GraP: platform for functional genomics analysis of *Gossypium raimondii*

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Abstract

Cotton (*Gossypium* spp.) is one of the most important natural fiber and oil crops worldwide. Improvement of fiber yield and quality under changing environments attract much attention from cotton researchers; however, a functional analysis platform integrating omics data is still missing. The success of cotton genome sequencing and large amount of available transcriptome data allows the opportunity to establish a comprehensive analysis platform for integrating these data and related information. A comprehensive database, Platform of Functional Genomics Analysis in *Gossypium raimondii* (GraP), was constructed to provide multi-dimensional analysis, integration and visualization tools. GraP includes updated functional annotation, gene family classifications, protein–protein interaction networks, co-expression networks and microRNA–target pairs. Moreover, gene set enrichment analysis and *cis*-element significance analysis tools are also provided for gene batch analysis of high-throughput data sets. Based on these effective services, GraP may offer further information for subsequent studies of functional genes and in-depth analysis of high-throughput data. GraP is publically accessible at http://structuralbiology.cau.edu.cn/GraP/, with all data available for downloading.

Introduction

As the most significant natural fiber and oil crop in the world, cotton (*Gossypium* spp.) is also an industrial raw material and military supply, and is widely used in daily life. The *Gossypium* genus comprises ~50 species (diploid and polyploid), and is ideal material to study polyplody and genome evolution. Improving fiber yield and quality under various kinds of abiotic and biotic stresses attracts most of the attention in cotton breeding research.
Some fiber developmental genes have been identified, such as \( E6 \) (1), \( GbTub1 \) (2), \( GbSusA1 \) (3) and GA20ox (4). Many genes controlling response to changing environments have been identified in model plants, and only a few stress-related genes have been reported in cotton, such as \( GbNHX1 \) (5), \( GbDREB1 \) (6), \( GbNAC1–GhNAC13 \) (7, 8), \( GbMKK1 \) (9), \( GbSnRK2 \) (10), \( GbCLPK6 \) (11), \( GbWRKY40 \) (12) and \( GbRLK \) (13). It was reported that overexpressing of \( G. \) \( hirsutum \) sucrose non-fermenting 1-related protein kinase 2 (\( GbSnRK2 \)), which acts as a positive regulator in stress responses, exhibited increased tolerance to drought, cold, abscisic acid (ABA) and salt stresses (10). In addition, Wang et al. (12) found that \( GbWRKY40 \) was a multiple stress-responsive cotton WRKY gene and played an important role in regulating wounding- and pathogen-induced responses, its overexpression down-regulated most defense-related genes. With the success of the cotton whole-genome sequencing (14, 15), research on functional genomics has become a major challenge for the scientific community. The widespread use of microarrays and next-generation sequencing is an epitome of high-throughput techniques, which produce massive amounts of omics data on cotton and offer biologists new ideas for cotton functional genomics research. Accordingly, integrating the genomic and transcriptomic data in an online database and mining from the integrated data is essential to maximize utility of these valuable data, and to give cotton researchers further understanding of the complex cellular networks.

Several cotton online databases are currently available, such as CottonDB (16), CMD (Cotton Marker Database) (17), TropGene-DB (18), Cotton expressed sequence tag (EST) database (19) and CottonGen (20). CottonDB contains genomic, genetic and taxonomic information for cotton. CMD provides publicly available cotton simple sequence repeat markers. Furthermore, the Cotton EST database is a platform for cotton EST-related information. CottonGen is an integration and update of publicly available cotton data from CottonDB, CMD and TropGene-DB. However, the databases mentioned above emphasize genomic, genetic, taxonomic and marker data, providing limited information on key functional genes and the relationships between them. These databases are also limited in their utilizing of high-throughput data sets.

