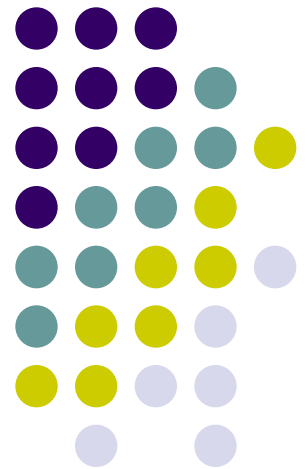


麦长管蚜唾液腺蛋白DSR33的 结构功能分析

小组：G09

汇报：蔡洵

组员：蔡洵 王平喜
魏景欣 李文爽

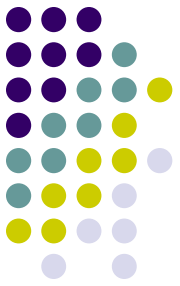


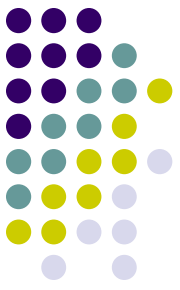
研究背景

麦蚜（尤其是麦长管蚜）是危害中国小麦生产的主要害虫之一。据统计，中国每年小麦蚜虫危害面积可高达0.17亿公顷，占小麦总种植面积的62%，造成减产15%—30%，严重时可高达50%。



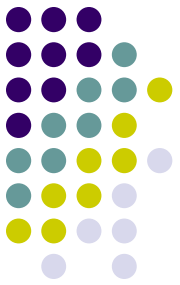
植物介导的RNAi技术已成为农作物抗虫基因工程的热点之一，通过寄主植物表达相应昆虫特异基因的dsRNA，昆虫取食植物后沉默其相应的基因从而达到控制害虫危害的目的。



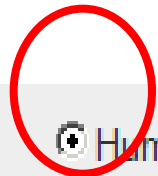


- 麦长管蚜唾液腺蛋白DSR33为本实验室通过对麦长管蚜(*Sitobion avenae Fabricius*)的全转录组测序，进而用dsRNA饲喂法筛选到的蚜虫唾液腺蛋白（致死效果显著），对其进行结构和功能分析对后续的RNAi载体构建是必要的。
- 为阐明致死机制： DSR33

分泌蛋白？ 膜蛋白？



1. Blast – 比对 *DSR33* 的核酸序列和其他物种的同源性



Human genomic + transcript Mouse genomic + transcript Others (nr etc.):

Human genomic plus transcript (Human G+T)

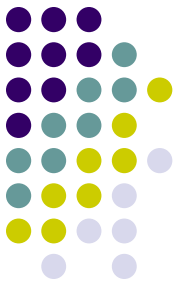
DSR33

Query ID |d|53499
Description DSR33
Molecule type nucleic acid
Query Length 439

Database Name Mouse G+T (2 databases)
Description [▶ See details](#)
Program BLASTN 2.2.27+ [▶ Citation](#)

No significant similarity found. For reasons why, [click here](#)

Other reports: [▶ Search Summary](#) [[Taxonomy reports](#)]



Human genomic + transcript Mouse genomic + transcript Others (nr etc.):

Mouse genomic plus transcript (Mouse G+T)

DSR33

Query ID |cl|53499
Description DSR33
Molecule type nucleic acid
Query Length 139

Database Name Mouse G+T (2 databases)
Description [▶ See details](#)
Program BLASTN 2.2.27+ [▶ Citation](#)

No significant similarity found. [For reasons why, click here](#)

Other reports: [▶ Search Summary](#) [\[Taxonomy reports\]](#)

Database

Human genomic + transcript Mouse genomic + transcript Others (nr etc.):

Nucleotide collection (nr/nt)

