# Peer

# Genome-wide identification of putative dihydroflavonol 4-reductase (DFR) gene family in eight Solanaceae species and expression analysis in Solanum lycopersicum

Wenjing Li<sup>1,2,3,\*</sup>, Yiming Zhang<sup>1,\*</sup>, Hualiang Liu<sup>4</sup>, Qiuping Wang<sup>1</sup>, Xue Feng<sup>1</sup>, Congyan Wang<sup>1</sup>, Yanxiang Sun<sup>1</sup>, Xinye Zhang<sup>1,2,3</sup> and Shu Zhu<sup>1</sup>

<sup>1</sup> College of Life Science, Langfang Normal University, Langfang, Hebei, China

<sup>2</sup> Hebei Key Laboratory of Animal Diversity, Langfang, Hebei, China

<sup>3</sup> Langfang Key Laboratory of Cell Engineering and Application, Langfang, Hebei, China

<sup>4</sup> Xingtai University, Xingtai, Hebei, China

\* These authors contributed equally to this work.

### ABSTRACT

Dihydroflavonol 4-reductase (DFR; EC1.1.1.219) is an important rate-limiting enzyme in the plant flavonoid pathway toward both anthocyanins and proanthocyanidins. Although DFR genes have been isolated from multiple plants and their functions have been well characterized in some plants, little is known about DFRs in Solanaceae species. Therefore, in this study, we performed genome-wide analysis and identified 6, 5, 4, 5, 5, 6, 6 and 5 DFR gene family members in eight Solanaceae species (S. lycopersicum, S. pennellii, S. tuberosum, S. melongena, C. annuum, N. tabacum, P. inflata, and P. axillaris) respectively. The putative DFR genes were systematically identified using bioinformatics to predict their protein properties, cellular location, phylogenetic relationships, gene structure, conserved motifs, and *cis*-acting elements in the promoters. Furthermore, quantitative real-time PCR (qRT-PCR) was used to identify the expression pattern of DFRs in tomato. We classified all DFRs into five groups based on their phylogenetic features. Sequence analysis showed that all encoded DFR protein sequences possess a highly conserved NAD-dependent epimerase/dehydratase. In addition, almost all the members of each group displayed similar gene structures and motif distributions, which might be related to their identical executive functions. All 42 DFRs possess a series of lightresponsive, phytohormone-responsive, MYB-responsive, stress-responsive, and tissue-specific expression-related cis-elements in the promoter sequences. qRT-PCR analysis showed that tomato DFRs were expressed in many different organs. This study will provide a theoretical basis for further investigation of the function of DFRs in Solanaceae.

**Subjects** Bioinformatics, Genomics, Molecular Biology, Plant Science **Keywords** Solanaceae, Dihydroflavonol 4-reductase (DFR), Gene expression patterns, Genomewide analysis

Submitted 8 March 2023 Accepted 27 August 2023 Published 20 September 2023

Corresponding author Shu Zhu, zhushu@lfnu.edu.cn

Academic editor Sushma Naithani

Additional Information and Declarations can be found on page 16

DOI 10.7717/peerj.16124

Copyright 2023 Li et al.

Distributed under Creative Commons CC-BY 4.0

#### **OPEN ACCESS**

### INTRODUCTION

In many plants, anthocyanins are major secondary metabolites that have been widely studied due to their important properties. They are involved in functions related to determining fruit and flower color and mitigating naturally occurring stresses to the plant (Grotewold, 2006; Qiu et al., 2016; Kim et al., 2017). Furthermore, anthocyanins were reported to delay over-ripening in tomato fruits and their over-expression resulted in a substantial increase in fruit shelf-life (Bassolino et al., 2013; Zhang et al., 2013). In recent years, the anthocyanin biosynthetic pathway has been well studied in several plants, such as Arabidopsis thaliana (Gonzalez et al., 2008), Zea mays (Petroni & Tonelli, 2011; Pourcel et al., 2012), Petunia hybrida, Antirrhinum majus (Winkel-Shirley, 2001), Malus domestica (Espley et al., 2013), and Brassica oleracea var. capitata f. rubra (Sasaki, 2020). Most of the genes involved in the flavonoid synthesis pathway have been identified, and it has been established that dihydroflavonol 4-reductase (DFR) is a pivotal multifunctional oxidoreductase involved in anthocyanin biosynthesis, which can selectively or unselectively catalyze the reduction of three colorless dihydroflavonols (DHFs)dihydrokaempferol (DHK), dihydroquercetin (DHQ), and dihydromyricetin (DHM)-to their corresponding leucoanthocyanidins in an NADPH-dependent manner regulated by the MYB-bHLH-WD40 (MBW) complex (Tian et al., 2017). Leucoanthocyanidins are subsequently converted into their respective anthocyanidins and other flavonoids (Li et al., 2017).

To date, many DFRs have been cloned from multiple plants and DFR mutations have been shown to cause the loss of anthocyanins and proanthocyanidins in plants (*Zhu et al.*, 2018). Although DFR proteins can catalyze the above three substrates in an appreciable number of plants, DHK cannot be used as a substrate in *Petunia* and *Cymbidium* species. As such, these species cannot produce pelargonidin-based orange flowers, indicating that DFRs from different species exhibit different substrate preferences (Forkmann & Ruhnau, 1987; Johnson et al., 1999). In view of its substrate specificity, DFR controls the flux into the three biosynthetic branches, leading to diverse anthocyanidins (cyanidin, delphinidin, pelargonidin) (Forkmann & Ruhnau, 1987; Johnson et al., 1999). The substrate preference of DFR from different plants can be determined by using recombinant proteins to analyze the enzyme activity (Johnson et al., 2001; Katsu et al., 2017; Zhu et al., 2018). Furthermore, we can elucidate the crystal structure to determine why most DFRs accept dihydroflavonols with different hydroxylation patterns. However, the purified DFR protein was described as very unstable; therefore, alignment of amino acid sequences—especially in the region responsible for substrate specificity—was the most effective way to determine the substrate preference (Petit et al., 2007).

Alignment and crystal structure studies characterized all DFRs containing an NADPH-binding Rossmann domain at the N-terminus and substrate-binding specificity in the variable C-terminus (*Petit et al., 2007*). The amino acid region from 131–156 has been characterized as the substrate-binding site. In particular, an asparagine (Asn, N) or aspartic acid (Asp, D) residue at position 134 has been shown to be associated with substrate recognition, although the variant N134D may not be specific only for recognizing

the three hydroxylation patterns in the B-ring of dihydroflavonols. Once we have identified the region that determines the substrate specificity of DFR, we can modulate the substrate specificity by mutating the amino acids in that region. To achieve this, scientists have generated chimeric *DFRs* using petunia *DFR*, which cannot reduce DHK, and gerbera *DFR*, which can reduce DHK, and introduced the chimeric *DFRs* to a mutant petunia line (*Johnson et al., 2001*). The first successful petunia flower color engineering was achieved using *DFR* cDNA cloned from maize (*Meyer et al., 1987*); Since then, multiple homologs have been cloned to modify anthocyanins (*Rosati et al., 2003*; *Davies et al., 2003*) and proanthocyanidins (*Bavage et al., 1997*; *Robbins et al., 1998*). The overexpression of functionally active DFR enzymes definitively increases anthocyanin accumulation in rice (*Takahashi et al., 2006*), tobacco (*Xie et al., 2004*), forsythia (*Rosati et al., 1997*), crabapple (*Tian et al., 2017*), and others.

To date, a few of gene families of Solanaceae, including argonautes (Liao et al., 2020), WOX (Li et al., 2018), and SAUR (Wu et al., 2012), have been identified by bioinformatics methods; in addition, the functions of some genes have been identified. However, despite many efforts, studies of the identification and expression pattern of DFR gene families in Solanaceae are scarce. In Solanaceae, different species display diverse fruit or flower colors due to various degrees of anthocyanin accumulation. In spiny solanums, variation in the DFR promoter region and the alternative splicing of DFR account for altered anthocyanin accumulation (Wang et al., 2022). In this study, we performed a systematic study to identify and characterize the DFR gene family in the genomes of eight Solanaceae species, including tomato (Solanum lycopersicum), wild tomato (Solanum pennellii), potato (Solanum tuberosum), eggplant (Solanum melongena), pepper (Capsicum annuum), tobacco (Nicotiana tabacum), and two petunia species (Petunia inflata and Petunia axillaris). Based on whole-genome sequencing results, the members of the putative DFR gene family in Solanaceae were identified by using bioinformatics analysis; subsequently, their sequence features, gene structure, evolutionary relationships, conserved motifs, chromosome distribution, cellular location, and *cis*-acting elements in the promoter were analyzed. Finally, the expression pattern of DFR in S. lycopersicum was analyzed by qRT-PCR methods. This fundamental research can provide a foundation for further research into the physiological and functional studies of the DFR family in S. lycopersicum and other related Solanaceae species.

# MATERIALS AND METHODS

### Plant materials and tissue collection

Solanum lycopersicum ('Micro-Tom') seeds were collected from Guangdong Ocean University and were sprouted in a greenhouse at 25 °C under a 16-h light/8-h dark cycle at Langfang Normal University. The seeds were sterilized for 10 min with 10% sodium hypochlorite and washing five times with sterile water. Five tomato major tissues—the 45day-old seedling roots, stems, leaves, flowers, and green ripening fruits—were collected and conserved at -80 °C after liquid nitrogen treatment; three biological replicates were performed for each sample.

### DFR sequence retrieval and data analysis

The DFR sequences of Arabidopsis thaliana, Solanum lycopersicum, and Vitis vinifera were retrieved from Phytozome v13 (https://phytozome-next.jgi.doe.gov/) using the KEGG codes (K13082). The sequences were At5g42800.1, Solyc02g085020.4.1, and VIT\_218s0001g12820.1 (Kim et al., 2017; Zhu et al., 2018; Li et al., 2019), which were previously reported and were used as queries to extract the DFR genes of eight Solanaceae species. The local BLAST program was performed against the genomic sequence of the eight Solanaceae species in the Solanaceae Genomics Network (www.solgenomics.net) with -5 expect (E) threshold. A total of 42 candidate members were found as listed in Table 1. All the candidate protein sequences were further checked for the presence of epimerase domains (PF01370) using the Pfam tool; the bit score between each member and at least two probes was not to be less than 240, and the candidate DFR genes were aligned to ensure that no gene was represented repeatedly. The number of amino acids, molecular weight (MW), isoelectric point (pI), instability index, and grand average of hydropathicity (GRAVY) index of the candidate DFR proteins were identified using ExPASy (http://web.expasy.org/protparam/). The cellular location was identified using CELLO v.2.5 (cello.life.nctu.edu.tw/).

### Phylogenetic analyses of the DFR gene family

The full-length DFR amino acid sequences of the eight species were aligned using MUSCLE or ClustalW (an inbuilt feature of MEGA 11.0) (*Edgar*, 2004). For phylogenetic analysis, the neighbor-joining (NJ) phylogenetic tree of the *DFR* gene family was constructed using MEGA 11.0 by performing 1,000 bootstraps (*Tamura*, *Stecher & Kumar*, 2021).

# Exon-intron structure and conserved motifs analysis

The exon-intron organization of the *DFR* genes were analyzed by comparing their respective coding and genomic sequence information in the Solanaceae Genomics Network database. Gene structure was presented using the Gene Structure Display Server (GSDS 2.0) (http://gsds.cbi.pku.edu.cn/) (*Hu et al., 2015*). Besides, MEME program 5.1.1 was used to identify finer motifs in the candidate DFR protein sequences (*Bailey et al., 2006*). The parameters were set as: site distribution, 0 or 1 site per sequence; number of motifs to find, 6; and width of the motif, 6–300 residues.

### In silico analysis of promoter sequences

To investigate the putative role of *cis*-acting elements that were responsible for gene expression, the upstream sequence (2,000 bp) of each coding sequence was retrieved from the Solanaceae Genomics Network. The sequences were analyzed by different bioinformatics programs, including PlantCARE (*Lescot et al., 2002*) and PLACE (*Higo et al., 1999*).

Table 1 D	FR genes identified from e	eight sequenced Solanaceae genom	es.
Index	Abbreviation	Species	Number of DFR genes
1	Sl	Solanum lycopersicum	6
2	Sp	Solanum pennellii	5
3	St	Solanum tuberosum	4
4	Sm	Solanum melongena	5
5	Ca	Capsicum annuum	5
6	Na	Nicotiana attenuata	6
7	Pi	Petunia inflata	6
8	Pa	Petunia axillaris	5
Total			42

### Gene expression analysis of SIDFRs in tomato

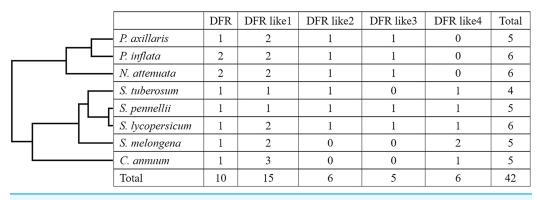
To verify the expression profiles of six *SlDFR* genes, qRT-PCR was used to measure the expression of *SlDFRs* in different tomato tissue samples, including the roots, stems, leaves, flowers, and fruits. Total RNA was isolated using RNAsimple total RNA kit according to the manufacturer's recommendations (TIANGEN, Beijing, China). The quantity and quality of total RNA samples were tested using a Nanodrop spectrophotometer (Thermo, Waltham, MA, USA) and RNA gel electrophoresis. The DNase I-treated RNA was reverse-transcribed using GoScript<sup>™</sup> Reverse Transcription System I (Promega, Madison, WI, USA), and qRT-PCR was performed using a CFX96 Touch thermocycler (Bio-Rad, Hercules, CA, USA). Gene-specific primers were designed using Primer 5.0 software to amplify 121–165 bp PCR products specific for each *SlDFR* gene (Table 2). The expression of the tomato Actin gene (GenBank accession no. FJ532351.1) was used as the internal control. Each reaction mixture contained 10  $\mu$ L of 2  $\times$  SYBR Green qPCR Mix (Low ROX) (Aidlab, Beijing, China), 1.0 µL of diluted cDNA sample, and 400 nM of gene-specific primers in a final volume of 20  $\mu$ L. The thermal cycling protocol used was as follows: 95 °C for 10 min, followed by 40 cycles of 95  $^\circ C$  for 15 s, 56  $^\circ C$  for 15 s, and 72  $^\circ C$  for 20 s. After the qRT-PCR reaction was completed, a melting curve was generated to analyze the specificity of each gene by increasing the temperature from 60 °C to 95 °C. Three independent biological replicates of the experiment were performed, and the significance was determined with IBM SPSS Statistics 20 software ( $p \le 0.05$ ).

