



## 棉花抗百草枯基因Ghpar1基因 生物信息学分析

汇报人：孙金秋

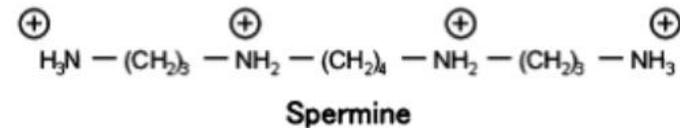
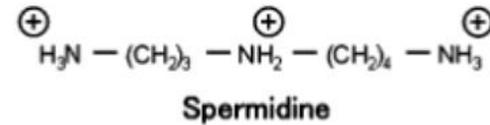
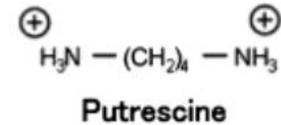
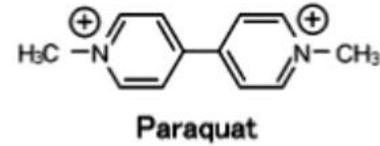
队员：4G04、4G05、4G06、4G10

授课老师：罗静初老师



# 背景介绍

百草枯，化学名称是1-1-二甲基-4-4-联吡啶阳离子盐，具有触杀作用和一定内吸作用。能迅速被植物绿色组织吸收，使其枯死。对非绿色组织没有作用。在土壤中迅速与土壤结合而钝化，对植物根部及多年生地下茎及宿根无效。





# 文献报道

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## **PARAQUAT RESISTANT1, a Golgi-Localized Putative Transporter Protein, Is Involved in Intracellular Transport of Paraquat<sup>1</sup>[C][W]**

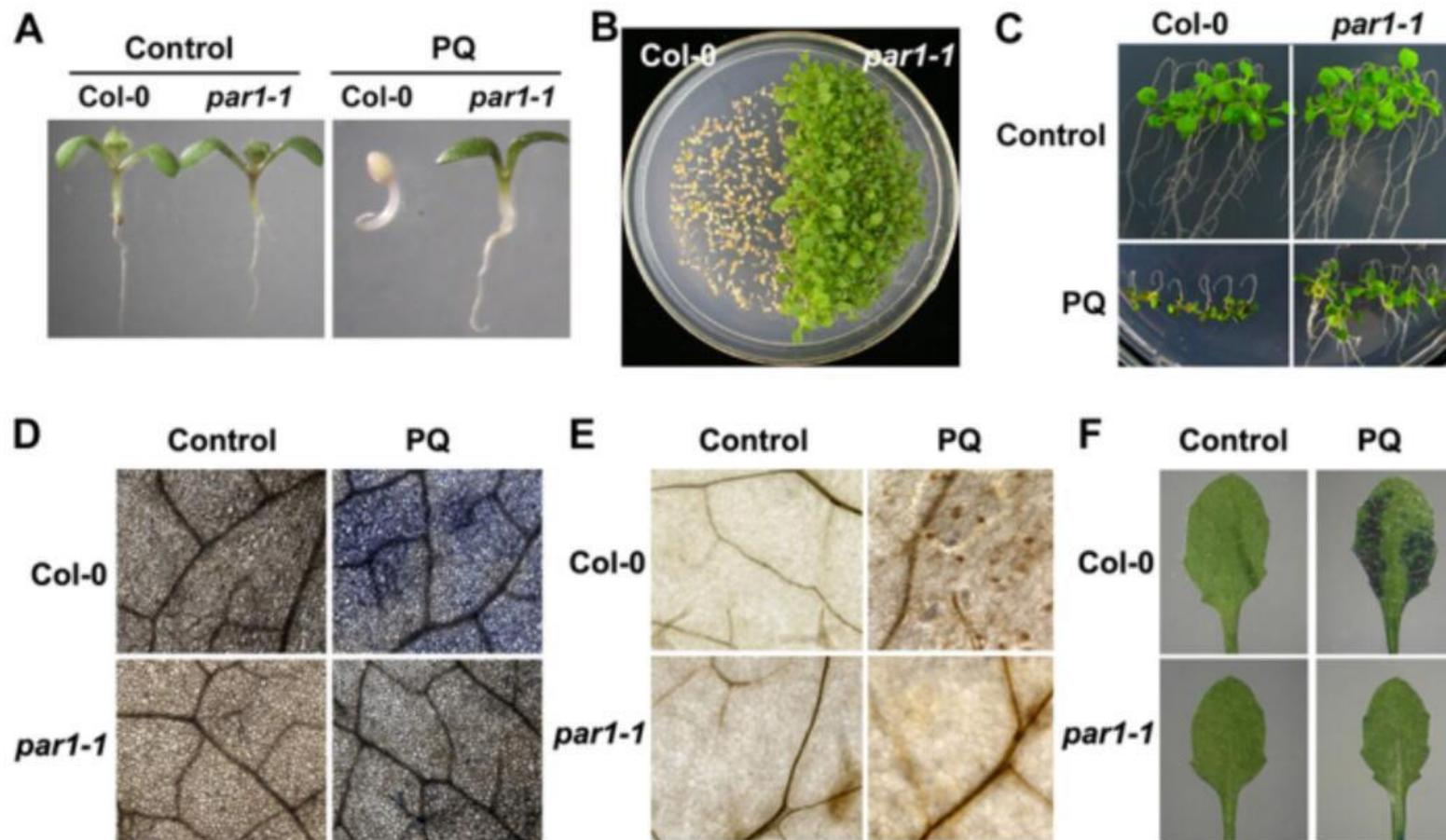
Jianyong Li<sup>2</sup>, Jinye Mu<sup>2</sup>, Jiaoteng Bai<sup>2</sup>, Fuyou Fu, Tingting Zou, Fengying An, Jian Zhang, Hongwei Jing, Qing Wang, Zhen Li, Shuhua Yang, and Jianru Zuo\*

State Key Laboratory of Plant Physiology and Biochemistry and National Plant Gene Research Center, College of Biological Sciences (J.L., S.Y.), and State Key Laboratory of Animal Nutrition, College of Animal Sciences and Technology (T.Z., Z.L.), China Agricultural University, Beijing 100193, China; State Key Laboratory of Plant Genomics and National Plant Gene Research Center, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China (J.M., J.B., F.F., F.A., J.Zh., H.J., Q.W., J.Zu.); and Graduate School, Chinese Academy of Sciences, Beijing 100049, China (J.B., H.J.)