Driven by this need, GraP (Platform of Functional genomics in \( Gossypium \) \( raimondii \)) was developed to provide an integration, multi-dimensional analysis and visualization platform for cotton functional genomics research. Up to 2 December 2014, GraP includes: (i) integrated information of two versions of genome sequences and microarray and mRNA-seq data; (ii) different analysis tools, including gene searching and genome browsing, metabolism analysis, protein–protein interaction (PPI) prediction, co-expression network and genome synteny analysis of relative species and (iii) other user-friendly interfaces such as GSEA (gene set enrichment analysis) (21) and \( cis \)-element significance analysis tools with downloadable results. With these integrated information and web tools, GraP will further broaden the omics data access and improve the accuracy and robustness of cotton functional genomics analysis. We also hope it will provide some inspiration for cotton researchers, and further speed up research on cotton yield and quality.

Data sources

The genome assembly sequences and gene structural annotations of \( G. \) \( raimondii \), which was the first completed sequence of cotton species, used in GraP are the JGI version from Phytozome (15, 22) and the BGI version from CottonGen (20), respectively (Supplementary Table S1). To get an updated annotation of predicted genes from these assemblies, we used \( Arabidopsis \) TAIR10 version (23) and closely related species to re-annotate the genome using BLAST (basic local alignment and search tool). The functional annotations of proteins were downloaded or performed respectively from MAPMAN (24), Kyoto Encyclopedia of Genes and Genomes database (KEGG) (25) and InterProScan (26). Furthermore, motif information was downloaded from the plant \( cis \)-acting regulatory DNA elements (PLACE) database (27). We also collected microRNA data from the miRBase (28) and published literature (29). The cotton microarray probes were downloaded from the Affymetrix official website (http://www.affymetrix.com/estore/). Microarray and RNA-seq data were respectively collected from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) in the National Center for Biotechnology Information (NCBI) (Supplementary Table S2).

Materials and Methods

Gene family classifications

Because transcription factors/regulators (TFs/TRs), protein kinases/phosphatases (PKs/PPs), ubiquitin proteasome system (UPS) members and CYP450s are functionally important in signaling pathways, related gene family classifications were carried out by the strategies summarized in Figure 1. Initially, Hidden Markov Model (HMM) models and well-trained parameters from UUCD (30) and unpublished iTAK databases (http://bioinfo.bti.cornell.edu/cgi-bin/itak/index.cgi) were used respectively to predict putative UPSs, TFs/TRs and PKs/PPs. Additionally,
HMMER 3.0 (31) was used to determine proteins that contained conserved domains, annotated with ‘protein phosphatases’, ‘histidine kinases’ and ‘tyrosine phosphatase’. Meanwhile, proteins containing the ‘PF00067’ domain were considered as CYP450s according to the Pfam (http://pfam.xfam.org/) annotation.

After that, homologs between G. raimondii and Arabidopsis were identified by bidirectional BLASTp (e-value < 1e-5). Information from public databases, such as CYPSI (32), plantsUPS (33), the Cytochrome P450 Homepage (http://drnelson.uthsc.edu/CytochromeP450. html), AGRIS (34) and plantTFDB (35) was further used to consolidate the primary results and filter out ambiguities. Only the member was retained when its family classification was accordant with that of their corresponding Arabidopsis homologs in at least one public database. Meanwhile, conserved functional domains searched by InterProScan (26) were adopted to curate the classification results (Table 1). Taking TFs/TRs as an example, 6629 proteins belonged to this category according to the results of a HMM search against the iTAK model. Then, we used information from AGRIS and plantTFDB to manually screen these putative TFs/TRs, and 223 proteins without supporting information from the two public databases were discarded.

In addition, family classifications for these categories in GraP were manually curated with the following two steps: (a) conserved functional-domain based method: we used InterProScan to identify the domains for all proteins, and then selected the candidates for specific gene families based on conserved functional domains; (b) homology-based method: For those identified candidates derived from step (a), we checked if their best homologs in other relative species (such as Arabidopsis) belong to the corresponding gene families or not. Especially, for some ambiguous proteins which have certain domains but with low scores, we further manually aligned these domains to their homologs to avoid some false positives. After that, the remained members were finally released. In the meanwhile, classifications and manual curations for other gene families are undergoing and the results will be released in near future.