# RESULTS

# Identification and physicochemical properties of *DFR* gene family members in Solanaceae species

We used the amino acid sequences of *DFR* genes in *Arabidopsis*, *Vitis bellula*, and *S. lycopersicum* as a target to query for related Solanaceae DFRs. Through comprehensive screening, 42 DFR protein sequences were retrieved, including six from tomato (*S. lycopersicum*), tobacco (*N. tabacum*), and petunia (*P. inflata*), four from potato (*S. tuberosum*), and five from wild tomato (*S. pennellii*), eggplant (*S. melongena*), pepper

Table 2	The primers used in this	s study.	
S. No	Name	Sequence	Product size (bp)
1	SIDFR	TTGGCTTGTCATGAGACTCC CCTTCCACTGCCAAGTCAGC	150
2	SlDFR like1-1	GATCATGGCTCATCATGAGG CTCTCTGATGCACCTTCTAG	121
3	SIDFR like1-2	CAGGATACCTAGCATCATGG TAGCCTTTGGGATGCTTCAG	137
4	SIDFR like2	GGACAGATGTTGAGTTCTTG AAGATCAACACTACTTGGAG	165
5	SIDFR like3	CAATGAGAGGTTGCATTGGC TTCAGAACATTCAAGGTCCC	136
6	SIDFR like4	AAGGGTAAAGTATGTGTGAC GCTCCTTGTAGCTTCCATAG	149



**Figure 1** Distribution of the *DFR* genes in different plant species and groups. The numbers in each column represent the number of genes in that species. Full-size DOI: 10.7717/peerj.16124/fig-1

(*C. annuum*), and petunia (*P. axillaris*) (Table 1, Fig. 1). For convenience, all genes were designated as *DFR*, *DFR like1*, *DFR like2*, *DFR like3*, and *DFR like4* in different species, according to their similarity (Table 3).

Table 3 lists the 42 *DFR* genes and the proteins they encode, including gene name, length, molecular weight, isoelectric point, GRAVY index, instability index, cellular location, and chromosome start and end location (Fig. 1, Table 3). The number of amino acid of *DFR* genes ranged from 282 to 427 aa with an average of 340 aa. Molecular weights varied from 31,855.70 to 46,908.53 Da, and the isoelectric points were distributed from 5.23 to 9.18, which indicates that DFRs, except for PiDFR1, PiDFR2, PiDFR like3, and PaDFR like3, are all acidic proteins. A large divergence in GRAVY indices was observed, from -0.325 to 0.114, with the average GRAVY index of -0.18, -0.18, 0.08, -0.06, and -0.15 in five subfamilies, respectively. The instability index of these genes varied from 23.33 to 44.59 with an average of 34.63; most of the proteins were classified as stable proteins (83.3%). Most of DFRs located in cytoplasmic, except for PaDFR like2, SmDFR like4-1 and PiDFR2 located in periplasmic, and PaDFR like1-2 located in outer membrane.

