can prepare data formatted according to the guidelines or download and use the preformatted Excel spreadsheets. The spreadsheets may contain macros that ensure that the proper data formats are used. To use the spreadsheets, macros must be enabled. TAIR will also accept direct submissions by email to *curator@arabidopsis.org* for small datasets and corrections to existing data, as well as very large datasets and those requiring special formats. Please contact us with any questions about data submission.

In addition, each data detail page includes a Community Comments section where community members can add additional information; click on the comment text to view the entire comment. Registered users can submit comments that are then immediately displayed in the Comments section of the detail page. On-line instructions for submitting comments are available at *http://arabidopsis.org/help/helppages/addcomment.jsp*.

BASIC PROTOCOL8

USING PHYLOGENES TO VISUALIZE GENE FAMILIES AND PREDICT FUNCTIONS

PhyloGenes (www.phylogenes.org) is a website for visualizing gene function data alongside phylogenetic trees (Zhang et al., 2019). It uses pre computed phylogenetic trees and multiple sequence alignments generated by the PANTHER project for the trees (Mi et al., 2021) and can be used to make phylogenetic based inferences about gene function for unknown members of the gene family. PHYLOGENES trees are updated annually when the PANTHER families are updated. PhyloGenes contains all plant species from PANTHER as well as 10 well annotated non plant reference species. The underlying concept of phylogenetic based inference is that gene functions that are shared by common ancestors can be attributed to their descendants. Visualizing functions alongside the tree can also indicate where evolutionary novelty arises in gene families. As described in *BASIC PROTOCOL 1*, the homology data from PhyloGenes will display links to the corresponding PhyloGenes tree. The following protocol explains some of the basic features of PhyloGenes that can be performed after retrieving the PhyloGenes tree from the TAIR locus page links

Necessary Resources

See Basic Protocol 2

Viewing the PhyloGenes gene family page from a TAIR locus page

1. Clicking the link to the PhyloGenes tree viewer from a TAIR locus page (Fig 1.11.2 item h) will open a new web page on the PhyloGenes website that displays the corresponding gene family page (Figure 1.11.11 A) centered on the TAIR locus which is also highlighted. The display has three main sections.

This is the submitted version. For the final, edited version see: Reiser, L., Subramaniam, S., Zhang, P., & Berardini, TZ., (2022) Using the Arabidopsis Information Resource (TAIR) to find information about Arabidopsis genes. Current Protocols in Bioinformatics. DOI:10.1002/cpz1.574

- a. Metadata section (Fig 1.11.11 A item a) contains the name of the PANTHER family along with a count of the total number of genes and the taxonomic range of the gene family (the last common ancestor).
- b. Tree Panel (Fig 1.11.11). This section is where the phylogenetic tree is displayed. The default display is a compact view in which branches having genes with known functions are expanded and those without known functions are collapsed (grey triangles). The tree panel includes a search box for searching within the family (Fig 1.11.11 A item b) as well as controls for customizing the tree and downloading data (Fig 1.11.11 A item c).
- c. The Data Panel (Fig 1.11.11 A). This section displays linked descriptive and gene function data. The descriptive data includes gene names, symbols, species, protein names and UniProt IDs (linked to UniProt records). Gene functions are experimentally determined and phylogenetically inferred Gene Ontology annotations to Molecular Functions or Biological Processes. The data panel can be customized to reduce or reorder columns (Fig 1.11.11 A item d).

Controlling and customizing the tree display

2. Users can collapse and expand individual nodes or expand the entire tree. Collapsed nodes are represented by a grey triangle. Clicking on a collapsed node will expand it to show all members. To expand all the nodes, click on the "Expand all" icon in the Operations menu.

Nodes in PhyloGenes are color coded as described in the legend which can be toggled on or off using the down arrows in the operations menu (Fig 11.1.11 A item b). Speciation events are green, duplication events are orange, horizontal transfer events are light blue and subfamily nodes are represented by dark blue diamonds.

3. To reduce the complexity of the tree, users can choose to prune or remove species from the display. Clicking on the 'Tools' icon in the Operations menu will display a dropdown list of functions including 'Prune tree by organism'. Selecting that option will open a new pop up window showing all the species in the tree; by default, all species are checked (Fig 11.1.11 B). To remove a species, uncheck the box and then click the Update button. This will redraw the tree, the topology will remain the same, but the display will be less crowded. Another quick way to display a small subset is to check the box in the header to uncheck all and then check the boxes for the species you wish to display.

Controlling and customizing the data display

- 4. The linked gene function data is displayed in a tabular format to the right of the tree panel. Linked data are aligned with the corresponding protein in the gene tree. The data displayed include:
 - a. Gene Symbol, The common name for the gene (e.g. PHOT2).

- b. Gene ID. The unique identifier for that gene sequence in the reference genome (e.g. AT5G58140). For Arabidopsis, these gene IDS also link back to the corresponding TAIR locus pages.
- c. Protein name. the full name for the protein (e.g. PHOTOTROPHIN-2)
- d. UniProtID. The unique identifier for the corresponding protein entry in Uniprot. This is also a hyperlink to the UniProt entry (e.g.P93025)
- e. PANTHER subfamily name. Subfamilies within each family are groups of genes that share a particularly high degree of similarity due to limited divergence from their common ancestor. Subfamilies are, in general, closely-related orthologs.
- f. Gene Ontology Annotations are displayed for Biological Process and Molecular Function Ontologies. Phylogenetically based annotations are indicated with a green tree icon (Fig 11.1.11 A item e). Annotations that are experimentally determined are indicated by a yellow flask icon (Fig 11.1.11 A item f). Clicking on the icon will display detailed information and links to the supporting reference (Fig 11.1.11 A item g).

PhyloGenes incorporates annotations from Gene Ontology Consortium that are filtered based on evidence codes. Experimentally determined annotations include those with the following evidence codes IDA: inferred from direct assay; IMP: inferred from mutant phenotype; IGI: inferred from genetic interaction; IPI: physical interaction; IEP: expression pattern; EXP: experimental evidence. Phylogenetically inferred annotations have the evidence code IBA: inferred from biological ancestry. Annotations are retrieved from the Gene Ontology Consortium (www.geneontology.org) and updated on a quarterly basis.

- 5. The display of data in columns can be adjusted by clicking on the gear icon in the table header (Fig 11.1.11 A item d). The text next to the icon indicates if and how many data columns are hidden. Clicking on the icon opens a popup configuration window with the following functions (Fig 11.1.11 C).
 - a. Show/hide columns by checking the box next to the name.
 - b. Reorder columns using the up or down arrows next to the column name.
- 6. Users can also opt to toggle the display to show the multiple sequence alignment for the underlying PANTHER tree. Clicking on the text 'Show MSA>'will replace the data table with the alignment. The MSA display also includes a legend that explains the different fonts and color coding.
- 7. Users have several options for saving the tree data. Clicking on the Downloads icon in the Operations menu (Fig 11.1.11 B item c) displays the following download options.
 - a. Download the multiple sequence alignment for the entire tree.
 - b. Download a CSV file of orthologs of a given gene within the tree.

- c. Download tree in PhyloXML format. The entire tree can be downloaded and saved locally in a standard data exchange format for use in other applications.
- d. Save tree as SVG or PNG. These options allow you to save the tree image for use in other graphics applications or for publication.
- e. Download gene table as CSV. Use this option to save all the data fields from the data panel in a single table.

Grafting a new sequence onto a gene tree

For users whose species are not represented in PhyloGenes, there is an option to graft single sequences onto the precomputed PhyloGenes trees. The TreeGrafter (Tang, 2019) tool will run HMM scoring and find the best matching gene family, if it exists, add the sequence to the MSA of that family, then run RAxML to insert the new protein sequence to the best location of the gene family tree. The inserted sequence will be labeled as 'grafted'.

8. From the PhyloGenes home page (www. phylogenes.org) click on the link 'Not seeing your species' above the list of included species (Fig 1.11.12 A) or go to http://www.PhyloGenes.org/grafting.

The option to graft a sequence can also be accessed in the search results when no match is found (Fig 1.11.12 B).