MicroRNAs and targets
MicroRNAs play important roles in transcriptional regulation through impacting the stability and fate of their binding target mRNAs (36). More and more functionally important microRNAs have been identified in various conditions, such as salt, cold and drought environments, with the help of high-throughput technology (e.g. microarray, miRNA-seq and sRNA-seq). Thus we collected 296 microRNAs from miRBase (28) and 127 microRNAs from the recently published literature (29). Because some of them were not identified under the genomic background of JGI version, the genomic locations of their precursors were relocated by the BLAT tool (37). In addition, RNAfold (38) was applied to obtain the pre-miRNA secondary structures and the positions of mature microRNA. We further removed the microRNAs with redundant genomic positions, and finally integrated 416 microRNAs from
those resources (shown in Supplementary Table S3). In order to establish the relationships between microRNAs and mRNAs, the psRNATarget web service (39), TargetFinder (40, 41) and TAPIR (42) were employed to find their downstream binding mRNA targets.

**PPI prediction**

Intracellular signals are mainly transmitted through PPIs (43). However, only a few PPIs have been experimentally identified, and so it is essential to perform high-quality prediction of PPIs, which will benefit understanding of regulatory relationships between genes. In total, 18 014 experimentally assayed PPIs in Arabidopsis were collected from BioGRID (44), TAIR (23), BAR (45) and CCSB (46).

We determined 15 142 ortholog pairs between Arabidopsis and G. raimondii using Inparanoid (Table 1), using a cutoff of bootstrap ≥ 0.60 (47). Meanwhile, orthologs were selected from homolog match results by bidirectional BLASTp between Arabidopsis and G. raimondii (e-value ≤ 1e−5). The top three hits from cotton-to-Arabidopsis BLASTp results were inspected and further divided into two levels: (i) level 1 if these hits occurred in the top three in the Arabidopsis-to-cotton BLASTp output and (ii) level 2 if these hits occurred in the top 10 (Table 1). Then, 29 399 cotton genes were mapped to 19 169 genes.

With these ortholog matches, PPIs were further predicted according to the collected experimentally verified PPIs in Arabidopsis. Since hubs containing closely connected nodes were usually functionally related to each other, the network was divided into 93 closely connected hubs (each hub with at least 10 nodes) using MCODE (fluff = 0.9) (48), which is a tool to find hubs by evaluating the topological structure of a network.

**Gene co-expression network**

Due to the lack of transcriptional profile data sets for G. raimondii and the high similarities between Gossypium subspecies, cotton microarray data sets related to different developmental stages and stresses (Supplementary Table S2) were collected from GEO to construct the cotton gene co-expression network. Microarray elements were also downloaded from the Affymetrix official website (http://www.affymetrix.com/estore/), and used to search for gene–probe matches.

First, the probe consensus sequences were aligned to transcripts by BLASTn with e-value ≤ 1e−3 and cumulative identity percentage of consensus ≥ 60, and 22 524 probes matched to 39 963 transcripts. Second, all CEL files were preprocessed by gcRMA (Guanine Cytosine Robust Multi-Array Analysis) algorithm (49). For each pair of probe sets i and j, all microarray expression values were used to
calculate PCC (Pearson’s correlation coefficient) values. Then, the probe-set pairs were retained as expression-correlated if their corresponding PCC $\leq -0.7$ or $\geq 0.7$. The expression patterns of two probe sets are similar or positively correlated if their corresponding PCC $\geq 0.7$ and vice versa.

Cis-element significance analysis

There were 394 different motif factors scanned in the upstream of 37 223 genes using the PLACE web service (27). Users can submit their gene sets to GraP to scan for motifs. In order to facilitate users to perform significance analysis of these motifs, the z-score of each scanned motif and its corresponding $P$-value are computed with the formulas that follow (50):

$$z \text{- score} = \frac{N_i - \text{mean}}{\sigma_i}$$
$$P\text{-value} = 1 - \text{pnorm}(N_i, \text{mean}, \sigma_i)$$

Where $N_i$ represents the number of occurrences of motif $i$ in the submitted gene batch, and mean, and $\sigma$ are the mean and standard deviation of the number of detected occurrence of motif $i$ in 1000 randomly selected genes, respectively. Except for the analysis of known genes, sequences of fasta format can also be submitted for de novo motif recognitions.

