

# 花生气生果针败育候选蛋白 PAD2的生物信息学初步分析

第11组

朱 伟 谭龙涛

郭 军 管庆丰



# 花生气生果针败育候选蛋白PAD2的生物信息学初步分析

- 1 研究背景
- 2 前期工作
- 3 进展分析
- 4 生物信息学分析

# 1. 研究背景

## 花生的生长特性

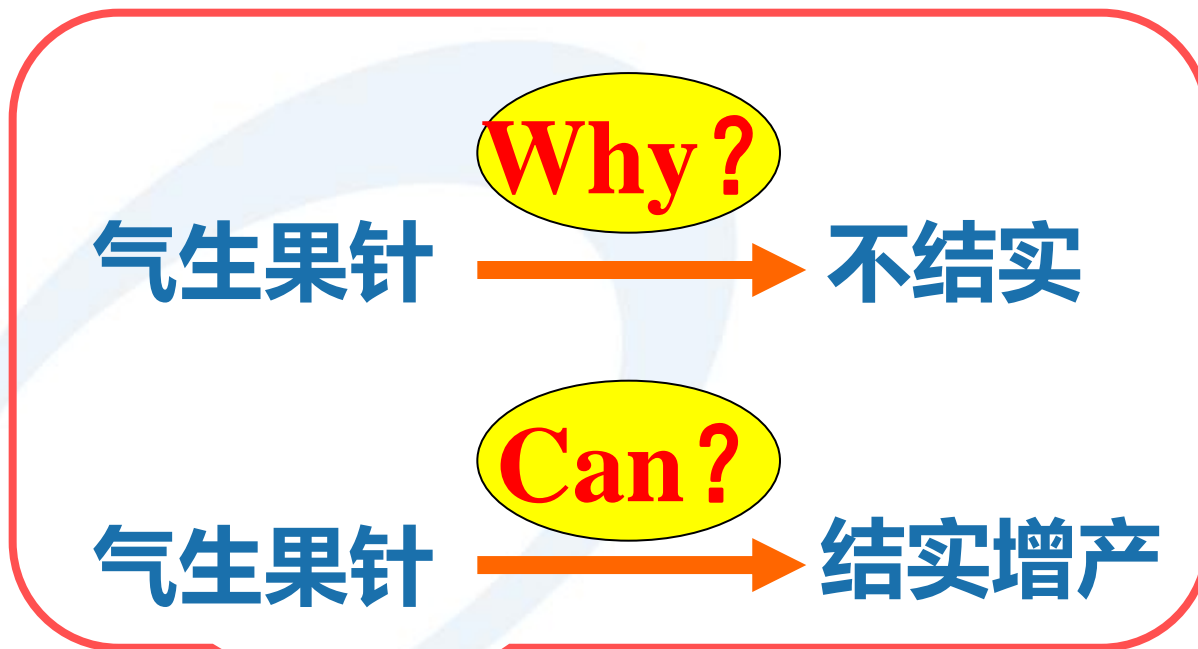


# 研究背景

气生果针的不结实性



# 研究意义



## 2. 前期工作

形态学观察

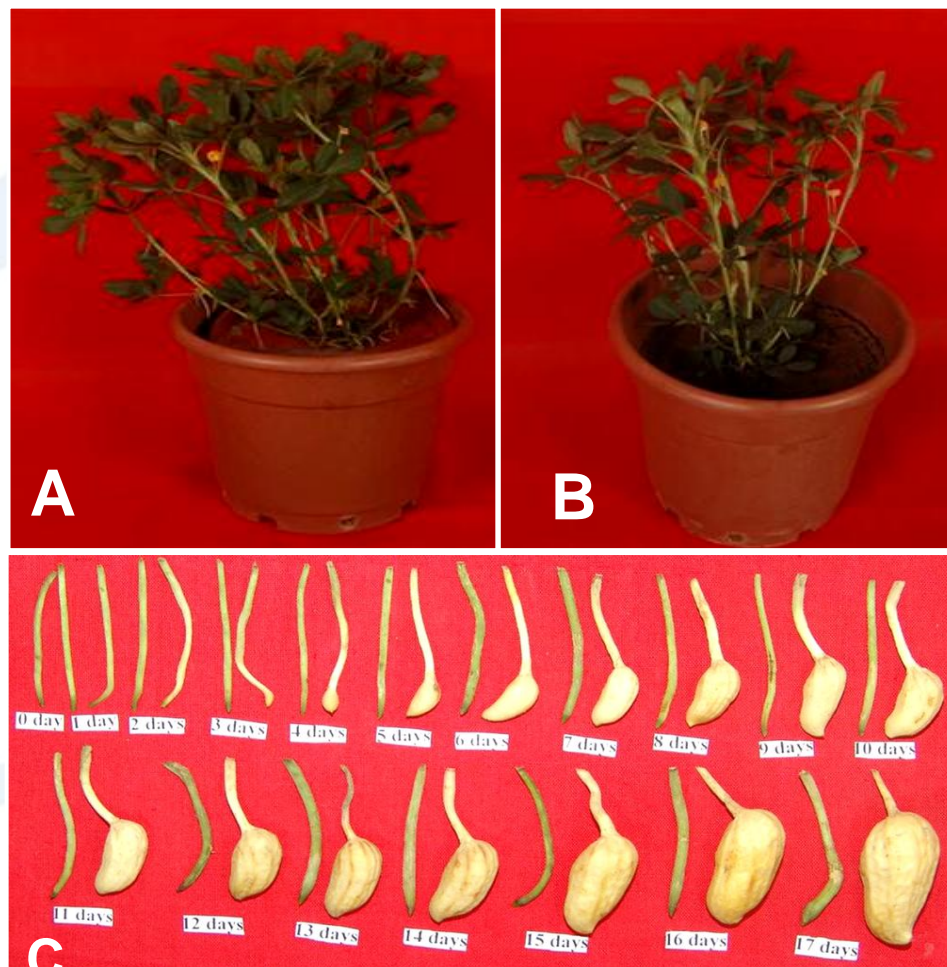


图3-1 气生果针与地下荚果的生长发育形态学观察

## 细胞显微结构差异分析

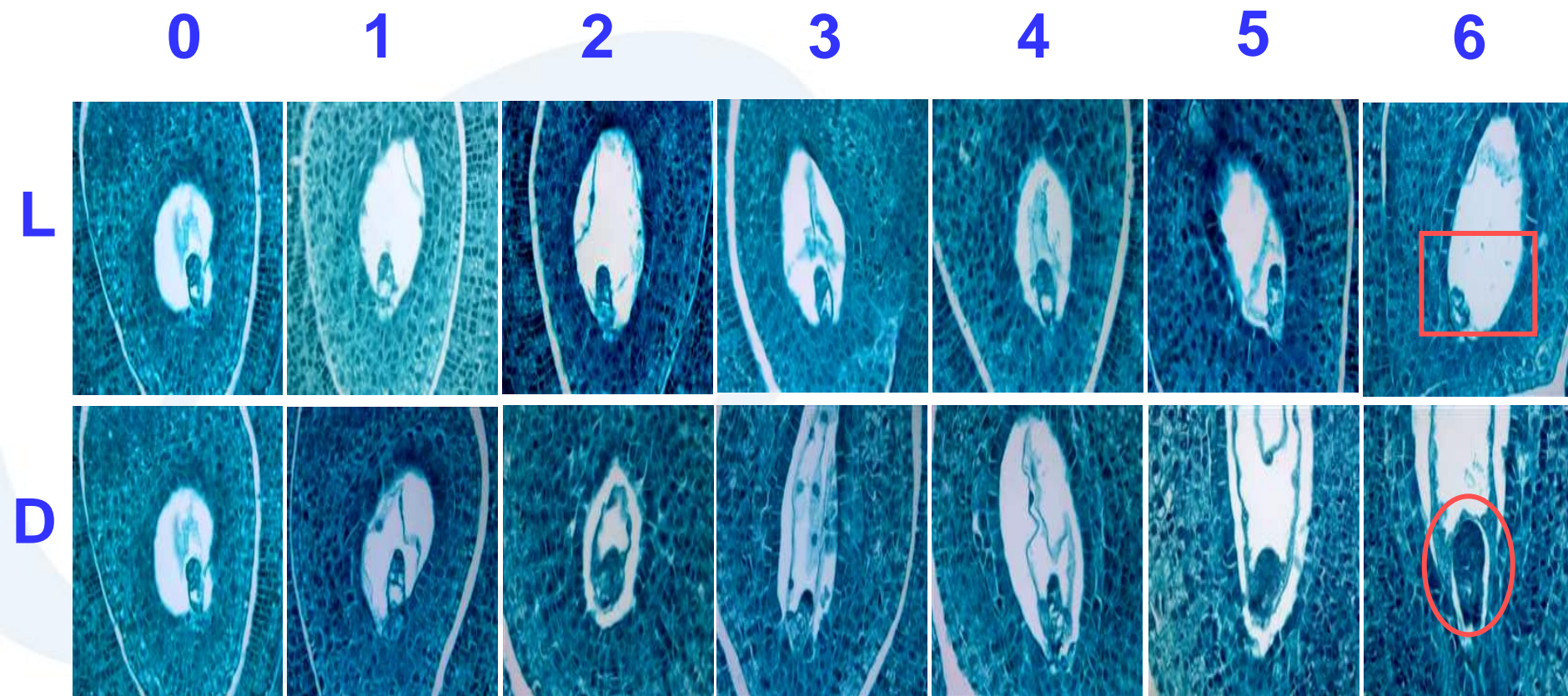


图3-2 标记后不同发育天数的气生果针和地下果针的细胞显微结构差异分析

2013/1/2 L代表气生果针，D表示地下果针，0-6表示标记后不同发育天数。

# 基因表达谱差异分析

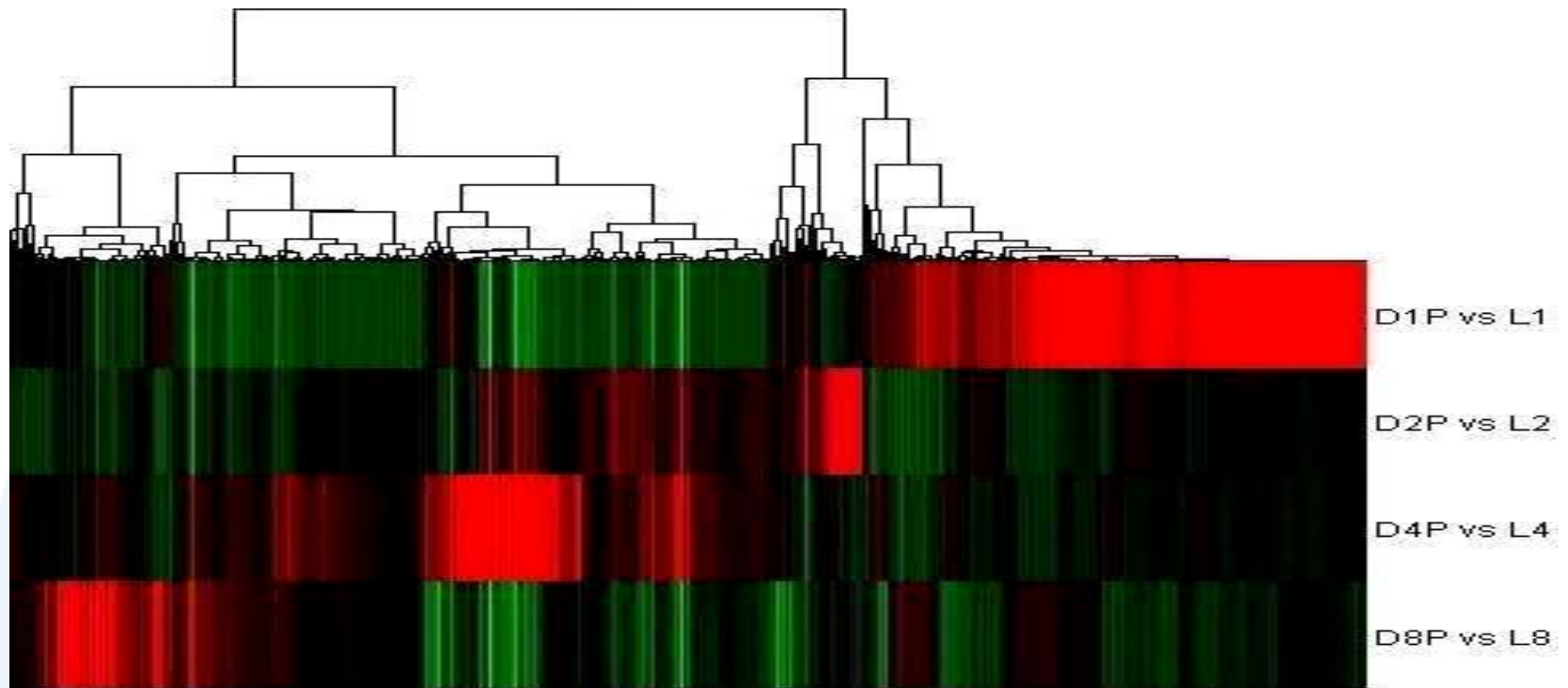


图3-3 不同发育天数的地下荚果比气生果针上调或下调3倍以上的差异基因聚类分析

L代表气生果针，D表示地下荚果，1-8表示标记后不同发育天数。



# 蛋白质表达谱差异分析

Transcriptome  $\neq$  Proteome



# 蛋白质表达谱差异分析

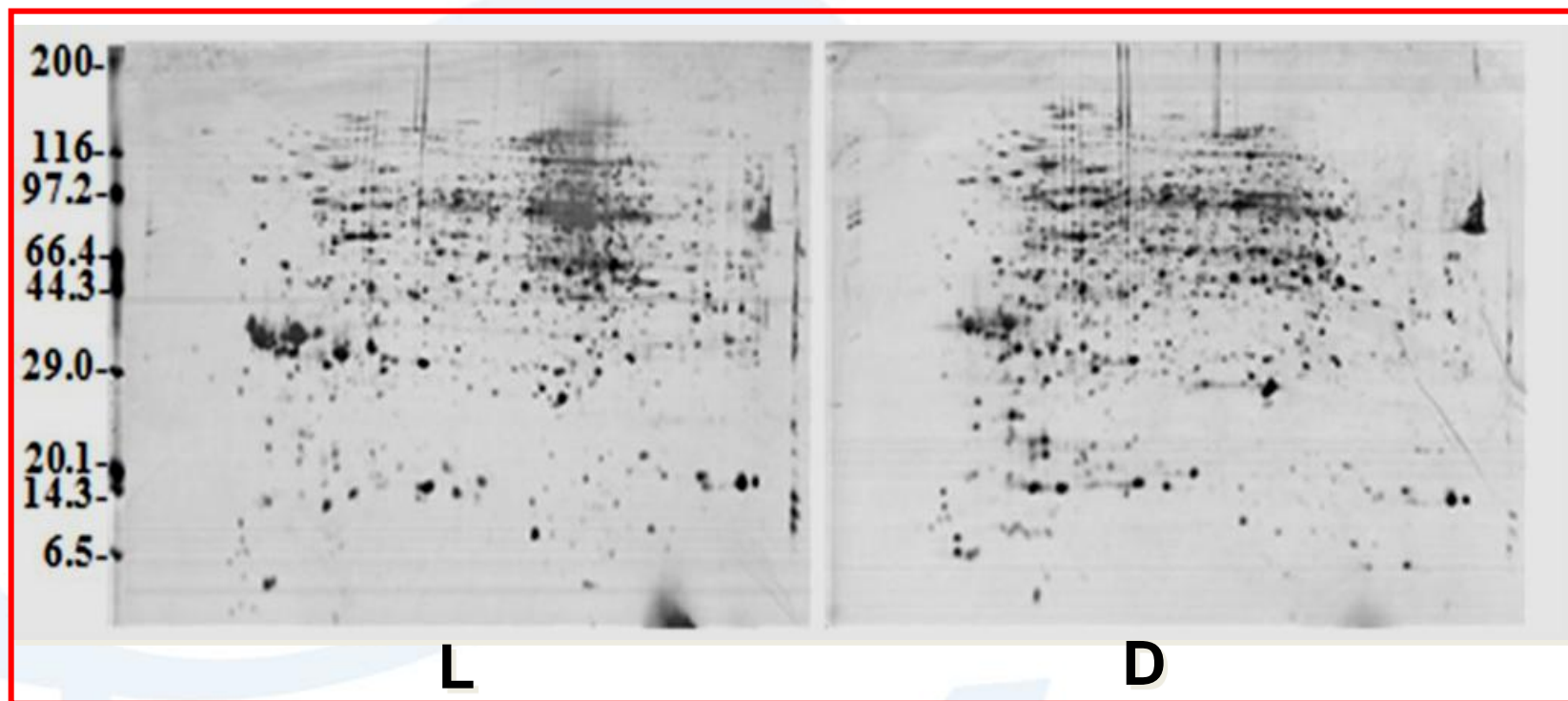


图 3-5 花生气生果针和地下荚果不同发育天数的蛋白质2-DE图谱

# 表3-1. MALDI-TOF-TOF对差异表达蛋白的鉴定

Spots ID	Homologous Protein name	Accession Number	Mascot score	Sequence coverage	Theoretical Mr(kDa)	Theoretical PI
<b>Photosynthesis</b>						
G1/S1	photosystem II type I chlorophyll a/b-binding protein [Glycine max]	Q43437	165	8%	30818	7.66
G2/S2	Oxygen-evolving enhancer protein 2(OEE2) [ Pisum sativum]	P16059	740	26%	44094	8.93
G10/S10	Plastocyanin[Arabidopsis thaliana]	P42699	472	73%	10521	4.34
G22/S11	Plastocyanin A [Ricinus communis]	B9R8G0	267	28%	20769	7.44
G11/S15	Oxygen-evolving enhancer protein 1(OEE1)	P14226	322	21%	38510	7.70
G20/S16	RecName: Full=Oxygen-evolving enhancer protein 1 (OEE1)[ Pisum sativum]	P14226	483	21%	38510	7.70
G21/S23	rubisco activase [Glycine max]	D4N5G3	272	41%	18121	5.09
H5/P6	rubisco activase [Glycine max]	D4N5G2	192	23%	29708	7.71
H6/P41	rubisco activase [Glycine max]	D4N5G3	125	14%	52727	5.74