- 9. Enter the raw amino acid sequence and click 'Graft'.
- 10. The software will return the matched tree with the new sequence included and labeled a 'Grafted'. (Fig 1.11.12 C).

Occasionally the grafting program will produce unexpected results. If this happens, please submit a report to info@PhyloGenes.org.

BASIC PROTOCOL 9

USING TAIR TO BROWSE ARABIDOPSIS LITERATURE

TAIR provides a number of ways for researchers to keep abreast of the literature. In addition to the curated links between genes and articles that are displayed on the locus detail pages (see *BASIC PROTOCOL 2*), the entire corpus of publications in TAIR (including abstracts and conference proceedings) can be searched using the Publication Search (http://www.arabidopsis.org/servlets/Search?action=new_search&type=publication) or Keyword browser (http://www.arabidopsis.org/servlets/Search?action=new_search&type=keyword). For users wanting to keep up with the latest Arabidopsis research, TAIR developed an additional tool for browsing recently added literature.

Necessary Resources

See Basic Protocol 1

Browsing Recently Added Literature

- 1. On the TAIR home page (*http://www.arabidopsis.org*) go to Browse and select Recently Added Literature. Alternatively go to http://www.arabidopsis.org/servlets/Search?pageNum=1&type=publication&acti on=search&recent=14&size=500&sort=journal.
- 2. The page will display a list of the research articles downloaded from PubMed and entered into TAIR during the time period specified in the header. Typically this is a one week period. Each article is displayed in a separate band of alternating background color. The contents of each band include basic citation information and links to associated resources.

The default display is sorted alphabetically by journal name. Choose author name from the drop down selector in the upper right corner to display the results in alphabetical order by the last name of the first author.

- a. Citation. The citation includes the authors, title, journal name, and publication year.
- b. Associated genes (may be empty). These are manually curated links to genes described in the paper. Clicking on the Gene name will display the corresponding locus detail page in TAIR where you can find more information about the locus (see *BASIC PROTOCOL 2*).
- c. Associated Keywords (may be empty). Keywords are generated by automatic text matching of GO terms to the text and are not curated. To find other objects in TAIR associated with that keyword, click on the term to display the keyword detail page (see *BASIC PROTOCOL 4*).
- d. Article views. Three options are provided that offer different views of the article.
 - i. Click on the Journal link to read the article on the journal's website.
 - ii. Click on PubMed link to view the corresponding record and abstract at NCBI's PubMed site.
 - iii. Click on the TAIR link to view the publication detail page in TAIR. The TAIR publication page displays the citation, which may include the abstract, as well as associated keywords, loci and GO and PO annotations. The annotations and linked loci are manually curated.

BASIC PROTOCOL 10

USING THE SYNTENY VIEWER TO FIND AND DISPLAY SYNTENIC

REGIONS FROM ARABIDOPSIS AND OTHER PLANT SPECIES

Synteny Viewer (https://www.arabidopsis.org/cgi-bin/syntenyviewer2/showSynteny.pl) is a tool that displays precomputed syntenic regions between *Arabidopsis thaliana* and over 35 different plant genomes. The syntenic regions between *Arabidopsis thaliana* and the other genomes have been precomputed using the SynMap tool at genomevolution.org. (https://www.arabidopsis.org/help/helppages/syntenyViewHelp/SyntenyViewHelp.pdf). The syntenic regions are displayed using the GEvo tool from CoGE (Lyons and Freeling, 2008; www.genomevolution.org). Users can search for a specific Arabidopsis gene of interest by AGI locus ID or by a chromosome region and view syntenic regions from another selected genome. It can be used to help researchers study and analyze homologous genes and other conserved elements and sequences. It can also be used to study genome duplication and evolution. By comparing newly sequenced or less studied genomes to the well-annotated *Arabidopsis* genome scientists can identify novel genes and putative regulatory elements.

Necessary Resources

See Basic Protocol 1

Searching for syntelogs

- 1. On the TAIR home page (http://www.arabidopsis.org) go to Tools and select Synteny Viewer. Alternatively go to https://www.arabidopsis.org/cgibin/syntenyviewer2/showSynteny.pl
- 2. To search by name enter the AGI locus ID (e.g. AT1G01010) or Gene Model ID (e.g. AT1G01010.1) into the search box in section 1. Alternatively, search by location by entering the Arabidopsis chromosomal location using the format Chromosome:Start position..Stop position (e.g. 1:3760..5630).
- 3. Select the species in which to identify the syntenic region from the drop-down menu in section 2. Then click submit.
- 4. If a syntelog is found the results will include a table displaying a list of the syntelogs, and a link to the full GEvo display at CoGE (Fig 1.11.13). Below the table results, is an iFrame containing the same High Score Segment Pairs (HSPs) and analysis functions from GEvo site. For details on using the Synteny viewer options, consult the CoGE tutorials (https://genomevolution.org/wiki/index.php/Tutorials).

GUIDELINES FOR UNDERSTANDING RESULTS

General Considerations for Using TAIR

As with any Web-based resource, some general guidelines should be observed when interpreting results. Databases are constantly changing; new information is incorporated and interfaces can also change from the time of publication of this unit.

Revisions to the Data in the Database

Over the course of genome annotation, many new genes have been added and existing genes have been made obsolete or updated (split or merged) to reflect new information (Haas et al., 2003; Swarbreck et al., 2008, Lamesch, et al., 2012; Cheng, et al., 2017). In 2004, TAIR inherited the responsibility of maintaining the genome sequence and annotation from the former Institute for Genomic Research (TIGR), now the J. Craig Venter Institute (JCVI), which provided the genome sequence and annotation from 2000 to 2004. TAIR produced five genome releases culminating in TAIR 10 (Lamesch, et al., 2012). The Arabidopsis Information Portal (Araport), which subsequently took on the responsibility of genome annotation, released Araport 11 in 2016 (Chang et al., 2017). The naming convention agreed upon by the AGI for adding new loci and updating existing loci (http://www.arabidopsis.org/portals/nomenclature/guidelines.jsp) is continued in TAIR and Araport releases. Users are encouraged to submit structural annotation updates to TAIR via email and to deposit the relevant supporting sequence data to GenBank (http://www.arabidopsis.org/submit/gene annotation.submission.jsp) for incorporation into future reannotation efforts. Changes in sequence annotation may affect the association of genes to related data such as protein domains, polymorphisms, and homologies. For example, domains associated to a locus that was subsequently split may then be associated to only one of the two resulting loci. The locus history, shown on the bottom of the locus page (Fig. 1.11.2, p item), summarizes all of the structural updates that have been made to the locus such as merges, splits or obsoletions. The locus history can also be searched independently by locus name using the Locus History Search (http://www.arabidopsis.org/tools/bulk/locushistory/index.jsp). For many data sets in Downloads section of the website, TAIR maintains older versions of the data. Users should always note the date or version information associated with any data files, such as BLAST data sets or GO annotations.

Evidence Codes in GO Annotations

When interpreting Gene Ontology annotations, it is essential to understand the process of annotation and the importance of evidence codes in interpreting the annotations. The GO Consortium has developed a set of evidence codes (The Gene Ontology Consortium, 2010) as a way of quickly assessing the basis for the assertion made in the annotation. In TAIR, annotations include an evidence description, in addition to the evidence code (Berardini et al., 2004). The evidence description is a set of controlled vocabularies that describe the type of experimental or computational evidence in greater detail. For example, an annotation having the evidence code "inferred from mutant phenotype" (IMP) may be further elaborated by including more specific information about the type of experiment done such as "RNAi experiments." Since more than one gene may be affected by RNA interference, the phenotype may be due to changes in expression of multiple loci. Thus, the GO annotation should be viewed with the understanding that the phenotype may be due to the loss of function of more than one homologous locus. When no information is found in the available published literature, annotations are made to the root terms "biological process," "molecular function," or "cellular component." Such "root" annotations indicate that at the time of annotation, no information for a more specific assignment was available for the associated gene. In contrast, a gene lacking annotations altogether might have available data but has not vet been curated. At TAIR, GO annotation is an ongoing process; annotations are updated as new information about genes is published (Berardini et al., 2004). Each annotation has an associated date, which refers to the date the annotation was made. Users

should carefully evaluate the source of any tools utilizing GO annotations (e.g. Term Enrichment) to ensure that the underlying annotations are current.