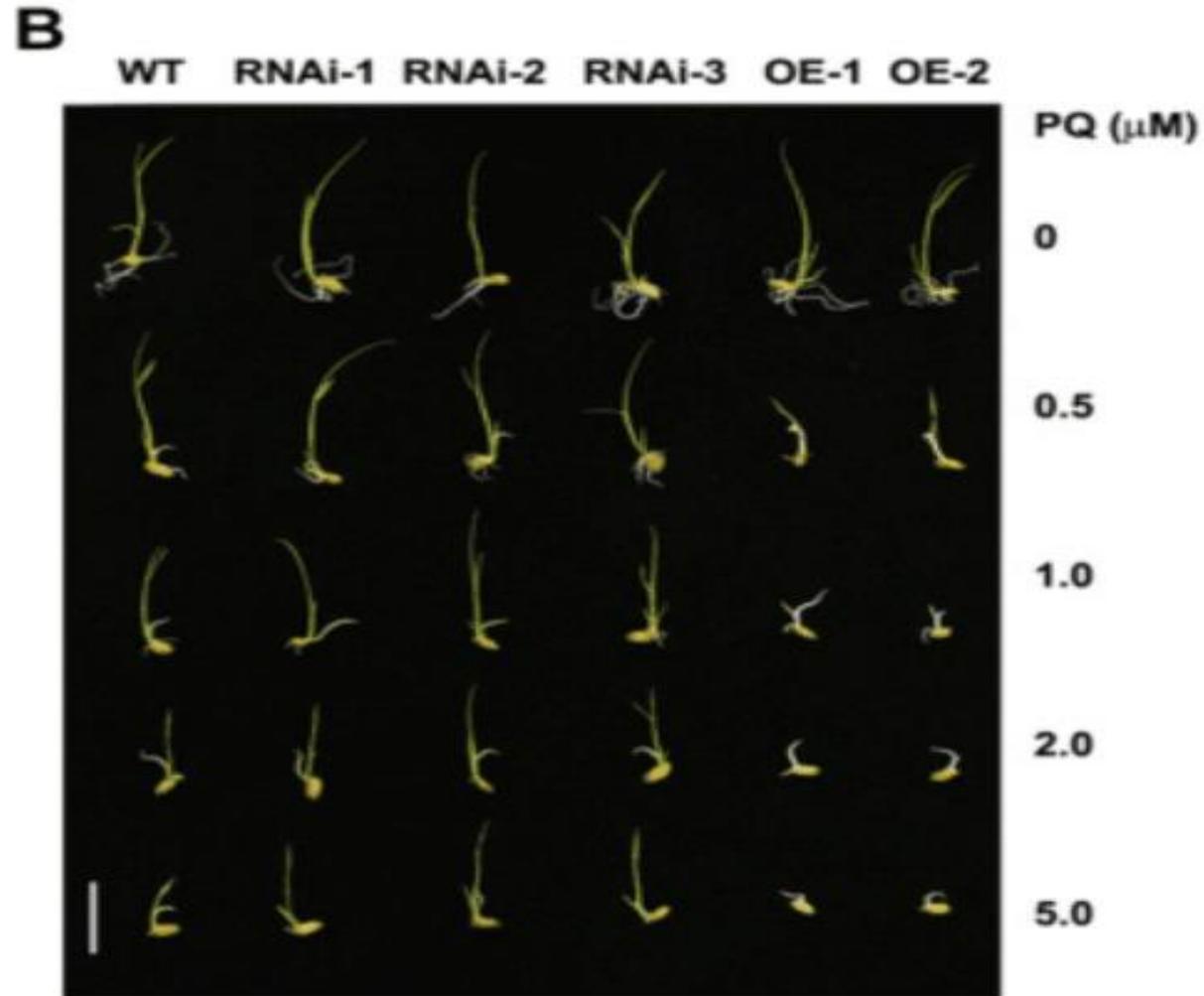
Jianyong Li Plant physiology, May 2013, vol, 162, pp. 470-483, www.



# 文献报道



# 文献报道





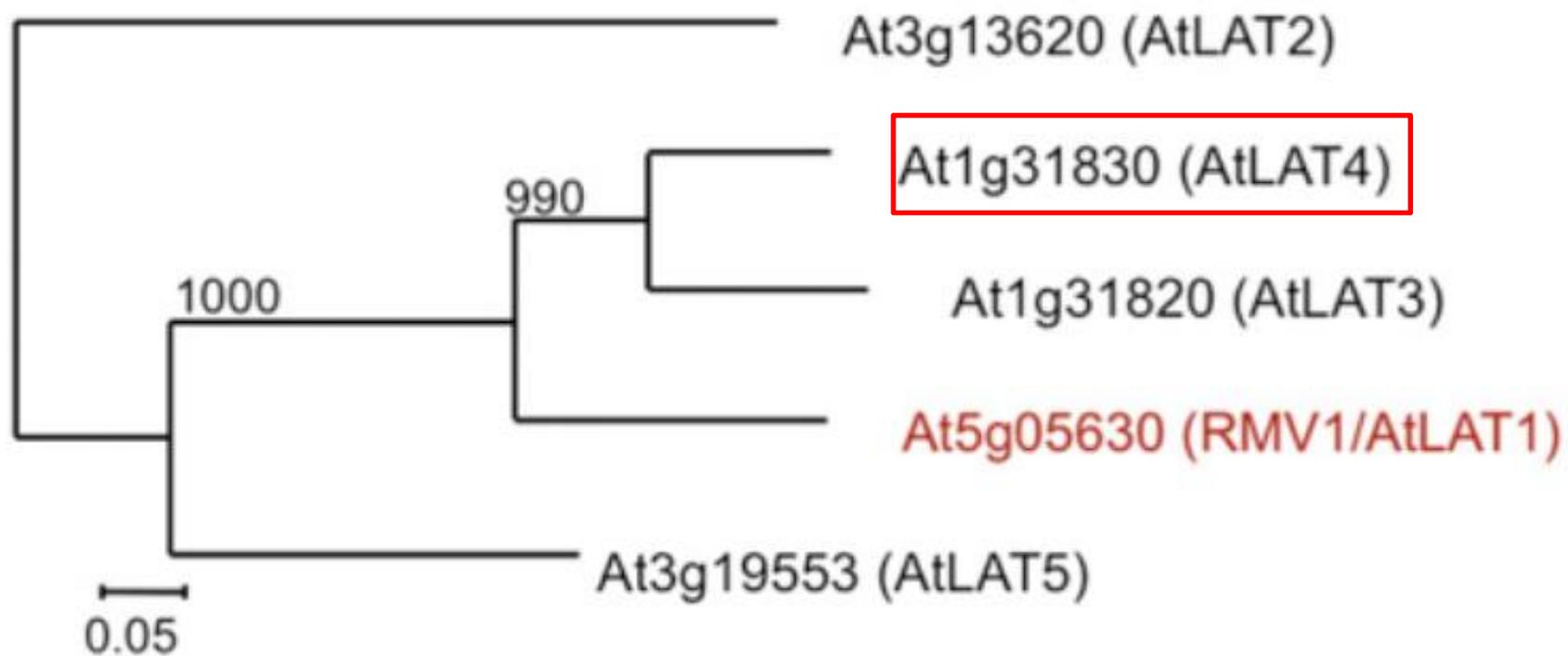
# Natural variation in a polyamine transporter determines paraquat tolerance in *Arabidopsis*

Miki Fujita<sup>a</sup>, Yasunari Fujita<sup>b,c</sup>, Satoshi Iuchi<sup>d</sup>, Kohji Yamada<sup>b,e,1</sup>, Yuriko Kobayashi<sup>d,2</sup>, Kaoru Urano<sup>a</sup>, Masatomo Kobayashi<sup>d</sup>, Kazuko Yamaguchi-Shinozaki<sup>b,e</sup>, and Kazuo Shinozaki<sup>a,c,3</sup>

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Edited\* by Maarten Koornneef, Max Planck Institute for Plant Breeding Research, Cologne, Germany, and approved March 9, 2012 (received for review December 26, 2011)

# 文献报道



Miki Fujitaa, Yasunari Fujitab, PNAS [www.pnas.org/cgi/doi/10.1073/pnas.1121406109](http://www.pnas.org/cgi/doi/10.1073/pnas.1121406109)



# 研究内容

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通过开展本研究最终实现：

- 克隆拟南芥PAR1棉花同源基因，并验证其在抗百草枯中的作用，为改良作物的抗逆能力的基因工程提供优良的基因源；
  - 通过改良和导入该编码基因，进一步提高棉花的综合抗逆能力，使其满足更大范围的推广种植，缓解粮棉争地的问题，实现棉花的稳产和丰产
-

# 数据库检索



 Home Help Contact About Us Subscribe Login Register Institution: Chinese Academy of Agricultural Sciences (subscribed)

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**Locus: AT1G31830** [What's new on this page](#) [Add a Comment](#)

Representative Gene Model **AT1G31830.1**

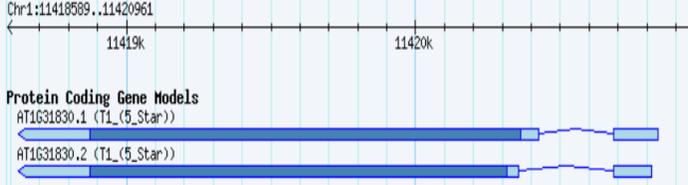
Gene Model Type protein\_coding

Other names: ATPUT2, PARAQUAT-RESISTANT 2, POLYAMINE UPTAKE TRANSPORTER 2, PQR2, PUT2

Description Encodes POLYAMINE UPTAKE TRANSPORTER 2, an amino acid permease family protein.