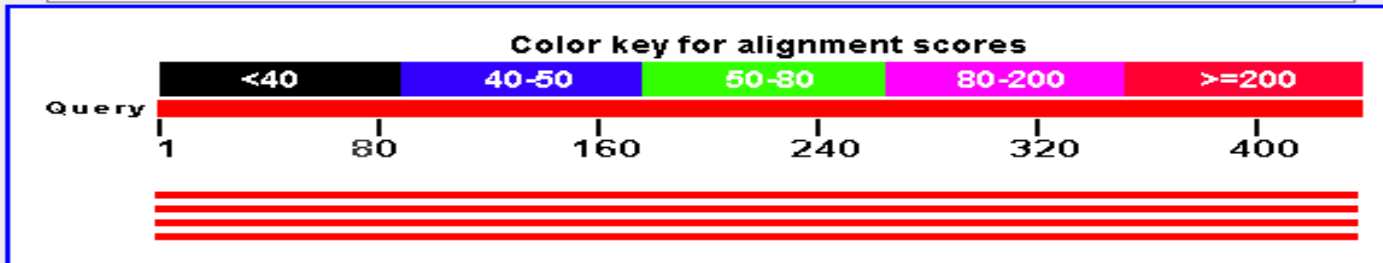
Organism

Exclude



Distribution of 4 Blast Hits on the Query Sequence

Mouse over to see the define, click to show alignments



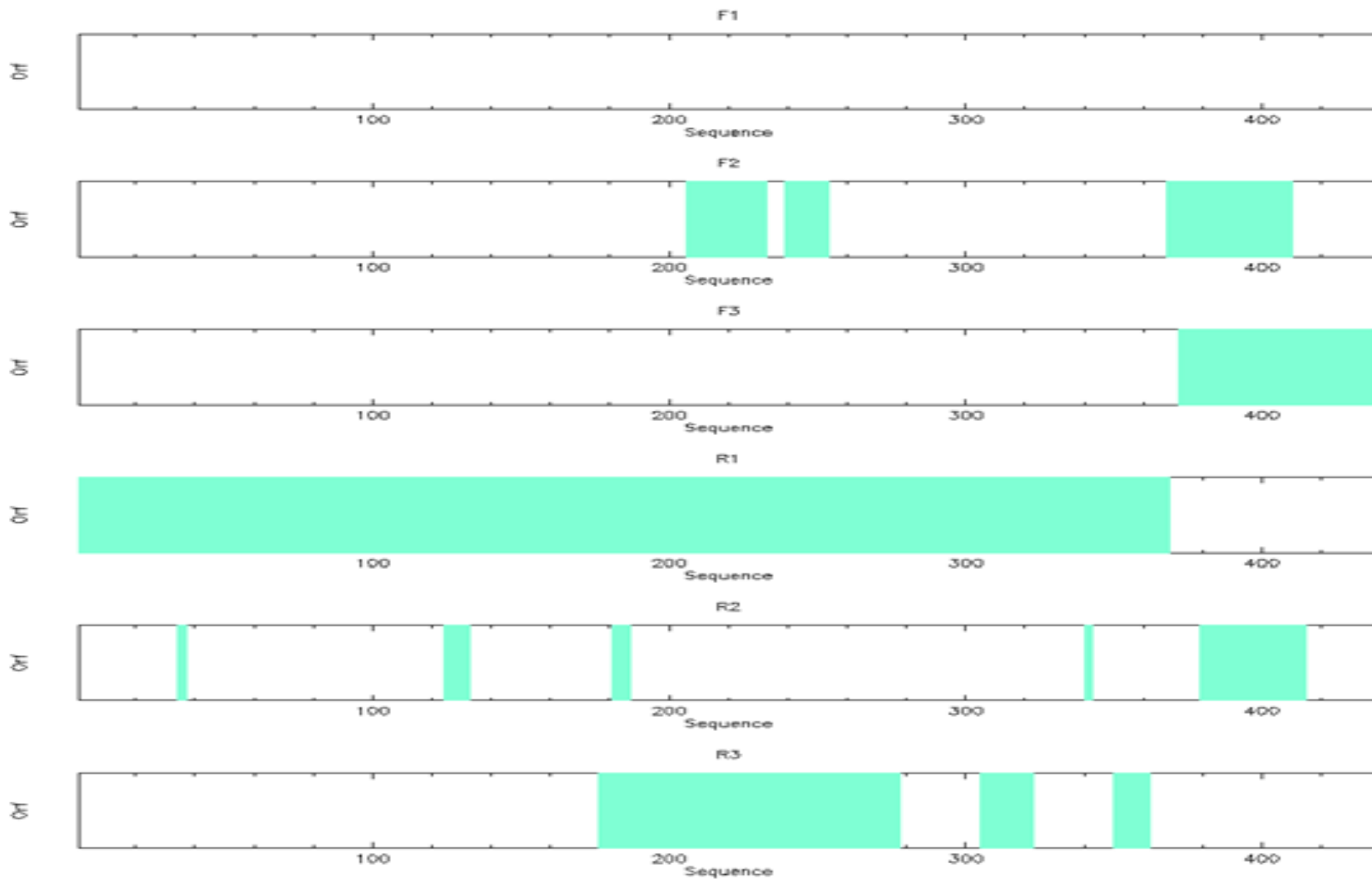
Sequences producing significant alignments:

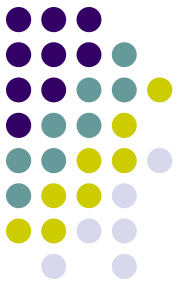
Select: [All](#) [None](#) Selected:0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input type="checkbox"/>	Acyrtosiphon pisum salivary protein MYS2 (LOC100302455), mRNA	771	771	99%	0.0	98%	NM_001163178.1
<input type="checkbox"/>	Acyrtosiphon pisum AC1743895 mRNA, clone: 358E5, complete cds, full-insert cDNA sequence based on the ESTs	771	771	99%	0.0	98%	AK342328.1
<input type="checkbox"/>	Acyrtosiphon pisum AC1743895 mRNA, clone: 676I3, complete cds, full-insert cDNA sequence based on the ESTs	771	771	99%	0.0	98%	AK341642.1
<input type="checkbox"/>	Acyrtosiphon pisum mRNA for hypothetical protein, clone: 211E17, complete cds, full-insert cDNA sequence based on	760	760	99%	0.0	98%	AK342005.1

2. 利用Jemboss中的plotorf预测潜在的读码框(ORF)





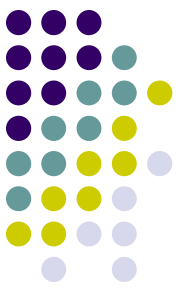
3. remap(v6.0.1) - 酶切位点分析

Enzymes that cut Frequency

AanI	1	AcI	1	AcIWI	2	AcoI	1	AcsI	1	AfaI	2	AfiI	1	AgeI	5		
AjuI	1	AflI	4	AluBI	1	AluI	1	AlwI	2	Ama87I	1	ApoI	1	AseI	1		
AssI	1	AsuHPI	1	AvaI	1	BalI	1	BbsI	1	BceAI	1	BdaI	2	BfaI	3		
BfuCI	2	BmcAI	1	BmeT110I	1	BpiI	1	BpuAI	1	BsaBI	1	BsaXI	1	BscAI	1		
BseI	1	Bse8I	1	BseGI	3	BseJ	1	BseLI	1	BseNI	1	BshFI	1	BsiHKCI	1	BsII	1
	1	BsmI	1	BsnI	1	BsoBI	1	Bsp143I	2	BspACI	1	BspHI	1	BspPI	2	BsrI	1
BsrSI	1	BssMI	2	Bst4CI	2	BstF5I	3	BstKTI	2	BstMBI	2	BstMWI	2	BstV2I	1		
BsuRI	1	BtsCI	3	CciI	1	CfrI	1	Csp6I	2	CspCI	3	CviAI	1	CviJI	4		
CviKI-1	4	CviQI	2	DpnI	2	DpnII	2	EaeI	1	Eco88I	1	FaeI	1	Fail	2	Fall	1
	2	FatI	1	FokI	3	FspBI	3	HaeIII	1	Hin1II	1	Hin4I	1	Hinfl	1	HphI	1
Hpy188III	1	Hpy99I	4	HpyCH4III	2	HpyCH4V	3	HpyF10VI	2	Hsp92II	1	Kzo9I	2				
LpnPI	3	LweI	2	MaeI	3	MaellI	1	MaiI	2	MboI	2	MbolI	3	MluCI	3		
MmeI	1	MnlI	2	MseI	1	MspJI	77	Mva1269I	1	MwoI	2	NdeI	2	NlaIII	1		
PaeR7I	1	PagI	1	PciI	1	PctI	1	PfeI	1	PhoI	1	PshBI	1	PsiI	1		