COMMENTARY

Background Information

TAIR was originally a collaborative project between biologists at the Carnegie Institution, Department of Plant Biology, and computer scientists at the National Center for Genome Resources, initiated in 1999. TAIR is the third incarnation of an *Arabidopsis* community database after AAtDB (An *Arabidopsis thaliana* Database, which continued from 1991 to 1994) and AtDB (*Arabidopsis thaliana* Database, which continued from 1991 to 1994) and AtDB (*Arabidopsis thaliana* Database, which continued from 1999; Flanders et al., 1998; Rhee et al., 1999). TAIR arose out of the need to accommodate genomic data such as the genome sequence, gene annotations, and integration of physical and genetic maps, in the context of the experimentally verified data in the literature. From its inception until early 2014, the National Science Foundation (NSF) funded TAIR. In late 2013, anticipating the end of NSF funding, four TAIR staff members founded the nonprofit organization Phoenix Bioinformatics (www.phoenixbioinformatics.org) and transitioned TAIR to a new, sustainable user fee model (Reiser, et al., 2016). The user fee structure was carefully crafted to distribute the costs equitably among the widespread and varied user community. With the support of the research community, TAIR continues to provide up to date, continuously curated data to its global users. Curated data is updated weekly. Data that have been in TAIR for one year are released on a quarterly basis and available for download and reuse (http://www.arabidopsis.org/download/index-

auto.jsp?dir=/download_files/Public_Data_Releases) under a CC-BY license. Users without a subscription can access a limited number of page views per month. Unlimited access to all TAIR pages and quarterly releases of recent data requires a subscription. ABRC stock detail and ordering pages are available free of charge. The data release policy was designed to encourage and support data reuse, while still providing an incentive to subscribe. More information on how institutions or individuals can help support this nonprofit effort is available by clicking on the Subscribe link on the TAIR home page.

Design principles and current limitations

TAIR has been designed and built as a Web tool to allow researchers to access all of the data housed in TAIR using a standard Web browser such as Google Chrome or Mozilla Firefox. It is built upon industry standards for database management systems, software architecture, and software design (Weems et al., 2004). The current system of interfaces described herein is due for a significant overhaul which will modernize the interfaces but the primary functions will remain the same. TAIR is primarily designed by biologists and, although the interfaces were created with biologists in mind, it has not always been possible to arrive at solutions that meet every user's requirements. A certain amount of familiarity with *Arabidopsis* and with basic concepts of molecular genetics and plant biology is assumed. Consequently, the breadth of information on the home page and myriad options on the search interfaces can be daunting to a novice user. More experienced users and developers may be frustrated by the difficulty in obtaining the entire database for retrieving specialized, custom data sets. Users are encouraged to contact us via email (curator@arabidopsis.org) for assistance in using any of the tools or in accessing large or specialized datasets.

Keeping up to date with TAIR and Arabidopsis research

Users can stay connected with TAIR by following the *Arabidopsis* Information Resource on Facebook (https://www.facebook.com/tairnews), or receiving tair_news twitter feeds (http://twitter.com/tair_news) or YouTube channel alerts (https://www.youtube.com/user/TAIRinfo). TAIR News and Job Postings are relayed through the TAIR Twitter feed.

Ensuring your published Arabidopsis data visible in TAIR and other resources by making it Findable, Accessible, Interoperable and Reusable

For researchers to maximize the value of published data, that data needs to be made available in standardized, machine-readable formats that are easily discoverable in accordance with the Findable, Accessible, Interoperable and Reusable (FAIR) data principles (Wilkinson et al., 2016). TAIR curators translate experimental findings into computationally accessible formats such as Gene Ontology annotations to help make these data FAIR. TAIR offers some basic guidelines for researchers to follow when preparing their data for publication to ensure that their data is FAIR (https://conf.phoenixbioinformatics.org/pages/viewpage.action?pageId=22807345). When published data is FAIR it can be more readily accessed by members of the research community and by curators from TAIR or other genomic resources who may perform further curation to integrate or add value to those data (Reiser, et al., 2018).

Additional tools at TAIR

In addition to the tools discussed in the protocols, TAIR hosts several other useful analysis tools. Some of these are briefly described below.

Textpresso

Textpresso is an information extracting and processing package for biological literature(https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-018-2103-8). Textpresso for *Arabidopsis* (*http://www.textpresso.org/arabidopsis*) allows users to search all abstracts and over 40,527 full-text Arabidopsis publications. Keyword searches can be narrowed by searching in specific categories. The individual matches are displayed showing each of the text snippets that match the query Textpresso was initially developed by Hans-Michael Muller, Eimear Kenny, and Paul W. Sternberg, with contributions from JuanCarlos Chan and David Chen. The most recent version, Textpresso 2.0, was developed by Hans-Michael Muller with contributions from Arun Rangarajan and Tracy K. Teal. The current version of Textpresso for Arabidopsis was updated in 2022.

Chromosome map tool

This tool (*http://www.arabidopsis.org/jsp/ChromosomeMap/tool.jsp*) allows the user to map genes on top of the five *Arabidopsis* chromosomes using a list of locus names (e.g., At1g01010). The list should contain one locus name per line. To display an alternate name, append the symbol after the locus identifier in the same row (e.g. AT1g01010 ANAC001). The resulting image, which displays the location of the queried list of genes on the five chromosomes, can be saved in a variety of formats.

Critical Parameters and Troubleshooting

No data found

A frequently reported problem is that searches do not retrieve any results. In some cases the data sought are not in the database, but in other cases the data are in TAIR but are not found because of problems arising from poorly formed queries or improper use of the search forms. The temptation to fill out all of the optional fields in the advanced searches can generate too many restrictions that limit the scope of the data retrieved. This can be overcome by using fewer, rather than more options. Another reason why searches fail is that the data are not accessible through the existing search interfaces. The categories under the Advanced Search section of the Web site (*http://arabidopsis.org/servlets/Search?type=general&action=new_search*) list data types that can be searched. To obtain data that are included in the TAIR database but are not easily accessible through any of the advanced searches, please send an e-mail to the curators to request the data.

Too much data found

While "no data found" is probably the most common problem encountered, retrieving too many results can also be a problem. There are two ways to handle this problem: (1) using the advanced searches and restricting parameters to retrieve a subset of the results, or (2) manipulating the results set to select a subset of data. Restricting the search parameters can be done on all the Advanced Search pages and detailed help on using these parameters is available (*http://arabidopsis.org/help/helpcontents.jsp*). Large results sets can be downloaded and reformatted to explore the data more efficiently. All of the search results can be downloaded as tab-delimited text files (see *BASIC PROTOCOL 2*). The results can be imported into software like Excel or Google Spreadsheets that allows manipulations such as sorting, reordering, reformatting columns, and graphing the results.

Layers of connected data that are hidden

TAIR's database structure exploits the relational database design and each data type has a high degree of association to other data types. This network of associated data is not easily represented in a two-dimensional, tabular format via hyperlinks. Consequently, associated data may be separated by one or more hyperlinks. For example, all gene models are associated to a given locus, but to view information for a specific gene model, such as a list of gene features (e.g. introns, exons, UTRs) and coordinates, it is necessary to click on the link to the individual gene model.

Reporting problems and requests to curators

Perhaps the most important thing to know about troubleshooting problems with TAIR is that users are encouraged to e-mail curators (*curator@arabidopsis.org*) to report problems, ask for help or request data. Users that want a particular set of customized data should contact the curators, who can then generate the requested file. Reporting problems may also lead to improvements to TAIR's data display or addition of new data or tools that benefit the whole community.

Advanced parameters

Despite the extensive content of this unit, it still does not cover all of the functionalities of the searches and tools that are offered at TAIR. Users familiar with the basic functionalities

and who are interested in more complicated queries or specialized views are encouraged to review the help documents or contact the curators.

Suggestions for Further Analysis

While there are no complete alternatives to TAIR, there are other web sites that provide a significant amount of *Arabidopsis* data and alternative ways to view, manipulate and analyze the data. All of these sites are linked extensively from TAIR, whereby the sites in the former category are linked from each locus detail pages and sites in both categories are listed and updated in the TAIR Portal pages (*http://www.arabidopsis.org/portals/index.jsp*). Many of these resources integrate TAIR curated functional annotations (e.g. gene summaries, names, literature) however, in accordance with TAIR's data release policy, the data on these sites will be at least one year out of date.