Other Gene Models **AT1G31830.2** (splice variant)

Map Detail Image



Annotations

category	relationship type	keyword
GO Biological Process	involved in	polyamine transport
GO Cellular Component	located in	plasma membrane
GO Molecular Function	has	basic amino acid transmembrane transporter activity, polyamine transmembrane transporter activity, symporter activity
Growth and Developmental Stages	expressed during	L mature pollen stage, LP.02 two leaves visible stage, LP.04 four leaves visible stage, LP.06 six leaves visible stage, LP.08 eight leaves visible stage, LP.10 ten leaves visible stage, LP.12 twelve leaves visible stage, M germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescence stage

## Function<sup>i</sup>

Probable cell membrane polyamine/proton symporter involved in the polyamine uptake in cells. [By similarity](#)

## GO - Molecular function<sup>i</sup>

- antiporter activity [Source: GO\\_Central](#)
- L-amino acid transmembrane transporter activity [Source: GO\\_Central](#)
- polyamine transmembrane transporter activity [Source: TAIR](#)
- symporter activity [Source: UniProtKB-KW](#)

[View the complete GO annotation on QuickGO ...](#)

## GO - Biological process<sup>i</sup>

- polyamine transport [Source: TAIR](#)

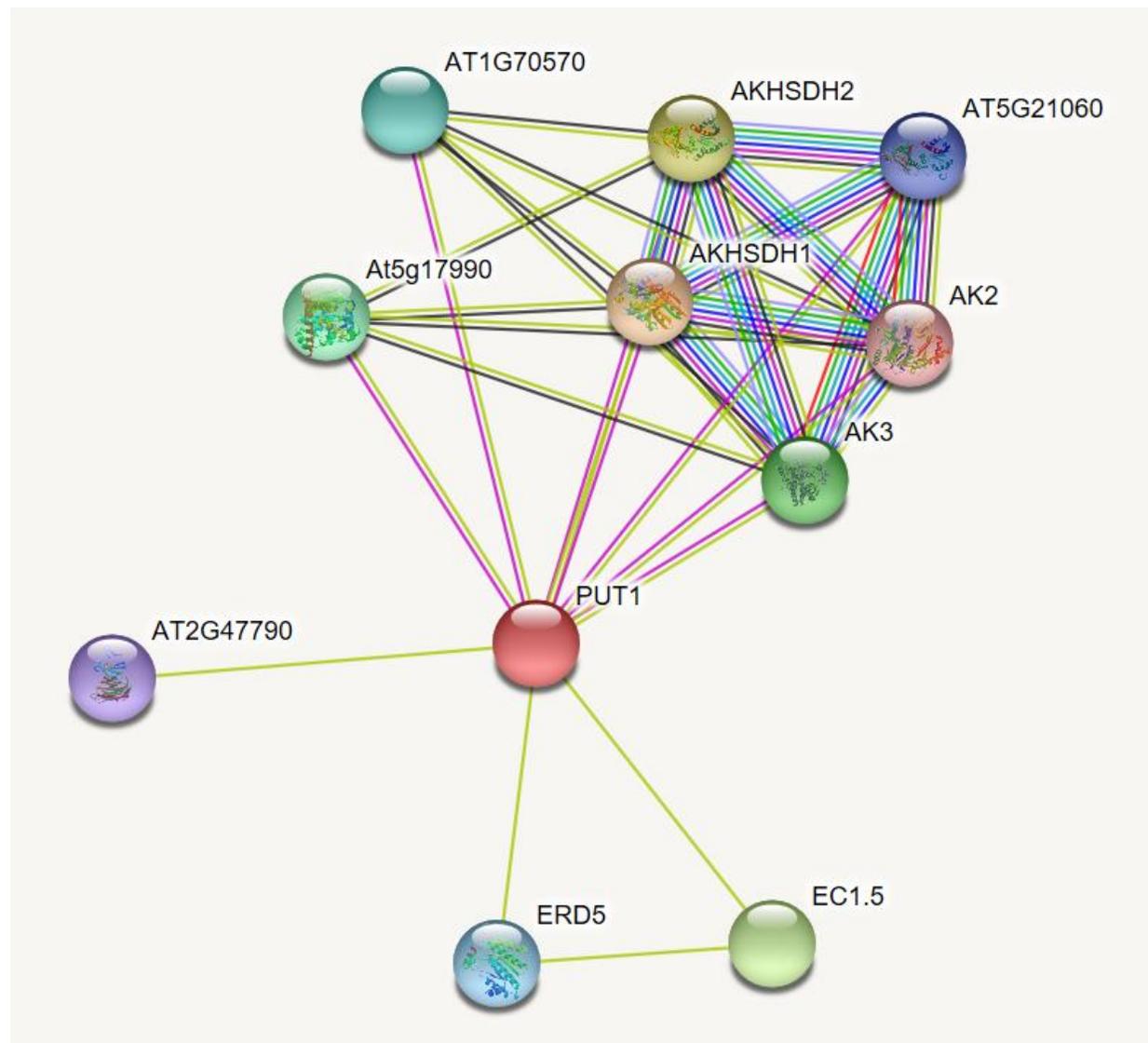
[View the complete GO annotation on QuickGO ...](#)

## Keywords<sup>i</sup>

Biological process [Symport, Transport](#)

## Names & Taxonomy<sup>i</sup>

# 互作蛋白质



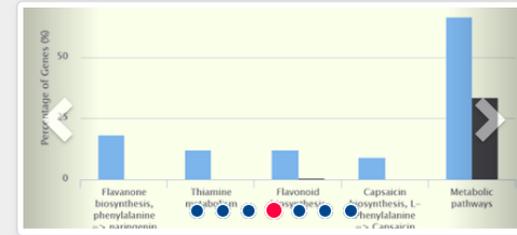


# BLAST 同源比对

CottonFGD [Home](#) [Search](#) [Analyze](#) [Tools](#) [About](#)

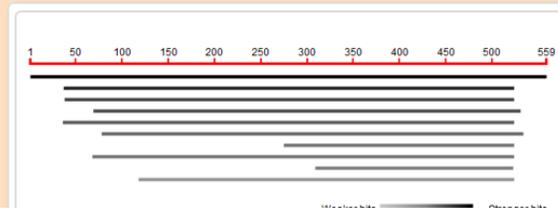
## Cotton Functional Genomics Database

Cotton Functional Genomics Database (CottonFGD) integrates with modern genomic/transcriptomic data and search/analysis/visualization modules. It aims at providing an easy, quick and visualized data analysis platform for cotton (*Gossypium* spp) researchers and other functional genomic researches.



