RESEARCH ARTICLE

DsTRD: Danshen Transcriptional Resource Database

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OPEN ACCESS

Citation: Shao Y, Wei J, Wu F, Zhang H, Yang D, Liang Z, et al. (2016) DsTRD: Danshen Transcriptional Resource Database. PLoS ONE 11(2): e0149747. doi:10.1371/journal.pone.0149747

Editor: Yu Xue, Huazhong University of Science and Technology, CHINA

Received: October 5, 2015

Accepted: February 4, 2016

Published: February 24, 2016

Data Availability Statement: Data are available from the supplemental files and NCBI database. The NCBI accession numbers are: SRR1043998, SRR1045051, SRR1020591, SRR946948, SRR946949, SRR946950, SRR946951, SRR037804, SRR1005880, SRR924322, SRR1557860, SRR1557861, SRR1557862 and SRR1557863.

Funding: This work was supported by the 521 Talent Foundation of Zhejiang Sci-Tech University, the website is http://www.zstu.edu.cn/. This work was supported by the Doctoral Scientific Research Foundation of Zhejiang Sci-Tech University, the website is http://www.zstu.edu.cn/. This work was supported by the grants of the Natural Science

Abstract

Salvia miltiorrhiza has been comprehensively studied as a medicinal model plant. However, research progress on this species is significantly hindered by its unavailable genome sequences and limited number of expressed sequence tags in the National Center for Biotechnology Information database. Thus, a transcript database must be developed to assist researchers to browse, search, and align sequences for gene cloning and functional analysis in *S*. *miltiorrhiza*. In this study, the Danshen Transcriptional Resource Database (DsTRD) was built using 76,531 transcribed sequences assembled from 12 RNA-Seq transcriptomes. Among these 12 RNA-seq data, ten were downloaded from NCBI database. The remaining two were enced on the Hiseq2000 platform using the stem and hairy-root of *S*. *miltiorrhiza*. The transcripts were annotated as protein-coding RNAs, long non-coding RNAs, microRNA precursors, and phased secondary small-interfering RNA genes through several bioinformatics methods. The tissue expression levels for each transcript were also calculated and presented in terms of RNA-Seq data. Overall, DsTRD facilitates browsing and searching for sequences and functional annotations of *S*. *miltiorrhiza*. DsTRD is freely available at http://bi.sky.zstu.edu.cn/DsTRD/home.php.

Introduction

Salvia miltiorrhiza Bunge is a perennial plant that belongs to the Lamiaceae family. The dried root or rhizome of this plant is called “Danshen” in traditional Chinese medicine. *S. miltiorrhiza* is comprehensively studied as a medicinal model plant because of its small genome, short life cycle, and stable genetic transformation system. Elucidation and regulation of biosynthesis pathways involving the active ingredients of *S. miltiorrhiza* are considered major research topics. However, the unavailable genome sequence and limited nucleotide sequences (only 1,048 nucleotides and 10,288 expressed sequence tags, ESTs) of *S. miltiorrhiza* in the National Center for Biotechnology Information (NCBI) database significantly hinder research progress on this species, specifically in terms of molecular growth mechanisms, developmental and stress responses, and biosynthesis of active ingredients.

Foundation of China (grant No. 81373908 and 81403033), and the website is http://isisn.nsfc.gov.cn/. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Numerous transcriptomes of *S. miltiorrhiza* have been extensively recovered through high-throughput sequencing and deposited into the NCBI Sequence Read Archive (SRA) database. Nevertheless, researchers should continuously analyze large amounts of raw sequencing reads. Despite the currently available routine processes and methods, considerable time and effort are expended to retrieve sequences and features and install diverse software packages. Thus, a transcript resource, which can assemble and annotate raw reads and can be easily searched by humans and computers, must be developed. In this regard, we developed the Danshen Transcriptional Resource Database (DsTRD), which is a simple but comprehensive transcript resource for *S. miltiorrhizae*.