PspXI	1	RcaI	1	RsaI	2	RsaNI	2	Sau3AI	2	Scal	1	SetI	3	SfaNI	2		
Sfr274I	1	SgeI	38	Slal	1	SmII	1	Smol	1	Sse9I	3	Ssil	1	StrI	1		
TaaI	2	TaqI	2	TasI	3	TatI	1	TfiI	1	TliI	1	Tru1I	1	Tru9I	1		
Tsp509I	3	TspDTI	2	TspEI	3	TspGWI	2	VspI	1	XapI	1	XhoI	1	XspI	3		
Zrml	1																

分析结果：没有发现我们实验室常用的**SacI**、**SpeI**、**HpaI**以及**EcoRV**

4. pepstats(v6.0.1) - Calculates statistics of protein properties



PEPSTATS of DSR33_p from 1 to 177

Molecular weight = 19373.97

Residues = 177

Average Residue Weight = 109.457

Charge = 0.5

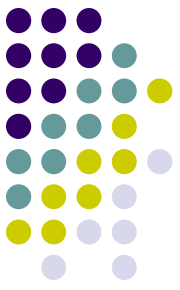
Isoelectric Point = 6.6955

A280 Molar Extinction Coefficient = 17780

A280 Extinction Coefficient 1mg/ml = 0.92

Improbability of expression in inclusion bodies = 0.761

蛋白等电点 = 6.6955



5. garnier(v6.0.1) - Predicts protein secondary structure using GOR method

```
      .      10      .      20      .      30      .      40      .      50
helix  MKLTASISLCLIALFGSSAGLKCYTCGWWSKSCGDPFLKDDYLLVECNTR
sheet  HHHHHHHH                                     H HHHHHHHHHHH
turns  EEEEEEE      EE      EEEEEEE      E
coil   TTTTTTT      TTTTTTTTTT      T T
      .      60      .      70      .      80      .      90      .      100
helix  AINDFNHELGNLNTASNALQNFANQVGFNINQNNNFNLPTISEDSVGCT
sheet  HH
turns  E      TT      E EEE      EE      EEE      E
coil   CC CCC CCCCCCCC C      C      CC      CCC      TTTTTT
      .      110     .      120     .      130     .      140     .      150
helix  KVVLTHGEDIVRVARGCVYNKADLCKGMQRLDDELKTLKYCGSCDDDGCN
sheet  HH      HHHHHHHHHHHHHHHHHHHHH
turns  EEEEE      EEEEE      TTTTTT      TTTTTTTTTTTT
coil   CC
      .      160     .      170
helix  GSRSLKSSAVATILTTMATCLFYRLQH
sheet  HHH      HHHHHHH
turns  TTT T      E      EEEEE      TT
coil   CC CC
```

