

**Function of a p24 heterodimer in morphogenesis and protein transport in**

***Penicillium oxalicum***

Fangzhong Wang <sup>1</sup>, Kuimei Liu <sup>1</sup>, Lijuan Han <sup>1</sup>, Baojie Jiang <sup>1</sup>, Mingyu Wang <sup>1</sup>, Xu  
Fang <sup>1,\*</sup>

<sup>1</sup> State Key Laboratory of Microbial Technology, School of Life Science, Shandong  
University, Jinan, Shandong, China

\* To whom correspondence is addressed. E-mail: fangxu@sdu.edu.cn

Tel: +86-531-88364004, Fax: +86-531-88364363

## Supplementary Information

### Fig. S1

Sequence alignment of p24 homologs in *P. oxalicum* 114-2 and *S.cerevisiae*.

The same amino acid residues are shaded.

### Fig. S2

SDS-PAGE analysis of intracellular proteins of p24 deletion strains grown in liquid media containing 2% Avicel, MM and 0.5% urea.

M: protein marker; 1: parent strain; 2: *P. oxalicum* 114-2  $\Delta p\delta\Delta PDE\_03657$ ;

3: *P. oxalicum* 114-2  $\Delta erp\Delta p\delta$ ; 4: *P. oxalicum* 114-2  $\Delta p\delta\Delta PDE\_00613$ ;

5: parent strain; 6: *P. oxalicum* 114-2  $\Delta PDE\_00613\Delta PDE\_03657$ ;

7: *P. oxalicum* 114-2  $\Delta erp\Delta PDE\_03657$ ; 8: *P. oxalicum* 114-2  $\Delta erp\Delta PDE\_00613$ .

### Fig. S3

Deletion of the *p $\delta$*  gene was confirmed by Southern blotting.

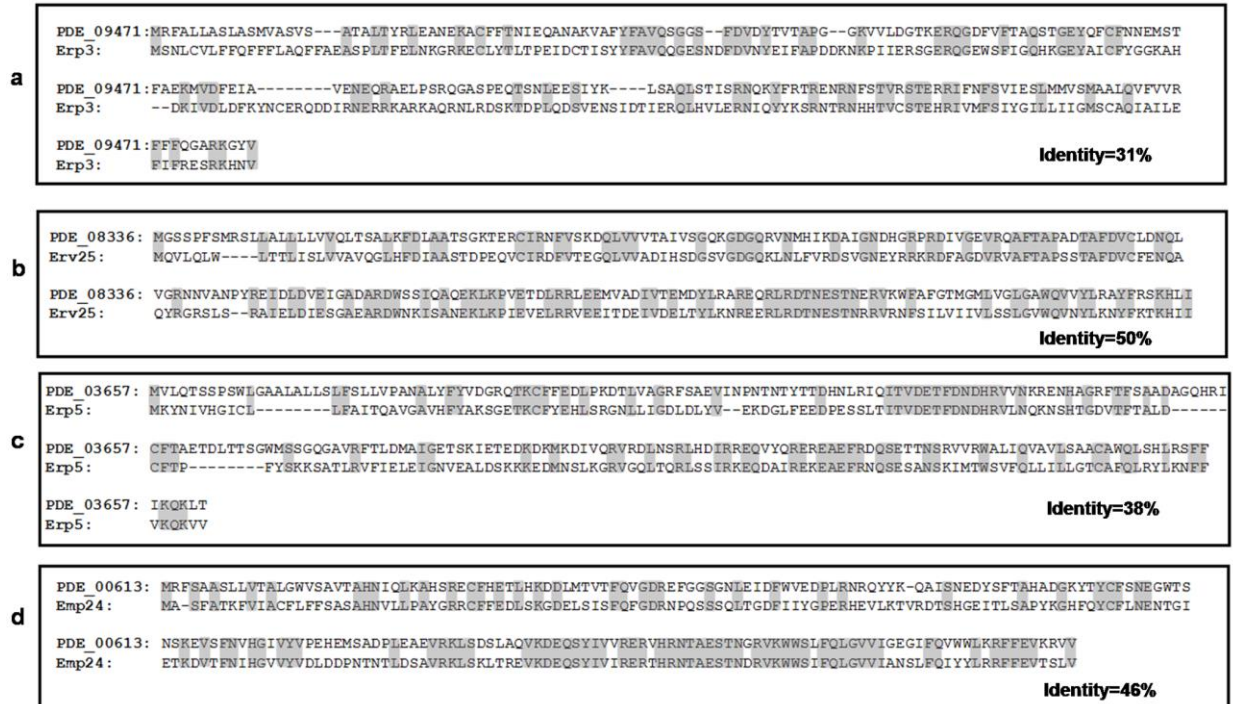
Panel a, schematic diagram of Southern blotting. Panel b, Southern blotting analysis of *p $\delta$*  deletion. M: 1 kb marker; 1: *P. oxalicum*  $\Delta erp$  strain; 2: *P. oxalicum*  $\Delta erp\Delta p\delta$  strain. Arrows are indicated the predicted band sizes of each strain.