II/P42	rubisco activase [Glycine max]	D4N5G3	359	17%	52727	5.74
<b>Lignin synthesis</b>						
G3/S3	cinnamyl-alcohol dehydrogenase-like protein [Arabidopsis thaliana]	Q8L9G4	97	16%	20389	6.49
G6/S31	caffeic acid methyl transferase [Arachis hypogaea]	Q850G5	269	20%	29340	5.59
<b>Protein catabolic process</b>						
H24/P25	Peptidyl-prolyl cis-trans isomerase[Arachis diogeni]	E3NYI6	393	29%	18332	8.68
H4/P32	threonine endopeptidase [Arachis hypogaea]	B4UWD9	113	28%	17418	6.82
H18/P22	polyubiquitin 1 [Phaseolus vulgaris]	P69325	60	28%	5543	4.99
H1/P23	ubiquitin carrier-like protein [Arachis hypogaea]	Q06H23	77	12%	27392	9.00
H22/P29	Proteasome subunit alpha type-7[Arabidopsis thaliana]	O24616	244	24%	27194	6.86

### 3. PubMed : PAD2研究进展

---

- The ubiquitin-proteasome system (**UPS**) is an important intermediate signaling step involving in plant developmental process, including embryogenesis, photomorphogenesis, hormone signaling and senescence (**Moon et al., 2004**).
- Some studies suggested that **phytochrome** was localized in tissue-specific of the developing embryo and integument tissues, which may play an important role in the underground phase during pod development, but not be involved in the elongation phase of the gynophore after fertilization (**Thompson et al., 1992; Moctezuma, 2003**).
- **Vierstra (2003)** revealed that UPS was crucial in removing multiple light regulators (such as phyA, HY5, COP) during photomorphogenesis, indicating that UPS might regulate aerial and subterranean pod growing under the light and dark conditions.