#

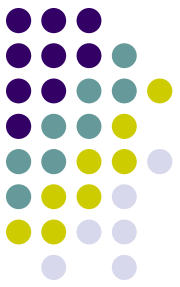
Residue totals: H: 51 E: 38 T: 58 C: 30

percent: H: 31.7 E: 23.6 T: 36.0 C: 18.6

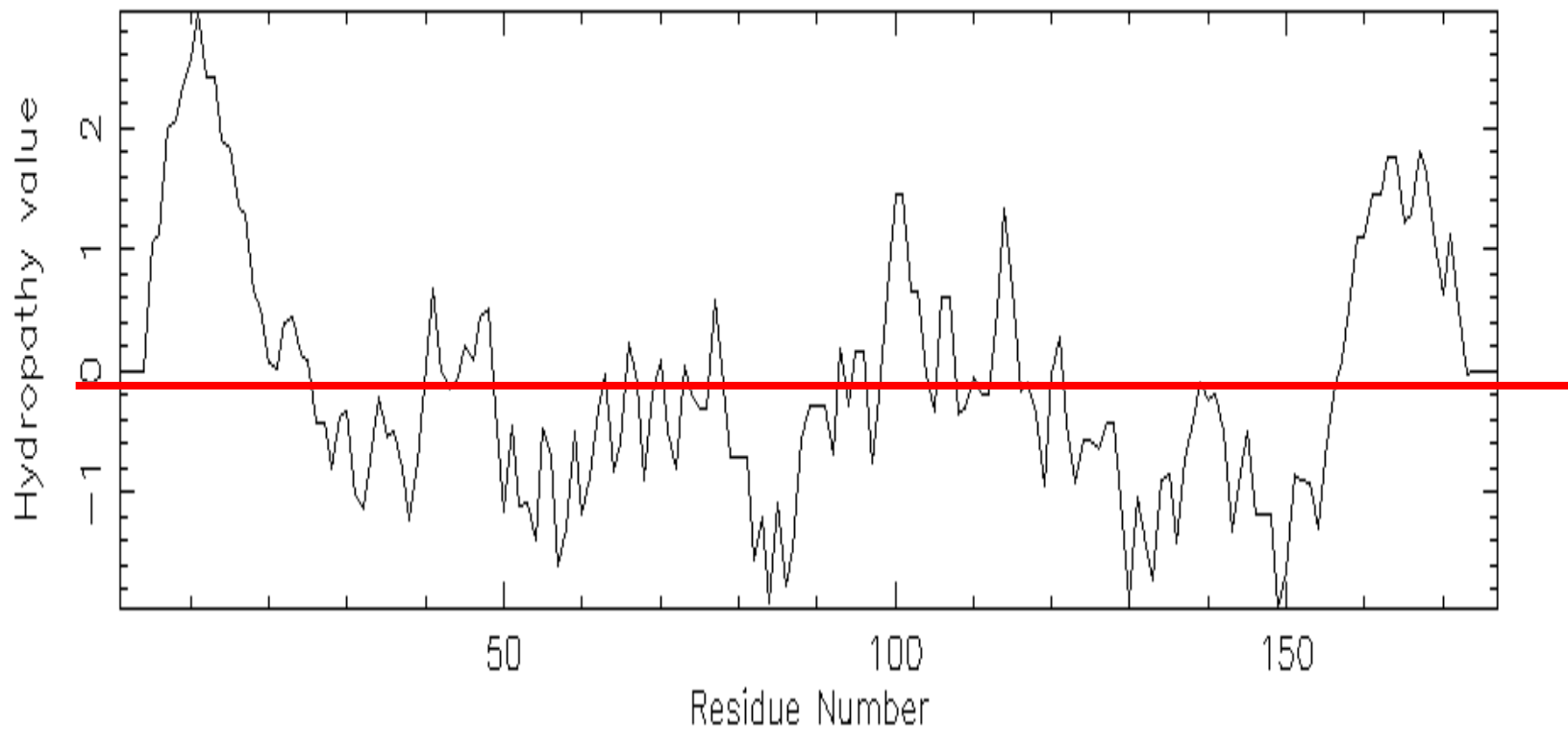
#

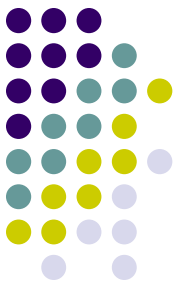
H: α -螺旋 E: β -折叠 T: 转角 C: 无规卷曲

6.pepwindow(v6.0.1) - Draw a Kyte-Doolittle hydropathy plot for a protein sequence