Materials and Methods

RNA-Seq data information

Ten RNA-seq data including 7 pair-end sequencing data based on Hiseq2000 platform and three single-end sequencing data based on 454 platform were downloaded from NCBI database with Sequence Read Archive (SRA) formation (S1 Table).

In addition, two RNA-seq data (RNAseq_1 and RNAseq_2) were added for transcripts assembly and tissue expression analysis by using the stem and hairy-root of *S. miltiorrhiza* and sequencing on the Hiseq2000 platform (S1 Table). The stems of *S. miltiorrhiza* were collected from a Salvia planting base in shangluo, China. And the hairy-root of *S. miltiorrhiza* were cultured by our lab.

Transcriptional assembly annotation and quantification

The assembly flowchart of *S. miltiorrhiza* RNA-Seq data is shown in Fig 1. The adapters and poor quality sequences were firstly removed from RNA-seq data using FASTX toolkit downloaded from http://hannonlab.cshl.edu/fastx_toolkit/download.html. Clean sequencing data were assembled into transcripts by using Trinity software with the parameters "—min_kmer_cov 3—min_glue 3" [1]. All assembled transcripts from the total samples were clustered by tgicl v2.1 with default parameters [2] (S2 Table). These RNAs were annotated and classified by the Blast2GO software (http://www.blast2go.com/) with the NR database and e-values < 1 e^{-5}. Unknown transcripts were classified as unknown coding RNA or noncoding RNA (ncRNA) based on the length of the longest open reading frame (> 100 amino acids = unknown coding RNA, < 100 amino acids = ncRNA). Finally, ncRNAs were considered as long ncRNAs (lncRNAs) if their length is > 200 nt. The remaining ncRNAs were considered as other ncRNAs. To profile the tissue expression features of the assembled transcripts, we derived the count and expression levels (fragments per kilobase per million mapped reads) from RNA-seq data by using the RSEM pipeline with default parameters [3]. RNA-seq datasets were selected from the 12 RNA-seq datasets (S1 Table). The selected sets were produced from the same sequencing platform (Hiseq2000) with pair-end sequencing, high-depth sequencing, and five different tissue samples (SRR1043998, SRR1045051, SRR1020591, RNAseq_1, and RNAseq_2) (S3 Table).

Annotation and quantification of miRNAs in *S. miltiorrhiza*

Four sRNA-Seq data were downloaded from the NCBI database with SRA formation (S3 Table). The other sRNA-Seq data (sRNAseq_1) were added for miRNA identification and tissue expression analysis; these data were sequenced on the Hiseq2000 platform by using the hairy root of *S. miltiorrhiza* (S3 Table).