5. b6. bColumnProtectProtectProtectEachColumn <th< th=""><th>Gene must be the first section in the first sectin the first section in the first section in the first section i</th><th>F</th><th>able 3 The det</th><th>Table 3 The details of DFR gene family identified</th><th></th><th>in Solanaceae.</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></th<>	Gene must be the first section in the first sectin the first section in the first section in the first section i	F	able 3 The det	Table 3 The details of DFR gene family identified		in Solanaceae.								
NormalizedAnd weight(NindexChromisomeShaffidd<	Name         Name <th< th=""><th>Ś</th><th></th><th></th><th>Protein/</th><th>Molecular</th><th>pI</th><th>GRAVY</th><th>Instability</th><th>Cellular</th><th>Instability</th><th>Location</th><th></th><th></th></th<>	Ś			Protein/	Molecular	pI	GRAVY	Instability	Cellular	Instability	Location		
GIPR         Salyca206500.4.1         379         4.21.8.49         5.57         -0.208         3.7.8         Cytoplasmic         3.7.8         2         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         6.10         0.023         0.05         0.055         1         7.79355         6.066535         1         7.79355         6.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.066535         4.0112         3.33         3.35712         3.35         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37731         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3.37331         3	BDFR         Skycloges00.4.1         579         4.218.49         557         -0.208         3.3.78         Cytoplasmic         3.3.78         2         4.0665535           SUPRF like1.1         Skyclog931470.3.11         324         36.655.29         6.10         0.03         35.51         Cytoplasmic         3.55         1         7.515.44           SUPRF like1.2         Skyclog95147.0.3.1         334         3.657.529         6.61         0.010         3.53         Cytoplasmic         3.55         1         7.515.44           SUPRF like2         Skyclog0550.0.1         334         3.734.52         6.64         0.010         3.65         Cytoplasmic         3.65         3.9177.14         1         37.545.64         3.946         Cytoplasmic         3.05         2.23211         31         37.545.64         3.946         Cytoplasmic         3.05         2.23211         31         37.545.66         0.01         3.246         Cytoplasmic         3.12         2.23211         31         3.246.67         0.46         0.01         3.23         Cytoplasmic         3.22         2.23211         3         3.23517         3.317.33         3.317.33         3.317.33         3.317.33         3.317.33         3.317.33         3.317.33         3.317.33	ž	•	4	AA	weight/D			index	localization	index	Chromosome /Scaffold	Start	End
SIDPR like1.         Solyclygol-070.3.1         324         56.55.2.9         6.10         0.023         30.5         Cytoplasmic         30.5         1         7791554         77           SIDPR like1.         Solyclygol10.0.4.1         3.27         35.57.9         5.51         Cytoplasmic         35.51         5         60050.35         6         60.00         36.57         Cytoplasmic         35.51         5         60050.35         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         3         31.77.1         31.77.1         31.77         3         31.77.1         31.77         3         31.77.1         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3         31.77         3 <t< td=""><td>SUPR like!         Solvolig06407.0.11         21         5655.2.9         610         0.029         35.5         Cyoplasmic         35.5         1         7731554           SUPR like!         Solyclig06407.0.11         324         55.77.69         55.3         0.102         35.51         5         06005195           SUPR like!         Solyclig061700.41         334         57.77.69         58.3         0.123         55.57         55</td><td>-</td><td>SIDFR</td><td>_</td><td>379</td><td>42,318.49</td><td>5.97</td><td>-0.208</td><td>33.78</td><td>Cytoplasmic</td><td>33.78</td><td>2</td><td>46065285</td><td>46067689</td></t<>	SUPR like!         Solvolig06407.0.11         21         5655.2.9         610         0.029         35.5         Cyoplasmic         35.5         1         7731554           SUPR like!         Solyclig06407.0.11         324         55.77.69         55.3         0.102         35.51         5         06005195           SUPR like!         Solyclig061700.41         334         57.77.69         58.3         0.123         55.57         55	-	SIDFR	_	379	42,318.49	5.97	-0.208	33.78	Cytoplasmic	33.78	2	46065285	46067689
SIDPR like1         Solyclogi010.11         37         35,3750         5.83         -0.12         35,51         5.57         5.57         5.57         6005195         6         0001195         6         0001195         6         0001195         6         0001195         5         0001195         5         0001195         5         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         0001195         6         00111         2133         2375123         249         250         0001195         3         391731         2         20111         2133         213315         2         20111         2133         2         20111         213315         2         20111         20111         213315         2         20111         213315         2         20111         213315         2         20111         20111         20111         20111         20111         20111         20111         20111         20111         20111         20111         201111         201111         20111<	BUFR like1         Solyclog(51010.4.1         327         35.8769         5.83         -0.192         35.51         Cytoplasmic         35.51         5         0000135           SUPR like1         Solyclog(5110.0.4.1         31         37.37126         6.67         0.114         2.33         Cytoplasmic         39.67         12         39.771           SUPR like1         Solyclog(9530.1.1)         314         37.9452         6.46         -0.00         3.967         Cytoplasmic         39.67         12         2.29271           SUPR like1         Sopen01g(97380.1.1)         318         7.74520         6.4         -0.017         3.747         Cytoplasmic         3.867         3.74116         3.96731         3.86532         6.4         -0.017         3.771         Cytoplasmic         3.827         2.44975         3.827         3.74120         3.86731         3.812         3.7451         2.99711         3.9731         3.9741	2	SIDFR like1-1		324	36,655.29	6.10	-0.029	30.5	Cytoplasmic	30.5	1	77915544	77917312
SIDTR like2         Solyclag031470.31         34 $56/71/59$ $667$ 0.114         2.333         Cytoplasmic         2.33         3 <td>BIFR like2         Solyclag031470.3.1         34         <math>567763</math> <math>667</math> <math>0.114</math> <math>2333</math> <math>Cytoplasmic</math> <math>2333</math> <math>397371</math>           SIDFR like3         Solyclag05780.1.1         341         <math>7794532</math> <math>646</math> <math>0.040</math> <math>3967</math> <math>72371</math> <math>22971</math>           SIDFR like1         Solyclag05780.1.1         329         <math>377722</math> <math>6.2</math> <math>-0.163</math> <math>3475</math> <math>77975</math> <math>2297121</math> <math>2297121</math>           SPDFR like1         Sopen01g077880.1         329         <math>3777226</math> <math>6.27</math> <math>-0.163</math> <math>30.71</math> <math>11</math> <math>12</math> <math>249106</math>           SPDFR like1         Sopen01g077880.1         334         <math>3463552</math> <math>6.4</math> <math>0.08</math> <math>30.71</math> <math>11</math> <math>12</math> <math>249106</math>           SPDFR like1         Sopen029073011         334         <math>3473712</math> <math>6.67</math> <math>0.116</math> <math>32.24</math> <math>(707)lasmic</math> <math>31.77</math> <math>249012</math> <math>243437</math>           SDFR like1         Sopen029003107140001390         331         <math>37371231</math> <math>3274</math> <math>249351</math> <math>249351</math> <math>249351</math>           SDFR like2         Sopen12g0013AT40000387         323         <math>347371</math></td> <td>Э</td> <td>SIDFR like1-2</td> <td></td> <td>327</td> <td>35,876.9</td> <td>5.83</td> <td>-0.192</td> <td>35.51</td> <td>Cytoplasmic</td> <td>35.51</td> <td>5</td> <td>60605195</td> <td>60608354</td>	BIFR like2         Solyclag031470.3.1         34 $567763$ $667$ $0.114$ $2333$ $Cytoplasmic$ $2333$ $397371$ SIDFR like3         Solyclag05780.1.1         341 $7794532$ $646$ $0.040$ $3967$ $72371$ $22971$ SIDFR like1         Solyclag05780.1.1         329 $377722$ $6.2$ $-0.163$ $3475$ $77975$ $2297121$ $2297121$ SPDFR like1         Sopen01g077880.1         329 $3777226$ $6.27$ $-0.163$ $30.71$ $11$ $12$ $249106$ SPDFR like1         Sopen01g077880.1         334 $3463552$ $6.4$ $0.08$ $30.71$ $11$ $12$ $249106$ SPDFR like1         Sopen029073011         334 $3473712$ $6.67$ $0.116$ $32.24$ $(707)lasmic$ $31.77$ $249012$ $243437$ SDFR like1         Sopen029003107140001390         331 $37371231$ $3274$ $249351$ $249351$ $249351$ SDFR like2         Sopen12g0013AT40000387         323 $347371$	Э	SIDFR like1-2		327	35,876.9	5.83	-0.192	35.51	Cytoplasmic	35.51	5	60605195	60608354
SIPR likelSolyc12g005350.11341 $3794.582$ $6.46$ $0.040$ $3.967$ $(7 c)$ $12$ $2.29271$ $2.29271$ SIDR likelSolyc04g008780.4.1338 $3.77322$ $6.2$ $-0.169$ $3.496$ $(7 c)$ qojasmic $3.496$ $4$ $2.4916$ $2.20119$ $5.20119$ SIDR likelSopen0g003580.1.1329 $4.26836$ $6.2$ $-0.238$ $3.122$ $C goplasmic$ $3.122$ $2.20271$ $2.293211$ SIDR likelSopen0g003580.1334 $36.5322$ $6.6$ $0.07$ $3071$ $2.499$ $2.499$ $2.4996$ $2.4996$ SIDR likelSopen03001013338 $3.7371286$ $6.67$ $-0.116$ $3.244$ $2.499$ $2.49963862$ $4.90039601$ SIDR likelSopen03DMT40009287382 $4.246978$ $5.71$ $0.171$ $3.277$ $2.499$ $2.4993862$ $4.90039601$ SIDR likelPGSC0003DMT40009287382 $4.246978$ $5.71$ $0.171$ $3.277$ $2.4993862$ $4.9003862$ SIDR likelPGSC0003DMT40009203382 $3.776507$ $5.794$ $0.177$ $2.946978$ $2.92362$ $4.9003862$ SIDR likelPGSC0003DMT40009203382 $3.74607$ $5.794$ $5.7708869$ $4.777$ $2.943732$ $2.34372$ $2.34862$ SIDR likelPGSC0003DMT40009201333 $3.7756079869990121$ $3.7756079899021$ $3.7756078990021$ $3.7756078990021$ $3.746078699090011$ $3.746078699000011$ $3.9460578$ $3.7476$ $2.94610156013666$	SIDFR likel         Solyc12g005350.11         341         3794.58         6.46         0.040         3.67         12         22921           SIDFR likel         Solyc04g0878.0.4.1         338         3737.22         6.2         -0.169         34.96         Cytoplasmic         31.82         2749106           SIDFR likel         Solyc04g0878.0.4.1         338         37,877.22         6.2         -0.183         31.82         Cytoplasmic         31.82         24990         207313           SyDFR likel         Sopen03g003360.1         334         36.535.2         6.2         -0.16         34.95         Cytoplasmic         31.82         207313           SyDFR likel         Sopen03g003360.1         341         38.27         -0.16         34.24         Cytoplasmic         31.82         264315           SyDFR likel         Sopen03g001330.1         341         38.27         0.11         31.77         24.99         264313           SyDFR likel         Sopen03g00330.1         333         37.87/172         6.67         0.17         31.77         2         26.3233           SyDFR likel         PGSC0003DMT40000337         333         37.4697         34.4         2         23.4341           Sopen0410017         333 </td <td>4</td> <td></td> <td>_</td> <td>334</td> <td>36,717.69</td> <td>6.67</td> <td>0.114</td> <td>23.33</td> <td>Cytoplasmic</td> <td>23.33</td> <td>3</td> <td>3917371</td> <td>3919983</td>	4		_	334	36,717.69	6.67	0.114	23.33	Cytoplasmic	23.33	3	3917371	3919983
SIDFR likel         Salyobagions780.11         338         37,87.2.52         6.2         -0.169         34.96         Cytoplasmic         31.82         2.449106         2           SpDRR likel         Sopen02g03720.11         382         2.4568.96         6.2         -0.283         31.82         Cytoplasmic         31.82         2.491016         3           SpDRR likel         Sopen02g0370.11         382         2.456.896         5.3         -0.073         30.71         1         1         93912119         93912119         93           SpDRR likel         Sopen03g0350.11         338         37,871.25         6.67         -0.116         3.244         Cytoplasmic         3.827         1         3.037         1         3.037         2.439382         4         3.039382         4         3.037         3.049         3.04115         3.04115         3.0411         1         3.077         2         3.071         2.04913886         5         3.04115         2         3.043215         3.24405         4         2.434315         3.24415         3.071         3.071         2         2.043386         4         3.0323415         4         3.03346         4         3.0376         4         3.0436666         0.014         3.075 <td>SIPTR         Solved-geors780.4.1         38         37,87.2.52         6.2         -0.169         34.96         Cytoplasmic         34.96         4         2.449106           SyDPR         Kspen02g03730.1.         32.9         24,666.96         5.2         -0.038         31.82         Cytoplasmic         31.82         2         2439105           SyDPR         Kikel         Sopen03g0350.1.         33.9         37,71236         5.95         -0.007         30.71         Cytoplasmic         31.77         1         1           SyDPR         Kikel         Sopen04g00350.1.         38         37,371.72         6.67         -0.16         32.94         Cytoplasmic         31.77         2         2635355           SyDPR         Kikel         Sopen04g00350.1         38         3,4737.72         6.67         -0.17         31.77         Cytoplasmic         31.77         2         2635355           SDPR         Kikel         PGSC0003DMT40000507         38         3,493607         6.67         -0.147         3.555         Cytoplasmic         31.77         2         2635365           SDPR         Kikel         PGSC0003DMT400005107         33         3,443607         6.67         -0.147         3.555         Cytoplas</td> <td>ŝ</td> <td></td> <td></td> <td>341</td> <td>37,945.82</td> <td>6.46</td> <td>-0.040</td> <td>39.67</td> <td>Cytoplasmic</td> <td>39.67</td> <td>12</td> <td>229271</td> <td>231650</td>	SIPTR         Solved-geors780.4.1         38         37,87.2.52         6.2         -0.169         34.96         Cytoplasmic         34.96         4         2.449106           SyDPR         Kspen02g03730.1.         32.9         24,666.96         5.2         -0.038         31.82         Cytoplasmic         31.82         2         2439105           SyDPR         Kikel         Sopen03g0350.1.         33.9         37,71236         5.95         -0.007         30.71         Cytoplasmic         31.77         1         1           SyDPR         Kikel         Sopen04g00350.1.         38         37,371.72         6.67         -0.16         32.94         Cytoplasmic         31.77         2         2635355           SyDPR         Kikel         Sopen04g00350.1         38         3,4737.72         6.67         -0.17         31.77         Cytoplasmic         31.77         2         2635355           SDPR         Kikel         PGSC0003DMT40000507         38         3,493607         6.67         -0.147         3.555         Cytoplasmic         31.77         2         2635365           SDPR         Kikel         PGSC0003DMT400005107         33         3,443607         6.67         -0.147         3.555         Cytoplas	ŝ			341	37,945.82	6.46	-0.040	39.67	Cytoplasmic	39.67	12	229271	231650
SpDFRlikelSopen020/2720.138.242.668.966.2-0.2383.1.82Cytoplasmic31.82220121155SpDFRlikelSopen01037380.133.43.66.35.526.00730.71Cytoplasmic31.8225.0121159SpDFRlikelSopen01037380.13343.66.35.526.40.0083.420.703.1.7295012199SpDFRlikelSopen04003780.13343.66.35.526.40.0882.4.99Cytoplasmic2.4.9932.4186534SpDFRlikelSopen04000305013383.37.371206.57-0.1113.17Cytoplasmic2.4.9932.6423524SDFRlikelPGSC0003DMT400002033323.7.456076.67-0.1143.5.59Cytoplasmic2.3.4932.532472SDFRlikelPGSC0003DMT400010003383.6.9.93815.330.0233.6.93.7.130.0213.7.33.7.73SDFRlikelPGSC0003DMT400051073333.5.990.01433.7.73Cytoplasmic2.4.493.7.732.4.4305SDFRlikelPGSC0003DMT400051073333.5.990.0173.7.130.0173.7.732.4.493.7.73SDFRlikelPGSC0003DMT400051073333.5.990.0143.7.90.01753.7.732.4.4900SDFRlikelPGSC0003DMT400050013333.3.3055656.7<	SpDFRSopend2g03720.138242.668.966.2-0.23831.82Cytoplasmic31.8222012113SpDFR like1Sopend1g037380.133436.53.526.40.0830.71Cytoplasmic30.711195912119SpDFR like2Sopend1g037380.134436.53.526.40.08824.99Cytoplasmic30.71119592119SpDFR like3Sopend9g0396.013333.8337.871.726.667-0.11632.94Cytoplasmic32.94426.334215SDFR like4Sopend9g00390.133837.871.726.67-0.11632.94Cytoplasmic31.771226.334215SDFR like4Sopend9g00390.133837.871.726.67-0.11731.77Cytoplasmic32.94426.34215SDFR like4PGSC0003DMT4000130033836.959345.74-0.17131.7727.940344.9003SDFR like4PGSC0003DMT4000130033836.959345.74-0.17131.7727.9403437.007SDFR like4PGSC0003DMT4000130033836.959365.71-0.17131.7727.9403437.007SDFR like4PGSC0003DMT4000130033836.959345.84-0.17331.9527.940324.9400PDFR like4PGSC0003DMT4000130033837.456076.67-0.14735.25Cytoplasmic30.7027.3473SDFR like4PGSC0003DMT4000150001331.35570031.950<	9			338	37,872.52	6.2	-0.169	34.96	Cytoplasmic	34.96	4	2449106	2452152
SpDFR like1         Sopen01g037880.1         329         37,129.86         5.95         -0.07         30.71         Cytoplasmic         30.71         1         9591119         959           SpDFR like2         Sopen03g003360.1         34         36.535.2         6.4         0.088         24.99         Cytoplasmic         38.27         12         2635.25           SpDFR like3         Sopen03900.1         34         36.53.22         6.4         0.088         24.99         7         12         2635.25         418463         4           SpDFR like4         Sopen03000.1         338         37.871.22         6.67         -0.117         31.77         Cytoplasmic         32.94         4         2634315         2           StDFR like1         PGSC0003DMT40001300         338         37.871.22         6.67         -0.147         35.59         5         4         2634315         2         4734765         4           StDFR like1         PGSC0003DMT40001300         338         3745607         6.67         -0.147         35.59         5         4         255.447         2         2         4         255.447         2         2         4         4         255.461         7         4         2	SpDFR like1Sopen01037880.132937.129.865.95-0.00730.71Cytoplasmic30.71195912110SpDFR like2Sopen03005560.131436.535226.40.08824.99Cytoplasmic24.99334184663SpDFR like3Sopen030050.131335.53226.40.0883.827Cytoplasmic24.9933418463SpDFR like4Sopen04003050.13183.7571.226.67-0.1163.294Cytoplasmic3418460SDFR like1PGSC0003DMT4000103038137.039545.71-0.1713.177Cytoplasmic3335.4375SDFR like1PGSC0003DMT4000130038137.039545.71-0.1713.177Cytoplasmic33448400SDFR like1PGSC0003DMT4000130038137.539545.71-0.1713.55Cytoplasmic33448400SDFR like1PGSC003DMT400051073833.456576.67-0.1473.652Cytoplasmic333SDFR like1Peinf01Scf005090210212833.456576.073.476Cytoplasmic3333PDFR like1Peinf01Scf00509021213833.416Cytoplasmic33433PDFR like1Peinf01Scf00509021213333.419Cytoplasmic333433PDFR like1Peinf01Scf00136600601113543.3305666.5<	4			382	42,668.96	6.2	-0.238	31.82	Cytoplasmic	31.82	2	52012315	52014356
SpDR like2Sopend3g003360.13436.53.526.40.0882.4995.4093441846634SpDR like3Sopend2g0130.134138.02.2916.86-0.06438.77Cytoplasmic38.27112265852265852SpDR like4Sopend3g00360.133837.877.126.67-0.1163.294Cytoplasmic38.27122658524SDPR like4Sopend3g003960.133837.877.126.67-0.11131.77Cytoplasmic33.74248576554SDPR like4PGSC0003DMT40000538733137.039545.81-0.1733.177Cytoplasmic35.59543576554SDPR like4PGSC0003DMT40006510733836.593815.930.0872.3.48Cytoplasmic35.595435705544SDPR like4PGSC0003DMT40006510733935.590.0872.3.48Cytoplasmic35.5943954057375557PIDPR like1Peinf01Sc00075g04051133935.64-0.1473.652-0.14736.52439752PIDPR like1.1Peinf01Sc001326g0061123333461623.445.53444003175244400PIDPR like1.2Peinf01Sc001326g00122333345656.7-0.16333.745.645.645.645.675.67435762PIDPR like1.2Peinf01Sc001326g00112333346566.57-0.12334.24Cytoplasmic36.7<	SpDR like2         Sepend§00360.1         34         36.55.2         6.4         0.088         2499         Cytoplasmic         24.9         3         418463           SpDR like3         Sopend§00360.1         34         36.55.2         6.4         0.084         38.27         12         268582           SpDR like4         Sopend§003960.1         38         37.871.72         6.67         -0.113         31.77         Cytoplasmic         38.27         12         268583           StDR like4         Sopend§003960.1         38         37.871.72         6.67         -0.113         31.77         Cytoplasmic         37.77         2         434605           StDR like1         PGSC0003DMT40000028         38         34.94678         57.1         -0.171         31.77         Cytoplasmic         35.59         4         25.34245           StDR like1         PGSC0003DMT4000510         33         37.77         5.748         7         4         25.3445           StDR like1         PGSC0003DMT4000510         33         37.77         5.74         7         4         2         4         2         4         2         4         4         2         4         4         4         4         4 <td< td=""><td>8</td><td></td><td></td><td>329</td><td>37,129.86</td><td>5.95</td><td>-0.007</td><td>30.71</td><td>Cytoplasmic</td><td>30.71</td><td>1</td><td>95912119</td><td>95913769</td></td<>	8			329	37,129.86	5.95	-0.007	30.71	Cytoplasmic	30.71	1	95912119	95913769
SpDFR like3Sopen12g001330.134138.02.2916.86-0.06438.77Cytoplasmic38.2712265882SpDFR like4Sopen4g003960.13337,871/726.67-0.1163.294Cytoplasmic31.772402938624StDFR like1PGSC0003DMT4000028738242.469785.71-0.17131.77Cytoplasmic31.772402938624StDFR like1PGSC0003DMT4000130033836959815.910.0872.348Cytoplasmic35.595455765554StDFR like2PEnft1015c00073g04012135337456076.67-0.14736.52Cytoplasmic35.5954557655545576555PIDFR like1Penft1015c00073g0401213533456576.67-0.14736.52Cytoplasmic36.6243924805PIDFR like1Penft1015c00132g0401612333456576.67-0.14336.72Cytoplasmic36.7443575755PIDFR like1Penft1015c00132g030061233345155736.740.02334.04Cytoplasmic36.0444.24321476PIDFR like1.2Penft1015c00132g03006133005666.25-0.2334030.0734.242.84924.0431.7752PIDFR like1.2Penft1015c00132g03006113434.04Cytoplasmic36.0424.0421.176231.7752PIDFR like1.2Penft1015c00132g03006113434.06Co118Cytoplasmic38.0	SpDFR like3Sopen12g00130.13138,022.916.86-0.06438.27Cytoplasmic38.2712263852SpDFR like4Sopen04g00360.13337,871.726.67-0.1163.294Cytoplasmic3.17722634215StDFR like1PGSC0003DMT400002373337,871.726.67-0.11731.77Cytoplasmic3.5595.5395.5492.040plasmic3.17722634215StDFR like2PGSC0003DMT40013003383.5993815.5390.0872.348Cytoplasmic3.559544400StDFR like2Peinf01Sc000730MT40013003383.5993815.315.539Cytoplasmic3.559544400StDFR like2Peinf01Sc000730MT400051073353.54373.559Cytoplasmic3.55943.94305PIDFR like1.1Peinf01Sc0073040211333.543653.745073.559Cytoplasmic3.65243.94305PIDFR like1.2Peinf01Sc0013060101333.3745603.17524.0593.0704.0491asmic3.0704.0490034.4400PIDFR like1.2Peinf01Sc0013060101333.3005566.25-0.1263.0705.0409asmic3.0704.0491034.02380PIDFR like1.2Peinf01Sc0013060011333.33005566.25-0.1264.0583.0705.4405.64704.0583.1752PIDFR like1.2Peinf01Sc00130600030333.33005566.25-0.1264.24 <td>6</td> <td></td> <td></td> <td>334</td> <td>36,635.52</td> <td>6.4</td> <td>0.088</td> <td>24.99</td> <td>Cytoplasmic</td> <td>24.99</td> <td>3</td> <td>4184663</td> <td>4187477</td>	6			334	36,635.52	6.4	0.088	24.99	Cytoplasmic	24.99	3	4184663	4187477
SpDFR liketSopendeg003960.133837,87.1726.67-0.1163.294Cytoplasmic3.294426342152StDFR liketPGSC0003DMT4000028738242,469785.71-0.17131.77Cytoplasmic31.7724029386245StDFR liketPGSC0003DMT40001805333137,039545.84-0.17935.59Cytoplasmic35.5954557655545StDFR liketPGSC0003DMT40001800533835959815.930.08723.48Cytoplasmic35.5954557655545576555StDFR liketPeinf101Sc000739040211353357456076.67-0.14736.52Cytoplasmic36.524324405PIDFR liket-1Peinf101Sc0073904021135339450557.13-0.01440.58Peinf101Sc00073448400PIDFR liket-1Peinf101Sc00132603006129233.315646.00-0.12636.04Cytoplasmic36.04Peinf101Sc001356438710PIDFR liket-1Peinf101Sc00132603006131333.315646.00-0.2334424Cytoplasmic36.04Peinf101Sc001360317752PIDFR liket-1Peinf101Sc00132603006131333.306566.22-0.23334.24Cytoplasmic36.04Peinf101Sc001396317752PIDFR liket-1Peinf101Sc00129900011135433.958015.310.06828.89Cytoplasmic36.42Peinf101Sc001396317752 <trr<tr>PIDFR liket-1Peinf101Sc001299</trr<tr>	SpDFR liketSopend4003960.133837,871/26.67-0.1163.294Cytoplasmic3.29442634215StDFR liketPGSC0003DMT4000028738242469785.71-0.17131.77Cytoplasmic31.77240293862StDFR liketPGSC0003DMT40001805933137,0395445.84-0.17935.59Cytoplasmic35.5954246978StDFR liketPGSC0003DMT40001300033836959815.84-0.17935.59Cytoplasmic35.59544400StDFR liketPeinf101Sc0007390402135337456076.67-0.14736.52Cytoplasmic36.5244400PIDFR liketPeinf101Sc000739040213533450557.13-0.02140.5855.5444400PIDFR liket/LPeinf101Sc00132690401612933016566.57-0.16330.70Peinf101Sc001326431762PIDFR liket/LPeinf101Sc00132690301212933016556.52-0.23334.247.00plasmic36.0495.0473147PIDFR liket/LPeinf101Sc001326903011353333065566.25-0.23334.24Cytoplasmic38.9495.94916760130591762032631752PIDFR liket/LPeinf101Sc001326903061313333980335310.06823.840.00828.89Cytoplasmic38.7473476PIDFR liket/LPeinf101Sc001326903061333333980335310.06823.8220.038 <t< td=""><td>10</td><td></td><td></td><td>341</td><td>38,022.91</td><td>6.86</td><td>-0.064</td><td>38.27</td><td>Cytoplasmic</td><td>38.27</td><td>12</td><td>268582</td><td>271680</td></t<>	10			341	38,022.91	6.86	-0.064	38.27	Cytoplasmic	38.27	12	268582	271680
StDFRFGSC0003DMT400002873242469785.71-0.17131.77Cytoplasmic31.77240293624StDFR like1PGSC0003DMT400018093337,939545.84-0.17935.595745765554StDFR like2PGSC0003DMT4000180033836,959815.930.08723.48Cytoplasmic35.595448400StDFR like4PGSC0003DMT4000510733537,456076.67-0.14736.52Cytoplasmic3.6.3439248053PtDFR1Peinf101Sc0003gq027135339,450557.13-0.01340.58Cytoplasmic3.6.3448.400PtDFR2Peinf101Sc0003gq02713339,450557.13-0.01340.58Cytoplasmic3.6.3448.400PtDFRPeinf101Sc0103gq02012129334,240.0655.0.330.70Peinf101Sc0103921198992PtDFR like1-1Peinf101Sc01032gg03006131333,95656.0.3-0.25334,24Cytoplasmic36.4Peinf101Sc0103931471PtDFR like1-1Peinf101Sc0103061113441.24Cytoplasmic36.4Peinf101Sc0103931476PtDFR like1-1Peinf101Sc0103060050131333,95656.3-0.2334,24Cytoplasmic43.2491607PtDFR like1-1Peinf101Sc010309001113434.24Cytoplasmic28.89Peinf101Sc0103973147PtDFR like1-1Peinf101Sc0103090011134<	StDFR         FGSC0003DMT40009287         322         42,469.78         5.71         -0.171         31.77         Cytoplasmic         31.77         2         4023362           StDFR likel         FGSC0003DMT400018053         331         37,03954         5.84         -0.179         35.59         Cytoplasmic         35.59         5         4557655           StDFR likel         FGSC0003DMT400018053         336         359581         5.93         0.087         23.48         Cytoplasmic         35.59         5         4557655           StDFR likel         FGSC0003DMT40005107         335         3456055         5.53         0.087         23.48         Cytoplasmic         35.59         4         3924805           PiDFR likel-1         Peinfl01Scf000739040211         353         3456355         5.014         40.58         Cytoplasmic         36.79         4         3924805           PiDFR likel-1         Peinfl01Scf0125600300011         313         30450         531         30.70         Peinfl01Scf00379         448400           PiDFR likel-1         Peinfl01Scf0135690300011         313         30730         Peinfl01Scf013569030011         333         30454         56.04         Peinfl01Scf01356         337703           PiDFR likel-1	11	SpDFR like4		338	37,871.72	6.67	-0.116	32.94	Cytoplasmic	32.94	4	2634215	2637585