- [Help](#)
- [Data Download](#)
- [Acknowledgement](#)

Search Cotton Genes



<http://10.122.114.30/about/acknowledgement.html>



# BLAST 同源比对

## BLAST: Search Homologs by Sequence Similarity in Cotton (*Gossypium* spp.)



```
>sp|Q9C6S5|PHSB_ARATH Probable polyamine transporter At1g31830 OS=Arabidopsis thaliana OX=3702 GN=At1g31830 PE=2 SV=
MQKRRITVNPASIEMSQYENNEVPYSSVGADEVSPSPPKATDKIRKVSMLPLVFLIFY
EVSGGPFQGVEDSVNAAGPLLALLGFVIFPFIWSIPEALITAEMGTMYPENGGYVWVSSA
LGPFWGFQQGWKWLKSGVIDNALYPVFLDYLDKSGVPALGSGLPRVASILVLTILLTYLN
YRGLTIVGWWAVLMGVFSILPFAVMGLISIPQLEPSRWLVMDLGNVWNLNLTFLWNLN
YWDSISTLAGEVENPNHTLPKALFYGVILVACSYIFPLLAGIGAIPLERKWTGDFSDV
AKALGGAWLRWVQAAAATSNMGMFIAEMSSDSFQLLGM AERGMLPEFFAKRSRYGTPLL
GILFSASGVVLLSWLSFQEI VAAENLLYCVGMILEFIAFVRMRMKHPAASRPYKIPIGTT
GSILMCIPPTILICAVVALSSLKVAAVSIVMMIIGFLIHPLLNHMDRKRWVKFSISSDLP
DLQQQTREYEETLIR
```

### Nucleotide databases

- NCBI Nucleotide collection (nt), LARGE !!!
- cDNA, *G. arboreum* (A<sub>2</sub>), CRI, 40,960 principle transcripts
- cDNA, *G. barbadense* (AD<sub>2</sub>), NAU, 77,358 principle transcripts
- cDNA, *G. hirsutum* (AD<sub>1</sub>), JGI, 66,577 principle transcripts
- cDNA, *G. hirsutum* (AD<sub>1</sub>), NAU, 70,478 principle transcripts
- cDNA, *G. raimondii* (D<sub>5</sub>), JGI, 37,505 principle transcripts
- genome, *G. arboreum* (A<sub>2</sub>), CRI, 13 Chrs + 4,503 scaffolds
- genome, *G. barbadense* (AD<sub>2</sub>), NAU, 26 Chrs + 2,013 scaffolds

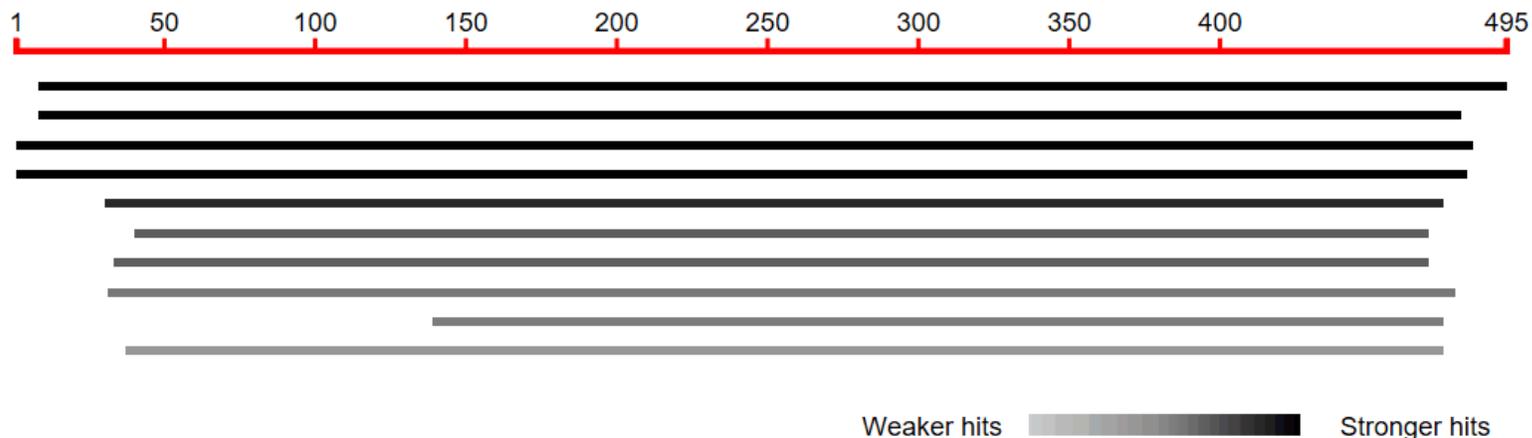
### Protein databases

- G. arboreum* (A<sub>2</sub>), CRI, 40,960 principle proteins
- G. barbadense* (AD<sub>2</sub>), NAU, 77,358 principle proteins
- G. hirsutum* (AD<sub>1</sub>), JGI, 66,577 principle proteins
- G. hirsutum* (AD<sub>1</sub>), NAU, 70,478 principle proteins
- G. raimondii* (D<sub>5</sub>), JGI, 37,505 principle proteins
- NCBI Non-redundant protein sequences (nr), EXTREMELY LARGE !!!
- UniProt (Swiss-Prot) Database



# BLAST 同源比对

Query= sp|Q9C6S5|PHSB\_ARATH Probable polyamine transporter At1g31830 1/1  
OS=Arabidopsis thaliana OX=3702 GN=At1g31830 PE=2 SV=1



Number	Sequences producing significant alignments	Total score	E value	Length
1.	Gohir.A10G067600.1	761.91	0.00	538
2.	Gohir.D10G107000.1	752.28	0.00	478
3.	Gohir.D04G168400.1	664.07	0.00	485
4.	Gohir.A04G131800.1	655.60	0.00	485

BLASTP: 1 query, 1 database

Query= sp|Q9C6S5|PHSB\_A...

Download FASTA, XML, TSV

[FASTA of all hits](#)

[FASTA of selected hit\(s\)](#)

[Standard tabular report](#)

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[Full XML report](#)



# 核苷酸序列比对

Center of Bioinformatics your lab on the web **WebLab**

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Result: 622 View: Pair-alignment view View Edit Download Friendly Print

### Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
546	1978.0	367/546 (67.2%)	429/546 (78.6%)	59/546 (10.8%)