**Table S1** Proteins were significantly increased or decreased in  $\Delta erp\Delta p\delta$  strain.

\* were predicted as GPI-anchor proteins

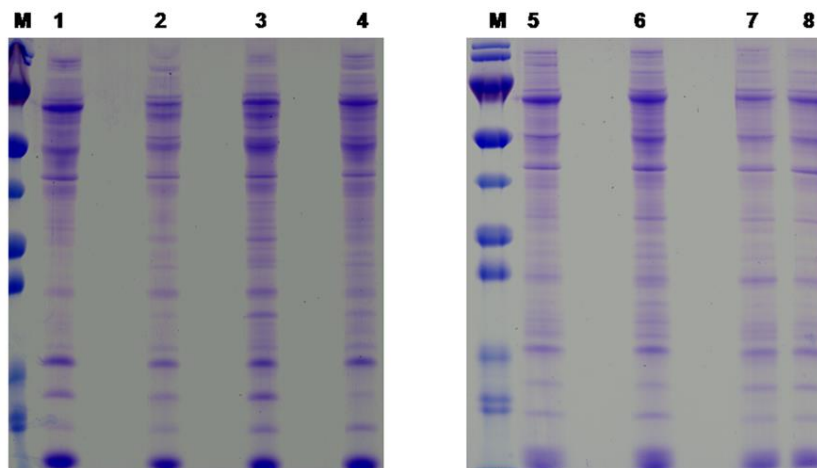
**Table S2** *P. oxalicum* strains that were used in this study.

**S1** Protein accession numbers in the phylogenetic tree in Fig. 1a.



**Fig. S1** Sequence alignment of p24 homologs in *P. oxalicum* 114-2 and *S. cerevisiae*.

The same amino acid residues are shaded.



**Fig. S2** SDS-PAGE analysis of intracellular proteins of p24 deletion strains grown in

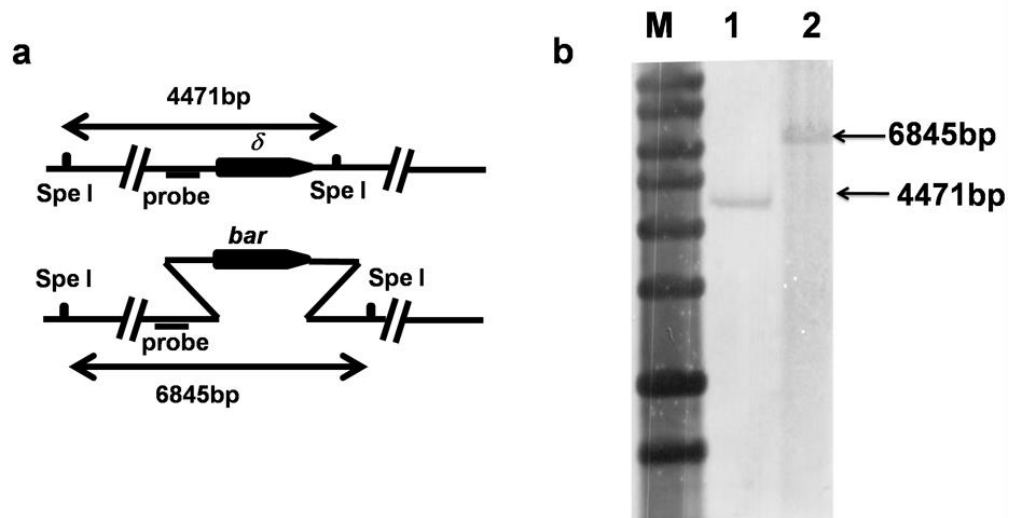
liquid media containing 2% Avicel, MM and 0.5% urea.