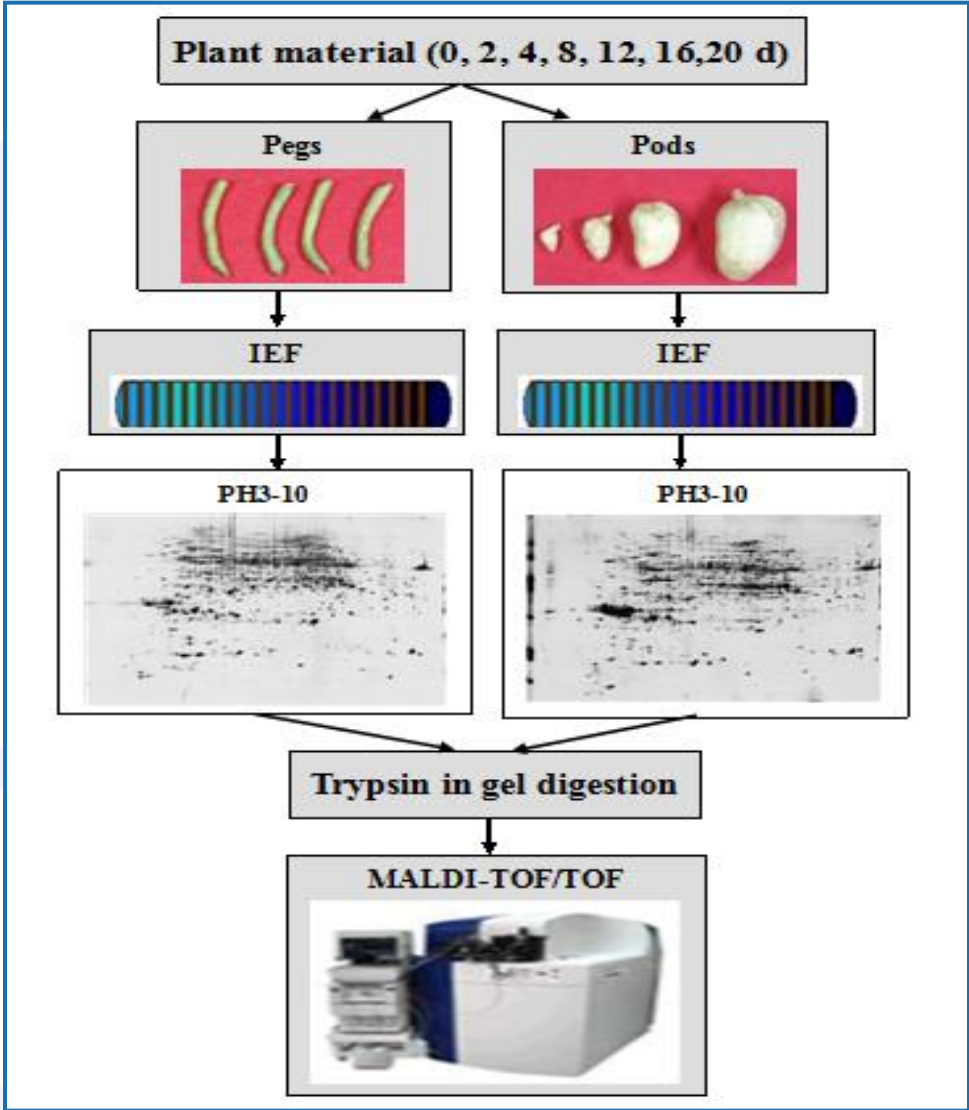
## PAD2研究进展

---

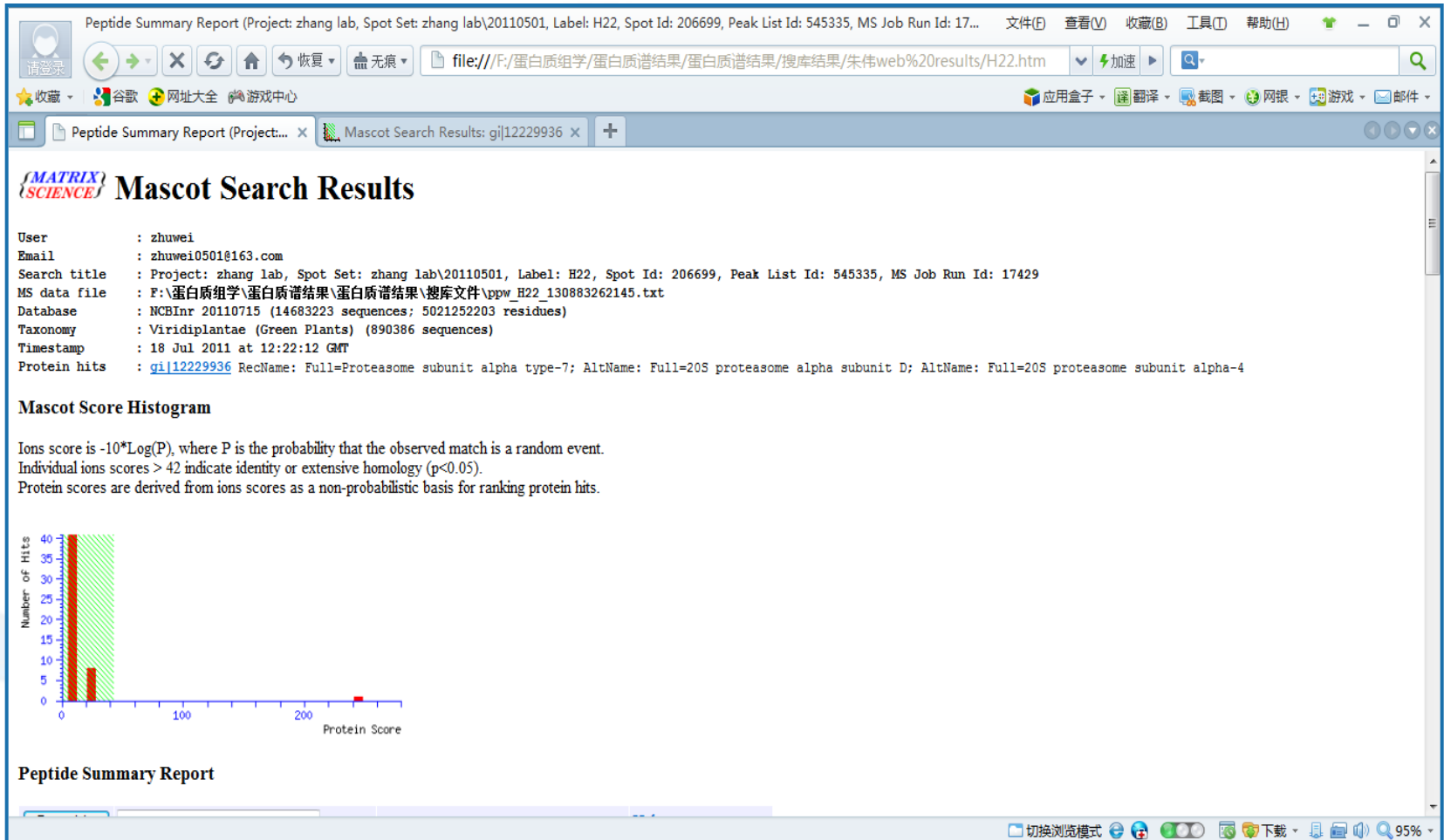
- It is well known that the failure of peg penetration into the soil leads to seed abortion in peanut (**Chen et al., 2012**).
- Recently, many studies revealed that the 26S proteasome might contribute to plant cell division cycle progression by actively associating with intracellular structures including mitotic spindles, the preprophase band, and the phragmoplast (**Farras et al., 2001; Yanagawa et al., 2002**), and also by affecting the half-life of many modulators, such as cyclins, CDK-activating and -inhibiting kinases, and several CDK inhibitors (**Dewitte and Murray, 2003; Smalle and Vierstra, 2004**).
- Moreover, several senescence mutants provided UPS with a direct connection to senescence signaling, such as ORE9 (**Woo et al, 2001**), and the UPS also appeared to involve in plant PCD (**Kim et al, 2003**).

# 4.生物信息学初步分析

## 蛋白质组学流程



# First Step : Mascot搜索结果



The screenshot shows a web browser window with the following content:

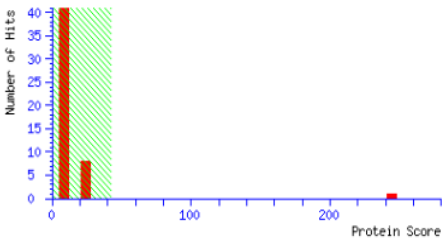
**Peptide Summary Report (Project: zhang lab, Spot Set: zhang lab\20110501, Label: H22, Spot Id: 206699, Peak List Id: 545335, MS Job Run Id: 17...**

**Mascot Search Results**

User : zhuwei  
Email : zhuwei0501@163.com  
Search title : Project: zhang lab, Spot Set: zhang lab\20110501, Label: H22, Spot Id: 206699, Peak List Id: 545335, MS Job Run Id: 17429  
MS data file : F:\蛋白质组学\蛋白质谱结果\蛋白质谱结果\搜库文件\ppw\_H22\_130883262145.txt  
Database : NCBIInr 20110715 (14683223 sequences; 502125203 residues)  
Taxonomy : Viridiplantae (Green Plants) (890386 sequences)  
Timestamp : 18 Jul 2011 at 12:22:12 GMT  
Protein hits : [gij12229936](#) RecName: Full=Proteasome subunit alpha type-7; AltName: Full=20S proteasome alpha subunit D; AltName: Full=20S proteasome subunit alpha-4

**Mascot Score Histogram**

Ions score is  $-10 \cdot \log(P)$ , where P is the probability that the observed match is a random event.  
Individual ions scores  $> 42$  indicate identity or extensive homology ( $p < 0.05$ ).  
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



**Peptide Summary Report**

Peptide Summary Report (Project: zhang lab, Spot Set: zhang lab)20110501, Label: H22, Spot Id: 206699, Peak List Id: 545335, MS Job Run Id: 17...

file:///F:/蛋白质组学/蛋白质谱结果/蛋白质谱结果/搜库结果/朱佳web%20results/H22.htm

### Peptide Summary Report

Format As: Peptide Summary [Help](#)

Significance threshold p < 0.05    Max. number of hits AUTO

Standard scoring  MudPIT scoring  Ions score or expect cut-off 0    Show sub-sets 0

Show pop-ups  Suppress pop-ups  Sort unassigned Decreasing Score    Require bold red

Select All    Select None    Search Selected     Error tolerant