StDFR like1PGSC0003DMT400180593137,039.545.84-0.17935.59Cytoplasmic35.5954557655545StDFR like2PGSC0003DMT4001300033836,959.815.930.08723.48Cytoplasmic23.48335542472StDFR like4PGSC0003DMT4001300033537,456.076.67-0.14736.52Cytoplasmic23.4833948053PIDFR1Peinf101Sc0007390407.135339450.557.13-0.02140.58Cytoplasmic23.483448400PIDFR1Peinf101Sc0073904027.123339450.557.13-0.02140.58Cytoplasmic36.70Peinf101Sc00739448400PIDFR like1-1Peinf101Sc0013604016.129733,01566.23-0.20334.24Cytoplasmic36.04Peinf101Sc00130211752PIDFR like1-2Peinf101Sc001306001131333,005666.25-0.23334.24Cytoplasmic28.89Peinf101Sc001209211752PIDFR like1-3Peinf101Sc0013060001135333,005666.25-0.23334.24Cytoplasmic28.89Peinf101Sc00120973147PIDFR like1-3Peinf101Sc0013060001135333,005666.23-0.23341.24Cytoplasmic28.89Peinf101Sc00120973147PIDFR like1-3Peinf101Sc001260011135433,005666.23-0.23341.98Peinf101Sc00120973147PIDFR like1Peinf101Sc0012900111354 <td< td=""><td>StDFR like1         FGSC0003DMT40001805         31         37,039,54         5.84         -0.179         35.59         Cytoplasmic         35.59         5         4557655           StDFR like2         FGSC0003DMT40001300         338         36,959,81         5,93         0.087         23,48         Cytoplasmic         35.59         4         33-35447           StDFR like4         FGSC0003DMT400065107         335         37,45607         6.67         -0.147         36.52         Cytoplasmic         36.78         7         34400           PIDFR         Peinf101scf00073g040271         353         39,450,55         7.13         -0.0147         36.52         Cytoplasmic         36.78         Peinf101scf001306         3185700         843         -0.163         36.04         Cytoplasmic         36.94         Peinf101scf001306         31752           PIDFR like1         Peinf101scf01326g030061         23         33,050         36.04         Cytoplasmic         34.24         Cytoplasmic         35.04         Peinf101scf001306         31762           PIDFR like1         Peinf101scf01326g030061         23         33,3056         6.25         -0.235         34.24         Cytoplasmic         35.04         Peinf101scf001306         31762           PIDFR like</td><td>12</td><td>StDFR</td><td></td><td>382</td><td>42,469.78</td><td>5.71</td><td>-0.171</td><td>31.77</td><td>Cytoplasmic</td><td>31.77</td><td>2</td><td>40293862</td><td>40297510</td></td<>	StDFR like1         FGSC0003DMT40001805         31         37,039,54         5.84         -0.179         35.59         Cytoplasmic         35.59         5         4557655           StDFR like2         FGSC0003DMT40001300         338         36,959,81         5,93         0.087         23,48         Cytoplasmic         35.59         4         33-35447           StDFR like4         FGSC0003DMT400065107         335         37,45607         6.67         -0.147         36.52         Cytoplasmic         36.78         7         34400           PIDFR         Peinf101scf00073g040271         353         39,450,55         7.13         -0.0147         36.52         Cytoplasmic         36.78         Peinf101scf001306         3185700         843         -0.163         36.04         Cytoplasmic         36.94         Peinf101scf001306         31752           PIDFR like1         Peinf101scf01326g030061         23         33,050         36.04         Cytoplasmic         34.24         Cytoplasmic         35.04         Peinf101scf001306         31762           PIDFR like1         Peinf101scf01326g030061         23         33,3056         6.25         -0.235         34.24         Cytoplasmic         35.04         Peinf101scf001306         31762           PIDFR like	12	StDFR		382	42,469.78	5.71	-0.171	31.77	Cytoplasmic	31.77	2	40293862	40297510
StDFR like2FGSC0003DMT4001300335,593.815,930.0872.348Cytoplasmic23.48332.3542472StDFR like4PGSC0003DMT4006510733537,456.076.67-0.14736.52Cytoplasmic36.52439,248053PiDFR1Peinf101Sc00073g04027.133339,450.557.13-0.02140.58Cytoplasmic36.52439,24805PiDFR2Peinf101Sc00073g04016.133339,450.557.13-0.02140.58Cytoplasmic30,70Peinf101Sc005902119809PiDFR like1-1Peinf101Sc001326g0306.12933,016.566.25-0.25334.24Cytoplasmic36.04Peinf101Sc001305438410PiDFR like2Peinf101Sc001326g0306.12933,006.566.25-0.25334.24Cytoplasmic28.89Peinf101Sc001305438710PiDFR like2Peinf101Sc001306g00630133333,980335.310.06828.89Cytoplasmic28.89Peinf101Sc00140817602PiDFR like1-1Peinf101Sc0014098006.131333,980335.310.06828.999.16Peinf101Sc001305231752PiDFR like1-1Peinf101Sc001306g00630135333,980335.310.06828.99Peinf101Sc001305213752PiDFR like1-1Peinf101Sc001306g00630135333,980335.310.06823.260.330.10323.26PiDFR like1-1Peinf101Sc001306g00630135333,88033	StDFR like2PGSC0003DMT4000130003836,959,815.930.0872.348Cytoplasmic2.34833StDFR like4PGSC0003DMT4000510733537,45607667-0.14736.52(Ytoplasmic36.5243924805PIDFR1Peinf101Sc00073g04027.133339,450557.13-0.02140.58(Ytoplasmic36.7243924805PIDFR1Peinf101Sc0003g04027.133339,450557.13-0.02140.58(Ytoplasmic36.74Peinf101Sc000730448400PIDFR like1-1Peinf101Sc001326g04016.129733,015646.00-0.20536.04Cytoplasmic36.04Peinf101Sc001326438710PIDFR like1-2Peinf101Sc001326g0306.131333,905566.2344.24Cytoplasmic34.24Peinf101Sc00132631762PIDFR like1Peinf101Sc001326g006.01.135433,005566.2344.24Cytoplasmic28.89Peinf101Sc00136653147PIDFR like2Peinf101Sc001306g00630.135333,905566.23-0.10341.24Cytoplasmic28.89Peinf101Sc00139673147PIDFR like1Peinf101Sc00129931333,980335.310.06828.89Cytoplasmic38.79Peinf101Sc00139673147PIDFR like1Peinf101Sc00129931333,980335.310.06828.89Cytoplasmic38.49Peinf101Sc00129973147PIDFR like1Peinf101Sc00129931335,180.018	13			331	37,039.54	5.84	-0.179	35.59	Cytoplasmic	35.59	5	45576555	45580152
StDFR like4         PGSC0003DMT400065107         335         37,456.07         6.67         -0.147         3.652         Cytoplasmic         3.6.52         4         3924805         3           PiDFR1         Peinf101Scf00073g04077.1         353         39,450.55         7.13         -0.021         40.58         Cytoplasmic         40.58         946101Scf00073         44400           PiDFR2         Peinf101Scf00590g21022.1         282         31,855.70         8.43         -0.163         30.70         Periplasmic         36.74         Peinf101Scf00730         448400           PiDFR2         Peinf101Scf01326g02005.1         282         31,855.70         8.43         -0.163         30.70         Periplasmic         36.74         Peinf101Scf01326         438710           PiDFR like1-2         Peinf101Scf01326g03006.1         313         33,015.64         6.00         -0.205         36.04         Cytoplasmic         36.34         Peinf101Scf01326         31762           PiDFR like1-2         Peinf101Scf0136g006301.1         353         33,32480         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf01329         73147           PiDFR like2         Peinf101Scf0136g005301.1         353         39,38942         6.37         <	StDFR like4         FGSC0003DMT40065107         35         37,456.07         6.67         -0.147         36.52         Cytoplasmic         36.52         4         32,4805           PiDFR1         Peinf101Scf00073g04027.1         33         39,450.55         7.13         -0.021         40.58         Cytoplasmic         46.58         Peinf101Scf00073         448400           PiDFR2         Peinf101Scf0073g04027.1         33         34,56.57         7.13         -0.023         36.04         Cytoplasmic         36.04         Peinf101Scf00730         448400           PiDFR like1.1         Peinf101Scf01326g04016.1         297         33,01564         6.00         -0.203         36.04         Cytoplasmic         36.04         Peinf101Scf01326         438710           PiDFR like1.2         Peinf101Scf01326g04016.1         213         33,01564         6.00         -0.203         34.24         Cytoplasmic         34.24         Peinf101Scf01326         314752           PiDFR like1.2         Peinf101Scf01326g04016.1         313         33,906.56         6.23         -0.103         34.24         Cytoplasmic         34.24         Peinf101Scf01326         314752           PiDFR like1.2         Peinf101Scf01299g00011.1         33         33,906.56         6.23         -0.033 <td>14</td> <td></td> <td></td> <td>338</td> <td>36,959.81</td> <td>5.93</td> <td>0.087</td> <td>23.48</td> <td>Cytoplasmic</td> <td>23.48</td> <td>3</td> <td>2354247</td> <td>2357891</td>	14			338	36,959.81	5.93	0.087	23.48	Cytoplasmic	23.48	3	2354247	2357891
PiDFR1Peinf101Scf00073g04027.135339,450.557.13-0.02140.58Cytoplasmic40.58Peinf101Scf0073448400PiDFR2Peinf101Scf00590g21022.128231,855.708.43-0.16330.70Peinf101Scf0059021198092PiDFR like1-1Peinf101Scf01326g04016.129733,015.646.00-0.20536.04Cytoplasmic36.04Peinf101Scf01326438710PiDFR like1-2Peinf101Scf01326g0306.121333,980.935.310.06828.89Cytoplasmic34.24Peinf101Scf01305321752PiDFR like2Peinf101Scf01299g00011.135333,980.935.310.06828.89Cytoplasmic28.89Peinf101Scf0140817602PiDFR like2Peinf101Scf01299g00011.135439,518.829.18-0.10444.24Cytoplasmic44.24Peinf101Scf0129973147PaDFR like2Peinf101Scf01299g00011.135339,389.426.37-0.03341.98Cytoplasmic36.17Peinf101Scf0129973147PaDFR like1-1Paxi162Scf00238g0125.131635,226.196.37-0.03341.98Cytoplasmic36.17Peinf101Scf002396655201PaDFR like1-2Peaxi162Scf00238g0125.1146,908.536.43-0.2336.17Cytoplasmic36.17Peaxi162Scf0023812585821PaDFR like1-2Peaxi162Scf00238g0121.114240.985.40.04481.98Peinf101Scf0129973147PaDFR like1-2Pea	PiDFR1         Peinf101Scf00073g04027.1         353         39,450.55         7.13         -0.021         40.58         Cytoplasmic         40.58         Peinf101Scf000739         448400           PiDFR2         Peinf101Scf00530g2102.1         282         31,855.70         8.43         -0.163         30.70         Peinf101Scf005309         2119809           PiDFR like1-1         Peinf101Scf01326g03006.1         297         33,01564         6.00         -0.205         36.04         Cytoplasmic         36.04         Peinf101Scf01326         438710           PiDFR like1-2         Peinf101Scf01326g03006.1         313         33,01564         6.00         42.35         34.24         Cytoplasmic         36.34         Peinf101Scf01326         438710           PiDFR like2         Peinf101Scf01129g03006.1         313         33,98093         5.31         0.068         28.89         Cytoplasmic         44.24         Peinf101Scf01326         37175           PiDFR like2         Peinf101Scf0129g00011.1         354         39,51882         9.18         -0.103         41.24         Cytoplasmic         41.43         Peinf101Scf01306         5701           PiDFR like2         Peinf101Scf0129g00011.1         354         39,51882         9.18         -0.103         41.24         C	15			335	37,456.07	6.67	-0.147	36.52	Cytoplasmic	36.52	4	3924805	3927853
PiDFR2         Peinf101Scf00590g21022.1         282         31,855.70         8.43         -0.163         30.70         Peinf101Scf00590         2119809         2           PiDFR like1-1         Peinf101Scf01326g04016.1         297         33,015.64         6.00         -0.205         36.04         Cytoplasmic         36.04         Peinf101Scf01326         438710           PiDFR like1-2         Peinf101Scf01326g04016.1         298         33,006.56         6.25         -0.2053         34.24         Cytoplasmic         34.24         Peinf101Scf01326         337752           PiDFR like2         Peinf101Scf01299g0001.1         354         33,389.93         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf0140         817602           PiDFR like3         Peinf101Scf01299g0011.1         354         39,518.82         9.18         -0.103         41.24         Cytoplasmic         28.89         Peinf101Scf0140         817602           PiDFR like3         Peinf101Scf01299g0011.1         354         39,518.82         9.18         -0.033         41.98         Peinf101Scf01299         73147           PaDFR like1         Peaxi162Scf00238g0131.1         316         35,226.19         6.37         -0.033         41.98         Peaxi162Scf00366	PiDFR2         Peinf101Scf00590g21022.1         282         31,855.70         8.43         -0.163         30.70         Peinf101Scf00590         2119809           PiDFR like1-1         Peinf101Scf01326g04016.1         297         33,015.64         6.00         -0.205         36.04         Cytoplasmic         36.04         Peinf101Scf01326         33710           PiDFR like1-2         Peinf101Scf01326g03006.1         298         33,006.56         6.25         -0.253         34.24         Cytoplasmic         34.24         Peinf101Scf01326         321752           PiDFR like2         Peinf101Scf00140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         34.24         Peinf101Scf01309         31752           PiDFR like2         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         34.24         Peinf101Scf01309         73147           PaDFR like1.1         Peaxi162Scf0036606301.1         353         39,389,42         6.37         -0.033         41.98         Cytoplasmic         34.24         Peinf101Scf01309         73147           PaDFR like1.1         Peaxi162Scf0036606301.1         353         39,389,42         6.37         -0.033         31.38	16			353	39,450.55	7.13	-0.021	40.58	Cytoplasmic	40.58	Peinf101Scf00073	448400	450165
PiDFR like1-1         Peinf101Scf01326g04016.1         297         33,015.64         6.00         -0.205         36.04         Cytoplasmic         36.04         Peinf101Scf01326         438710           PiDFR like1-2         Peinf101Scf01326g03006.1         298         33,006.56         6.25         -0.253         34.24         Cytoplasmic         34.24         Peinf101Scf01326         331752           PiDFR like2         Peinf101Scf01326g03006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf01299         31475           PiDFR like2         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         58.99         Peinf101Scf01299         73147           PaDFR like1         Peaxi162Scf00366g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         36.17         Peaxi162Scf00366         655201           PaDFR like1.1         Peaxi162Scf00238g01311.1         45         35,226.19         6.82         -0.35         36.17         Cytoplasmic         36.17         Peaxi162Scf00366         655201           PaDFR like1.2         Peaxi162Scf00238g01311.1         45         45.98         Oute	PiDFR like1.1         Peinf101Scf01326g04016.1         297         33,015.64         6.00         -0.205         36.04         Cytoplasmic         36.04         Peinf101Scf01326         438710           PiDFR like1.2         Peinf101Scf01326g03006.1         298         33,006.56         6.25         -0.253         34.24         Cytoplasmic         34.24         Peinf101Scf01306         31752           PiDFR like1.2         Peinf101Scf0129g0006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.99         Peinf101Scf0140         817602           PiDFR like2         Peinf101Scf01299g0001.1         354         39,51882         9.18         -0.033         41.24         Cytoplasmic         44.24         Peinf101Scf01299         73147           PaDFR like1.1         Peaxi162Scf0036g0630.1         353         39,389.42         6.37         -0.033         41.98         Peari162Scf00366         655201           PaDFR like1.1         Peaxi162Scf00238g00125.1         316         35,226.19         6.82         -0.033         31.98         Peari162Scf00366         655201           PaDFR like1.2         Peaxi162Scf00238g01311.1         427         44.24         Cytoplasmic         36.17         Cytoplasmic         36.17         2566	17	PiDFR2		282	31,855.70	8.43	-0.163	30.70	Periplasmic	30.70	Peinf101Scf00590	2119809	2135294
PiDFR like1-2         Peinf101Scf01326g0306.1         298         33,006.56         6.25         -0.233         34.24         Cytoplasmic         34.24         Peinf101Scf01326         321752         321752           PiDFR like2         Peinf101Scf00140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf00140         817602           PiDFR like3         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         28.89         Peinf101Scf01299         73147           PaDFR like1         Peaxil62Scf00366g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         44.24         Peinf101Scf01299         73147           PaDFR like1.1         Peaxil62Scf00366g00630.1         353         35,39,42         6.37         -0.033         41.98         Cytoplasmic         36.17         Peaxil62Scf00366         655201           PaDFR like1.2         Peaxil62Scf00238g01311.1         427         46.908.53         6.43         -0.201         38.78         Peaxil62Scf00238         1258582         1           PaDFR like1.2         Peaxil62Scf00738g01311.1         427         46.908.53         6.43 <td>PiDFR like1-2         Peinf101Scf01326g03006.1         298         33,006.56         6.25         -0.253         34.24         Cytoplasmic         34.24         Peinf101Scf01326         321752           PiDFR like2         Peinf101Scf0140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.99         Peinf101Scf0140         817602           PiDFR like2         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         28.99         Peinf101Scf01299         73147           PiDFR like1         Peaxil62Scf0036g00630.1         353         39,518.82         9.18         -0.103         41.98         Cytoplasmic         41.98         Peinf101Scf01299         73147           PaDFR like1-1         Peaxil62Scf0036g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         555600366         555600366         555600366         555600366         555600366         55580           PaDFR like1-1         Peaxil62Scf00238g01311.1         427         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         55582           PaDFR like1-2         Peaxil62Scf00781g00211.1</td> <td>18</td> <td></td> <td></td> <td>297</td> <td>33,015.64</td> <td>6.00</td> <td>-0.205</td> <td>36.04</td> <td>Cytoplasmic</td> <td>36.04</td> <td>Peinf101Scf01326</td> <td>438710</td> <td>440897</td>	PiDFR like1-2         Peinf101Scf01326g03006.1         298         33,006.56         6.25         -0.253         34.24         Cytoplasmic         34.24         Peinf101Scf01326         321752           PiDFR like2         Peinf101Scf0140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.99         Peinf101Scf0140         817602           PiDFR like2         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         28.99         Peinf101Scf01299         73147           PiDFR like1         Peaxil62Scf0036g00630.1         353         39,518.82         9.18         -0.103         41.98         Cytoplasmic         41.98         Peinf101Scf01299         73147           PaDFR like1-1         Peaxil62Scf0036g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         555600366         555600366         555600366         555600366         555600366         55580           PaDFR like1-1         Peaxil62Scf00238g01311.1         427         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         55582           PaDFR like1-2         Peaxil62Scf00781g00211.1	18			297	33,015.64	6.00	-0.205	36.04	Cytoplasmic	36.04	Peinf101Scf01326	438710	440897
PiDFR like2         Peinf101Scf00140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf00140         817602           PiDFR like3         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         24.24         Peinf101Scf01299         73147           PaDFR         Peaxi162Scf00366g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.24         Peinf101Scf00366         655201           PaDFR         Ikel-1         Peaxi162Scf00238g0135.1         316         35,226.19         6.82         -0.235         36.17         Cytoplasmic         36.17         Peaxi162Scf00238         1258582         1           PaDFR         like1-2         Peaxi162Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxi162Scf00238         1258582         1           PaDFR         like1-2         Peaxi162Scf00238g01311.1         427         46,908.53         5.54         0.021         38.78         Peaxi162Scf00238         1258582         1315303           PaDFR         like1-2         Peaxi162Scf	PiDFR like2         Peinf101Scf00140g08006.1         313         33,980.93         5.31         0.068         28.89         Cytoplasmic         28.89         Peinf101Scf00140         817602           PiDFR like3         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         28.89         Peinf101Scf01299         73147           PaDFR         Peaxi162Scf00366g0630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         44.24         Peaxi162Scf00366g0630.1         353         35,226.19         6.37         -0.033         41.98         Cytoplasmic         36.17         Peaxi162Scf00366g0630.1         355         35.226.19         58.78         0.35.79         135303           PaDFR like1-1         Peaxi162Scf00238g0131.1.1         427         46,908.53         6.43         -0.201         38.78         Peaxi162Scf00238         135303           PaDFR like1-2         Peaxi162Scf00238g0131.11         427         46,908.53         6.43         -0.201         38.78         Peaxi162Scf00238         1315303           PaDFR like1-2         Peaxi162Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Peaxi162Scf00738         1315303 </td <td>19</td> <td></td> <td>Peinf101Scf01326g03006.1</td> <td>298</td> <td>33,006.56</td> <td>6.25</td> <td>-0.253</td> <td>34.24</td> <td>Cytoplasmic</td> <td>34.24</td> <td>Peinf101Scf01326</td> <td>321752</td> <td>323931</td>	19		Peinf101Scf01326g03006.1	298	33,006.56	6.25	-0.253	34.24	Cytoplasmic	34.24	Peinf101Scf01326	321752	323931
PiDFR like3         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         44.24         Peinf101Scf01299         73147           PaDFR         Peaxil62Scf00366g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         655201           PaDFR like1-1         Peaxil62Scf00238g01351.1         316         35,226.19         6.82         -0.325         36.17         Cytoplasmic         36.17         Peaxil62Scf00238         1258582         1           PaDFR like1-2         Peaxil62Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303         1           PaDFR like2         Peaxil62Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Periplasmic         28.60         Peaxil62Scf00781         212259	PiDFR like3         Peinf101Scf01299g00011.1         354         39,518.82         9.18         -0.104         44.24         Cytoplasmic         44.24         Peinf101Scf01299         73147           PaDFR         Peaxil62Scf0036g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         655201           PaDFR like1-1         Peaxil62Scf00238g00125.1         316         35,226.19         6.82         -0.325         36.17         Cytoplasmic         31.7         Peaxil62Scf00238         1258582           PaDFR like1-2         Peaxil62Scf00238g01121.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303           PaDFR like2         Peaxil62Scf00238g0111.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303           PaDFR like2         Peaxil62Scf0078lg00211.1         313         33,826.80         5.54         0.094         28.60         Peaxil62Scf0078l         21259	20			313	33,980.93	5.31	0.068	28.89	Cytoplasmic	28.89	Peinf101Scf00140	817602	819839
PaDFR         Peaxil62Scf00366g00630.1         353         39,389,42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         655201           PaDFR like1.1         Peaxil62Scf00238g0125.1         316         35,226.19         6.82         -0.215         36.17         Cytoplasmic         36.17         Peaxil62Scf00238         1258582         1           PaDFR like1.2         Peaxil62Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303         1           PaDFR like1.2         Peaxil62Scf00781g00211.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303         1           PaDFR like2         Peaxil62Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Periplasmic         28.60         Peaxil62Scf00781         212259	PaDFR         Peaxil62Scf00366g00630.1         353         39,389.42         6.37         -0.033         41.98         Cytoplasmic         41.98         Peaxil62Scf00366         655201           PaDFR like1-1         Peaxil62Scf00238g00125.1         316         35,226.19         6.82         -0.325         36.17         Cytoplasmic         41.98         Peaxil62Scf00238         125858           PaDFR like1-1         Peaxil62Scf00238g00125.1         316         35,226.19         6.82         -0.325         36.17         Cytoplasmic         36.17         Peaxil62Scf00238         1258582           PaDFR like1-2         Peaxil62Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303           PaDFR like2         Peaxil62Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Periplasmic         28.60         Peaxil62Scf00781         212259	21	PiDFR like3		354	39,518.82	9.18	-0.104	44.24	Cytoplasmic	44.24	Peinf101Scf01299	73147	79583
PaDFR like1-1         Peaxil62Scf00238g0125.1         316         35,226.19         6.82         -0.325         36.17         Cytoplasmic         36.17         Peaxil62Scf00238         1258582           PaDFR like1-2         Peaxil62Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         OuterMembrane         38.78         Peaxil62Scf00238         1315303           PaDFR like2         Peaxil62Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Periplasmic         28.60         Peaxil62Scf00781         212259	PaDFR like1-1       Peaxil62Scf00238g0125.1       316       35,226.19       6.82       -0.325       36.17       Cytoplasmic       36.17       Peaxil62Scf00238       1258582         PaDFR like1-2       Peaxil62Scf00238g01311.1       427       46,908.53       6.43       -0.201       38.78       OuterMembrane       38.78       Peaxil62Scf00238       1315303         PaDFR like1-2       Peaxil62Scf00781g00211.1       313       33,826.80       5.54       0.094       28.60       Periplasmic       28.60       Peaxil62Scf00781       212259	22			353	39,389.42	6.37	-0.033	41.98	Cytoplasmic	41.98	Peaxi162Scf00366	655201	626979
PaDFR like1-2         Peaxil62Scf00238g01311.1         427         46,908.53         6.43         -0.201         38.78         DuterMembrane         38.78         Peaxil62Scf00238         1315303           PaDFR like2         Peaxil62Scf00781g00211.1         313         33,826.80         5.54         0.094         28.60         Periplasmic         28.60         Peaxil62Scf00781         212259	PaDFR like1-2 Peaxi162Scf00238g01311.1 427 46,908.53 6.43 –0.201 38.78 OuterMembrane 38.78 Peaxi162Scf00238 1315303 PaDFR like2 Peaxi162Scf00781g00211.1 313 33,826.80 5.54 0.094 28.60 Periplasmic 28.60 Peaxi162Scf00781 212259	23		Peaxi162Scf00238g00125.1	316	35,226.19	6.82	-0.325	36.17	Cytoplasmic	36.17	Peaxi162Scf00238	1258582	1260813
PaDFR like2 Peaxi162Scf00781g00211.1 313 33,826.80 5.54 0.094 28.60 Periplasmic 28.60 Peaxi162Scf00781 212259	PaDFR like2 Peaxi162Scf00781g00211.1 313 33,826.80 5.54 0.094 28.60 Periplasmic 28.60 Peaxi162Scf00781 212259	24		Peaxi162Scf00238g01311.1	427	46,908.53	6.43	-0.201	38.78	OuterMembrane	38.78	Peaxi162Scf00238	1315303	1320945
	(Cont	25			313	33,826.80	5.54	0.094	28.60	Periplasmic	28.60	Peaxi162Scf00781	212259	214581