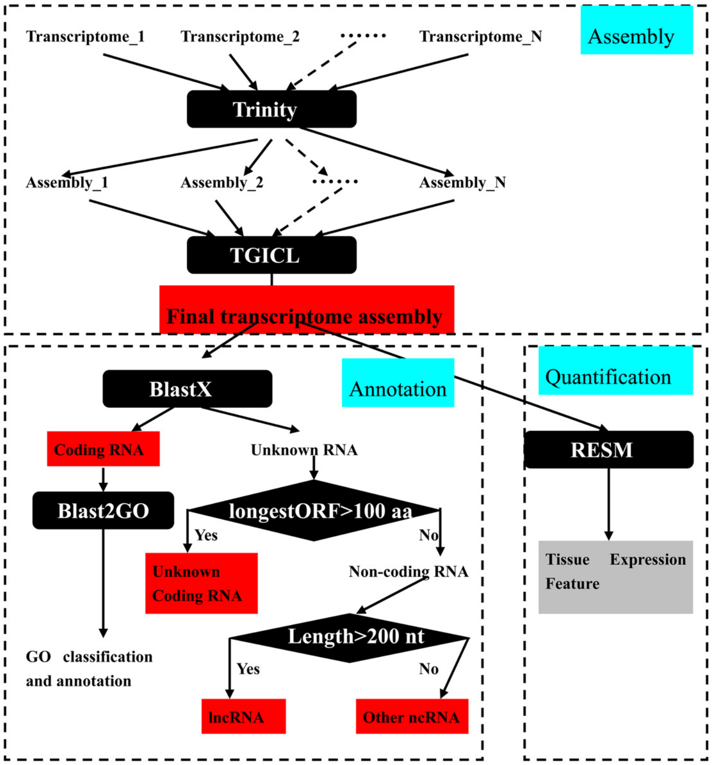


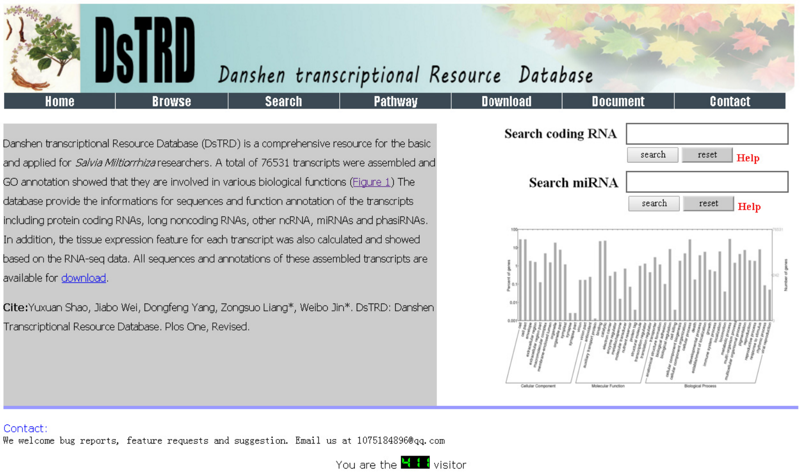
Fig 1. Flowchart of the assembly, annotation, and quantification of RNA-seq in *S. miltiorrhiza*.

doi:10.1371/journal.pone.0149747.g001

In DsTRD, miRNAs were classified into three types. The first type is named as "miRxxx-like," with a sequence similar to the known "miRxxx" in other plants (published in miRBase with the version 21.0). The precursor with stem-loop structure of this miRNA could not be retrieved from the assembled transcripts. The second type is named as "Smi-miRxxx," with a sequence also similar to the known "miRxxx" in other plants (published in miRBase with the version 21.0). But the precursor with stem-loop structure of this miRNA could be retrieved from the assembled transcripts. The third is novel miRNA identified in a plant and satisfied the criteria established by Meyers et al. [4] and Thakur et al [5]. Among novel miRNAs, those supported with the reads of miRNA* were annotated as real novel miRNAs, whereas the unsupported reads were annotated as novel miRNA candidates. To profile the tissue expression features of miRNAs, we derived the counts and expression levels (reads per million, RPM) from sRNA-seq data by using an in-house Perl script.

Annotation and quantification of the phased secondary small interfering RNAs (phasiRNA) in *S. miltiorrhiza*

Phased, secondary small-interfering RNAs (phasiRNAs) are important in post-transcriptional regulatory networks in plants [6]. Well-characterized trans-acting siRNAs (tasiRNAs), as a special subgroup of phasiRNAs, are initiated by miRNA-mediated cleavage and converted to dsRNA to yield siRNAs in a 21-nt phase [6–8]. All sRNA-Seq data applied in the miRNA annotation were used for phasiRNA identification and quantification through the PhaseTank pipeline with default parameters [9]. The identified phasiRNA genes triggered by miRNAs were also considered as tasiRNAs and named "TASxxx," whereas the remaining phasiRNA



Danshen transcriptional Resource Database (DsTRD) is a comprehensive resource for the basic and applied for *Salvia Miltiorrhiza* researchers. A total of 76531 transcripts were assembled and GO annotation showed that they are involved in various biological functions (Figure 1) The database provide the informations for sequences and function annotation of the transcripts including protein coding RNAs, long noncoding RNAs, other ncRNA, miRNAs and phasiRNAs. In addition, the tissue expression feature for each transcript was also calculated and showed based on the RNA-seq data. All sequences and annotations of these assembled transcripts are available for download.

Cite: Yuxuan Shao, Jiabo Wei, Dongfeng Yang, Zongsuo Liang*, Weibo Jin*. DsTRD: Danshen Transcriptional Resource Database. Plos One, Revised.