点击此处进入蛋白质全序列

- [gi|12229936](#) ~~Mass: 27194~~    Score: 244    Matches: 4 (3)    Sequences: 4 (3)    emPAI: 0.84  
 RecName: Full=Proteasome subunit alpha type-7; AltName: Full=20S proteasome alpha subunit D; AltName: Full=20S proteasome subunit alpha-4  
 Check to include this hit in error tolerant search

Query	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
<input checked="" type="checkbox"/> <a href="#">18</a>	920.5482	919.5409	919.5127	0.0282	0	28	2.7	1	U	R.YIAGLQQK.Y
<input checked="" type="checkbox"/> <a href="#">62</a>	1634.9012	1633.8939	1633.8563	0.0376	0	64	0.00039	1	U	R.LTVEDPVTVYEIYTR.Y
<input checked="" type="checkbox"/> <a href="#">75</a>	2000.0531	1999.0458	1998.9997	0.0462	0	101	6.5e-08	1	U	R.QLEEAIEDAIVAEIEAEK.A
<input checked="" type="checkbox"/> <a href="#">100</a>	2462.3364	2461.3291	2461.2642	0.0650	0	52	0.0036	1	U	R.AITVFPDGHLPQVEYALEAVR.K

Proteins matching the same set of peptides:

[gi|1224078277](#)    Mass: 27066    Score: 244    Matches: 4 (3)    Sequences: 4 (3)  
 predicted protein [Populus trichocarpa]

[gi|1224105175](#)    Mass: 27106    Score: 244    Matches: 4 (3)    Sequences: 4 (3)  
 predicted protein [Populus trichocarpa]

[gi|1225428005](#)    Mass: 27306    Score: 244    Matches: 4 (3)    Sequences: 4 (3)  
 PREDICTED: hypothetical protein [Vitis vinifera]

Peptide matches not assigned to protein hits: (no details means no match)





# Third Step : 进入NCNI数据库

Protein BLAST: search protein databases using a protein query - 360安全浏览器 4.1 正式版

文件(F) 查看(V) 收藏(B) 工具(T) 帮助(H)

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?ALIGNMENTS=50;ALIGNMENT\_VIEW=Pair

NCBI BLAST/ blastp suite

Standard Protein BLAST

BLASTP programs search protein databases using a protein query. [more...](#) [Reset page](#) [Bookmark](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)  Clear Query subrange

Or, upload file

Job Title

Align two or more sequences

Choose Search Set

Database: Non-redundant protein sequences (nr)

Organism:   Exclude

Exclude:  Models (XM/XP)  Uncultured/environmental sample sequences

Entrez Query:

Program Selection

Algorithm

- blastp (protein-protein BLAST)
- PSI-BLAST (Position-Specific Iterated BLAST)
- PHI-BLAST (Pattern Hit Initiated BLAST)
- DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

**BLAST** Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

Show results in a new window

Algorithm parameters **Note:** Parameter values that differ from the default are highlighted in yellow and marked with + sign

完成

切换浏览模式

70%

# Fourth Step : blast 分析核酸同源序列

NCBI Blast: Protein Sequence (249 letters) - 360安全浏览器 4.1 正式版

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

Sequences producing significant alignments:

Select: All None Selected: 0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/> RecName: Full=Proteasome subunit alpha type-7; AltName: Full=20S proteasome alpha	431	431	100%	9e-151	100%	<a href="#">gil12229936 Q9SXU1.1</a>
<input type="checkbox"/> PREDICTED: proteasome subunit alpha type-7-like [Glycine max]	421	421	100%	2e-146	98%	<a href="#">gil356559270 XP_003547923.1</a>
<input type="checkbox"/> PREDICTED: proteasome subunit alpha type-7-like isoform 1 [Glycine max] >gil3565027	420	420	100%	2e-146	97%	<a href="#">gil356502736 XP_003520172.1</a>
<input type="checkbox"/> Proteasome subunit alpha type-7 [Medicago truncatula] >gil355523301 gb AF103755.1 Pro	420	420	87%	3e-146	98%	<a href="#">gil357517981 XP_003629279.1</a>
<input type="checkbox"/> PREDICTED: proteasome subunit alpha type-7 [Vitis vinifera] >gil297734987 emb CBI173	417	417	100%	3e-145	95%	<a href="#">gil225431090 XP_002264052.1</a>
<input type="checkbox"/> PREDICTED: proteasome subunit alpha type-7 [Vitis vinifera] >gil297744621 emb CBI373	417	417	100%	5e-145	95%	<a href="#">gil225428005 XP_002278162.1</a>
<input type="checkbox"/> proteasome-like protein alpha subunit-like [Solanum tuberosum]	410	410	100%	1e-142	94%	<a href="#">gil82621184 ABB86280.1</a>
<input type="checkbox"/> proteasome-like protein alpha subunit [Solanum tuberosum]	410	410	100%	1e-142	94%	<a href="#">gil77999287 ABB16990.1</a>
<input type="checkbox"/> proteasome subunit alpha type-7 [Solanum lycopersicum] >gil3334299 sp O24030.1 PSA	410	410	87%	3e-142	94%	<a href="#">gil350538237 NP_001234333.1</a>
<input type="checkbox"/> proteasome, alpha subunit-like protein [Solanum tuberosum]	409	409	100%	8e-142	94%	<a href="#">gil76160964 ABA40445.1</a>

# Fifth Step : blast分析氨基酸同源序列

Accession	Entry name	0Query hit137	0Match hit (sqrt scale)767	Name (Organism)
<input type="checkbox"/> Query 2013010760M4BK0SLE				
<input type="checkbox"/> B4UWD5	B4UWD5_ARAHY			Proteasome subunit alpha type (Arachis hypogaea)
<input type="checkbox"/> I3SJA9	I3SJA9_LOTJA			Proteasome subunit alpha type (Lotus japonicus)
<input type="checkbox"/> I1MN39	I1MN39_SOYBN			Proteasome subunit alpha type (Glycine max)
<input type="checkbox"/> Q9SXU1	PSA7_CICAR			Proteasome subunit alpha type-7 (Cicer arietinum)
<input type="checkbox"/> D7SNX0	D7SNX0_VITVI			Proteasome subunit alpha type (Vitis vinifera)
<input type="checkbox"/> D7U564	D7U564_VITVI			Proteasome subunit alpha type (Vitis vinifera)
<input type="checkbox"/> B7FJB6	B7FJB6_MEDTR			Proteasome subunit alpha type (Medicago truncatula)
<input type="checkbox"/> G7LB82	G7LB82_MEDTR			Proteasome subunit alpha type (Medicago truncatula)
<input type="checkbox"/> C0PTN5	C0PTN5_PICSI			Proteasome subunit alpha type (Picea sitchensis)
<input type="checkbox"/> A9NX29	A9NX29_PICSI			Proteasome subunit alpha type (Picea sitchensis)
<input type="checkbox"/> A9NK08	A9NK08_PICSI			Proteasome subunit alpha type (Picea sitchensis)
<input type="checkbox"/> Q38HT8	Q38HT8_SOLTU			Proteasome subunit alpha type (Solanum tuberosum)
<input type="checkbox"/> Q2V988	Q2V988_SOLTU			Proteasome subunit alpha type (Solanum tuberosum)
<input type="checkbox"/> K4B407	K4B407_SOLLC			Proteasome subunit alpha type-7 (Solanum lycopersicum)
<input type="checkbox"/> O24030	PSA7_SOLLC			Proteasome subunit alpha type-7 (Solanum lycopersicum)
<input type="checkbox"/> G5DW59	G5DW59_SILLA			Proteasome subunit alpha type (Silene latifolia)
<input type="checkbox"/> G5DW58	G5DW58_SILLA			Proteasome subunit alpha type (Silene latifolia)
<input type="checkbox"/> B9SXV7	B9SXV7_RICCO			Proteasome subunit alpha type (Ricinus communis)
<input type="checkbox"/> Q3HVM9	Q3HVM9_SOLTU			Proteasome subunit alpha type (Solanum tuberosum)
<input type="checkbox"/> K3ZWD4	K3ZWD4_SETIT			Uncharacterized protein (Setaria italica)
<input type="checkbox"/> I1IS98	I1IS98_BRADI			Proteasome subunit alpha type (Brachypodium distachyon)
<input type="checkbox"/> B6TIY7	B6TIY7_MAIZE			Proteasome subunit alpha type (Zea mays)
<input type="checkbox"/> Q6YT00	PSA7A_ORYSJ			Proteasome subunit alpha type-7-A (Oryza sativa subsp. japonica)
<input type="checkbox"/> A2YXU2	PSA7A_ORYSI			Proteasome subunit alpha type-7-A (Oryza sativa subsp. indica)
<input type="checkbox"/> I1QL69	I1QL69_ORYGL			Proteasome subunit alpha type (Oryza glaberrima)

# Sixth Step : Uniprot信息交叉链接

The screenshot shows a web browser window displaying the UniProt entry for B4UWD5. The browser's address bar shows the URL <http://www.uniprot.org/uniprot/B4UWD5>. The page title is "Proteasome subunit alpha type - Arachis hypogaea (Peanut)". The browser tabs include "Peptide Summary Report (Project...)", "Mascot Search Results: gi|12229936", "NCBI Blast:Protein Sequence (249 ...)", and "Proteasome subunit alpha type - ...".

The UniProt interface shows a search bar with "Protein Knowledgebase (UniProtKB)" selected. Below the search bar, the entry "B4UWD5 (B4UWD5\_ARAHY)★ Unreviewed, UniProtKB/TrEMBL" is displayed, with a "Contribute" button and "Send feedback" and "Read comments (0) or add your own" links.

A red box highlights the navigation menu, which includes the following items: Names, Attributes, General annotation, Ontologies, Sequence annotation, Sequences, References, Cross-refs, Entry info, and Customize order. The "Names" item is currently selected.

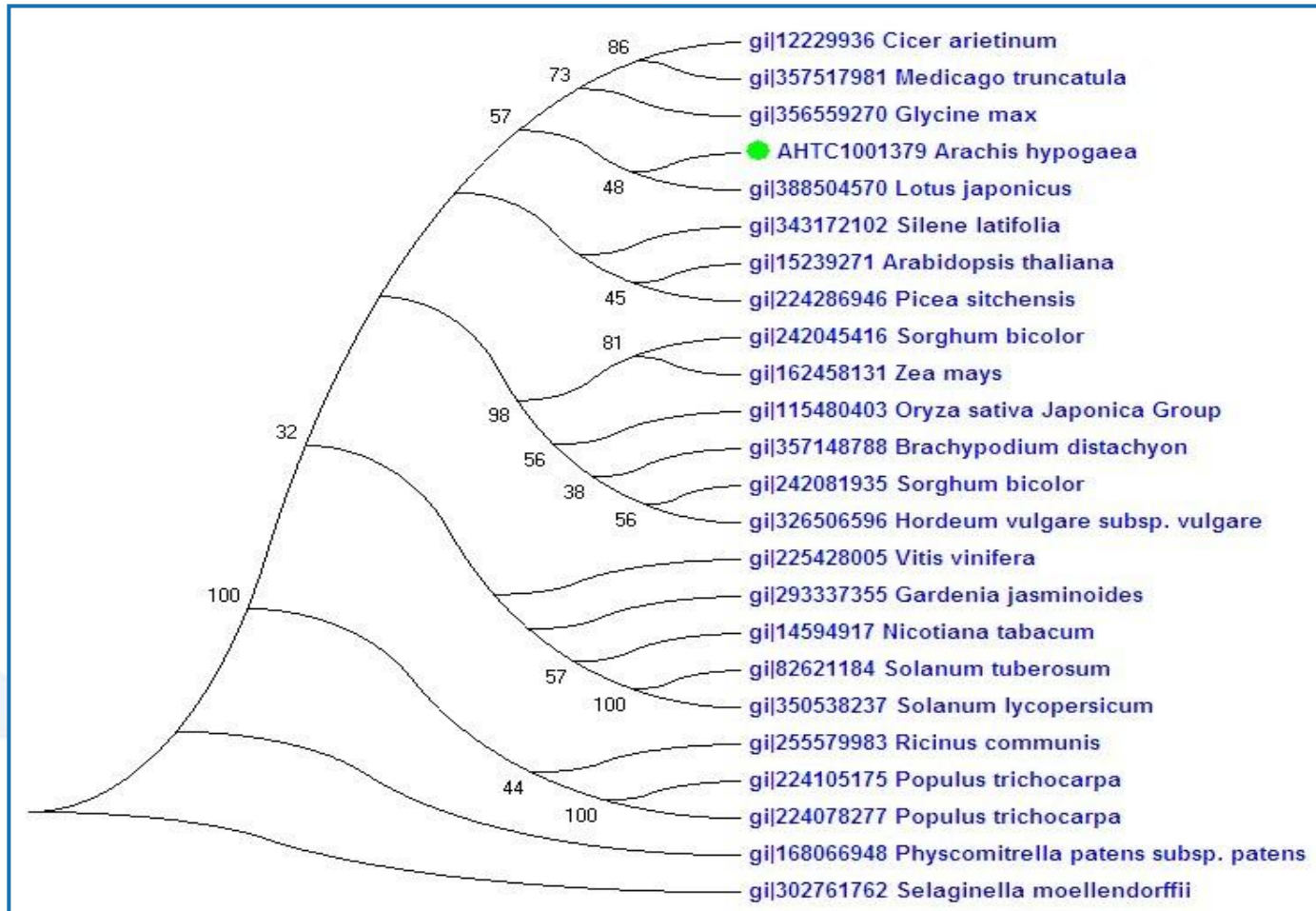
The "Names and origin" section contains the following information:

Protein names	<i>Recommended name:</i> <b>Proteasome subunit alpha type</b> (RuleBase RU000551) EC=3.4.25.1 (RuleBase RU000551)
Organism	<b>Arachis hypogaea (Peanut)</b> (EMBL ACF74355.1)
Taxonomic identifier	3818 [NCBI]
Taxonomic lineage	Eukaryota > Viridiplantae > Streptophyta > Embryophyta > Tracheophyta > Spermatophyta > Magnoliophyta > eudicotyledons > core eudicotyledons > rosids > fabids > Fabales > Fabaceae > Papilionoideae > Dalbergieae > Arachis

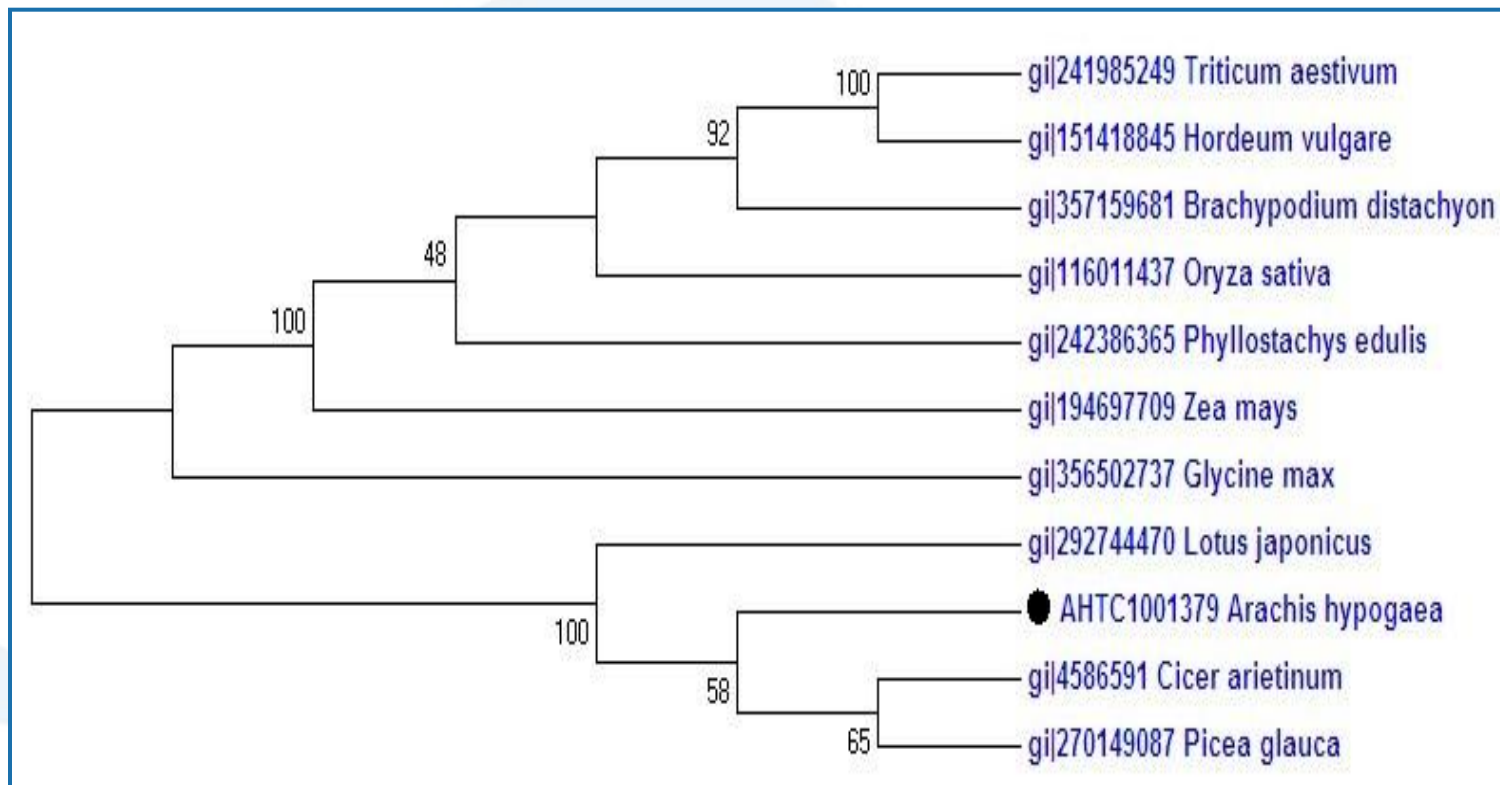
The "Protein attributes" section contains the following information:

Sequence length	137 AA.
Sequence status	Fragment.
Protein existence	Evidence at transcript level

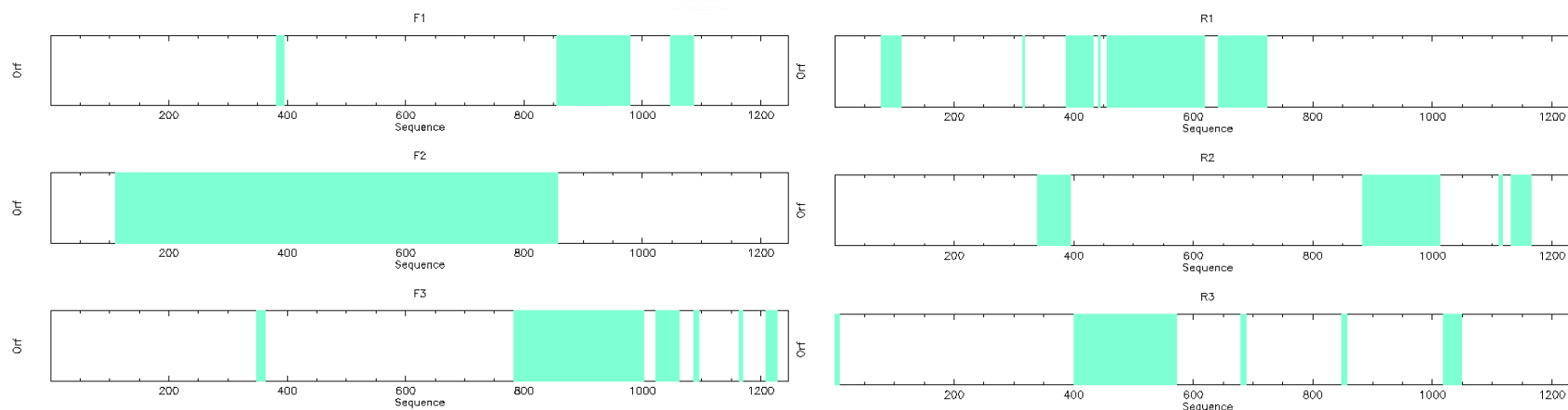
# Seventh Step : 系统进化树分析



# Seventh Step : 系统进化树分析



# Eighthth Step : ORF分析



## >AHTC1001379 [95 - 856] Proteasome subunit alpha

IAPPPMARYDRAITVFSPDGHLFQVEYALEAVRKGNAAVGVRGTDNVVLGVVEKKSTAK  
LQDSRTVRKIVNLDDHIALACAGLKADARVLINRARVECQSHRLTVEDPVTVEYITRYIA  
GLQQKYTQSGGVRPFGLSTLIVGFDPYTGTPSLYQTDPSTGTFSAWKANATGRNSNSI  
REFLEKNYKEVSGQETIKLAIRALLEVVESGGKNIEVAVMTKEQGLRQLEEAEIDAIVAEI  
EAEKAAAEAAKKAPPKET