Arabidopsis genome annotation resources

There are other resources that offer views of the Arabidopsis genome that complement the TAIR resource. Users can search, download and analyze Arabidopsis genome data with ThaleMine, an InterMine instance created by the Araport project ((Krishnakumar, et al., 2015; Cheng et al. 2017) and now maintained by the Provart group (Pasha et al. 2020)). Another view of the Arabidopsis genome can be found as one of the databases in Ensembl Plants (http://plants.ensembl.org/Arabidopsis thaliana/Info/Index). Ensembl provides its own genome browser for visualization, as well as plant gene families generated using Compara. Users can access variant data for Arabidopsis ecotypes generated by the 1001 Genomes project (http://1001genomes.org/) within Ensembl. Experienced users will find it useful to be able to generate their own custom datasets using Ensembl's BioMart or ThaleMine. SIGnAL (http://signal.salk.edu/) from the Salk Institute offers a genome viewer (T-DNA Express, *http://signal.salk.edu/cgi-bin/tdnaexpress*) that is decorated with all of the T-DNA and transcript data that are generated from Salk and other laboratories around the world. Often, SIGnAL displays data that are not yet displayed at TAIR; therefore, it is a good idea to check this site to get the latest mapping of T-DNA insertions and cDNA clones. Users should pay attention to the sources of data including gene functional annotations, genome annotation versions, assembly versions and the currency of the data. Because TAIR is updated on a weekly basis (mostly for functional annotations) the information in these resources may differ from what is shown in TAIR. There are also many sites that provide detailed information about a subset of genes of Arabidopsis such as chromatin remodeling factors, transcription factors, and small RNAs. TAIR tries to maintain up-to-date links to these resources from the TAIR Portal pages. Please contact TAIR by e-mail (*curator@arabidopsis.org*) if there are missing or nonfunctional links.

Arabidopsis Gene Expression Resources

There are a number of databases and tools that have been developed for storing, accessing and analyzing public Arabidopsis gene expression data that includes RNA seq, microarray and single cell RNA seq. TAIR stopped accepting microarray data in 2005 as ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) and the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) emerged as centralized community repositories. TAIR still provides access to the data via the Microarray Experiment

(http://www.arabidopsis.org/servlets/Search?type=expr&search_action=new_search) and Microarray Expression searches

(http://www.arabidopsis.org/servlets/Search?action=new_search&type=expression) for archival

This is the submitted version. For the final, edited version see:

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purposes. In addition to the data and tools available at ArrayExpress, The BioAnalytic Resource for Plant Biology (http://bar.utoronto.ca/) hosts a number of user friendly tools for visualizing and analyzing Arabidopsis tissue RNA seq, microarray and single cell RNA seq expression data, motif analysis and more. It provides a virtual graphical gene expression map (eFP browser;http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi, see also Fig 1.11.2 A, item f) an Expression Angler tool which can be used to find similarly expressed genes (http://bar.utoronto.ca/ExpressionAngler/) and Expressolog TreeViewer (http://bar.utoronto.ca/expressolog_treeviewer/cgi-bin/expressolog_treeviewer.cgi) for finding expression orthologs. Another popular tool is GENEVESTIGATOR (https://genevestigator.com/), which contains most of the publicly available high-density array data from AtGenExpress (http://arabidopsis.org/info/expression/ATGenExpress.jsp) and other laboratories, and allows searching and displaying of the data (Zimmermann et al., 2004). Academic users must create a basic account, after which they can search for genes that are expressed in specific conditions, growth stages, or organs, or for genes of particular interest to them, and get a comprehensive view of the expression profiles in the different environmental conditions, growth stages, and organs. GENEVESTIGATOR requires subscriptions to access additional data and tools.

Arabidopsis Metabolic Pathways

There are several excellent resources for visualizing, analyzing and accessing information about biochemical pathways in Arabidopsis and other species. AraCyc is a curated metabolic pathway database specifically for Arabidopsis thaliana and is included in the Plant Metabolic Network (PMN, www.plantcyc.org) database. PMN includes over 350 plant species. The AraCyc database was initially built using the Pathologic module in the Pathway Tools software developed for MetaCyc (Karp et al., 2002; Mueller et al., 2003). Pathologic predicts possible metabolic pathways based upon the set of annotated enzymes available for a particular species. Following the initial computational build of AraCyc, pathways were manually validated and some were supplemented with additional experimental evidence. AraCyc includes tools to search and browse metabolic pathways drilling down to individual reactions and products, ways to visualize gene expression data superimposed on a global pathway map, and options to save data in Smart Tables. Unification links to MetaCyc and PlantCyc facilitate comparison of pathways from different organisms. TAIR includes extensive links out to AraCyc from the locus pages (see BASIC PROTOCOL 2, Figure 1.11.2 item m). Another resource is the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/kegg2.html) that includes pathways, reactions, enzymes, genome and other information for Arabidopsis and many other plant species. Plant Reactome (http://plantreactome.gramene.org; Naithani, et al., 2017) contains information about biochemical, genetic and other pathways, and tools for data visualization and analysis. Reactome curates pathways for the reference genome Oryza sativa, which is presented along with data for many other species, including Arabidopsis.

CONFLICT OF INTEREST STATEMENT:

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT:

TAIR data and tools are available to the public at www.arabidopsis.org according to the detailed

Terms of Use (https://www.arabidopsis.org/doc/about/tair_terms_of_use/417).

ACKNOWLEDGMENTS

The authors of this unit are grateful for the continued support of members of the research community who share their expertise, ideas, data and criticisms, all of which improve TAIR immensely. We thank all of the curators and programmers past and present who helped make TAIR such a valued resource. TAIR is supported by individual, institutional, corporate and government subscriptions. TAIR is a project of Phoenix Bioinformatics (www.phoenixbioinformatics.org), which is supported, in part, by a grant from the Alfred P. Sloan Foundation. PhyloGenes was co-developed by Phoenix Bioinformatics and the PANTHER project at the University of Southern California. It was supported by the National Science Foundation (DBI-1661543).

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Figure Legends

Figure 1.11.1 TAIR's home page (*http://arabidopsis.org*) is the main entry point to the database and Web site. The general search (item a) can be used for a quick search of database entities by name

Figure 1.11.2 A sample of a locus page from TAIR showing the major data included in the detail page. A portion of the germplasm section has been deleted for simplicity. Each of the data types displayed in the alternating colored bands can be grouped into one or more the following categories: (**A**) (a) general descriptive locus information, (b) gene model information, (c)

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functional annotations, (d) nucleotide and protein sequences (e) gene expression data,(f) interactive BAR eFP browser image, (g) protein data, (h) plant homolog data; **B** (i) mapping data, (j) markers, polymorphisms and alleles, (k) germplasm information, (l) clones, (m) links to resources outside of TAIR, (n) community comments about the locus, (o) papers and abstracts and (p) locus history.

Figure 1.11.3 Overview of TAIR's JBrowse instance. (**A**) JBrowse display showing the track panel (left) and genome view panel (right). (**B**) Close up view showing the genome view controls including (a) Genome selector for choosing between different genome assemblies, (b) nucleotide sequence track, (c) left and right scroll buttons, (d) zoom controls, (e) search entry box, (f) highlighter, (g) track selector for adding new tracks, and (h) view control for modifying the display of tracks.

Figure 1.11.4 JBrowse genome view and track controls. (A) JBrowse genome view showing highlighting function (yellow) and pop-up detail (item a). (B) JBrowse page with track panel expanded to show different categories and options. The Epic CoGe link (item a) is used to retrieve tracks from CoGE. In addition to genome annotation data, JBrowse includes pre-loaded community tracks (item b) and whole genome alignments (item c). The arrow links from the selected community track to the actual display in the genome view.

Figure 1.11.5 JBrowse genome view options. (**A**) Example of a combined track generated by merging two different versions of the TAIR genome annotation (TAIR 10 and Araport 11). (**B**) SeqLighter pop up display.