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Fig 2. DsTRD home page.

doi:10.1371/journal.pone.0149747.g002

genes were named “PASxxx” in the DsTRD. We also derived the counts and RPM expression levels to profile the tissue expression features of phasiRNAs by using an in-house Perl script.

Database design and implementation

The web pages of DsTRD were constructed using a Hypertext Preprocessor (PHP) language and run on a Linux system (Centos 6.4). All data were stored in a MySQL (5.1.66) database (http://www.mysql.com), whereas the assembled transcripts, miRNAs, and phasiRNAs for download were stored as files in FASTA format.

Results and Discussion

Web interface of DsTRD

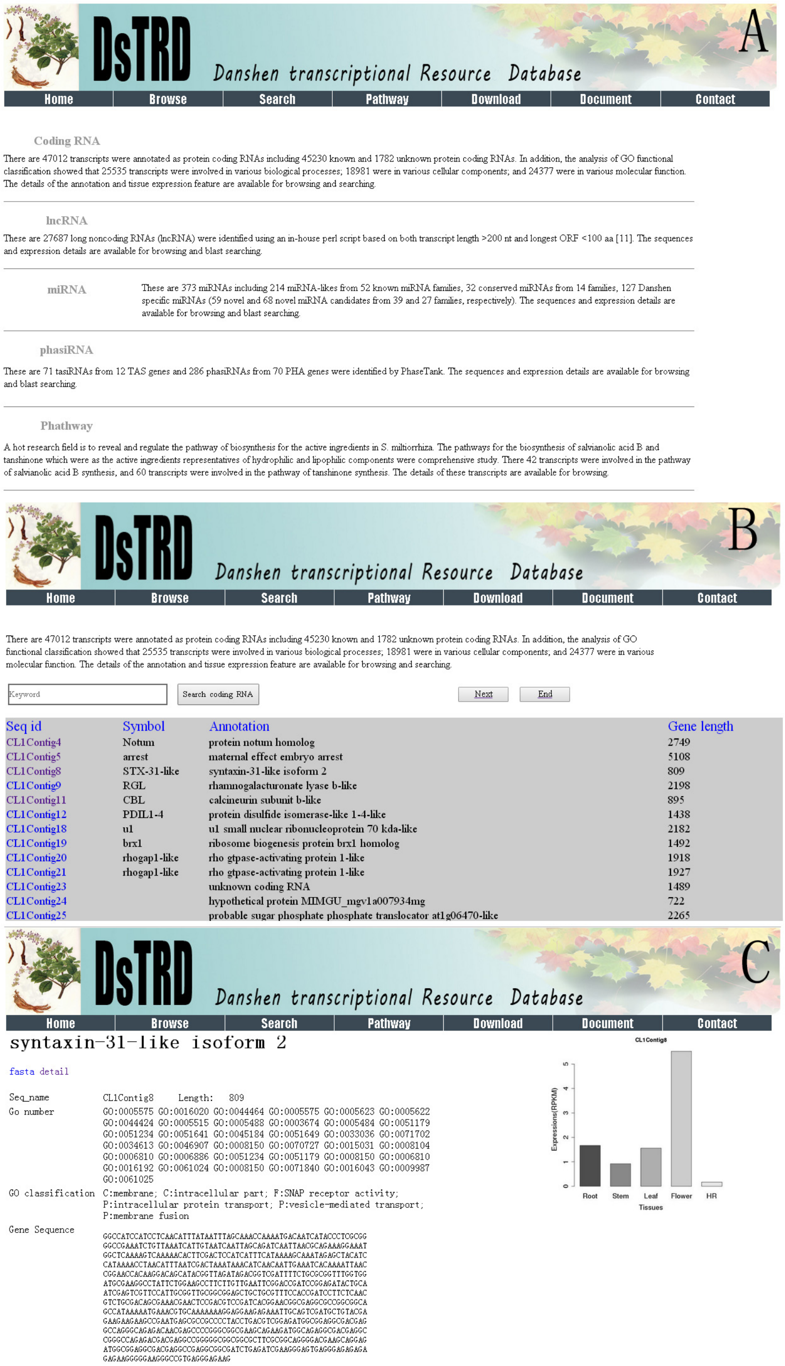
We created a freely accessible web interface for DsTRD. The interface comprises six core-page sections: “Home,” “Browse,” “Search,” “Pathway,” and “Download” (Fig 2).