High-throughput data integrations

Although genome-wide PPIs and co-expression networks are well constructed, understanding gene expression profiles in tissues under different environmental conditions will greatly increase researchers’ knowledge of gene functions. Since ambiguity still existed for intracellular expression levels of interested genes of not-good-enough matches between cotton microarray and G. raimondii RNA-seq data sets of 16 available RNA-seq data sets of G. raimondii collected from GEO (Supplementary Table S2) were analysed. The quality of reads were evaluated using FASTX-Toolkit and reads of high quality (at least 80% of bases per read have a score of $\geq 20$) were retained. Then TopHat was utilized to align these reads to the reference genome permitting four mismatches considering their germplasm variances. FPKMs (Fragments Per Kilobase of transcript per Million mapped reads) of transcripts or genes were calculated by cufflinks employing default parameters.

Results

All the processed and analysed results were well integrated. This included gene families comprising 6406 TFs/TRs, 2284 UPS members, 490 CYP450s and 4292 PKs/PPs, 103743 PPIs between 12483 proteins (Supplementary Figure S1), the gene co-expression network containing 1 419 237 positively and 1 127 237 negatively correlated pairs between 20 480 probe sets, and 416 microRNAs as well as their corresponding precursors (Table 1). PHP + MySQL + Javascript + Python/Perl were used to develop application platforms facilitating cotton research, and Cytoscape Web (51) was employed to supply users with a retrieval device to search for PPIs or co-expressed genes. Furthermore, BLAST and GBrowse (52) as well as other widgets were developed for users to freely and directly find the integrated information related to their genes or proteins of interest, comprising all integrated knowledge mentioned above and other fundamental functional annotations (GO, KEGG and MapMan), conserved protein domain, homologs or orthologs of relative species and gene expression levels in different tissues at various time points (Figure 2A).

With the help of GraP, users can directly search genes in GBrowse (52), gene structures and their expression levels are displayed and more details (as mentioned above) are also available (Figure 2B and 3). Furthermore, annotation items or keywords can also be submitted to find specific information through the search tools provided in GraP. Apart from this fundamental information in GraP, every gene is hyperlinked to CottonCyc pathways integrated in CottonGen (Figure 2B). In addition to this static information, dynamic clues including PPIs and co-expressed probe sets (Figures 2B and 4A) can further benefit understanding of the biological processes that a gene is involved in. This would benefit key gene selections and relevant experimental designs.

Compared with single gene verification, high-throughput technologies provide large amounts of omics data and give biologists more related information, usually related to specific phenotypes or agronomic traits. Subsequent statistical analysis of functions of the detected differentially expressed (RNA-seq or microarray) or modification enriched (TF binding, histone modification or DNA methylation) genes is routinely carried out to illustrate the related biological phenomena. Therefore, GraP offers users GSEA and cis-element significance analysis tools for relevant analysis of their gene batches. Taking PPI hubs as an example, GSEA analyses on them are mainly related to various signaling-mediated or stress-responsive physiological processes, including cell reproduction, defense response, ubiquitin-mediated protein degradation and regulation of gene expression (Supplementary Table S4).

To make query results more believable, homologs in relative species are mapped to cotton genes using BLAST and also linked to other public databases for more
information (Figure 2B). Because there were already two different genomic assemblies of *G. raimondii* (14, 15), syntenic blocks between them were scanned with SyMAP (53). Meanwhile, transcripts in each pair matched with BLASTn were retained if both of them belonged to the same block. GBrowse_syn was used to visualize these results in GraP (Supplementary Table S5) (54), which will help cotton biologists to take full advantage of two genome assemblies. Additionally, users can carry out personal searching or update requests through e-mail or leaving messages in GraP, and this will undoubtedly accelerate GraP improvement.