Figure 1.11.6 Keyword results and detail page. (**A**) Keyword search results after querying for the GO Biological Process terms containing the words "root development". (**B**) A tree view of the term "root development" with expanded children nodes and associated gene annotations.

Figure 1.11.7 TAIR Functional Categorizaton. (**A**) Results display for functional categorization of *WRKY* genes. The members of this family are grouped into pre selected high level plant specific GO Slim categories based on their annotations to more granular GO terms. A complete list of plant GO Slim terms and IDs can be found on the TAIR website (https://www.arabidopsis.org/download_files/GO_and_PO_Annotations/Gene_Ontology_Annota tions/TAIR_GO_slim_categories.txt). The results list can be re-sorted by choosing Gene count from the "re-sort by:" drop-down menu and clicking on the "re-sort" button. The keywords grouped by ontology aspect; Cellular Component, Biological Process and Molecular Function. The frequency of annotations to each category is listed in the last column; the number is linked to a list of genes annotated to the terms that are children of that category. (**B**) The drop down menu is used to select a graphical output format showing the distribution and frequency of annotations to each of the GO slim terms as either a bar graph or pie chart. A different graph/chart is created for each aspect of the GO ontologies.

Figure 1.11.8 GO Term Enrichment Tool at PANTHER (www.pantherdb.org). Results display for sample data from query input form, after it has been received and processed via the PANTHER web service. Results display (**A**) query parameters and identifier mapping, and (**B**) hierarchcal ordered table of term enrichment results.

Figure 1.11.9 Motif Finder tool. (**A**) Users can type in or upload a list of genes and select the promoter length to be analyzed. (**B**) The resulting motifs are listed with the corresponding genes in which they are found. Items in columns a-g are the values columns that match the headers listed in the summary.

Figure 1.11.10 Generic Online Annotation Tool (GOAT). (**A**) GOAT data submission page after logging in using ORCiD and clicking the Submission tab (item a). Users can click to additional genes (item b) and add additional annotation data entry fields (item c). After adding all of the desired annotations click on the review submission button (item d). (**B**) GOAT submission review screen. Users are asked to review their submission before finalizing. At this point it is possible to revise the submission (item a) or continue with the submission (item b).

Figure 1.11.11 PhyloGenes tree display. (**A**) Phylogenetic tree display for PANTHER family 45637 in PhyloGenes showing the results after entering the query 'PHOT1' into the search box (item a), the matching gene names are highlighted in the tree panel. The operations menu (item b) includes controls for tree pruning, downloading data files, expanding and collapsing nodes. The data panel controls (item c) allow for showing/hiding and reordering data columns or swapping between multiple sequence alignment (MSA) and data displays. The presence of icons in the data panel indicates the type of annotation; green trees indicate phylogenetic based annotations (item d) and yellow flasks (item e) indicate experimental annotations (f) boxed area shows annotation detail pop up displayed after clicking on the annotation icon. (**B**) Expanded view of tree pruning pop up selector. (**C**) Expanded view of data display configuration pop up.

Figure 1.11.12 PhyloGenes grafting. (**A**) PhyloGenes home page showing how to access the tree grafter (red circle). (**B**) Accessing the tree grafter from a search result (red circle). (**C**) Display of grafted sequence within an existing tree.

Figure 1.11.13 Results display from SyntenyViewer when there are matches. The upper table lists the query gene (item a) linked to the corresponding detail page in TAIR, the list of matched genes (item b) and links to the full display in GEVo (item c). The iframe below (item d) previews the alignment in GEVo.



The Arabidopsis Information Resource



About TAIR

Phoeni

The Arabidopsis Information Resource (TAIR) maintains a database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana*. Data available from TAIR includes the complete genome sequence along with gene structure, gene product information, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, publications, and information about the Arabidopsis research community. Gene product function data is updated every week from the latest published research literature and community data submissions. TAIR also provides extensive linkouts from our data pages to other Arabidopsis resources.

TAIR is located at Phoenix Bioinformatics and funded by subscriptions.

Full access to TAIR requires a subscription. Please see our subscription page for further details.

Note: This site has been tested with Chrome, Firefox, Safari, and Edge browsers. Some pages may not work as expected if you are using Internet Explorer. For best results, update your browser and enable Javascript and cookies (see help). **Scheduled Maintenance:** This site may be down for maintenance on any Saturday from 8 am to 10 am PDT.

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Breaking News

See you in Belfast at ICAR2022 [Jun 7, 2022]

Come find us in Belfast at ICAR2022! Learn more at the Arabidopsis Informatics Session Thursday June 23, 2022 4PM GMT in person or on line. Or visit our booth.

Help AgBioData chart a course towards FAIR agricultural data! [Apr 25, 2022]

Please take 10 minutes to complete this **important survey**

Your opinion is essential and can help AgBioData define better genomic, genetic, and breeding data curation practices.

30th public release of TAIR@Phoenix data

[Apr 1, 2022] 30th public release of data curated under TAIR's subscription-based funding model. Files contain new publications, annotations, gene symbols and other data through March 31, 2021.

Locus Page Updates: Plant Homologs

[Mar 22, 2022] TAIR's locus pages have been updated with **new ways to view and download plant homologs**.

InterPro domain update [Feb 23, 2022] TAIR protein domains have been updated to InterPro 87.0

ICAR2022-Funding

opportunities and deadlines [Feb 11, 2022] Upcoming deadlines for ICAR2022 including funding opportunities.



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General comments or questions: curator@arabidopsis.org

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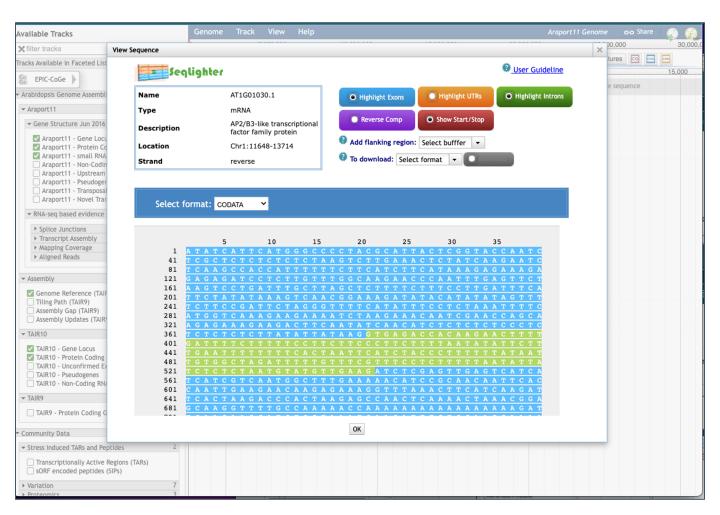
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□ TAIR10 - Gene Locia ☑ TAIR10 - Protein Coding Genes □ TAIR10 - Inconfirmed Exon □ TAIR10 - Preudogenes □ TAIR10 - Renc Coding RNA	Combination Tra	ack 1	-		-		•					+	-
▼ TAIR9 1													
TAIR9 - Protein Coding Genes													
Community Data 97													
Stress induced TARs and Peptides 2													