Home page

Users can access DsTRD at http://bi.sky.zstu.edu.cn/DsTRD/home.php. The home page contains an introduction to DsTRD and a search engineer (Fig 2). This section provides users an outline of DsTRD and can be conveniently used to search gene information.

Browse

Browse facilitates users to browse the details of various transcripts, including coding RNAs, lncRNAs, miRNAs, and phasiRNAs (Fig 3A). Users can click the coding RNA, lncRNA, miRNA, and phasiRNA to browse the summaries (Seq id, annotation, and the length of the transcripts) of the corresponding RNAs. Moreover, data source can be selected in the search results to download the sequences and information in FASTA format (Fig 3B). The details of each transcript is displayed by clicking on “Seq id.” The tissue expression features for each transcript is also shown in the information page (Fig 3C).



Coding RNA

There are 47012 transcripts were annotated as protein coding RNAs including 45230 known and 1782 unknown protein coding RNAs. In addition, the analysis of GO functional classification showed that 25535 transcripts were involved in various biological processes; 18981 were in various cellular components; and 24377 were in various molecular function. The details of the annotation and tissue expression feature are available for browsing and searching.

lncRNA

These are 27687 long noncoding RNAs (lncRNA) were identified using an in-house perl script based on both transcript length >200 nt and longest ORF <100 aa [11]. The sequences and expression details are available for browsing and blast searching.

miRNA

These are 373 miRNAs including 214 miRNA-likes from 52 known miRNA families, 32 conserved miRNAs from 14 families, 127 Danshen specific miRNAs (59 novel and 68 novel miRNA candidates from 39 and 27 families, respectively). The sequences and expression details are available for browsing and blast searching.

phasiRNA

These are 71 tasiRNAs from 12 TAS genes and 286 phasiRNAs from 70 PHA genes were identified by PhaseTank. The sequences and expression details are available for browsing and blast searching.

Phathway

A hot research field is to reveal and regulate the pathway of biosynthesis for the active ingredients in S. miltiorrhiza. The pathways for the biosynthesis of salvianolic acid B and tanshinone which were as the active ingredients representatives of hydrophilic and lipophilic components were comprehensive study. There 42 transcripts were involved in the pathway of salvianolic acid B synthesis, and 60 transcripts were involved in the pathway of tanshinone synthesis. The details of these transcripts are available for browsing.

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Seq id	Symbol	Annotation	Gene length
CL1Contig4	Notum	protein notum homolog	2749
CL1Contig5	arrest	maternal effect embryo arrest	5108
CL1Contig8	STX-31-like	syntaxin-31-like isoform 2	809
CL1Contig9	RGL	rhamnogalacturonate lyase b-like	2198
CL1Contig11	CBL	calcineurin subunit b-like	895
CL1Contig12	PDIL1-4	protein disulfide isomerase-like 1-4-like	1438
CL1Contig18	u1	u1 small nuclear ribonucleoprotein 70 kda-like	2182
CL1Contig19	brx1	ribosome biogenesis protein brx1 homolog	1492
CL1Contig20	rhogap1-like	rho gtpase-activating protein 1-like	1918
CL1Contig21	rhogap1-like	rho gtpase-activating protein 1-like	1927
CL1Contig23		unknown coding RNA	1489
CL1Contig24		hypothetical protein MIMGU_mgv1a007934mg	722
CL1Contig25		probable sugar phosphate phosphate translocator at1g06470-like	2265