M: protein marker; 1: parent strain; 2: *P. oxalicum* 114-2  $\Delta p\delta\Delta PDE\_03657$ ;

3: *P. oxalicum* 114-2  $\Delta erp\Delta p\delta$ ; 4: *P. oxalicum* 114-2  $\Delta p\delta\Delta PDE\_00613$ ; 5: parent

strain; 6: *P. oxalicum* 114-2  $\Delta PDE\_00613\Delta PDE\_03657$ ;

7: *P. oxalicum* 114-2  $\Deltaerp\Delta PDE\_03657$ ; 8: *P. oxalicum* 114-2  $\Deltaerp\Delta PDE\_00613$ .



**Fig. S3** Deletion of the  $p\delta$  gene was confirmed by Southern blotting.

Panel a, schematic diagram of Southern blotting. Panel b, Southern blotting analysis of  $p\delta$  deletion. M: 1 kb marker; 1: *P. oxalicum*  $\Deltaerp$  strain; 2: *P. oxalicum*  $\Deltaerp\Delta p\delta$  strain. Arrows are indicated the predicted band sizes of each strain.

**Table S1** Proteins were significantly increased or decreased in  $\Delta erp\Delta p\delta$  strain.

\* were predicted as GPI-anchor proteins

**Table S 1.1 Cytoplasm (Secreted protein)**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_09285	$\alpha$ -L-rhamnopyranohydrolase, GH28	positive infinity	1.24e-04	Rhamnogalacturonan hydrolysis
PDE_02805*	Hypothetical protein	151.66	2.21e-04	Unknown
PDE_07124	Cellobiohydrolase, GH6	64.4	2.23e-03	Cellulose hydrolysis
PDE_07936	Metallo peptidase	29.53	1.43e-03	Polypeptide hydrolysis
PDE_09226	Endo- $\beta$ -1,4-glucanase, GH5	14.45	3.97e-04	Cellulose hydrolysis
PDE_07929	Endo- $\beta$ -1,4-glucanase, GH7	11.29	1.63e-03	Cellulose hydrolysis
PDE_05263*	Chitin glucanosyltransferase, GH16	9.09	2.63e-03	The transfer of chitin to $\beta$ -1, 3- and $\beta$ -1, 6-glucans
PDE_05445	Cellobiohydrolase, GH7	8.83	9.68e-05	Cellulose hydrolysis
PDE_07897	$\alpha$ -L-arabinofuranosidase, GH62	8.79	2.61e-04	Arabinoxylan hydrolysis
PDE_06439	Endo- $\beta$ -1,4-glucanase, GH12	7.38	4.02e-05	Cellulose hydrolysis
PDE_06649	Feruloyl esterase, CE1	6.91	9.12e-03	Arabinoxylan hydrolysis

PDE_02536*	Hypothetical protein	3.96	4.64e-03	Unknown
PDE_07945	Cellobiohydrolase, GH7	3.59	1.00e-02	Cellulose hydrolysis
PDE_08094	Endo- $\beta$ -1,4-xylanase, GH10	3.43	3.32e-04	Xylan hydrolysis
PDE_03112*	Exo- $\beta$ -1,3-glucanase, GH17	2.83	7.96e-03	$\beta$ -1, 3-glucans hydrolysis
PDE_08122	Chitinase, GH18	2.61	5.09e-03	Chitin hydrolysis
PDE_00500	Non-hemolytic phospholipase C	1.7	8.38e-03	Phosphatidylcholine and phosphatidylserine hydrolysis
PDE_09417	Glucoamylase, GH15	-1.97	1.84e-03	$\alpha$ -1, 4-glucose hydrolysis
PDE_00615	$\beta$ -1,3-glucanosyltransferase, GH17	-2	1.00e-02	Transferring the newly generated reducing end to the non-reducing end of another $\beta$ -1, 3-glucan molecule, and generating a new $\beta$ -1, 6-linkage
PDE_06770	Hypothetical protein	-2.03	8.77e-03	Unknown
PDE_03966	$\alpha$ -glucosidase, GH31	-3.18	2.03e-04	Maltose and other small maltooligosaccharides hydrolysis