**Discussion**

There is no doubt that a novel and comprehensive method of integration and analysis for cotton omics data is essential to maximize utility of publicly available data, and will help to accelerate cotton research. However, a powerful mining tool for omics data has not previously been available. Differing from CottonGen, CottonDB or CMD, GraP is believed to fill the vacant fields for functional analysis. GraP is more suitable for molecular mechanism-driven investigations of gene batches derived from high-throughput sequencing or microarrays, especially for signal transductions under stresses, and will offer researchers some insights into their studies. GraP provides comprehensive knowledge about a specific gene, including family categories, potential interactive partners, *cis*-elements, microRNAs and their downstream targets, as well as possible regulatory pathways.

Taking SnRKs as an example, in *G. raimondii*, Gorai.011G121900 is a member of the SnRK family (Figure 3) and the ortholog of *GhSnRK2* (Figure 4B). It was shown that in the PPI network (Figure 4A), Gorai.011G121900 could directly interact with 100 other proteins, including F-box (Gorai.013G113800), CUL1 (Gorai.008G009700), EBF1 (Gorai.009G058000), bZIP TFs (Gorai.013G258300 and Gorai.006G111600), COI1 (Gorai.011G279900) and AREB3 (Gorai.009G301400). Most of these PPIs have already been verified in *Arabidopsis* (46, 55) (Supplementary Table S6).

In addition, GSEA analysis of all interactors of Gorai.011G121900, such as Tify, WRKY TFs and various E3 ligases, showed significant enrichment in the response to light stimulus and other important hormones, including ABA and jasmonic acid (Fisher exact test, FDR ≤ 0.05) (Figure 4D and Supplementary Table S8). Statistical analysis of motifs in the 3000 bp upstream sequences of these protein-coding genes showed that they were significantly related to stress and hormone signaling as well as partially tissue-specific motif factors, such as WRKY71OS, WBOXNTERF3, RAV1AAT and ARBELATERD1 (*P*-value ≤ 0.05; Figure 4C). This showed that these interactors might be transcribed by WRKY TFs and impacted by ABA or other stress-related key elements. It had also been reported that the overexpression of *GhSnRK2* in *Arabidopsis* led to reduced water loss under salt and drought stresses as well as the up-regulation of other key elements, such as AtABI3, AtCBF1 and AtABI5 (10). Furthermore, *GhSnRK2* silencing in cotton plants results in a phenotype of alleviated drought tolerance (10). Gra.16.2.A1_x_at was the corresponding probe set of Gorai.011G121900 and it had 157 positively co-expressed genes, which included the expression of *Gorai.011G121900* itself.
Probe sets. Gene ontology enrichment analysis of these probe sets using agriGO (56) exhibited that response to water and chemical stimuli as well as regulation of gene expression were significantly enriched (P-value ≤ 0.05) (Supplementary Figure S2 and Table S7). Moreover, MIR393 is involved in auxin signaling and controlled leaf development under salt stress, and MIR482 is a family of disease resistance-related miRNAs (57, 58). Both MIR393 and MIR482 participate in the posttranscriptional regulation of transcribed mRNAs from the interactors of Gorai.011G121900 (Figure 3). In summary, all the mined information illustrated that Gorai.011G121900 was involved in tissue development, hormone and stress resistance (Supplementary Table S8).

However, information in GraP is mainly constructed in silico. False-positives or false-negatives can exist due to
parameter and method selections during prediction processes and lack of experimental evidence. Therefore, subsequent manual updates are important for error rectification and knowledge supplementation, guaranteeing a good service for user requests. Users can also leave messages or write e-mails to GraP for personal problems.