В



	Keyword S	earch		
keyword starts with	•			
Restrict your search to keyword category by che	cking the box below			
	-	Plant Gro	wth and Developmental Stages	
	Anatomical Entity		ental Method	User def
] Experime	entar method	
reset				submit qu
TAIR Keyword Search Results				
Your query for keywords where contains roo	ot development resulted	in 15 ma	tches.	
Displaying 1 - 15 of 15 records on page 1 of 1 pa				
Displaying 1 - 15 of 15 records on page 1 of 1 pa	iges.			
Keyword 🕼	Keyword Category	Tree V	iew @ Associated Data(to this terr	m and to children t
root development	GO Biological Process	treevie	•	
post-embryonic root development	GO Biological Process	treevie	w 162 loci, 793 publications,	199 annotations
lateral root development	GO Biological Process	treevie	w 145 loci, 768 publications,	182 annotations
root development stage	Growth and Developmer Stages	ntal treevie	w 18 loci, 529 publications, 2	7 annotations
3 establishment of tissue systems stage	Growth and Developmer Stages	ntal treevie	w 4 publications	
4 root elongation stage	Growth and Developmer Stages			2 annotations
5 root hair formation stage	Growth and Developmer Stages	ntal treevie	w 1 loci, 77 publications, 1 ar	notations
adventitious root development	GO Biological Process	treevie	w 6 loci, 20 publications, 9 ar	notations
primary root development	GO Biological Process	treevie		
regulation of lateral root development	GO Biological Process	treevie		
regulation of post-embryonic root development	GO Biological Process	treevie		
regulation of root development	GO Biological Process	treevie	······································	
negative regulation of lateral root development	GO Biological Process	treevie		
positive regulation of lateral root development	GO Biological Process	treevie		otations
cell wall polysaccharide catabolic process involved in lateral root development	GO Biological Process	treevie	W	
involved in lateral root development				
TAIR Keyword Browser [Help]				
	annotations O micro	array expe	rimonto	
			annents	
Check the box and click the display button to see	e numbers of associated d	ata		
Keyword: Oroot development ID: O GO:0048364 Definition: The process whose specific outcom is the water- and mineral-absorbing				
and is typically derived from the rad		and and a	.g. =	

regu ates relationship positively regu Keyword Categories - Click on the link to generate a treeview for the category. GO Cellular Component GO Biological Process Plant Growth and Developmental Stages GO Molecular Function Plant Anatomical Entity **Experimental Method** 🛨 🔳 all biological_process developmental process anatomical structure development 🖭 multicellular organism development E system development • Toot system development Proot development 🖭 🕑 root morphogenesis (23 loci to term + 322 loci to children) post-embryonic root development (14 loci to term + 148 loci to children) adventitious root development (6 loci to term) Proot cap development (15 loci to term) Proot meristem growth (14 loci to term + 37 loci to children) e stele development (1 loci to term) I primary root development (33 loci to term)

Primary root development (of loci to term)
 Proot radial pattern formation (9 loci to term)

💷 🖬 regulation of root development (54 loci to term + 31 loci to children)

Toot regeneration (2 loci to term)

i root development

Functional Categorization Listing [Help]

Annotation Pie Chart V Draw

new search Charts for Functional Categorization [Help] re-sort by Annotation Count ~

Gene Count

Α

				-
Displaying 99 r	records.			
Keyword Cate		Functional Category	Annotation Count	(
GO Cellular Co		nucleus	71	1
GO Cellular Co		cytoplasm	3	1.1
GO Cellular Co		chloroplast	3	2
GO Cellular Co		mitochondrion	2	2
GO Cellular Co GO Cellular Co		vacuole	1	1
GO Cellular Co GO Cellular Co		other intracellular components	1	1
GO Cellular Co		plastid	1	
GO Cellular Co			0	(
GO Cellular Co			0	(
GO Cellular Co	omponent	cytoskeleton	0	(
GO Cellular Co		lysosome	0	(
GO Cellular Co		nucleoplasm	0	(
GO Cellular Co		endoplasmic reticulum cell wall	0	0
GO Cellular Co GO Cellular Co		other membranes	0	0
GO Cellular Co		peroxisome	0	0
GO Cellular Co		thylakoid	0	(
GO Cellular Co		cytosol	0	(
GO Cellular Co	omponent		0	(
GO Cellular Co			0	(
GO Cellular Co		other cellular components	0	0
GO Cellular Co		ribosome	0	(
GO Cellular Co GO Cellular Co		endosome	0	-
GO Cellular Co GO Cellular Co		nuclear envelope Golgi apparatus	0	0
GO Molecular		DNA binding	93	1
GO Molecular		DNA-binding transcription factor activity	71	1
GO Molecular	Function	protein binding	51	2
GO Molecular	Function	nucleic acid binding	47	4
GO Molecular		other binding	3	1
GO Molecular		nucleotide binding	2	1
GO Molecular		transferase activity	1	1
GO Molecular GO Molecular		catalytic activity kinase activity	1	ļ
GO Molecular GO Molecular		translation factor activity, RNA binding	0	1
GO Molecular GO Molecular		RNA binding	0	0
GO Molecular		carbohydrate binding	0	(
GO Molecular		transporter activity	0	0
GO Molecular	Function	oxygen binding	0	(
GO Molecular		translation regulator activity	0	0
GO Molecular		other molecular functions	0	0
GO Molecular		structural molecule activity	0	(
GO Molecular GO Molecular		chromatin binding enzyme regulator activity	0	1
GO Molecular GO Molecular		enzyme regulator activity signaling receptor activity	0	1
GO Molecular GO Molecular		transcription regulator activity	0	0
GO Molecular		motor activity	0	(
GO Molecular	Function	signaling receptor binding	0	0
GO Molecular	Function	unknown molecular functions	0	(
GO Molecular		lipid binding	0	0
GO Molecular		nuclease activity	0	(
GO Molecular GO Biological		hydrolase activity other cellular processes	0 131	0
GO Biological		response to stress	116	1
GO Biological		other metabolic processes	90	1
GO Biological		response to chemical	80	1
GO Biological		biosynthetic process	79	7
GO Biological	Process	nucleobase-containing compound metabolic process	74	Ì
GO Biological		response to external stimulus	72	1.1
GO Biological		response to biotic stimulus	69	100
GO Biological		anatomical structure development response to abiotic stimulus	35 34	-
GO Biological GO Biological		multicellular organism development	33	
GO Biological		signal transduction	28	1
GO Biological		response to endogenous stimulus	26	1
GO Biological		cell communication	15	6
GO Biological		reproduction	10	0
GO Biological		post-embryonic development	10	9
GO Biological		secondary metabolic process	7 7	6
GO Biological		cell differentiation	6	1
GO Biological GO Biological		other biological processes catabolic process	4	1
GO Biological		flower development	3	11
GO Biological		transport	2	1
GO Biological	Process	embryo development	2	-
GO Biological		response to light stimulus	2	2
GO Biological		cell death	2	2
GO Biological		growth	1	1
GO Biological GO Biological		cellular component organization pollination	1	ļ
GO Biological			0	(
GO Biological			0	0
GO Biological		regulation of gene expression, epigenetic	0	(
GO Biological	Process	lipid metabolic process	0	0
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GO Biological		carbohydrate metabolic process	0	(
GO Biological	Process	cell cycle	0	0
GO Biological	Process	DNA metabolic process	0	(
GO Biological			0	(
GO Biological			0	0
GO Biological	FICESS	abscission	0	0

3 2 Functional Categorization by annotation for : GO Cellular Component nucleus: 85.542% (raw value = 71) cytoplasm: 3.614% (raw value = 3) chloroplast: 3.614% (raw value = 3) mitochondrion: 2.41% (raw value = 2) 0 vacuole: 1.205% (raw value = 1) nucleolus: 1.205% (raw value = 1) other intracellular components: 1.205% (raw value = 1) $\label{eq:post} plastid: 1.205\% \mbox{ (raw value = 1)}$ 0 0 Functional Categorization by annotation for : GO Biological Process 0 0 70 other cellular processes: 13.936% (raw value = 131) response to stress: 12.34% (raw value = 116) 71 45 other metabolic processes: 9.574% (raw value = 90) response to chemical: 8.511% (raw value = 80) 44 biosynthetic process: 8.404% (raw value = 79) nucleobase-containing compound metabolic process: 7.872% (raw value) 2 response to external stimulus: 7.66% (raw value = 72) response to biotic stimulus: 7.34% (raw value = 69) anatomical structure development: 3.723% (raw value = 35) response to abiotic stimulus: 3.617% (raw value = 34) multicellular organism development: 3.511% (raw value = 33) signal transduction: 2.979% (raw value = 28) 0 response to endogenous stimulus: 2.766% (raw value = 26) cell communication: 1.596% (raw value = 15) reproduction: 1.064% (raw value = 10) post-embryonic development: 1.064% (raw value = 10) secondary metabolic process: 0.745% (raw value = 7) 0 cell differentiation: 0.745% (raw value = 7) other biological processes: 0.638% (raw value = 6) 0 catabolic process: 0.426% (raw value = 4) flower development: 0.319% (raw value = 3) 0 0 transport: 0.213% (raw value = 2) embryo development: 0.213% (raw value = 2) 0 0 response to light stimulus: 0.213% (raw value = 2) 0 70 cell death: 0.213% (raw value = 2) growth: 0.106% (raw value = 1) 43 cellular component organization: 0.106% (raw value = 1) pollination: 0.106% (raw value = 1) 70 36 70 32 30 23 Functional Categorization by annotation for : GO Molecular Function 14 25 17 DNA binding: 34.444% (raw value = 93) DNA-binding transcription factor activity: 26.296% (raw value = 71) protein binding: 18.889% (raw value = 51) 17 nucleic acid binding: 17.407% (raw value = 47) other binding: 1.111% (raw value = 3) nucleotide binding: 0.741% (raw value = 2) transferase activity: 0.37% (raw value = 1) catalytic activity: 0.37% (raw value = 1) kinase activity: 0.37% (raw value = 1) 0 0 0 0 0 0 0