**Table S 1.2 Cytoplasm**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_02929	D-xyllose reductase	positive infinity	2.95e-03	Reducing D-xyllose into xylitol

PDE_01713	Formamidase	13.19	4.23e-05	Formamide hydrolysis
PDE_02372	Coproporphyrinogen III oxidase	12.66	1.00e-02	The heme biosynthesis
PDE_09398	Transaldolase	11.47	1.80e-03	Pentose-phosphate pathway
PDE_03821	Mannitol-1-phosphate 5-dehydrogenase	9.1	5.96e-03	Fructose and mannose metabolism
PDE_08726	Ribosome biogenesis protein	7.53	1.07e-03	20S pre-rRNA processing; 40S ribosomal subunit synthesis
PDE_05515	$\beta$ -1,3-glucanase	7.44	5.88e-05	Hydrolysis of 1, 3- linkages in $\beta$ -glucans
PDE_02988	PLC-like phosphodiesterase	7.28	9.09e-03	Phosphatidylinositol metabolism
PDE_04575	Member of the PUF protein family	6.25	3.25e-04	Binding mRNAs encoding nucleolar ribosomal RNA-processing factors
PDE_04048	X-Pro aminopeptidase	4.42	2.13e-04	Peptidase hydrolysis
PDE_02117	Guanyl-specific ribonuclease	4.23	1.41e-04	Endoribonuclease activity
PDE_04364	GDSL esterase/lipase	3.69	6.84e-03	Ester bonds hydrolysis
PDE_01218	ABC transporter	3.39	1.44e-03	Translocation of a variety of compounds across transmembrane
PDE_04898	Chitinase	2.51	2.15e-03	Chitin hydrolysis
PDE_05480	Aminopeptidase	2.31	5.30e-03	Peptidase hydrolysis

PDE_03243	Esterase/lipase	1.8	1.00e-02	Ester bonds hydrolysis
PDE_04618*	$\beta$ -1,3-glucanosyltransferase, GH72	1.51	7.97e-03	Transferring the newly generated reducing end to the non-reducing end of another $\beta$ -1, 3-glucan molecule and elongating the $\beta$ -1, 3-linkage
PDE_04468	$\alpha$ -1,6-mannanase	-1.55	1.14e-03	Random hydrolysis of $\alpha$ -1, 6-mannosidic linkages in unbranched 1, 6-mannans
PDE_02839	DNA replication licensing factor Mcm3	-1.72	8.61e-03	DNA replication; Component of the Mcm2-7 hexameric helicase complex
PDE_08209	Dienelactone hydrolase family protein	-2.03	8.77e-03	Aromatic compounds degradation
PDE_09561	Lanosterol synthase	-2.9	1.96e-03	Ergosterol biosynthesis
PDE_06127	Ribonucleoside-diphosphate reductase subunit M1	-4.56	3.83e-03	Catalyzing rate-limiting step in dNTP synthesis
PDE_04823	Hydroxymethylglutaryl-CoA synthase	-4.69	6.03e-03	Mevalonate biosynthesis
PDE_02141	Polyketide synthase	-5.9	1.08e-04	Polyketides biosynthesis
PDE_01235	Polyketide synthase	-14.79	6.41e-05	Polyketides biosynthesis
PDE_02162	The dioxygenase family protein	-22.32	7.55e-04	Betalain biosynthesis
PDE_01429	Cytochrome P450 family protein	-319.88	1.92e-03	Oxidoreductase activity



**Table S 1.3 proteasome**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_02603	26S proteasome regulatory subunit	5.63	4.98e-04	Degrading proteins that have been ubiquitinated

**Table S 1.4 Nucleus**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_08726	Ribosome biogenesis protein	7.53	1.07e-03	20S pre-rRNA processing; 40S ribosomal subunit synthesis
PDE_06111	DNA mismatch repair protein	3.59	4.31e-04	DNA mismatch repair
PDE_05989	Kinesin family member 11	3.58	1.28e-03	Spindle assembly and chromosome segregation
PDE_02839	DNA replication licensing factor Mcm3	-1.72	8.61e-03	Protein involved in DNA replication; Component of the Mcm2-7 hexameric helicase complex
PDE_06220	DNA polymerase epsilon subunit 2	-2.04	5.31e-03	The maintenance of fidelity of chromosomal replication
PDE_07239	Quinate repressor protein	-2.39	1.00e-02	Transcription of the quinate utilization pathway genes
PDE_02391	Chromosome segregation protein	-2.48	1.45e-04	Sister chromatid cohesion and linking DNA synthesis to sister chromatid cohesion
PDE_07738	rRNA biogenesis protein	-3.33	1.99e-04	18S and 5.8S rRNA biogenesis