Conclusions

GraP is a user-friendly and up-to-date database and analysis platform for functional genomic studies in *G. raimondii*. At present, we have integrated two versions of *G. raimondii* genome data, microarray and mRNA-seq data in the database (Table 1). We have also developed a series of functional analysis tools such as cotton gene family analysis, PPI prediction, co-expression network web service, GSEA analysis, cis-element significance analysis toolbox, genome synteny analysis among relative species and other general tools in the database. We hope this will improve the accuracy of cotton functional genomics analysis, and further deepen understanding of gene regulatory networks for effective crop improvement. GraP is freely available at http://structuralbiology.cau.edu.cn/GraP/, and will be updated every 3–4 months with the development of cotton research, as well as the manual curated gene family available.
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Supplementary Data
Supplementary data are available at Database Online.

Conflict of interest. None declared.

References
1. John,M.E. and Crow, L.J. (1992) Gene expression in cotton (Gossypium hirsutum L.) fiber: cloning of the mRNAs. Proc. Natl. Acad. Sci. USA., 89, 5769–5773.
2. Li,Y.L., Sun,J., Li,C.H. et al. (2003) Specific expression of a beta-tubulin gene (GhTub1) in developing cotton fibers. Sci. China C Life Sci., 46, 235–242.
3. Jiang,Y., Gao,W., Zhu,H. et al. (2012) Overexpression of GhSusA1 increases plant biomass and improves cotton fiber yield and quality. Plant Biotechnol. J., 10, 301–312.
4. Bai,W.Q., Xiao,Y.H., Zhao,J. et al. (2014) Gibberellin overproduction promotes sucrose synthase expression and secondary cell wall deposition in cotton fibers. PLoS One, 9, e96537.
5. Wu,C.A., Yang,G.D., Meng,Q.W. et al. (2004) The cotton GhNHIK1 gene encoding a novel putative tonoplast Na(+)/H(+) antiporter plays an important role in salt stress. Plant Cell Physiol., 45, 600–607.
6. Gao,S.Q., Chen,M., Xia,L.Q. et al. (2009) A cotton (Gossypium hirsutum) DRE-binding transcription factor gene, GhDREB, confers enhanced tolerance to drought, high salt, and freezing stresses in transgenic wheat. Plant Cell Rep., 28, 301–311.
7. Meng,C.M., Cai,C.P., Zhang,T.Z. et al. (2009) Characterization of six novel NAC genes and their responses to abiotic stresses in Gossypium hirsutum. Plant Sci., 176, 352–359.
8. Huang,G.Q., Li,W., Zhou,W. et al. (2013) Seven cotton genes encoding putative NAC domain proteins are preferentially expressed in roots and in responses to abiotic stress during root development. Plant Growth Regul., 71, 101–112.
9. Lu,W., Chu,X., Li,Y. et al. (2013) Cotton GhMKK1 induces the tolerance of salt and drought stress, and mediates defence responses to pathogen infection in transgenic Nicotiana benthamiana. PLoS One, 8, e68503.
10. Bello,B., Zhang,X., Liu,C. et al. (2014) Cloning of Gossypium hirsutum sucrose non-fermenting 1-related protein kinase 2 gene (GhsnRK2) and its overexpression in transgenic Arabidopsis escalates drought and low temperature tolerance. PLoS One, 9, e112269.
11. He,L., Yang,X., Wang,L. et al. (2013) Molecular cloning and functional characterization of a novel cotton CBL-interacting protein kinase gene (GhCIPK6) reveals its involvement in multiple abiotic stress tolerance in transgenic plants. Biochem. Biophys. Res. Commun., 435, 209–215.