Ta	Table 3 (continued)	ied )										
Ś	S. Gene name	Protein ID	Protein/	Molecular	pI	GRAVY	GRAVY Instability	Cellular	Instability	Location		
2°			AA	weight/D			index	localization	index	Chromosome /Scaffold	Start	End
26	PaDFR like3	Peaxi162Scf00000g00839.1 355	355	39,421.49	8.58	8.58 -0.107	43.60	Cytoplasmic	43.60	Peaxi162Scf00000	8346643	8351171
27	CaDFR	PHT91252	348	39,223.98	5.91	-0.3	31.38	Cytoplasmic	31.38	2	155915163	55915163 155916746
28	CaDFR like1-1 PHT82354	PHT82354	327	36,381.85	6.6	-0.135	32.87	Cytoplasmic	32.87	5	233082910	233084595
29	CaDFR like1-2 PHT65511	PHT65511	327	36,758.21	6.27	-0.181	40.15	Cytoplasmic	40.15	12	42280995	42287068
30	CaDFR like1-3	PHT93586	331	37,098.48	6.42	-0.162	32.03	Cytoplasmic	32.03	1	60090121	60092229
31	CaDFR like4	PHT81181	340	37,969.55	6.03	-0.189	39.46	Cytoplasmic	39.46	5	23953961	23959080
32	SmDFR	SMEL_000g030720.1.01	382	42,572.61	5.46	-0.231	34.83	Cytoplasmic	34.83	SMEL3Ch00.06499	2363470	2365434
33	SmDFR like1-1	SmDFR like1-1 SMEL_005g225130.1.01	332	36,652.86	5.94	-0.190	31.98	Cytoplasmic	31.98	SMEL3Ch05	1716918	1720087
34	SmDFR like1-2	SmDFR like1-2 SMEL_004g205360.1.01	305	34,380.93	5.23	-0.242	39.74	Cytoplasmic	39.74	SMEL3Ch04	16495633	16500722
35	SmDFR like4-1	SmDFR like4-1 SMEL_001g150260.1.01	328	35,877.25	6.96	-0.069	30.55	Periplasmic	30.55	SMEL3Ch01	131345410 131350921	131350921
36	SmDFR like4-2	SmDFR like4-2 SMEL_011g375900.1.01	351	39,425.29	6.76	-0.196	37.52	Cytoplasmic	37.52	SMEL3Ch11	66373647	66376655
37	NaDFR1	OIS98434	349	39,069.87	6.42	-0.197	42.96	Cytoplasmic	42.96	6	9300451	9307387
38	NaDFR2	OIT31825	380	42,282.40	5.87	-0.202	32.38	Cytoplasmic	32.38	scaffold01190	169105	171023
39	NaDFR like1-1	OIT07851	331	36,723.15	6.32	-0.138	32.55	Cytoplasmic	32.55	1	37268172	37270851
40	NaDFR like1-2	OIT29772	328	36,625.86	6.27	-0.214	35.57	Cytoplasmic	35.57	scaffold01693	184391	186705
41	NaDFR like2	OIT35484	329	35,865.36	6.25	0.032	28.11	Cytoplasmic	28.11	scaffold00554	418106	420780
42	NaDFR like3	OIT01988	365	40,015.94	6.01	-0.033	44.59	Cytoplasmic	44.59	9	33244851	33250819