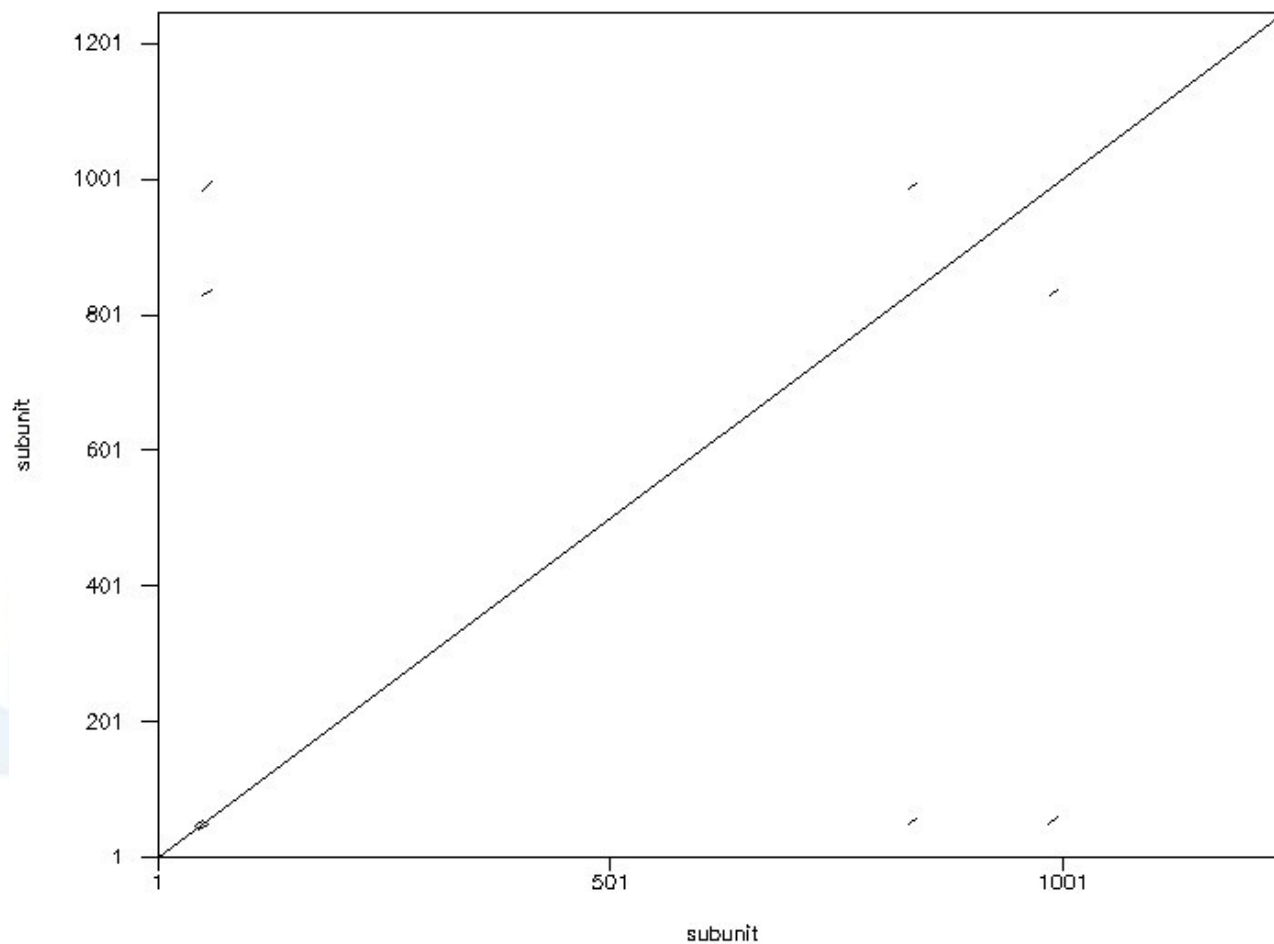


# Nineth Step : 氨基酸组成分析

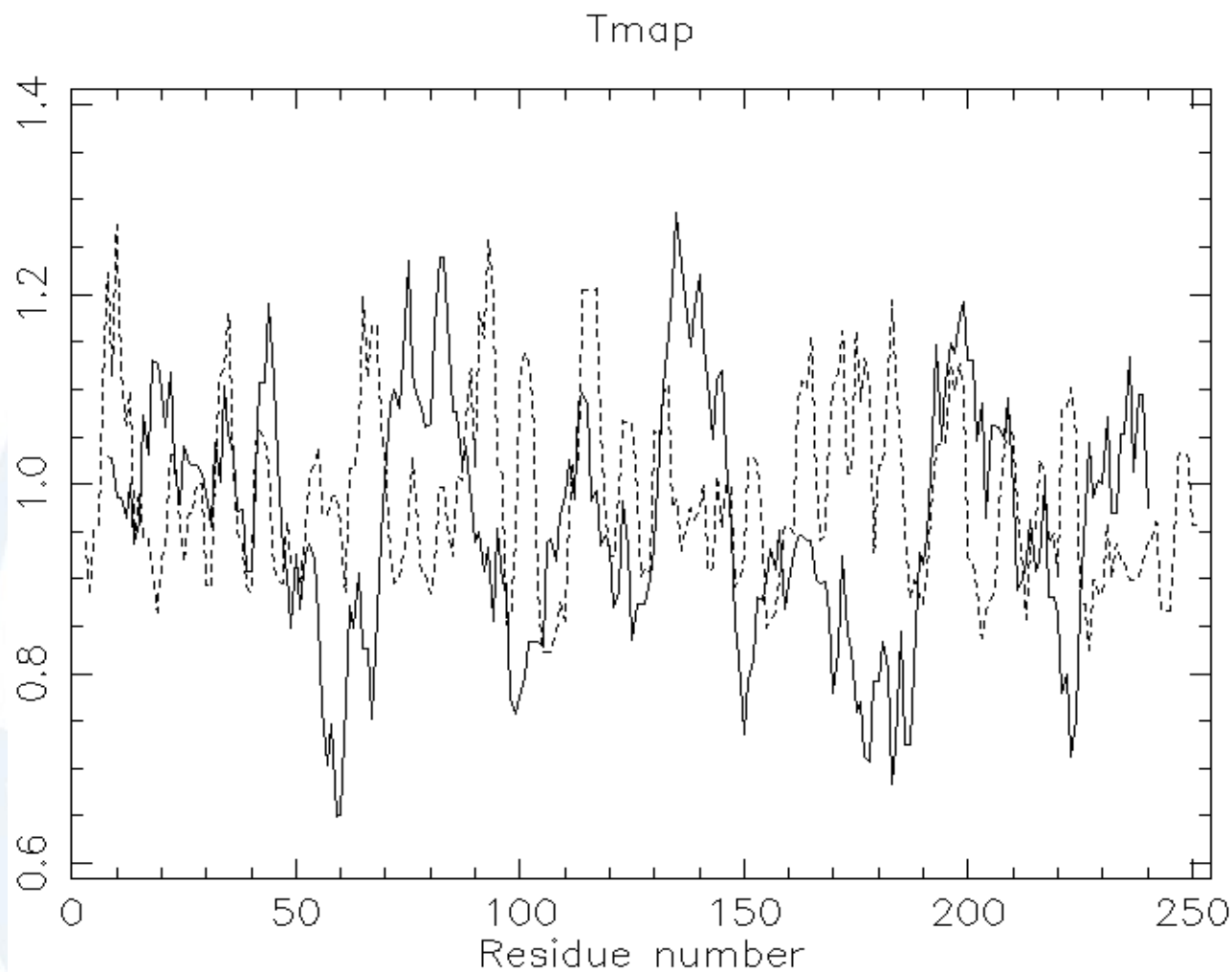
Residue	Number	Mole%	DayhoffStat
A = Ala	31	12.205	1.419
C = Cys	2	0.787	0.272
D= Asp	11	4.331	0.787
E = Glu	22	8.661	1.444
F = Phe	6	2.362	0.656
G = Gly	18	7.087	0.844
H= His	3	1.181	0.591
I= Ile	15	5.906	1.312
K= Lys	17	6.693	1.014
L = Leu	19	7.480	1.011
M= Met	2	0.787	0.463
N= Asn	9	3.543	0.824
P= Pro	11	4.331	0.833
Q = Gln	10	3.973	1.009
R = Arg	16	6.299	1.286
S= Ser	13	5.118	0.731
T= Thr	17	6.693	1.097
V= Val	23	9.055	1.372
W= Trp	1	0.394	0.303
Y = Tyr	8	3.150	0.926