of annotations to terms in this GOslim category x 100

of total annotations to terms in this ontology

= %

	ER Tools PANTHER Servic	es Workspace Dow	nloads Help/Tuto	rial					
PANTHER17.0 Released.									
alysis Summary: Please report in publication ③									
Analysis Type: PANTHER Overre	Analysis Type: PANTHER Overrepresentation Test (Released 20220202)								
Annotation Version and Release	Date: CO Optology data	DOI: 10.5281/7	anada 6300063 P.	alaasad 201	22.03.22				
	Date. GO Ontology data	Dase DOI: 10.5261/26	51000.0555505 K	eleaseu 202	22-03-22				
Analyzed List: uplo	ad_1 (Arabidopsis thalian	a)		0	Change				
Reference List: Arab	idopsis thaliana (all gene	s in database)			Change				
Annotation Data Set: GO biologic	al process complete	✔ ?							
Test Type: O Fisher's Exact	Binomial								
Correction: O Calculate False D	scovery Rate OUse th	e Bonferroni correctio	on for multiple test	ting 🕐 🔾	No correction				
Results 🕐	Defense list	untered d							
Uninvalu Mannad IDO	Reference list	upload_1	^						
Uniquely Mapped IDS: <u>27430</u> out of 27430 <u>2429</u> out of 2432									
Unmapped IDs: Multiple mapping information:	0	3							

Displaying only results for Bonferroni-corrected for P < 0.05, <u>click here to display all results</u>

B

	Arabidopsis thaliana (REF)		upload_	<u>1</u> (▼ <u>Hierarchy</u> NI	EW!	(?)
GO biological process complete	<u>#</u>	<u>#</u>	expected	Fold Enrichment	+/-	P value
cellular response to hypoxia	<u>238</u>	<u>119</u>	21.10	5.64	+	5.16E-39
<u> ←cellular response to stress</u>	<u>1200</u>	<u>229</u>	106.39	2.15	+	3.14E-20
+ <u>cellular response to stimulus</u>	<u>3901</u>	<u>680</u>	345.87	1.97	+	6.08E-59
+ <u>cellular process</u>	<u>15276</u>	<u>1647</u>	1354.40	1.22	+	1.03E-27
+response to stimulus	<u>9347</u>	<u>1398</u>	828.72	1.69	+	8.55E-109
<u> →response to stress</u>	<u>5305</u>	<u>971</u>	470.35	2.06	+	7.50E-106
+ <u>response to hypoxia</u>	<u>325</u>	<u>142</u>	28.82	4.93	+	1.09E-41
<u> </u>	<u>333</u>	<u>143</u>	29.52	4.84	+	2.62E-41
4 <u>response to oxygen levels</u>	<u>335</u>	<u>143</u>	29.70	4.81	+	4.54E-41
	<u>4145</u>	<u>698</u>	367.50	1.90	+	1.72E-55
<u>cellular response to decreased oxygen levels</u>	<u>240</u>	<u>120</u>	21.28	5.64	+	2.31E-39
<mark> </mark>	<u>241</u>	<u>120</u>	21.37	5.62	+	3.19E-39
4cellular response to chemical stimulus	<u>2081</u>	<u>479</u>	184.51	2.60	+	2.69E-69
<mark> <u> </u></mark>	<u>5130</u>	<u>894</u>	454.84	1.97	+	3.42E-84
response to chitin	<u>320</u>	<u>159</u>	28.37	5.60	+	8.81E-53
Presponse to oxygen-containing compound	<u>3119</u>	<u>616</u>	276.54	2.23	+	1.50E-69
4response to organonitrogen compound	<u>681</u>	<u>215</u>	60.38	3.56	+	1.56E-45
+ <u>response to organic substance</u>	<u>3557</u>	<u>665</u>	315.37	2.11	+	8.66E-68
→ <u>response to nitrogen compound</u>	<u>825</u>	<u>242</u>	73.15	3.31	+	5.17E-47
regulation of increasing acid mediated airmating methylau	A A	20	2.00	5.40		0.745.04

Home > Tools > Motif Analysis

Statistical Motif Analysis in Promoter or Upstream Gene Sequences

The program compares the frequencies of 6-mer "words" in your query set of sequences (on both strands) with the frequencies of the words in the current genomic sequence set of 33518 sequences, each containing 500 (or 1000) bp upstream of the start codon of each gene. You can type in sets of AGI locus identifiers (e.g. At1g01030) or sets of fasta sequences. Make sure each fasta header is formated as such, fasta symbol (>), immediatly followed by a unique ID, a space, then all other descriptions (e.g. >ABCD1.1 my gene). Ensure that there are no sequences appearing more than once in your query set.

At3g46230	
At5g12020	
At4g10250	
At5g12030	
At1g69920	
At5g52760	
At2g26150	
At1g59860	
At2g28210	
At1g66090	
At3g02840	
At3g54150	
At1g53540	
At5g42380	
	11
Upload file: Choose File No file chosen	

Dataset:

```
ullet 500 bp upstream \bigcirc 1000 bp upstream \bigcirc 3000 bp upstream
```

submit

Output type:

```
O HTML O Text Reset
```

В

А

Motif Analysis in Promoter/Upstream Sequences

Only oligos occurring in 3 or more of sequences in the query set are reported, and are sorted by p-value. Columns are as follows (left to right):

```
oligoMer
Absolute number of this oligoMer in query set
Absolute number in genomic set
Number of sequences in query set containing oligoMer
Number of sequences (out of 34187 in genomic set) containing oligoMer
p-value from binomial distribution
Query sequences containing this oligoMer
             C
                    d
                                                 q
         D
   а
                            e
AGGCCC 9 4917
                   8/15
                         3948/34187
                                    8.62e- AT3G46230 AT5G12020 AT4G10250 AT5G12030AT2G26150
                                     05
                                            AT1G59860 AT3G54150 AT1G53540
GGGCCT 9 4917
                   8/15 3948/34187
                                    8.62e- AT3G46230 AT5G12020 AT4G10250 AT5G12030AT2G26150
                                            AT1G59860 AT3G54150 AT1G53540
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CCAAGA 11 8014
                    10/15 6906/34187
                                    1.10e- AT1G69930 AT3G46230 AT5G12030 AT1G69920AT5G52760
                                     04
                                            AT1G59860 AT2G28210 AT3G02840AT3G54150 AT1G53540
TCTTGG 11 8014
                    10/15 6906/34187
                                    1.10e- AT1G69930 AT3G46230 AT5G12030 AT1G69920AT5G52760
                                     04
                                            AT1G59860 AT2G28210 AT3G02840AT3G54150 AT1G53540
GGCCCA 13 8386
                   9/15 5644/34187
                                    1.54e- AT3G46230 AT5G12020 AT4G10250 AT5G12030AT2G26150
                                     04
                                            AT1G59860 AT3G54150 AT1G53540AT5G42380
TGGGCC 13 8386
                   9/15 5644/34187 1.54e- AT3G46230 AT5G12020 AT4G10250 AT5G12030AT2G26150
```