# PeerJ\_

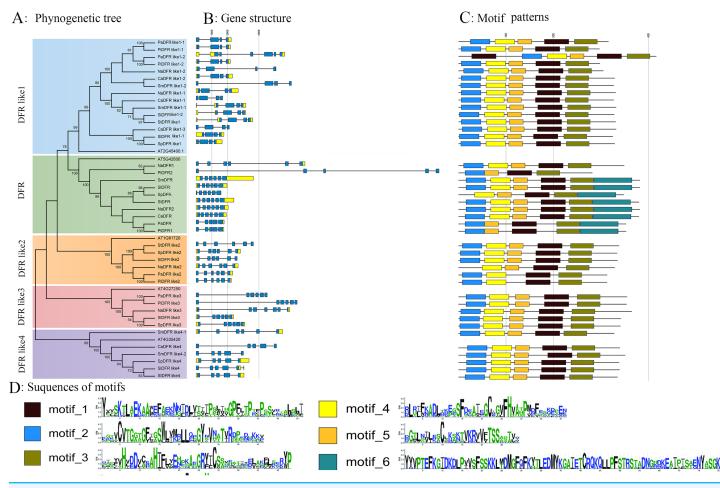


Figure 2 Phylogenetic relationship, gene structure, and composition of conserved motifs of the *DFR* genes in Solanaceae plants. (A) The phylogenetic tree was constructed using neighbour-joining method with 1,000 bootstrap replicates by MEGA11.0, and the bootstrap values >50 were indicated. The five major groups (DFR, DFR like1-4) were marked with different coloured backgrounds. Sl, Sp, St, Sm, Pi, Pa, Ca, Na in (A) representing *S. lycopersicum, S. pennellii, S. tuberosum, S. melongena, P. inflata, P. axillaris, C. annuum,* and *N. tabacum* respectively. (B) Exon/ intron structures of *DFRs* from Solanaceae plants. The UTR, exons were marked with yellow and blue boxes, respectively. The introns were marked with black lines. (C) Motif composition of the *DFR* proteins in plants. Each motif was indicated by a coloured boxes numbered at the bottom. (D) Sequence logos of the six motifs identified using the MEME search tool (E-value < 0.00001). The height of the letter represents the degree of amino acid conservation at the corresponding position. The numbers on the x-axis and y-axis represent the residue positions in the motifs and the information content measured in bits, respectively.

Although the DNA length of *DFRs* varied over a wide range, the length of each CDS and protein was similar within each subfamily.

# Gene structure and phylogenetic analysis of the *DFR* gene family in Solanaceae

To investigate the phylogenetic relationships of DFR proteins, a NJ tree was constructed based on the full length of all 42 DFR sequences from the eight species listed in Table 1. As shown in the phylogenetic tree (Fig. 2A), all 42 proteins were clustered into five groups (DFR, DFR like1, DFR like2, DFR like3, and DFR like4) with high bootstrap values consisting of 10, 15, six, five and six members, respectively. It was found that *S. lycopersicum* and *S. pennellii* contained all five groups of DFR proteins, *S. tuberosum*,

*Nicotiana attenuata*, *P. inflata*, and *P. axillaris* contained four, and *S. melongena* and *C. annuum* contained three. With the exception of *N. attenuata* and *P. inflata*, which contained two DFR proteins, all species contained one DFR protein. The results above suggested that the Solanaceae *DFRs* were derived from one ancestor gene and that they developed into different branches after their lineages diverged.

In addition, to gain an insight into the variation in the *DFR* genes, we analyzed the exon–intron structure. The structure of the *DFR* genes was relatively conserved within each subfamily (Fig. 2B). The number of exons ranged from 4 to 6; among them, six, five, and four exons were identified in 20, 8, and 14 genes, respectively. The *DFR like1* subfamily was characterized by four exons (93.3%), except for *PaDFR like1-2*, which had six exons. The *DFR* subfamily was characterized by six exons (70%), similar to other *DFR* genes from arabidopsis, petunia, snapdragon, morning glory, and onion plants, except for *PiDFR2*, *PaDFR*, *PiDFR1*, which had five exons. The *DFR like4* subfamily featured five exons (83.3%), except for *CaDFR like4*. Both the *DFR like2* and *DFR like3* subfamilies featured five exons (100%) (Fig. 2B). The number of exons within each subfamily agreed with that of *DFRs* in the tea plant (*Mei et al., 2019*). The conservation of the *DFR* gene structure revealed the ancient features of the evolution.

### Analysis of conserved motifs in DFR proteins

The patterns of conserved motifs were predicted using MEME5.1.1, six conserved motifs in Solanaceae were captured (Fig. 2C). Motif 1 (175–225 aa in SlDFR labeling) amounted to the NAD-dependent epimerase/dehydratase family, Motif 2 (19–60 aa) encoded a NAD(P) H-binding domain, Motif 5 (117–145 aa) corresponded to 3-beta hydroxysteroid dehydrogenase/isomerase family, Motif 6 (282–362 aa) encoded a domain of unknown function (DUF1731), and Motif 3 (234–281 aa) and 4 (69–109 aa) did not match any functional annotation (Fig. 2D). Of the 42 DFRs, all the proteins contained Motif 1 and 3; CaDFR and NaDFR like2 had lost Motif 2; PaDFR, PiDFR1, and PiDFR2 had lost Motif 4; and PaDFR like1-1, PiDFR like1-1, NaDFR like1-2, CaDFR like1-2, SlDFR like1-1, PaDFR like2, and PiDFR1 and PiDFR2. SlDFR contained the above six conserved motifs, similar to VvDFR.

Multiple sequence alignment of Solanaceae DFR proteins was carried out using Genedoc software. All of the six SIDFR proteins contain conserved NADPH-binding domains, showing that they belong to the NAD-dependent epimerase/dehydratase family. Consistant with DFRs in other plants, only SIDFR possessed a conserved substrate specificity-determining region (Fig. 3), which showed that maybe SIDFRs was unique. The 138<sup>th</sup> asparagine residue (ZmDFR lableing, *i.e.*, N133 of VvDFR in Fig. 3) is said to be extremely important for choosing substrate. However, in Solanaceae plants, N is substituted for D. Thus, SIDFR fell into Asp-type DFR, which converts DHK inefficiently. The remaining putative SIDFRs are neither Asn nor Asp types.

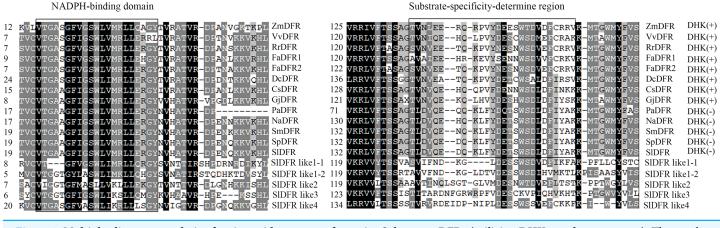


Figure 3 Multiple alignment analysis of amino acid sequence of putative Solanaceae DFRs (utilizing DHK as substrate or not). The numbers on the left represent residuals of different DFRs. DHK (+) or DHK (-) indicate whether these typical DFRs accept DHK as substrate (Asp-type) or not (Asn-type). The shading in different colors indicates the conserved percent of amino acid residues. The accession numbers of the protein sequences are as follows: Zm (*Zea mays*), NP\_001152467.2; Vv (*Vitis vinifera*), CAA53578.1 or P93799; Rr (*Rosa rugosa*), ALR74719.1; Fa (*Fragaria X ananassa*), AHL46444.1 (FaDFR1), AHL46451.1 (FaDFR1); Dc (*Dianthus caryophyllus*), P51104.1; Cs (*Camellia sinensis*), AB018685.1; Gj (*Gerbera jamesonii*), AHF58605.1; Pa (*Petunia axillaris*), Peaxi162Scf00366g00630.1; Na (*Nicotiana attenuata*), OIT31825; Sm (*Solanum melon-gena*), SMEL\_000g030720.1.01; Sp (*Solanum pennellii*), Sopen02g029720.1; Sl (*Solanum lycopersicum*), Solyc02g085020.4.1 (SlDFR, Solyc01g094070.3.1 (SlDFR like1-1), Solyc05g051010.4.1 (SlDFR like1-2), Solyc03g031470.3.1 (SlDFR like2), Solyc12g005350.2.1 (SlDFR like3), Solyc04g008780.4.1 (SlDFR like4).

# Tissue specificity of tomato DFRs

In order to get some idea of where the *DFR* genes function in the plant, the expression of six *SlDFR* genes was examined by means of qRT-PCR in five different organs: 45-day-old seedling root, stem, leaf, flower, and green ripening fruit. The expression profiles of each gene greatly differed (Fig. 4). Results showed that the six *SlDFR* genes were widely expressed in different organs at both the seedling stage and reproductive growth stage. This expression pattern reflected their physiological functions in each tissue. Among these genes, *SlDFR*, *SlDFR like1-1*, and *SlDFR like1-2*, with similar expression patterns, displayed high expression in stem, leaf, and flower; moreover, *SlDFR like1-2* showed high expression in root. *SlDFR like2* was preferentially expressed in the flowers. The expression of *SlDFR like3* was relatively high in all organs, except for the leaf. Meanwhile, transcripts of *SlDFR like4* were detected in all organs that we examined. These results suggested that there was a high correlation between qRT-PCR data and the data from the database.