12. Wang,X., Yan,Y., Li,Y. et al. (2014) GhWRKY40, a multiple stress-responsive cotton WRKY gene, plays an important role in the wounding response and enhances susceptibility to Ralstonia solanacearum infection in transgenic Nicotiana benthamiana. PLoS One, 9, e93577.
13. Zhao,J., Gao,Y., Zhang,Z. et al. (2013) A receptor-like kinase gene (GhRLK) from Gossypium barbadense enhances salinity and drought-stress tolerance in Arabidopsis. BMC Plant Biol., 13, 110.
14. Wang,K., Wang,Z., Li,F. et al. (2012) The draft genome of a diploid cotton Gossypium raimondii. Nat. Genet., 44, 1098–1103.
15. Paterson,A.H., Wendel,J.F., Gundlach,H. et al. (2012) Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature, 492, 423–427.
16. Yu,J., Kohel,R., Hinze,L. et al. (2012) CottonDB. In: Proceedings of the International Plant and Animal Genome Conference, 2012, San Diego, CA, USA, 14–18.
17. Blenda,A., Scheffler,J., Scheffler,B. et al. (2006) CMD: a cotton microsatellite database resource for Gossypium genomics. BMC Genomics, 7, 132.
18. Ruiz,M., Rouard,M., Raboin,L.M. et al. (2004) TropGENE-DB, a multi-tropical crop information system. Nucleic Acids Res., 32, D364–D367.
19. Xie,F., Sun,G., Stiller,J.W. et al. (2011) Genome-wide functional analysis of the cotton transcriptome by creating an integrated EST database. PLoS One, 6, e26980.
20. Yu,J., Jung,S., Cheng,C.H. et al. (2014) CottonGen: a genomics, genetics and breeding database for cotton research. Nucleic Acids Res., 42, D1229–D1236.
21. Yi,X., Du,Z., Su,Z. (2013) PlantGSEA: a gene set enrichment analysis toolkit for plant community. Nucleic Acids Res., 41, W98–W103.
22. Goodstein,D.M., Shu,S., Howson,R. et al. (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res., 40, D1178–D1186.
23. Poole,R.L. (2007) The TAIR database. Methods Mol. Biol., 406, 179–212.
24. Thimm,O., Blasing,O., Gibon,Y. et al. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J., 37, 914–939.
25. Kanehisa,M., Goto,S., Sato,Y. et al (2012) KEGG for integration and interpretation of large-scale molecular datasets. Nucleic Acids Res., 40, D109–D114.
26. Hunter,S., Apweiler,R., Attwood,T.K. et al. (2009) InterPro: the integrative protein signature database. Nucleic Acids Res., 37, D211–D215.
27. Higo,K., Ugawa,Y., Iwamoto,M. et al. (1998) PLACE: a database of plant cis-acting regulatory DNA elements. Nucleic Acids Res., 26, 358–359.
28. Griffiths-Jones,S., Grocock,R.J., van Dongen,S. et al. (2006) miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res., 34, D140–D144.
29. Gong,L., Kakrana,A., Arkit,S. et al. (2013) Composition and expression of conserved microRNA genes in diploid cotton (Gossypium) species. Genome Biol. Evol., 5, 2449–2459.
30. Gao,T., Liu,Z., Wang,Y. et al. (2013) UUCD: a family-based database of ubiquitin and ubiquitin-like conjugation. Nucleic Acids Res., 41, D445–D451.
31. Zhang,Z. and Wood,W.I. (2003) A profile hidden Markov model for signal peptides generated by HMMER. Bioinformatics, 19, 307–308.
32. Zhang, G., Zhang, Y. and Su, Z. (2012) CYPSI: a structure-based interface for cytochrome P450s and ligands in Arabidopsis thaliana. BMC Bioinformatics, 13, 332.
33. Du, Z., Zhou, X., Li, L. et al. (2009) plantsUPS: a database of plants’ Ubiquitin Proteasome System. BMC Genomics, 10, 227.
34. Yilmaz, A., Mejia-Guerra, M.K., Kurz, K. et al. (2011) AGRIS: the Arabidopsis Gene Regulatory Information Server, an update. Nucleic Acids Res., 39, D1118–D1122.
35. Jin, J., Zhang, H., Kong, L. et al. (2011) A predicted interactome for Arabidopsis. Plant Physiol., 154, 317–329.