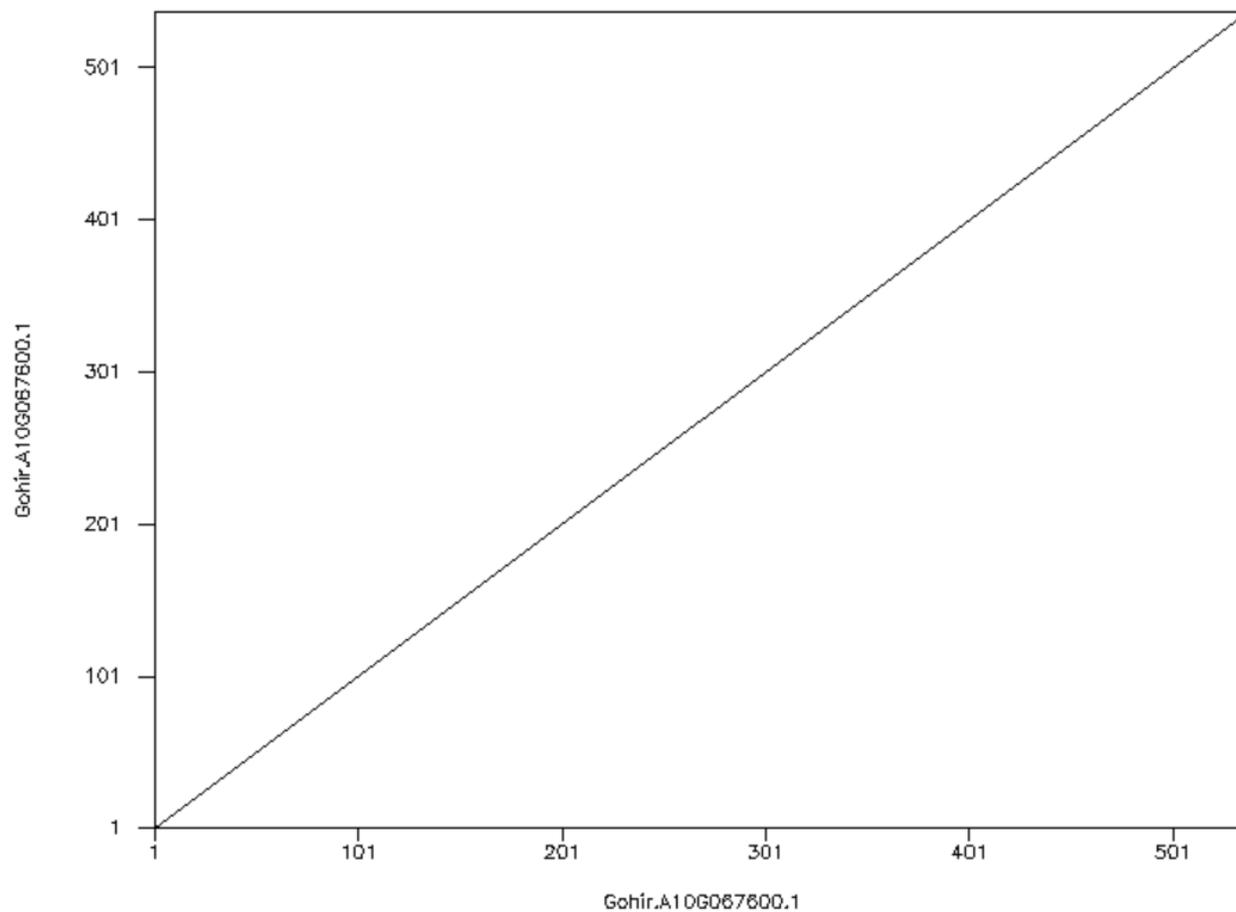
PHSB_ARATH	1	-----	-----MQ	2
Gohir.A10G067	1	MVEVVSEVSGDPQTSGQPAAGCVSPQNQLVSVSQQIQTTVDTKHPSSSEDE		50
PHSB_ARATH	3	KRRIITVNPASIASIEMSQYENNEVPYSSVGADEVPSPPKATDKIRKVSML		52
Gohir.A10G067	51	KLR-HTPNARASSAMGQYDSAE--YVTIG--EVPTPP---ADNFKKVSVL		92
PHSB_ARATH	53	PLVFLIFYEVS GGFVGVEDSVNAAGPLLALLGFVIFPFIWSIPEALITAE		102
Gohir.A10G067	93	PLIFLIFYEVS GGFVGVEDSVQAAGPFLALLGFLVFPFIWSIPEALITAE		142
PHSB_ARATH	103	MGTMPYENG GYVWVSSALGPFWGFQQGWMKWLSGVIDNALYPVFLFDYL		152
Gohir.A10G067	143	MGTMPYENG GYVWVASALGPYWGFQQGWMKWLSGVIDNALYPVFLFDYL		192



# 核苷酸序列分析

Dottup: fasta::1055047:Gohir.A10G067600.1 vs fasta::1055...

Sat 23 Jun 2018 05:33:02







# 载体构建

Vector NTI - [DNA/RNA Molecule from document PBI121sequence]

File Edit View Primer Design Analyses Cloning Gel Analysis List Window Align Assemble Tools Help

AF485783

- General Description
- Standard Fields
- References
- Comments
- Annotations
- Feature Map
- Imported Features Not Shown on Map
- Restriction/Methylation Map
  - ApaI: 2 sites
  - AvaI: 7 sites
  - BamHI: 1 site
  - ClaI: 1 site
  - EcoRI: 1 site
  - HindIII: 1 site
  - NcoI: 4 sites
  - PstI: 3 sites**
  - SmaI: 1 site
  - XmaI: 1 site

AF485783  
14758 bp

Restriction sites: *AvaI* (14005), *AvaI* (159), *PstI* (12855), *PstI* (3020), *NcoI* (9946), *NcoI* (9418), *HindIII* (4951), *ClaI* (4538), *NcoI* (3399).