# Cis-elements in the promoter sequences of DFR genes

To identify putative *cis*-elements in the promoter region of *DFRs*, genomic sequences located approximately 2,000 bp from the translational start site were analyzed in the PlantCARE and PLACE databases. The locations of *cis*-regulatory elements in the promoter sequences of *DFRs* were predicted to understand the possible roles of *DFRs* in response to abiotic stresses (Fig. 5). There were 48 *cis*-elements with 12 types in *PaDFR's* promoter region, which contained the most among all the putative *DFRs*. For other *DFRs*, 11–45 elements with 5–15 types were found (File S1). All these promoters, especially *PiDFR like1-1* and *StDFR*, were rich in light-responsive elements, such as GT-1 motif, Box II, Box4, AE box, I-Box, Sp1, ABRE, ABRE4, G-Box, ATC-motif, GA-motif, TCT-motif,

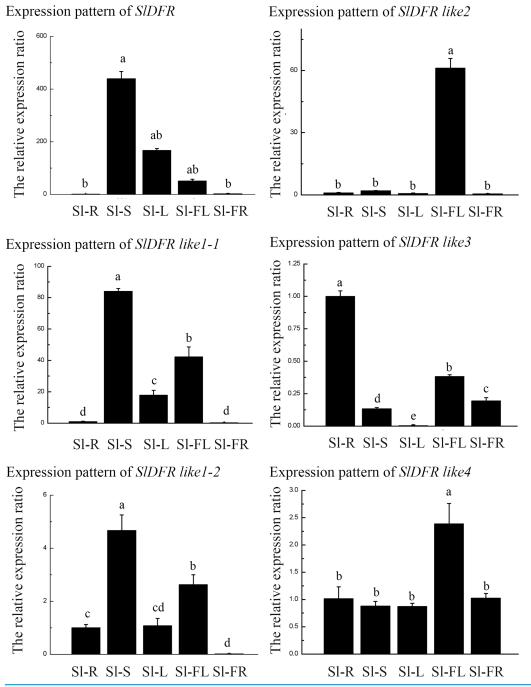


Figure 4 The relative expression ratio of *SlDFR* genes. The name of the gene was written on the top of each bar diagram (error bars indicate the standard deviation from three replicates). Sl-R, Sl-S, Sl-L, Sl-FR in the X-axis representing tomato roots, stems, leaves, flowers, and fruits respectively. Different lowercase letters indicate significantly different values at P < 0.05 (least significant difference, LSD). Full-size  $\square$  DOI: 10.7717/peerj.16124/fig-4

and GATA-motif. Furthermore, some promoters had several MYB and MYC elements. *StDFR*, *PaDFR like1-1*, and *PiDFR like1-1* contained two MYB-binding sites (CAACAG), and *PaDFR like3*, *PaDFR like1-2*, *PaDFR like2*, *PiDFR1*, *PiDFR2*, *SmDFR like1-1*, and *SpDFR like2* contained one MYB-binding site. *PaDFR* contained three MYB-recognition

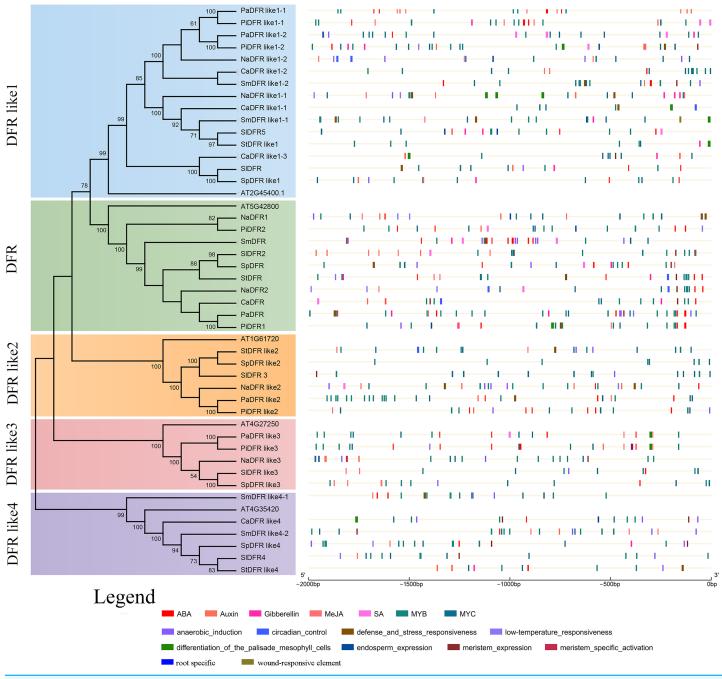


Figure 5 Predicted *cis*-regulatory elements in the promoters of Solanaceae *DFR* genes. The phylogenetic tree of the *DFR* family is replotted from Fig. 2. The *cis*-regulatory elements (CREs) in the 2 kb upstream regions of the 42 Solanaceae *DFR* genes were predicted using the PlantCARE and PLACE database. Black lines indicate promoter regions. CREs involved in response to phytohormones and induction of tissue specific expression are represented by color boxes. Full-size DOI: 10.7717/peerj.16124/fig-5

sites (CCGTTG), *PiDFRlike1-1* contained two MYB-recognition sites, *NaDFR like2*, *SmDFR* and *PaDFR like3* contained one MYB-recognition site. In addition, *cis*-elements involved in root-specific expression were only found in the promoter of *PiDFR like3*.

Figure 5 presents the distribution and numbers of *cis*-elements responding to phytohormones, stresses, and tissue-specific expression.

# DISCUSSION

Recent studies have suggested that *DFRs* in plant species are encoded by a gene family, and studies of the *DFR* gene family focusing on *Brassica rapa* (*Ahmed et al., 2014*), *Freesia hybrida* (*Li et al., 2017*), tea plant (*Mei et al., 2019*) and *Brassica napus* (*Qian et al., 2023*) have been reported. Different DFR copies are responsible for diverse functions. For example, different copies can be expressed in different tissues or at different times, or different but related substrates could be used to form three products. However, there have been no reports about the family members and functions of DFRs in Solanaceae plants. This study systematically identified 42 putative DFRs within the eight Solanaceae species and performed genome-wide identification and phylogenetic analysis and determined the gene structure, conserved motifs, expression patterns, cellular location, and *cis*-acting elements. The Solanaceae DFR members, together with five *Arabidopsis* DFR proteins, were divided into five subfamilies (DFR, DFR like1, DFR like2, DFR like3, and DFR like4) based on the phylogenetic analysis (Fig. 2).

The analysis of the physicochemical properties of the protein and the number and MW of amino acids were found to be quite different from each other, indicating some differences in their structure and function. The number of introns ranged from three to five, which was consistant with the results that the firstly cloned maize DFR contained three introns, while Petunia hybrida and Antirrhinum majus DFR contained five introns (O'Reilly et al., 1985; Beld et al., 1989). Previous studies have shown that different DFR subtypes in the same plant share 25.5%-59.6% amino acid sequence identity (Mei et al., 2019). In this study, the alignment of the 42 protein sequences was performed by CLUSTAL-W using MEGA 11.0. The results revealed that the DFRs of Solanaceae had little homology. Within a single species (e.g., S. lycopersicum), SIDFR shared 42.41%, 36.8%, 40.18%, 39.52%, and 37.67% identity with SIDFR like1-1, SIDFR like1-2, SIDFR like2, SIDFR like3, and SIDFR like4, respectively (Table S1). Although the sequences of tomato DFR like proteins greatly differ from SIDFR, they all have a conserved NADPH-binding domain, but only SIDFR has the substrate-binding domain (Fig. 3). Therefore, it is unclear whether they have the ability to form leucoanthocyanidins, similar to the outcomes of a previous study (*Mei et al., 2019*). Only one typical SIDFR has been reported so far, and the gene sequence was similar to that previously reported (Bongue-Bartelsman et al., 1994). Furthermore, a homology of over 96.04% among different plants was observed, such as tomato and potato, suggesting that these genes are highly conserved. Collectively, although 4–6 DFRs were found in every species of Solanaceae plants, only 1–2 DFRs were typical. For the other putative DFR proteins, further investigation is necessary.

DFR proteins can catalyze DHK, DHQ, and DHM to form their corresponding leucoanthocyanidins in many plants, such as *Gerbera (Johnson et al., 2001), Z. mays* (*Meyer et al., 1987*), *V. vinifera (Sparvoli et al., 1994*), and *V. bellula (Zhu et al., 2018*). However, *Petunia* and *Cymbidium* DFRs cannot reduce DHK efficiently, indicating that DFRs from different species exhibit diverse substrate preferences (*Gerats et al., 1982*;

Forkmann & Ruhnau, 1987; Johnson et al., 1999). Homologous sequence alignment is an effective method to determine the relationships between the substrate preference and amino acids in the region responsible for substrate specificity. It has been reported that in Gerbera DFR, residues 134 and 145 play important roles in the substrate specificity (Johnson et al., 2001). The glutamic acid (Glu, E) at position 145 is conserved in almost all DHK-accepting dicot DFRs, and its mutation (Glu to Leu) results in white flowers, although this is not the case for petunia DFR. In this article, all DFRs have E at residue 149, except for PaDFR. Recent investigations support that the presence of N at position 134 would determine the acceptance of DHK as substrate, whereas those with an D have a marked preference for DHQ (Gosch et al., 2014); in addition, a different mutation at site 134 changed the preference of DFR and modified its flux-controlling role. The Lathyrus japonicus and Medicago truncatula DFRs containing N at position 133, reduce DHK more efficiently than DHQ, while Petunia and Cymbidium DFRs containing a D at the same position, reduce DHK inefficiently. As the assumption mentioned above, whether the amino acid sequence at the specific location determining the substrate specificity should be verified in other ways. All the Solanaceae DFRs listed here have a D residue at position 145 (tomato numbering), suggesting that perhaps all the Solanaceae plants used DHQ and DHM as a substrate (Fig. 3).

Promoters of all the DFR genes of the eight Solanaceae species were screened for *cis*regulatory elements, and 11-48 elements of 5-15 types were found. Among them, hormone-responsive, light-responsive, abiotic stress-responsive, development stage-related, and MYB-responsive elements were found, suggesting that DFR genes may play important roles in plant development and adaptation to environmental conditions. To date, different transcriptional studies have shown that the expression of DFR is regulated by different factors. A single SNP at -301 in S. melongena DFR promoter, which belongs to MYB recognition site (CCGTTG) at the second nucleotide, influenced the interaction of the DFR promoter with MYB113, affecting the transcription of DFR in S. melongena, thereby abolishing anthocyanin production in this species (Wang et al., 2022). It has reported that the mutations in *MYB113* rather than in *DFR* causing a decrease in expression of both MYB113 and DFR in the S. melongena accessions with purple flowers and green or white fruit peels (Babak et al., 2020). Most domesticated tomato cultivars lack of anthocyanins in tomato fruits, contain a splicing site variation in SlAN2-like that causes the production of a non-functional SIAN2-like protein (Colanero et al., 2020; Colanero, Perata & Gonzali, 2020; Sun et al., 2020). Furthermore, all the tomato DFRs lacking MYB recognition site is the likely cause for the anthocyanin-free phenotype of tomato. High temperatures during night-time and a synthetic auxin, 2,4-dichlorophenoxyacetic acid, were found to inhibit the expression of VvDFR (Mori, Sugaya & Gemma, 2005; Ban et al., 2003). Light and abscisic acid were found to induce activation of the DFR promoter (Ban et al., 2003). The identification of the expression profiles will be useful for the classification of genes involved in the regulation of the precise nature of individual tissue. The expression profiles of *SlDFRs* revealed in this study agree with those in previous publications.

# **CONCLUSIONS**

In this study, we performed a comprehensive genome-wide analysis of *DFRs* in eight Solanaceae species. A total of 42 DFRs were identified, and they could be divided into five subfamilies. Analysis of the sequences and comparison of these genes with *DFR* genes from other species validated them as *DFR* genes. After analysis of phylogenetics and conserved motifs, we found that almost all of the DFR proteins contained NAD-dependent epimerase/dehydratase and an NAD(P)H-binding domain, but only the functional DFR proteins had substrate specificity-determining regions. The analysis of *cis*-acting elements revealed that the *DFRs* from eight Solanaceae species were involved in hormone response, light induction response, abiotic stress, and development stages. The expression patterns of the selected *SlDFR* genes were shown to be different in diverse tomato tissues. The elaborate results conferred here would provide valuable data for the useful research and application of DFR family in Solanaceae plants.

# **ADDITIONAL INFORMATION AND DECLARATIONS**

### Funding

This work was financially supported by the Fundamental Research Funds for the Universities in Hebei Province (Grant No. JYQ202101), the Science and Technology Project of Hebei Education Department (Grant No. ZD2020122), and the Langfang Normal University Undergraduate innovation and entrepreneurship training program fund (Grant No. S202010100016). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

# **Grant Disclosures**

The following grant information was disclosed by the authors: Fundamental Research Funds for the Universities in Hebei Province: JYQ202101. Science and Technology Project of Hebei Education Department: ZD2020122. Langfang Normal University Undergraduate Innovation and Entrepreneurship Training program: S202010100016.

# **Competing Interests**

The authors declare that they have no competing interests.

# **Author Contributions**

- Wenjing Li conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Yiming Zhang performed the experiments, prepared figures and/or tables, and approved the final draft.
- Hualiang Liu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Qiuping Wang performed the experiments, prepared figures and/or tables, and approved the final draft.

- Xue Feng analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Congyan Wang analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yanxiang Sun analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Xinye Zhang analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Shu Zhu conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.

# **Data Availability**

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

# **Supplemental Information**

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/ peerj.16124#supplemental-information.