PDE_05592	DEAD/DEAH box helicase	-4.39	5.95e-03	Nuclear RNA processing and degradation
PDE_06767	Nucleolar protein	-5.42	1.00e-02	35S pre-RNA processing; 40S ribosomal subunit biogenesis
PDE_09925	Nuclear condensin complex subunit 3	-5.9	5.95e-05	The establishment and maintenance of chromosome condensation; the segregation and chromatin binding of the condensin complex

**Table S 1.5 Cytoskeleton**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_08653	Dynactin, 150 kDa	2.47	5.48e-03	Dynactin is a multisubunit complex required for cytoplasmic dynein to efficiently transport vesicles along microtubules.
PDE_00169	Arp2/3 complex subunit 2	-12.02	1.63e-05	The regulation of actin polymerization and mediating the formation of branched actin networks

**Table S 1.6 Golgi-like apparatus**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_04430	Guanyl-nucleotide exchange factor	2.45	1.24e-03	Arl1p activation and vesicular transport
PDE_03529	Aminophospholipid translocase	2.15	8.94e-03	Endocytosis and vacuolar biogenesis

**Table S 1.7 Mitochondria**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_09399	Superoxide dismutase [Cu-Zn]	6.28	4.15e-04	Detoxifying superoxide
PDE_05733	3-hydroxybutyryl-CoA dehydrogenase	5.53	5.78e-03	Catalyzing the NAD-dependent dehydration of $\beta$ -hydroxybutyryl-CoA to acetoacetyl-CoA

**Table S 1.8 Endoplasmic reticulum**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_08512	Alkaline ceramidase	7	7.37e-03	Sphingolipid ceramide hydrolysis
PDE_07729	Phosphatidylethanolamine N-methyltransferase	6.06	4.82e-04	The conversion of phosphatidylethanolamine to phosphatidylcholine
PDE_03488	Fatty acid transporter protein	5.89	7.55e-04	Long chain fatty acids transport
PDE_06282	ER quality control lectin	1.89	2.12e-03	The bridging protein between the machinery that extracts misfolded proteins from the ER lumen and the machinery involved in their retro-translocation across the ER-membrane

**Table S 1.9 Plasma membrane**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_04996	Putative sensor/transporter protein	2.55	1.13e-03	Cell wall biogenesis

**Table S 1.10 Hypothetical protein**

<b>Gene name</b>	<b>Protein name</b>	<b>Fold change</b>	<b>P value</b>	<b>Predicted protein function</b>
PDE_02805*	Hypothetical protein	151.66	2.21e-04	Unknown
PDE_02536*	Hypothetical protein	3.96	4.64e-03	Unknown
PDE_08596	Hypothetical protein	2.03	2.81e-03	Unknown
PDE_05474	Hypothetical protein	1.73	1.49e-03	Unknown
PDE_06770	Hypothetical protein	6.49	4.14e-03	Unknown
PDE_07440	Hypothetical protein	-2.65	8.42e-03	Unknown
PDE_09712	Hypothetical protein	-3.09	1.00e-02	Unknown
PDE_05911	Hypothetical protein	-3.12	9.30e-04	Unknown
PDE_06953	Hypothetical protein	-3.41	4.40e-03	Unknown
PDE_00315	Hypothetical protein	-5.64	5.82e-04	Unknown
PDE_03744	Hypothetical protein	-13.12	1.46e-04	Unknown

PDE_06675	Hypothetical protein	minus infinity	6.74e-04	Unknown
-----------	----------------------	----------------	----------	---------

Table S2 *P. oxalicum* strains that were used in this study.