syntaxin-31-like isoform 2

```
Seq_name           CL1Contig8     Length:   809
Go number          GO:0005575 GO:0016020 GO:0044464 GO:0005575 GO:0005623 GO:0005622
                   GO:0044424 GO:0005515 GO:0005488 GO:0003674 GO:0005484 GO:0051179
                   GO:0051234 GO:0051641 GO:0045184 GO:0051649 GO:0033036 GO:0071702
                   GO:0034613 GO:0046907 GO:0008150 GO:0070727 GO:0015031 GO:0008104
                   GO:0006810 GO:0006886 GO:0051234 GO:0051179 GO:0008150 GO:0006810
                   GO:0016192 GO:0061024 GO:0008150 GO:0071840 GO:0016043 GO:0009987
                   GO:0061025
GO classification  C:membrane; C:intracellular part; F:SNAP receptor activity;
                   P:intracellular protein transport; P:vesicle-mediated transport;
                   P:membrane fusion
Gene Sequence
                   GGCCATCCATCCTCAACATTTATAATTTAGCAAACCAAAATGACAATCATACCCTCGCGG
                   GGCCGAAATCTGTTAAATCATTGTAATCAATTAGCAGATCAATTAACGCAGAAAGGAAAT
                   GGCTCAAAAGTCAAAAACACTTCGACTCCATCATTTCATAAAAGCAAATAGAGCTACATC
                   CATAAAACCTAACATTTAATCGACTAAATAAACATCAACAATTGAAATCACAAAATTAAC
                   CGGAACCACAAGGACAGCATACGGTTAGATAGACGGTCGATTTTCTGCGCGGTTTGGTGG
                   ATGCGAAGGCCTATTCTGGAAGCCTTCTTGTTGAATTCGGACCGATCCGGAGATACTGCA
                   ATCGAGTCGTTCCATTGCGGTTGCGGCGGAGCTGCTGCGTTTCCACCGATCCTTCTCAAC
                   GTCTGCGACAGCGAAACGAACTCCGACGTCCGATCACGGAACGGCGAGGCGCCGGCGGCA
                   GCCATAAAAATGAAACGTGCAAAAAAAGGAGGAAGAGAAATTGCAGTCGATGCTGTACGA
                   GAAGAAGAAGCCGAATGAGCGCCGCCCCTACCTGACGTCGGAGATGGCGGAGGCGACGAG
                   GCCAGGGCAGAGACAACGAGCCCCGGGCGGCGAAGCAGAAGATGGCAGAGGCGACGAGGC
                   CGGGCCAGAGACGACGAGGCCGGGGGCGGCGGCGCTTCGCGGCAGGGGACGAAGCAGGAG
                   ATGGCGGAGGCGACGAGGCCGAGGCGGCGATCTGAGATCGAAGGGAGTGAGGGAGAGAGA
                   GAGAAGGGGGAAGGGCCGTGAGGGAGAAG
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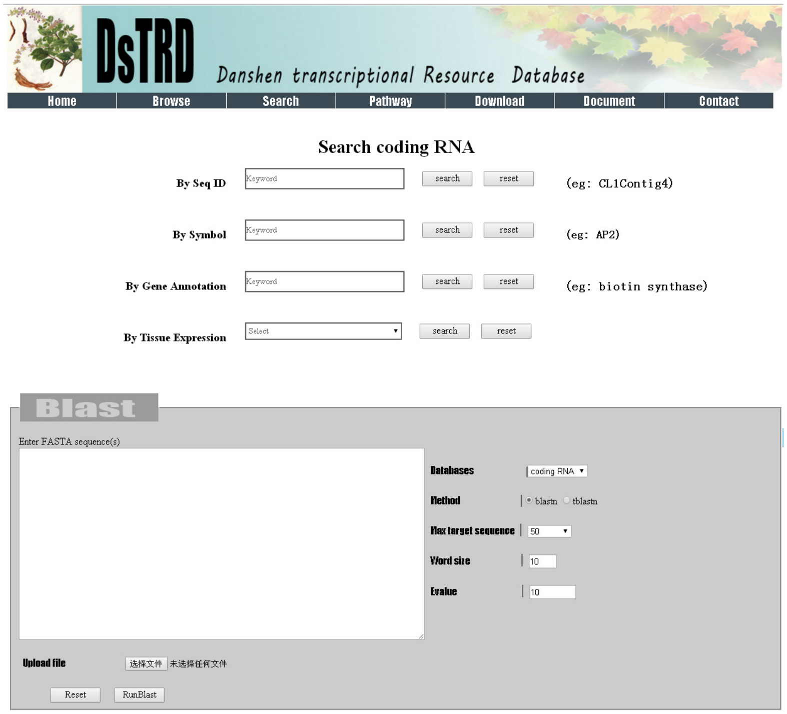
Fig 3. Information on various RNAs. (A) Users can browse the summaries of four kinds of RNAs. (B) Users can search and view the features of coding RNA. (C) Information page of RNA.

doi:10.1371/journal.pone.0149747.g003

Search

Search functionalities are designed to facilitate users to retrieve useful information. In the search frame (Fig 4), users can search for information regarding id, gene name, gene annotation, gene ontology (GO) number, and GO classification by encoding one or more key words. As DsTRD maintained the nucleotide-sequence data of various RNAs, a sequence alignment



By Seq ID: Keyword (eg: CL1Contig4)

By Symbol: Keyword (eg: AP2)