# REFERENCES

- Ahmed NU, Park JI, Jung HJ, Yang TJ, Hur Y, Nou IS. 2014. Characterization of dihydroflavonol 4-reductase (*DFR*) genes and their association with cold and freezing stress in *Brassica rapa*. *Gene* **550**:46–55 DOI 10.1016/j.gene.2014.08.013.
- Babak O, Gibson J, Meyer RS, Chapman MA. 2020. Identification of DNA markers of anthocyanin biosynthesis disorders based on the polymorphism of anthocyanin 1 tomato ortholog genes in pepper and eggplant. *Crop Breed Genet Genom* 2(3):e200011 DOI 10.20900/cbgg20200011.
- Bailey TL, Williams N, Misleh C, Li WW. 2006. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Research* 34:W369–W373 DOI 10.1093/nar/gkl198.
- Ban T, Ishimaru M, Kobayashi S, Shiozaki S, Goto-Yamamoto N, Horiuchi S. 2003. Abscisic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. *Journal of Horticultural Science & Biotechnology* 78:586–589 DOI 10.1080/14620316.2003.11511668.
- Bassolino L, Zhang Y, Schoonbeek HJ, Kiferle C, Perata P, Martin C. 2013. Accumulation of anthocyanins in tomato skin extends shelf life. *New Phytologist* 200(3):650–655 DOI 10.1111/nph.12524.
- Bavage AD, Davies IG, Robbins MP, Morris P. 1997. Expression of an Antirrhinum dihydroflflavonol reductase gene results in changes in condensed tannin structure and accumulation in root cultures of Lotus corniculatus (bird's foot trefoil). *Plant Molecular Biology* 35:443–458 DOI 10.1023/a:1005841214470.
- Beld M, Martin C, Huits H, Stuitje AR, Gerats AGM. 1989. Flavonoid synthesis in *Petunia hybrida*: partial characterization of dihydroflavonol-4-reductase genes. *Plant Molecular Biology* 13:491–502 DOI 10.1007/BF00027309.
- Bongue-Bartelsman M, O'Neill SD, Tong Y, Yoder JI. 1994. Characterization of the gene encoding dihydroflavonol 4-reductase in tomato. *Gene* 138(1-2):153–157 DOI 10.1016/0378-1119(94)90799-4.

- **Colanero S, Perata P, Gonzali S. 2020.** What's behind purple tomatoes? Insight into the mechanisms of anthocyanin synthesis in tomato fruits. *Plant Physiology* **182(4)**:1841–1853 DOI 10.1104/pp.19.01530.
- **Colanero S, Tagliani A, Perata P, Gonzali S. 2020.** Alternative splicing in the anthocyanin fruit gene encoding an R2R3 MYB transcription factor affects anthocyanin biosynthesis in tomato fruits. *Plant Communications* 1(1):100006 DOI 10.1016/j.xplc.2019.100006.
- **Davies KM, Schwinn KE, Deroles SC, Manson DG, Lewis DH, Bloor SJ, Bradley JM. 2003.** Enhancing anthocyanin production by altering competition for substrate between flavonol synthase and dihydrofflavonol 4-reductase. *Euphytica* **131**:259–268.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5):1792–1797 DOI 10.1093/nar/gkh340.
- Espley RV, Bovy A, Bava C, Jaeger SR, Tomes S, Norling C, Crawford J, Rowan D, McGhie TK, Brendolise C, Putterill J, Schouten HJ, Hellens RP, Allan AC. 2013. Analysis of genetically modifified red-fleshed apples reveals effects on growth and consumer attributes. *Plant Biotechnology Journal* 11(4):408–419 DOI 10.1111/pbi.12017.
- Forkmann G, Ruhnau B. 1987. Distinct substrate specificity of dihydroflavonol 4-reductase from flowers of *Petunia hybrida*. *Zeitschrift Für Naturforschung C* **42(9–10)**:1146–1148 DOI 10.1515/znc-1987-9-1026.
- Gerats AG, de Vlaming P, Doodeman M, Al B, Schram AW. 1982. Genetic control of the conversion of dihydroflavonols into flavonols and anthocyanins in flowers of *Petunia hybrida*. *Planta* 155:364–368 DOI 10.1007/BF00429466.
- Gonzalez A, Zhao M, Leavitt JM, Lloyd AM. 2008. Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/MYB transcriptional complex in Arabidopsis seedlings. *Plant Journal* 53(5):814–827 DOI 10.1111/j.1365-313X.2007.03373.x.
- Gosch C, Nagesh KM, Thill J, Miosic S, Plaschil S, Milosevic M, Olbricht K, Ejaz S, Rompel A, Stich K, Halbwirth H. 2014. Isolation of dihydroflavonol 4-reductase cDNA clones from *angelonia* × *angustifolia* and heterologous expression as GST fusion protein in *Escherichia coli*. *PLOS ONE* 9(9):e107755 DOI 10.1371/journal.pone.0107755.
- Grotewold E. 2006. The genetics and biochemistry of floral pigments. *The Annual Review of Plant Biology* 57:761–780 DOI 10.1146/annurev.arplant.57.032905.105248.
- Higo K, Ugawa Y, Iwamoto M, Korenaga T. 1999. Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research* 27(1):297–300 DOI 10.1093/nar/27.1.297.
- Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G. 2015. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* 31(8):1296–1297 DOI 10.1093/bioinformatics/btu817.
- Johnson ET, Ryu S, Yi HK, Shin B, Cheong H, Choi G. 2001. Alteration of a single amino acid changes the substrate specificity of dihydroflavonol 4-reductase. *Plant Journal* 25:325–333 DOI 10.1046/j.1365-313x.2001.00962.x.
- Johnson ET, Yi H, Shin B, BJ O, Cheong H, Choi G. 1999. *Cymbidium hybrida* dihydroflavonol 4-reductase does not efficiently reduce dihydrokaempferol to produce orange pelargonidin-type anthocyanins. *Plant Journal* 19(1):81–85 DOI 10.1046/j.1365-313x.1999.00502.x.
- Katsu K, Suzuki R, Tsuchiya W, Inagaki N, Yamazaki T, Hisano T, Yasui Y, Komori T, Koshio M, Kubota S, Walker AR, Furukawa K, Matsui K. 2017. A new buckwheat dihydroflavonol 4-reductase (DFR), with a unique substrate binding structure, has altered substrate specificity. *Bmc Plant Biology* 17:239 DOI 10.1186/s12870-017-1200-6.
- Kim J, Lee WJ, Vu TT, Jeong CY, Hong SW, Lee H. 2017. High accumulation of anthocyanins via the ectopic expression of *AtDFR* confers significant salt stress tolerance in *Brassica napus* L. *Plant Cell Reports* **36(8)**:1215–1224 DOI 10.1007/s00299-017-2147-7.

- Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S. 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research* **30**(1):325–327 DOI 10.1093/nar/30.1.325.
- Li XX, Hamyat M, Liu C, Ahmad S, Gao XM, Guo C, Wang YY, Guo YF. 2018. Identification and characterization of the WOX family genes in five Solanaceae species reveal their conserved roles in peptide signaling. *Genes* 9:260 DOI 10.3390/genes9050260.
- Li YQ, Liu XX, Cai XQ, Shan XT, Gao RF, Yang S, Han TT, Wang SC, Wang L, Gao X. 2017. Dihydroflavonol 4-reductase genes from *Freesia hybrida* play important and partially overlapping roles in the biosynthesis of flavonoids. *Frontiers in Plant Science* **8**:428 DOI 10.3389/fpls.2017.00428.
- Li Z, Vickrey TL, McNally MG, Sato SJ, Clemente TE, Mower JP. 2019. Assessing anthocyanin biosynthesis in Solanaceae as a model pathway for secondary metabolism. *Genes* 10:559 DOI 10.3390/genes10080559.
- Liao Z, Hodén KP, Singh RK, Dixelius C. 2020. Genome-wide identification of argonautes in Solanaceae with emphasis on potato. *Scientific Reports* 10:20577 DOI 10.1038/s41598-020-77593-y.
- Mei X, Zhou CB, Zhang WT, Rothenberg DO, Wan SH, Zhang LY. 2019. Comprehensive analysis of putative dihydroflavonol 4-reductase gene family in tea plant. *PLOS ONE* 14(12):e0227225 DOI 10.1371/journal.pone.0227225.
- Meyer P, Heidmann I, Forkmann G, Saedler H. 1987. A new petunia flower colour generated by transformation of a mutant with a maize gene. *Nature* 330:677–678 DOI 10.1038/330677a0.
- Mori K, Sugaya S, Gemma H. 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae* 105:319–330 DOI 10.1016/j.scienta.2005.01.032.
- O'Reilly C, Shepherd NS, Pereira A, Schwarz-Sommer Z, Bertram I, Robertson DS, Peterson PA, Saedler H. 1985. Molecular cloning of the A1 locus of *Zea mays* using the transposable elements en and Mu1. *EMBO Journal* 4:877–882 DOI 10.1002/j.1460-2075.1985.tb03713.x.
- Petit P, Granier T, D'Estaintot BL, Manigand C, Bathany K, Schmitter J-M, Lauvergeat V, Hamdi S, Gallois B. 2007. Crystal structure of grape dihydroflavonol 4-reductase, a key enzyme in flavonoid biosynthesis. *Journal of Molecular Biology* 368(5):1345–1357 DOI 10.1016/j.jmb.2007.02.088.
- Petroni K, Tonelli C. 2011. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Science* 181(3):219–229 DOI 10.1016/j.plantsci.2011.05.009.
- **Pourcel L, Bohórquez-Restrepo A, Irani NG, Grotewold E. 2012.** Anthocyanin biosynthesis, regulation, and transport: new insights from model species. In: *Recent Advances in Polyphenol Research*. New Jersey, NJ: Wiley-Blackwell, 143–160.
- Qian XZ, Zheng WY, Hu J, Ma JX, Sun MY, Li Y, Liu N, Chen TH, Wang MQ, Wang L, Hou XZ, Cai QG, Ye ZS, Zhang FG, Zhu ZH. 2023. Identification and expression analysis of DFR gene family in Brassica napus L. Plants 12(13):2583 DOI 10.3390/plants12132583.
- Qiu Z, Wang X, Gao J, Guo Y, Huang Z, Du Y. 2016. The tomato Hoffman's anthocyaninless gene encodes a bHLH transcription factor involved in anthocyanin biosynthesis that is developmentally regulated and induced by low temperatures. *PLOS ONE* **11(3)**:e0151067 DOI 10.1371/journal.pone.0151067.
- **Robbins MP, Bavage AD, Strudwicke C, Morris P. 1998.** Genetic manipulation of condensed tannins in higher plants. II. Analysis of birdsfoot trefoil plants harboring antisense

dihydroflflavonol reductase constructs. *Plant Physiology* **116(3)**:1133–1144 DOI 10.1104/pp.116.3.1133.

- Rosati C, Cadic A, Duron M, Renou JP, Simoneau P. 1997. Molecular cloning and expression analysis of dihydroflavonol 4-reductase gene in flower organs of *Forsythia* × *intermedia*. *Plant Molecular Biology* **35(3)**:303–311 DOI 10.1023/a:1005881032409.
- Rosati C, Simoneau P, Treutter D, Poupard P, Cadot Y, Cadic A, Duron M. 2003. Engineering of flower color in *forsythia* by expression of two independently transformed dihydroflflavonol 4reductase and anthocyanidin synthase genes of flavonoid pathway. *Molecular Breeding* 12(3):197–208 DOI 10.1023/A:1026364618719.
- Sasaki N. 2020. Identification of the biosynthetic pathway for anthocyanin triglucoside, the precursor of polyacylated anthocyanin, in red cabbage. *Journal of Agricultural and Food Chemistry* 68(36):9750–9758 DOI 10.1021/acs.jafc.0c03480.
- Sparvoli F, Martin C, Scienza A, Gavazzi G, Tonelli C. 1994. Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). *Plant Molecular Biology* 24(5):743–755 DOI 10.1007/BF00029856.
- Sun CL, Deng L, Du MM, Zhao JH, Chen Q, Huang TT, Jiang HL, Li CB, Li CY. 2020. A transcriptional network promotes anthocyanin biosynthesis in tomato flesh. *Molecular Plant* 13(1):42–58 DOI 10.1016/j.molp.2019.10.010.
- Takahashi H, Hayashi M, Goto F, Sato S, Soga T, Nishioka T, Tomita M, Kawai-Yamada M, Uchimiya H. 2006. Evaluation of metabolic alteration in transgenic rice overexpressing dihydroflavonol-4-reductase. *Annals of Botany* 98(4):819–825 DOI 10.1093/aob/mcl162.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 7:3022–3027 DOI 10.1093/molbev/msab120.
- Tian J, Chen MC, Zhang J, Li KT, Song TT, Zhang X, Yao YC. 2017. Characteristics of dihydroflavonol 4-reductase gene promoters from different leaf colored *Malus crabapple* cultivars. *Horticultural Research* 13(4):17070 DOI 10.1038/hortres.2017.70.
- Wang X, Chen XP, Luo SX, Ma W, Li N, Zhang WW, Tikunov Y, Xuan SX, Zhao JJ, Wang YH, Zheng GD, Yu P, Bai YL, Bovy A, Shen SX. 2022. Discovery of a DFR gene that controls anthocyanin accumulation in the spiny Solanum group: roles of a natural promoter variant and alternative splicing. *Plant Journal* 111(4):1096–1109 DOI 10.1111/tpj.15877.
- Winkel-Shirley B. 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology* 126(2):485–493 DOI 10.1104/pp.126.2.485.
- Wu J, Liu SY, He YJ, Guan XY, Zhu XF, Cheng L, Wang J, Lu G. 2012. Genome-wide analysis of *SAUR* gene family in Solanaceae species. *Gene* **509**:38–50 DOI 10.1016/j.gene.2012.08.002.
- Xie DY, Jackson LA, Cooper JD, Ferreira D, Paiva NL. 2004. Molecular and biochemical analysis of two cDNA clones encoding dihydroflavonol-4-reductase from *Medicago truncatula*. *Plant Physiology* **134(3)**:979–994 DOI 10.1104/pp.103.030221.
- Zhang Y, Butelli E, De Stefano R, Schoonbeek HJ, Magusin A, Pagliarani C, Wellner N, Hill L, Orzaez D, Granell A, Jones JD, Martin C. 2013. Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. *Current Biology* 23(12):1094–1100 DOI 10.1016/j.cub.2013.04.072.
- Zhu Y, Peng QZ, Li KG, Xie DY. 2018. Molecular cloning and functional characterization of a dihydroflavonol 4-reductase from *Vitis bellula*. *Molecules* 23(4):861 DOI 10.3390/molecules23040861.