vnload Data Admin Curation Submissio	חכ							
New Annotation Submission								
1. Publication	۱							
Enter a PubMed ID or a DOI.	Publication ID 10.1007/s11103-	• 022-01275-8						
	URL	https://link.springer.com/10.1007/s11103-022-01275-8						
2. Genes								
Enter genes with a UniProt ID, AGI locus ID, or RNA Central ID. Optionally enter a gene symbol and full name.	Gene 1 AT1G28580	✓ ×						
enter a gene symbol and full hame.	Gene Symbol	AXE1						
	Full Gene Symbol	ACETYL XYLAN ESTERASE 1						
		+ Add Another Gene						
3. Annotations								
Select an annotation format and a gene. All fields are required.	Annotation 1 Molecular Functi	ion (GO Function)						
gene. All fields are required.	Gene	AT1G28580 V						
	Molecular Function (GO Function)	acetylxylan esterase activity						
	Method	enzymatic activity assay evidence used in manual assertion						
	Annotation 2 Biological Proces	ss (GO Process)						
	Gene	AT1G28580 ~						
	Biological Process (GO Process)	polysaccharide metabolic process						
	Method	mutant visible phenotype evidence used in manual assertion						
	Annotation 3 Subcellular Loca	ation (GO Component)						
	Gene	AT1G28580						
	Subcellular Location (GO Component)	plasma membrane						
	Method	green fluorescent protein fusion protein localization evidence used in manual assertion						
		d +Add Another Annotation						
©Reset Form	l	e Review Submission>						
New Annotation Submission								
blication ID	10.1007/s11103-022-01275-8							
enes	AT1G28580, ACETYL XYLAN ESTERAS	SE 1, AXE1						

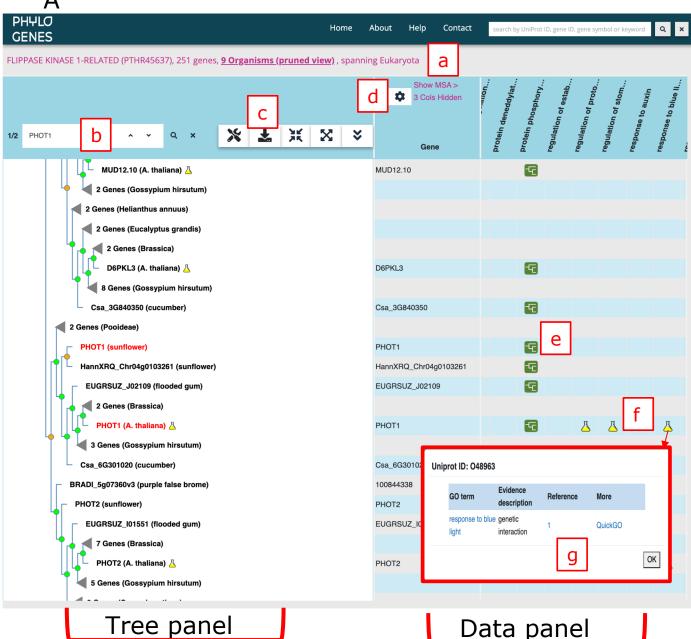
Α

mEdit Form

а

 Genes
 ATIG28580, ACETYL XYLAN ESTERASE 1, AXE1

 Annotations
 ATIG28580 functions in acetylxylan esterase activity @0:00485553 inferred from direct assay (IDA), inferred from enzymatic activity assay evidence used in manual assertion @0:000485553 inferred from Mutant Phenotype (IMP), inferred from mutant visible phenotype evidence used in manual assertion @0:0000580123 ATIG28580 located in plasma membrane, @0:000058862 inferred from direct assay (IDA), inferred from green fluorescent protein fusion protein localization evidence used in manual assertion @0:000058862 inferred from direct assay (IDA), inferred from green fluorescent protein fusion protein localization evidence used in manual assertion @0:000058662



С

В

Organisms (uncheck an organism to remove from tree)

	Organism	Number of genes
	Amborella trichopoda (A. trichopoda)	13
~	Arabidopsis thaliana (A. thaliana)	23
~	Brachypodium distachyon (purple false brome)	21
~	Brassica napus (rapeseed)	35
~	Brassica rapa subsp. pekinensis (Chinese cabbage)	32
	Capsicum annuum (pepper)	20
	Chlamydomonas reinhardtii (C. reinhardtii)	1
	Citrus sinensis (orange)	12
~	Cucumis sativus (cucumber)	16
	Erythranthe guttata (yellow monkeyflower)	22
~	Eucalyptus grandis (flooded gum)	17
	Glycine max (soybean)	43
 Image: A set of the set of the	Gossypium hirsutum (cotton)	51
✓	Helianthus annuus (sunflower)	35

Update tree

Close

Data panel

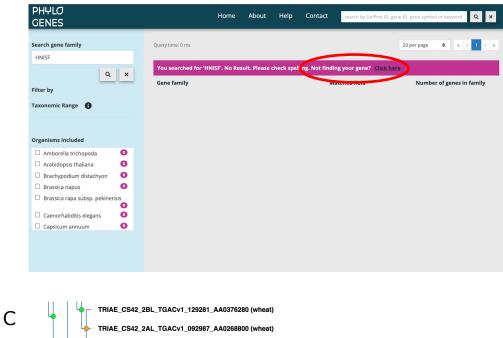
Customize gene info table (3 Cols Hidden)

		•	
✓ Organism	^	*	
 Molecular function 	^	•	
blue light photoreceptor activity	^	•	
DNA-directed 5'-3' RNA polymerase activity	y ^	•	
FMN binding	^	•	
□ identical protein binding	^	•	
metallopeptidase activity	^	•	
□ NEDD8-specific protease activity	^	•	

Update Table Close

oteins Photosians Phot	oteln sequences from these plant species are included in the during the VioCenter streake (version 4.0): In the version of which is chapted in the second
roteins Anno Anno Anno Anno Anno Anno Anno An	Not seeing your sy devila trichopade Marchantia polymorphilic to:
ecies Ara ant model organisms and tuny Control of the control of	bibliopsit thahinan Musia acuminata (Dananan) xchypoollum distachyon (purple false brome) Nelumba nacifyrar (sacred lotus) siscira napus (rapaseed) (Ricotana tabacum (tobacco)
Int model organisms Brain Br Brain Brain B	issica napus (rapeseed) Nicotiona tabacum (tobacco)
family Col	
family Chi	
	osicum annuum (pepper) Physicomitrella patens lamydomonas reinhardtii Populus trichocarpa (black cottonwood)
	rus sinensis (orange) Prunus persico (peach) cumis sotivus (cucumber) Ricinus communis (castor bean)
JniProt ID, gene ID, gene symbol or keyword Ery	thranthe guttata (yellow monkey flower) Selaginella moeilendorffi
Gly	ratipitus grandis (flooded gum) Setaria italica (foxtail millet) cine max (saybean) Solanum lycopersicum (tomato)
	ssypium hirsutum (cotton) Solanum tuberosum (potato) lianthus annuus (sunflower) Sorghum bicolor (sorghum)
	rdeum vulgare (barley) Spinacia oleracea (spinach) fans resia (walnut) Theobroma cacoa (cocoa)
CERTIFICITIER OF PHYTO ECHOS (Up CHITCH) CERTIFICITIER OF State Organisms, sparring LUCA State Organisms, sparring LUCA	bsormidium nitens Triticum aestivum (wheat)
	ttuca sativa (lettuce) Vitis vinifera (grape) nihot esculenta (cassava) Zea mays (corn)
	dicago truncatula (barrelclover) Zostera marina (eelgrass)
	otein sequences from the following non-plant model organisms are include to provide
4 Genes (Russectory)	nctional information that can be useful for when no experimental plant data is available
	znorhabditis elegans (nematode worm) nio rerio (zebrafish)
	tyostelium discoideum
	isophila melanogaster (fruit fly) herichia coli
LOC10459F4H (sacred inter)	mo sopiens (human)
	ttus norvegicus (rat) schoromyces cerevisioe (budding yeast)
- VT. 00x201g00000 (grape)	sizosaccharomyces pombe (fission yeast)
af 2 Oares (Sunatypus gra	

В



2145_0049_201_TGACv1_158945_AA0529290 (wheat)

SYP81 (sunflower)

Grafted

MIMGU_mgv1a010233mg (E. guttata)

101249911 (tomato) 101247988 (tomato)

А