By Gene Annotation: Keyword (eg: biotin synthase)

By Tissue Expression: Select

Databases: coding RNA

Method: ● blastn ○ tblastn

Max target sequence: 50

Word size: 10

Evalue: 10

Upload file: 未选择任何文件

Fig 4. Search and Blast page.

doi:10.1371/journal.pone.0149747.g004

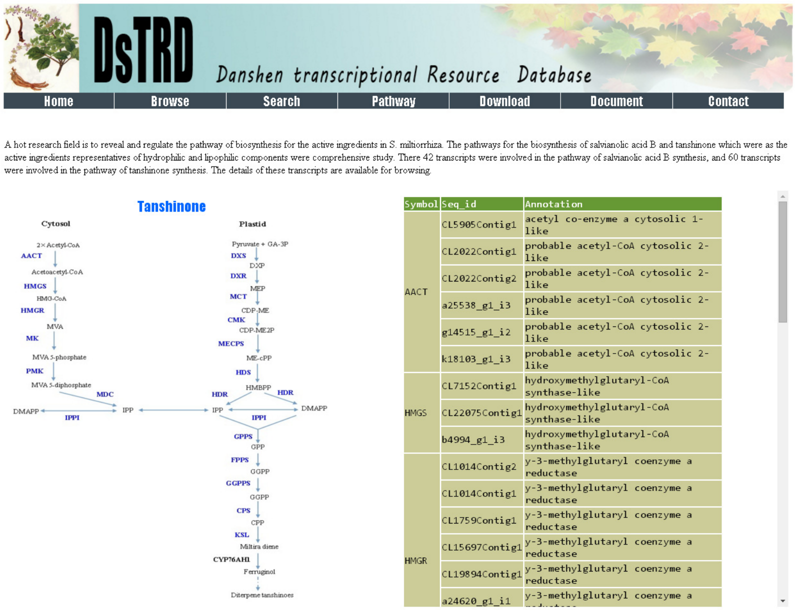
tool, namely, the Basic Local Alignment Search Tool (BLAST), was integrated into DsTRD. Users can compare a query sequence with a library of coding RNA, lncRNA, miRNA, and phasiRNA sequences by using BLAST search and identify the sequences that closely resemble the query sequence. BLAST will meet the users' requirements in finding homologous transcripts of interest. In the BLAST frame (Fig 4), users can supply one or more query sequences by uploading or directly pasting these sequences to search against the available databases using BLAST default parameters. Users can also specify additional parameters for BLAST search to control search sensitivity and result format as desired. The BLAST results are displayed in another page in a pairwise format by default.

Pathway

A hot research field is included to reveal and regulate biosynthesis pathways involving the active ingredients of *S. miltiorrhiza*. The biosynthesis pathways for salvianolic acid B and tanshinone, which are active ingredient representatives of the hydrophilic and lipophilic components, respectively, were comprehensively studied. By searching DsTRD, we found that 46 transcripts participate in salvianolic acid B synthesis, and 60 transcripts are involved in tanshinone synthesis (Fig 5). In the pathway page, users can view the outlines of these two pathways. Users can also view detailed information on each RNA of the enzyme by clicking the enzyme name (showed in blue font) on the pathways.

Downloads

DsTRD data can be downloaded to perform local analysis. In the download page, users can download coding RNAs, lncRNAs, miRNAs and their precursors, and phasiRNA and their precursors in FASTA format.



A hot research field is to reveal and regulate the pathway of biosynthesis for the active ingredients in S. miltiorrhiza. The pathways for the biosynthesis of salvianolic acid B and tanshinone which were as the active ingredients representatives of hydrophilic and lipophilic components were comprehensive study. There 42 transcripts were involved in the pathway of salvianolic acid B synthesis, and 60 transcripts were involved in the pathway of tanshinone synthesis. The details of these transcripts are available for browsing.

Symbol	Seq_id	Annotation
AACT	CL5905Contig1	acetyl co-enzyme a cytosolic 1-like
	CL2022Contig1	probable acetyl-CoA cytosolic 2-like