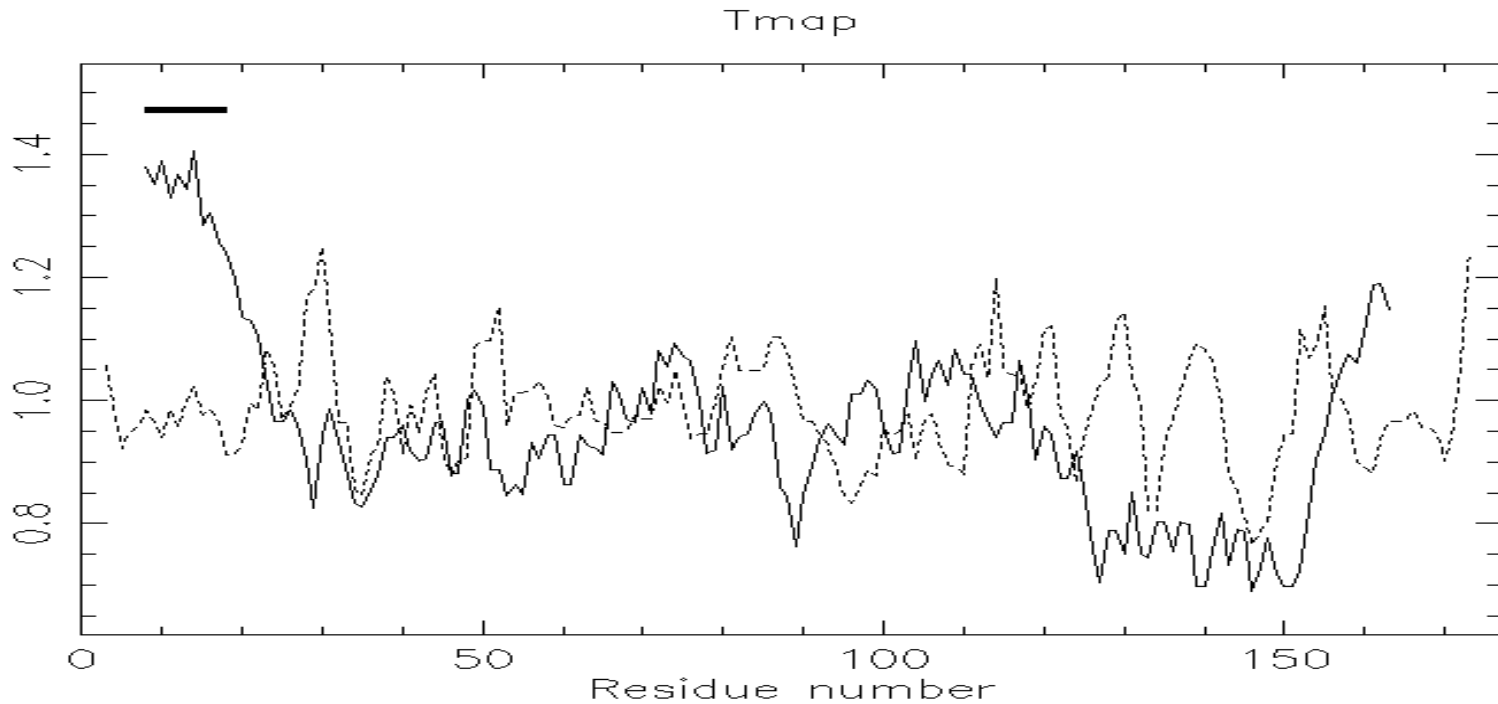


Hydropathy plot of residues 1 to 177 of sequence DSR33 using Kyte & Doolittle hydropathy parameters