36. Alvarez-Garcia, I., Miska, E.A. (2005) MicroRNA functions in plants’ Ubiquitin Proteasome System. Gene Regulatory Information Server, an update. Nucleic Acids Res., 39, D1118–D1122.
37. Kent, W.J. (2002) BLAT–the BLAST-like alignment tool. Genome Res., 12, 666–664.
38. Gruber, A.R., Lorenz, R., Bernhart, S.H. et al. (2008) The Vienna RNA web suite. Nucleic Acids Res., 36, W70–W74.
39. Dai, X. and Zhao, P.X. (2011) psRNATarget: a plant small RNA target analysis server. Nucleic Acids Res., 39, W155–W159.
40. Allen, E., Xie, Z., Gustafson, A.M. et al. (2005) microRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell, 121, 207–221.
41. Fahlgren, N., Howell, M.D., Kasschau, K.D. et al. (2007) High-throughput sequencing of Arabidopsis microRNAs: evidence for frequent birth and death of MiRNA genes. PLoS One, 2, e219.
42. Bonnet, E., He, Y., Billiau, K. et al. (2010) TAPIR, a web server for the prediction of plant microRNA targets, including target mimics. Bioinformatics, 26, 1566–1568.
43. Mayer, B.J. (2006) Protein-protein interactions in signaling cascades. Methods Mol. Biol., 332, 79–99.
44. Chatr-Aryamontri, A., Breitkreutz, B.J., Heinicke, S. et al. (2013) The BioGRID interaction database: 2013 update. Nucleic Acids Res., 41, D816–D823.
45. Geisler-Lee, J., O’Toole, N., Ammar, R. et al. (2007) A predicted interactome for Arabidopsis. Plant Physiol., 145, 317–329.
46. Arabidopsis Interaction Mapping Consortium. (2011) Evidence for network evolution in an Arabidopsis interactome Map. Science, 333, 601–607.
47. O’Brien, K.P., Remm, M., Sonnhammer, E.L. (2005) Inparanoid: a comprehensive database of eukaryotic orthologs. Nucleic Acids Res., 33, D476–D480.
48. Bader, G.D. and Hogue, C.W. (2003) An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics, 4, 2.
49. Gharabihe, R.Z., Fodor, A.A. and Gibas, C.J. (2008) Background correction using dinucleotide affinities improves the performance of GCRMA. BMC Bioinformatics, 9, 452.
50. Nemhauser, J.L., Mockler, T.C. and Chory, J. (2004) Interdependency of brassinosteroid and auxin signaling in Arabidopsis. PLoS Biol., 2, E258.
51. Lopes, C.T., Franz, M., Kazi, F. et al. (2010) Cytoscape Web: an interactive web-based network browser. Bioinformatics, 26, 2347–2348.
52. Stein, L.D. (2013) Using GBrowse 2.0 to visualize and share next-generation sequence data. Brief. Bioinform., 14, 162–171.
53. Soderlund, C., Nelson, W., Shoemaker, A. et al. (2006) SyMAP: A system for discovering and viewing syntenic regions of FPC maps. Genome Res., 16, 1159–1168.
54. McKay, S.J., Vergara, I.A. and Staigh, J.E. (2010) Using the Generic Synteny Browser (GBrowse_syn). Curr. Protoc. Bioinformatics, Chapter 9, Unit 9.12.
55. Marrocco, K., Zhou, Y., Bury, E. et al. (2006) Functional analysis of EID1, an F-box protein involved in phytochrome A-dependent light signal transduction. Plant J., 45, 423–438.
56. Du, Z., Zhou, X., Ling, Y. et al. (2010) agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res., 38, W64–W70.
57. Si-Ammour, A., Windels, D., Arn-Bouldoires, E. et al. (2011) miR393 and secondary siRNAs regulate expression of the TIR1/AFB2 auxin receptor clade and auxin-related development of Arabidopsis leaves. Plant Physiol., 157, 683–691.
58. Zhu, Q.H., Fan, L., Liu, Y. et al. (2013) miR482 Regulation of miR482. Plant Physiol., 162–171.
59. Supek, F., Bošnjak, M., Škunca, N. et al. (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One, 6, e21800.