Features: Misc Feature 1-14, Rep Origin 1, *nptII*.

```
6901  GCTGCTGTGC  GCTTTAACTT  CTCTTAGGCG  ATTGTTTTCG  AAGCGGGCAA  CAAGCCGAAA  GAACGTGACA  GCGAAGAGGC  AGTCAACGGG  GAAACTCASC
CGACGACAGC  CGAAATTGGA  GAGAAATCCG  TAACCAAAAG  TTCCGCCGTT  GTTCGGCTTT  CTTGACATGT  CGTTCTCCGG  TCAGTTGCCG  CTTTGAGTGG

7001  AAGCGCACTT  ACAGGCGATT  AAAGAGCTGA  TAGCGCGTGA  CAAAAACCAC  CCAAGCGTGG  TGATGTGGAG  TATTGCCAAC  GAACCGGATA  CCGTCCGCA
TTCCGCTGAA  TGTCCGCTAA  TTCTCGACT  ATCCGCGACT  GTTTTGGTGT  GATTCCGACC  ACTACACCCT  ATAACGGTTG  CTGGCCTAT  GGGCAGGCGT

7101  AAGTGCACGG  GAAATATTTC  CGCCACTGGC  GGAAGCAACG  CGTAAACTCG  ACCCGACGGC  TCCGATCACC  TGCGTCAATG  TAATGTTCTG  CGACGCTCAC
TCCACGTGCC  CTTATAAAGC  GCGGTGACCG  CCTTCGTTGC  GCATTTGAGC  TGGGCTGCGC  AGGCTAGTGG  ACGCAGTTAC  AITACAAGAC  GCTGCGAGTG

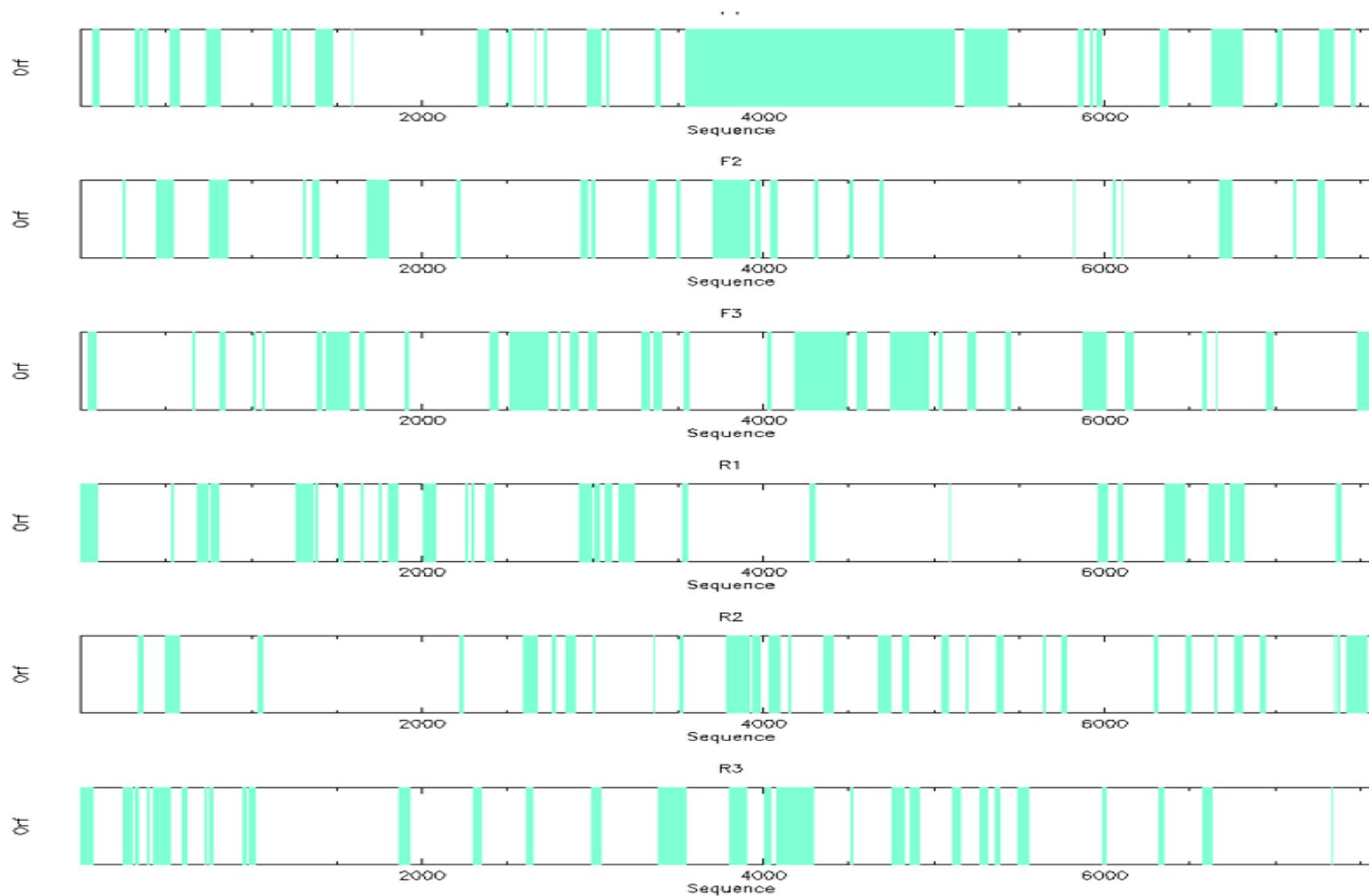
7201  ACCGATACCA  TCMGCGATCT  CTTTGATGTG  CTGTCGCTGA  ACCGTTATTA  CGGATGGTAT  GTCCAAAGCG  GCGATTGGGA  AACGGCAGAG  AAGGTACTGG
TGGCTATGGT  AGTCCGCTAG  GAAACTACAC  GACACGGACT  TGGCAATAT  GCCTACCATA  CAGSTTTCGC  CGCTAAACCT  TTGCCGCTCG  TTCCATGACC

7301  AAAAAAAGCT  TCTGGCCTGG  CAGGAGAAAC  TGCATCAGCC  GATTATCATC  ACCGAATACG  GCGTGGATAC  GTTAGCCGGG  GTGCACTCAA  TGTACACCGA
TTTTCTTGG  AGTCCGGACC  GTCCCTTTTG  ACGTAGTCGG  CTAATAGTAG  TGGCTTATCG  CGCACTATCG  CAATCGGCCG  GACGTGAGTT  ACATGTGGCT

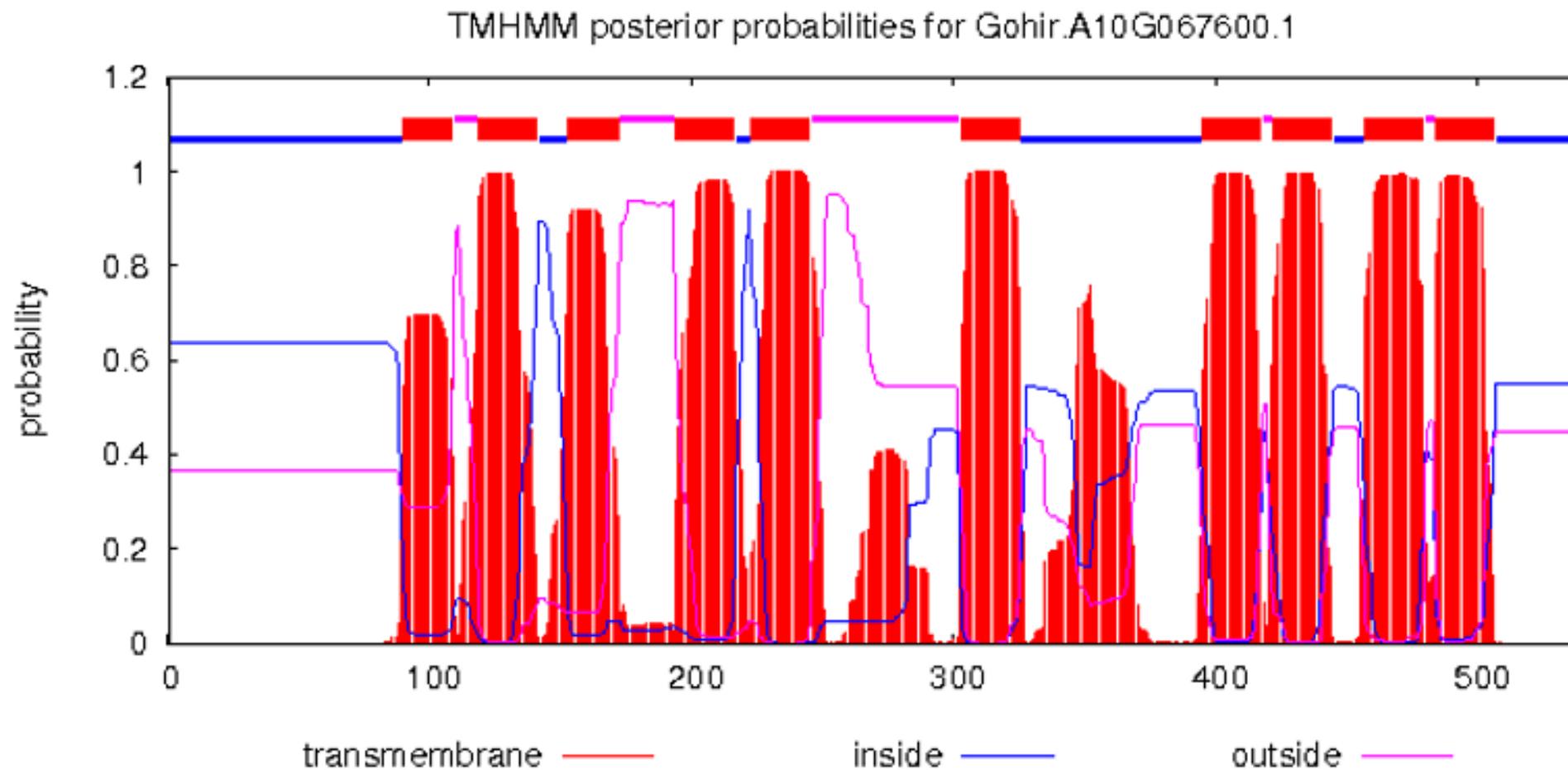
7401  CATGTGGAGT  GAAGAGTATC  AGTGTGCATG  GCTGATATG  TATCACCGCG  TCTTTGATCG  CGTCAGCGCC  GTCGTCGGTG  AACAGGTATG  GAATTTCCGC
```