strains	Genotype	Source
<i>P. oxalicum</i> 114-2	Wildtype	Reference 6
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph$	Reference 51
$\Delta pku70$		
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph; \Delta erp::ptrA$	Reference 27
$\Delta erp$		
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta erp\Delta p\delta$	$\Delta erp::ptrA; \Delta p\delta::bar$	
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta erp\Delta PDE\_00613$	$\Delta erp::ptrA; \Delta PDE\_00613::bar$	
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta erp\Delta PDE\_03657$	$\Delta erp::ptrA; \Delta PDE\_03657::bar$	
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta p\delta\Delta PDE\_00613$	$\Delta p\delta::ptrA; \Delta PDE\_00613::bar$	
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta p\delta\Delta PDE\_03657$	$\Delta p\delta::ptrA; \Delta PDE\_03657::bar$	
<i>P. oxalicum</i> 114-2	$\Delta pku70::hph;$	This study
$\Delta PDE\_00613\Delta PDE\_03657$	$\Delta PDE\_03657::ptrA; \Delta PDE\_00613::bar$	
BiFC- <i>erp/p</i> $\delta$	$Pgpd::erp::eyfpN::TtrpC::hph;$	This study
	$Pgpd::p\delta::eyfpC::TtrpC::ptrA;$	
BiFC- <i>erp/eyfpC</i>	$Pgpd::erp::eyfpN::TtrpC::hph;$	This study

---

	<i>Pgpd::eyfpC::TrpC::ptrA;</i>	
BiFC- <i>pδ/eyfpN</i>	<i>Pgpd::eyfpN::TrpC::hph;</i>	This study
	<i>Pgpd::pδ::eyfpC::TrpC::ptrA;</i>	
BiFC- <i>eyfpN/eyfpC</i>	<i>Pgpd::eyfpN::TrpC::hph;</i>	This study
	<i>Pgpd::eyfpC::TrpC::ptrA;</i>	

---

**S1.** Protein accession numbers in the phylogenetic tree in Fig. 1a.

Hp24 $\gamma$ 2 accession number: NP\_057124.2, Hp24 $\gamma$ 1 accession number: NP\_006849.1,  
 Hp24 $\gamma$ 4 accession number: NP\_031390.1, Hp24 $\gamma$ 3 accession number: NP\_861974.1,  
 Hp24 $\gamma$ 5 accession number: NP\_653277.1, Hp24 $\beta$ 1 accession number: NP\_006806.1,  
 Hp24 $\delta$ 1 accession number: NP\_006818.3, Hp24 $\alpha$ 2 accession number: NP\_059980.2;  
 Dcg31787 accession number: NP\_724105.1, Dcg9053A accession number:  
 NP\_572994.1, Dcg1967 accession number: NP\_572754.1, Dlogjam accession number:  
 AAL28932.1, Dcg3564 accession number: NP\_572165.1, Dcg9308 accession number:  
 NP\_611629.1, Declair accession number: NP\_788616.1, Dbaiser accession number:  
 NP\_651323.3; Serp2 accession number: NP\_009395.1, Serp4 accession number:  
 NP\_014659.1, Serp3 accession number: NP\_010266.1, Semp24 accession number:  
 NP\_011315.1, Serv25 accession number: NP\_013701.1, Serp1 accession number:  
 NP\_009402.1, Serp6 accession number: NP\_011513.1, Serp5 accession number:  
 NP\_011978.1; Xp24 $\gamma$ 3 accession number: NP\_989261.1, Xp24 $\gamma$ 5 accession number:  
 AAH87777.1, Xp24 $\gamma$ 1 accession number: NP\_001039263.1, Xp24 $\gamma$ 2 accession  
 number: NP\_001016560.1, Xp24 $\alpha$ 3 accession number: NP\_001015779.1, Xp24 $\delta$ 2  
 accession number: NP\_001007925.1; Pp24 $\alpha$ 1 accession number: XP\_517060;

A3g07680.1 accession number: NP\_187425.1, A3g29070.1 accession number:  
NP\_189550.2, A2g03290.X accession number: AAD17445.1, A1g69460.1 accession  
number: NP\_177105.2, A1g21900.1 accession number: NP\_173608.1, A1g09580.1  
accession number: NP\_172429.1. The tree was constructed by the neighbor-joining  
method. Bootstrap values are shown at each node, and are calculated from 1000 trees.  
Bar, evolutionary distance of 0.2.