Property	Residue	Number	Mole%
Tiny	A+C+G+S+T	81	31.890
Small	A+B+C+D+G+N +P+S+T+V	135	53.150
Aliphatic	A+I+L+V	88	34.646
Aromatic	F+H+W+Y	18	7.087
Non-polar	A+C+F+G+I+L+M +P+V+W+Y	136	53.543
Polar	D+E+H+K+N+Q +R+S+T+Z	118	46.457
Charged	B+D+E+H+K+R+Z	69	27.165
Basic	H+K+R	36	14.173
Acidic	B+D+E+Z	33	12.992

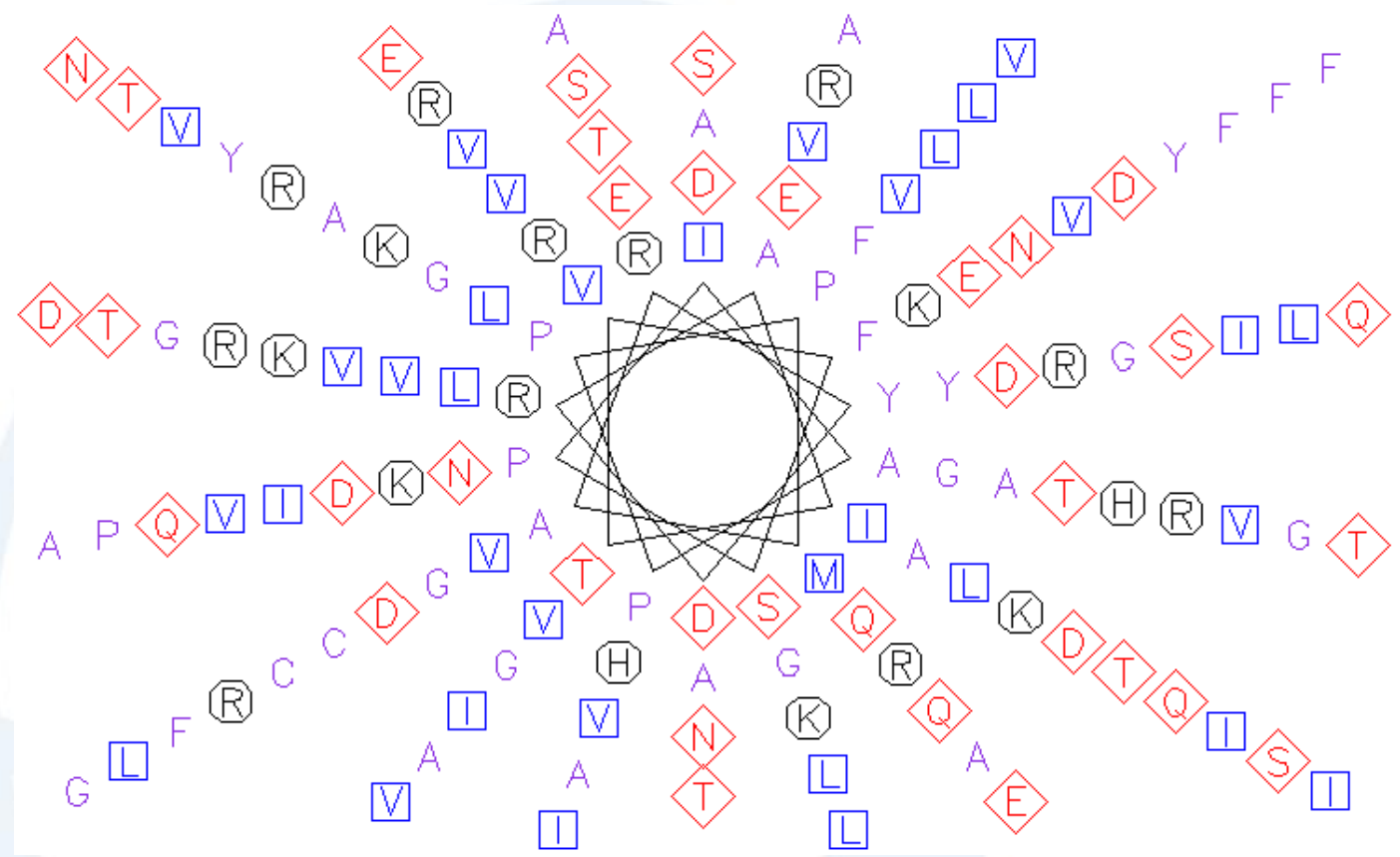
# Tenth Step : 重复序列点阵分析



# Eleventh Step : 跨膜区预测



# Twelveth Step : $\alpha$ 螺旋预测

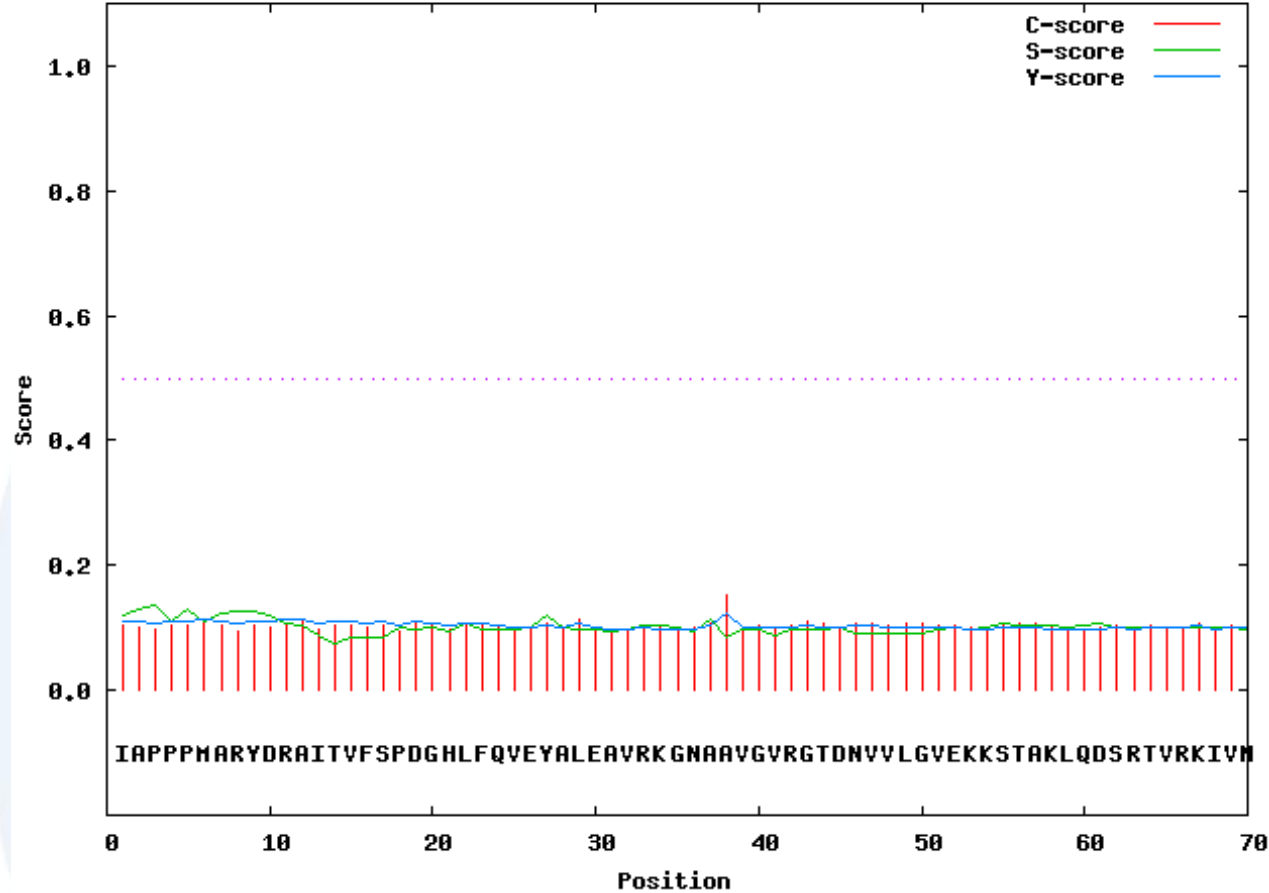


## Thirteenth Step : 三维结构模型预测



# Fourteenth Step : 信号肽预测

SignalP-4.1 prediction (euk networks): AHTC1001379



## Fifteenth Step : 亚细胞定位

Name	Len	cTP	mTP	SP	other	Loc	RC
AHTC1001379	254	0.046	0.266	0.084	0.681	_	3
cutoff		0.000	0.000	0.000	0.000		

# 致 谢

---

- **感谢罗老师的辛勤指导！**
- **敬请大家提出宝贵意见！**



**Thank you!**