	CL2022Contig2	probable acetyl-CoA cytosolic 2-like
	a25538_g1_i3	probable acetyl-CoA cytosolic 2-like
	g14515_g1_i2	probable acetyl-CoA cytosolic 2-like
	k18103_g1_i3	probable acetyl-CoA cytosolic 2-like
HMGS	CL7152Contig1	hydroxymethylglutaryl-CoA synthase-like
	CL22075Contig1	hydroxymethylglutaryl-CoA synthase-like
	b4994_g1_i3	hydroxymethylglutaryl-CoA synthase-like
HMGR	CL1014Contig2	y-3-methylglutaryl coenzyme a reductase
	CL1014Contig1	y-3-methylglutaryl coenzyme a reductase
	CL1759Contig1	y-3-methylglutaryl coenzyme a reductase
	CL15697Contig1	y-3-methylglutaryl coenzyme a reductase
	CL19894Contig1	y-3-methylglutaryl coenzyme a reductase
	a24620_g1_i1	y-3-methylglutaryl coenzyme a

Fig 5. Pathway page.

doi:10.1371/journal.pone.0149747.g005

Conclusion

S. miltiorrhiza is an extensively investigated medicinal model plant because of its small genome, short life cycle, and stable genetic transformation system. However, the lack of available genome sequences and the limited EST sequences stored in NCBI for *S. miltiorrhiza* considerably restrict study progress on the molecular mechanisms of this species. In this study, we aim to produce large numbers of transcript sequences with corresponding annotation information and make these data freely accessible to users. As such, we developed DsTRD, which exhibits simplicity of use for researchers and contains 76531 transcribed sequences assembled from the RNA-seq data. This database provides information regarding the sequences and functional annotations of the transcripts, including protein-coding RNAs, lncRNAs, other ncRNA, miRNAs, and phasiRNAs. The database also includes the tissue expression feature for each transcript, which was calculated and shown based on RNA-seq data. As an efficient bioinformatics tool, DsTRD is important in studying various kinds of molecular processes in *S. miltiorrhiza*.

Availability and Requirements

DsTRD is publicly available at http://bi.sky.zstu.edu.cn/DsTRD/home.php. DsTRD supports all the latest major web browsers, preferably Mozilla Firefox, Google Chrome, or Apple Safari, for visualization and performance purposes.

Supporting Information

S1 Table. Transcriptome data downloaded from the NCBI database according to the accession numbers.
(DOC)

S2 Table. All assembled transcripts in fasta format.
(ZIP)

S3 Table. sRNA-Seq data downloaded from the NCBI database according to the accession numbers.
(DOC)

Author Contributions

Conceived and designed the experiments: ZL WJ. Performed the experiments: YS JW. Analyzed the data: YS. Contributed reagents/materials/analysis tools: HZ DY. Wrote the paper: FW WJ.

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