# 开放阅读框分析



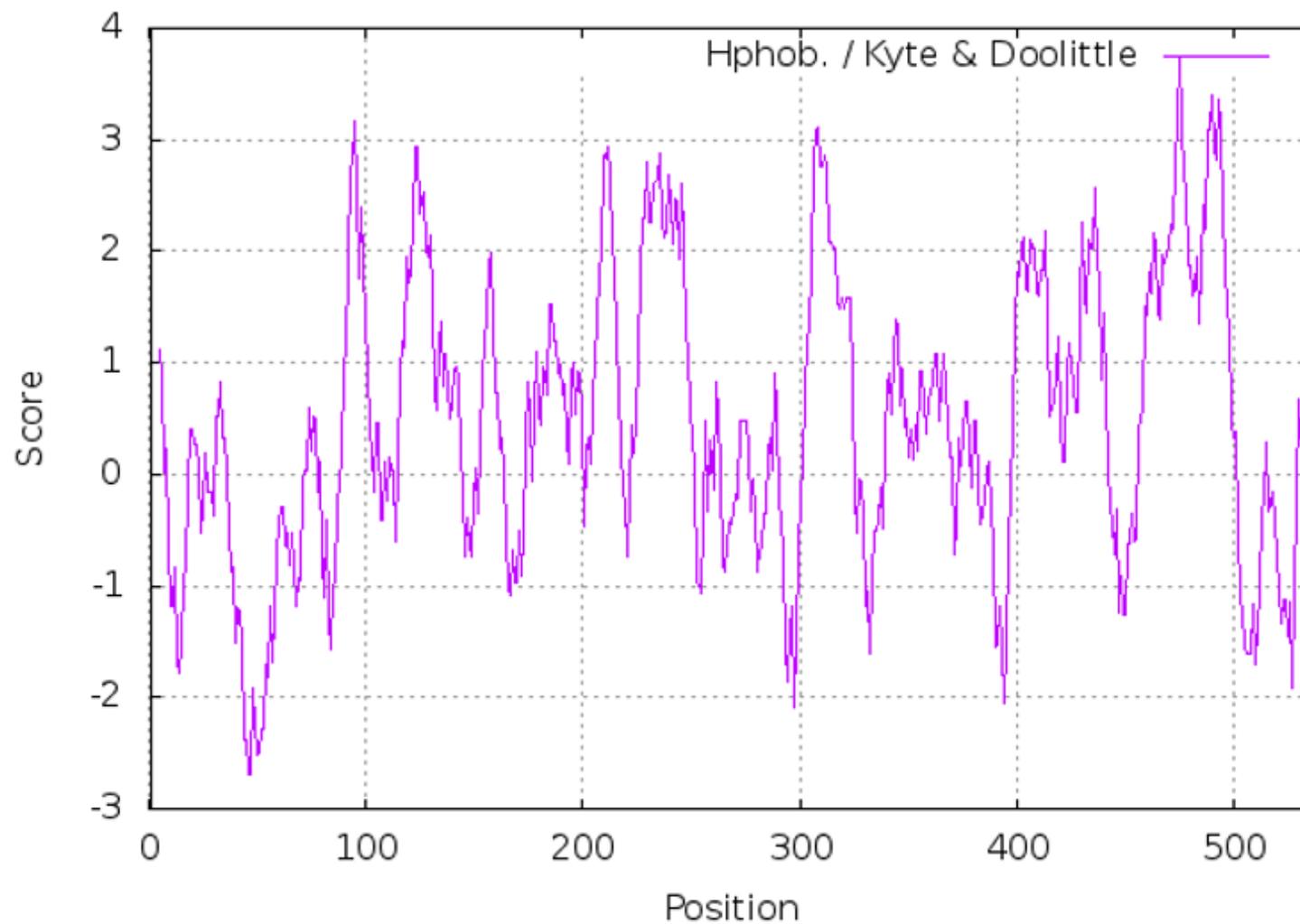
# 跨膜域预测





# 疏水性分析

ProtScale output for user\_sequence





# 亚细胞定位

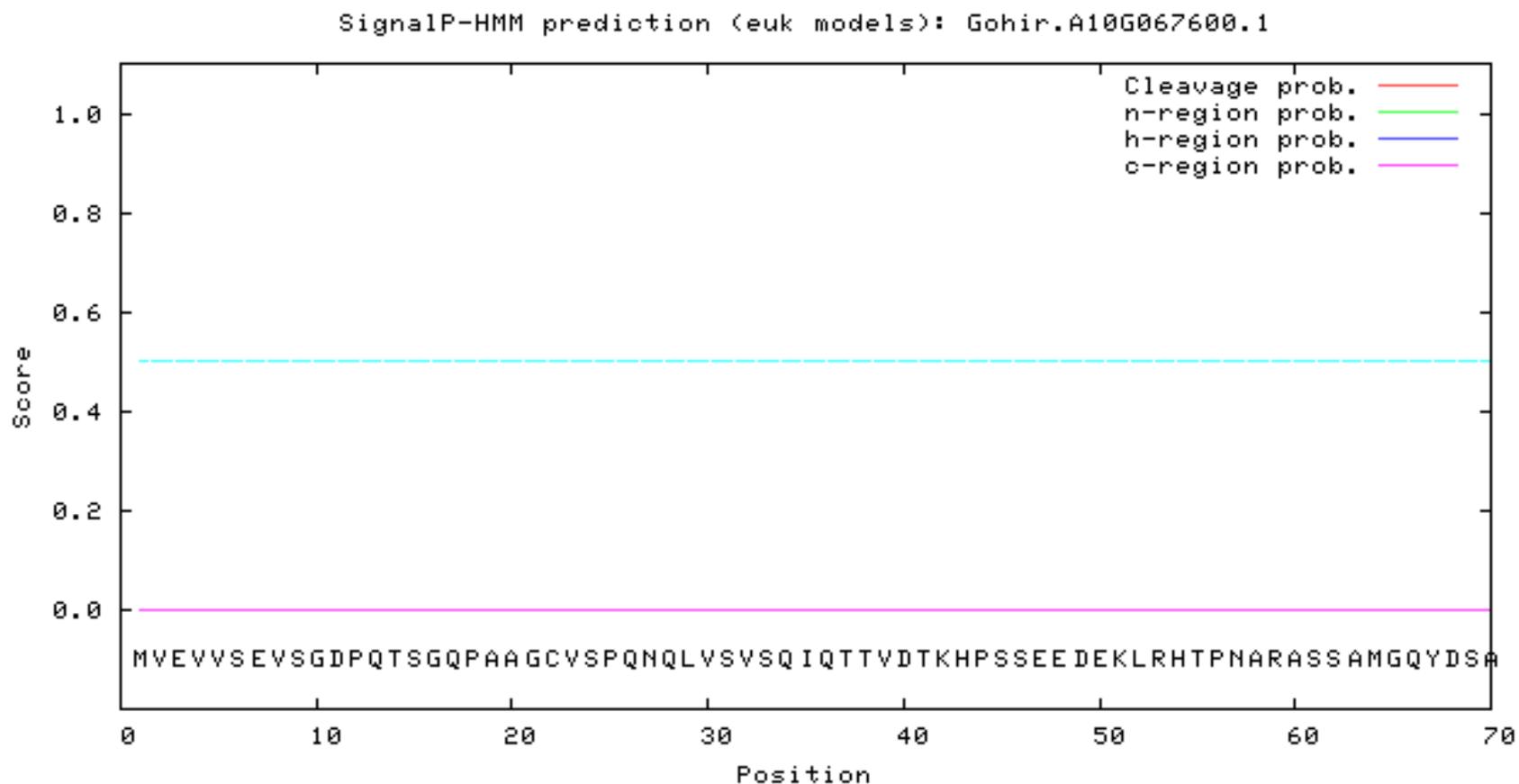


真核生物域的预测定位：质膜

Predicted localization for the Eukarya domain: Plasma Membrane (GO term ID: [GO](#))



# 信号肽分析



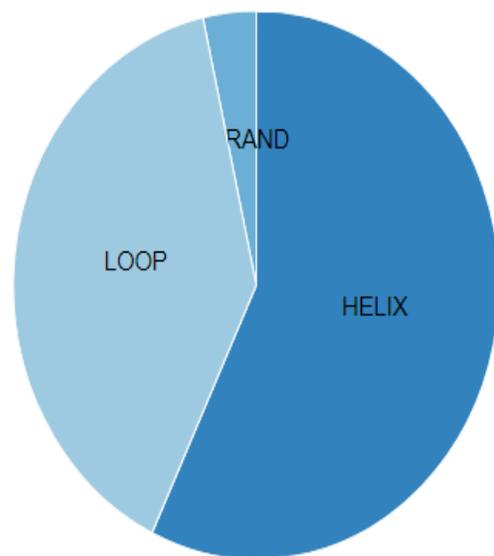
预测：非分泌蛋白    信号肽概率：0.000    信号锚定概率：0.000  
最大卵裂位点概率：位于0.000之间。



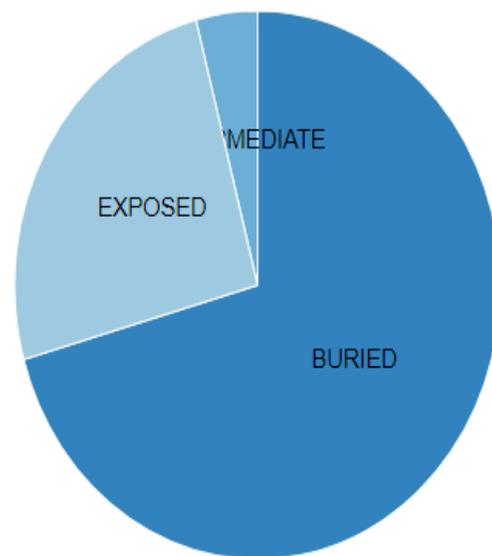
# 蛋白质二级结构



Secondary Structure Composition



Solvent Accessibility



Secondary Structure Composition

LOOP: 39.41% (折叠)

HELIX: 57.06% (螺旋)

STRAND: 3.53%

Solvent Accessibility

EXPOSED: 25.28% (膜外)

BURIED: 70.63% (膜内)

INTERMEDIATE: 4.09% (镶嵌)



# 跨膜螺旋预测

- Secondary Structure and Solvent Accessibility >
- Transmembrane Helices >**
- Protein Disorder and Flexibility >
- Disulphide Bridges >
- FUNCTION ANNOTATION
- Effect of Point Mutations >
- Gene Ontology Terms >
- Subcellular Localization >
- Binding Sites >
- ADDITIONAL SERVICES
- Literature Search >
- HELP
- Site Tutorial >

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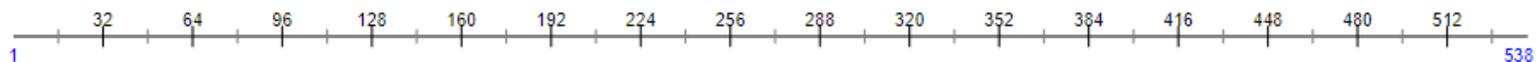
Visual [Help for TMSEG](#) [Help for PHDhtm](#)

**What am I seeing Here?** This viewer lays out predicted features that correspond to regions within the queried sequence. Mouse over the different colored boxes to learn more about the annotations. **Note that this panel may show results from two prediction methods: TMSEG (new) and PHDhtm (veteran). The PHDhtm method will be retired by the end of 2014. See references below and help sections for more information.**

Zoom - Start:1, End:538



Export to image



跨膜螺旋



# 结合位点预测

## Protein-Protein and Protein-Polynucleotide Binding Sites Prediction for

Export

Visual

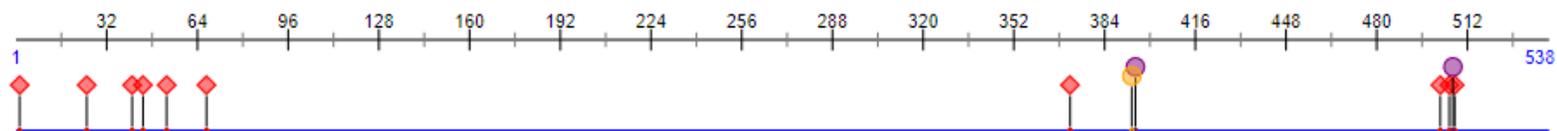
[Help for ISIS2](#)

[Help for SomeNA](#)

**What am I seeing Here?** This viewer lays out predicted features that correspond to regions within the queried sequence. Mouse over the different colored boxes to learn more about the annotations. **NOTE:** this section shows prediction from two methods: ISIS2 (protein-protein binding sites) and SomeNA (polynucleotide binding sites). Refer to the respective help pages for more information.

Zoom - Start:1, End:538

Export to image



◆ 蛋白结合位点

● RNA结合位点

● DNA结合位点

### VIEWS

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### STRUCTURE ANNOTATION

[Secondary Structure and Solvent Accessibility](#)

[Transmembrane Helices](#)

[Protein Disorder and Flexibility](#)

[Disulphide Bridges](#)

### FUNCTION ANNOTATION

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[Subcellular Localization](#)

[Binding Sites](#)

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# phyre2蛋白质结构分析



## Summary

Top model



Image coloured by rainbow N → C terminus

Model dimensions (Å): X:66.365 Y:59.012 Z:60.997

Model (left) based on template [c4djiA](#)

Top template information

**PDB header:**transport protein

**Chain:** A: **PDB Molecule:**probable glutamate/gamma-aminobutyrate antiporter;

**PDB Title:** structure of glutamate-gaba antiporter gadc

Confidence and coverage

Confidence: **100.0%** Coverage: **83%**

449 residues ( 83% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

3D viewing

[Interactive 3D view in JSmol](#)

For other options to view your downloaded structure offline see the [FAQ](#)

# 总结与展望

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- 通过生物信息学分析，我们发现基因Ghpar1与Atpar1序列高度相似，蛋白质结构类似是位于质膜上疏水氨基酸，因此推测它是一种多胺转运体，参与了百草枯在植物体内的运输过程。
  - 通过改良和导入该编码基因，验证其在抗百草枯中的作用，为改良作物的抗逆能力的基因工程提供优良的基因源
-

# 感谢

---

指导老师：罗静初

组员：

➤ 4G04：孙金秋 刘雨佳 胡志程 武亚红

➤ 4G05：王 鹏 吕立军 郑佳怡 牛雨萌

➤ 4G06：蒲伟军 赵官涛 郭位军 邢利娟

➤ 4G10：张旭欢 李鹏程 马旭辉 何 杉

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**Thanks for listening**