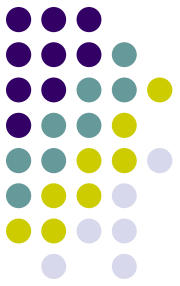
7.tmap(v6.0.1) - 预测跨膜结构



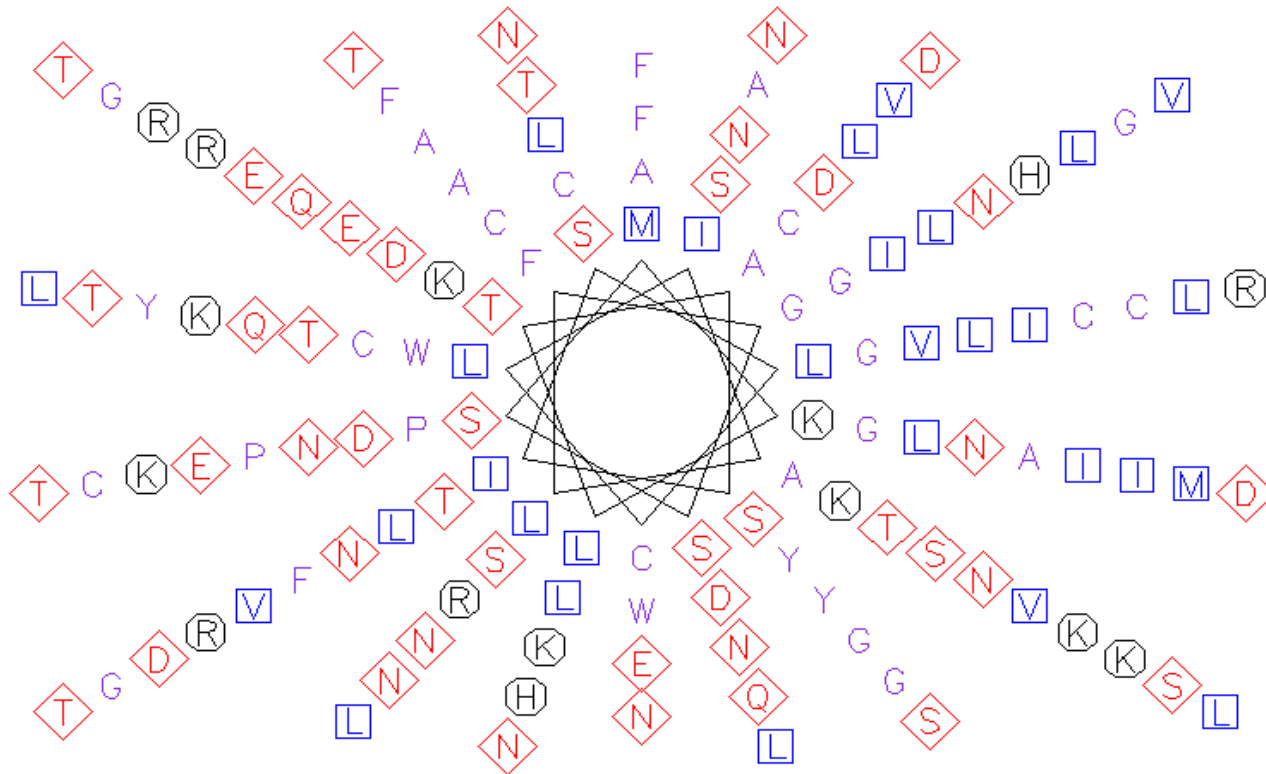
预测有一个跨膜结构:

Start	End	TransMem	Sequence
4	22	1	TASISLCLIALFGSSAGLK

8. pepwheel(v6.0.1) - Draw a helical wheel diagram for a protein sequence



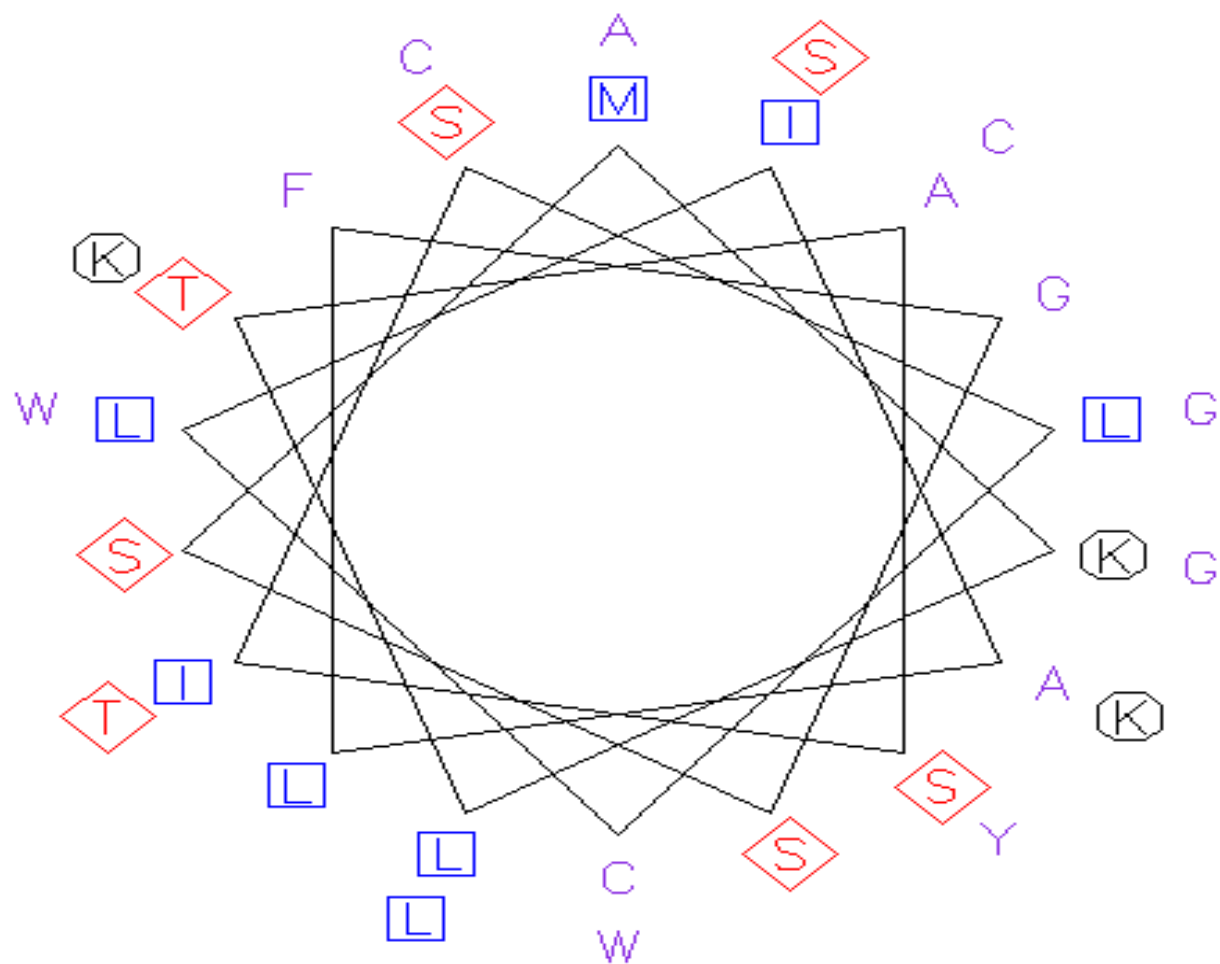
Helical wheel of fasta::586798:DSR33.p
Sat 19 Jan 2013 19:51:46



- 使用pepwheel, 需要限定序列的某一片段才有意义?

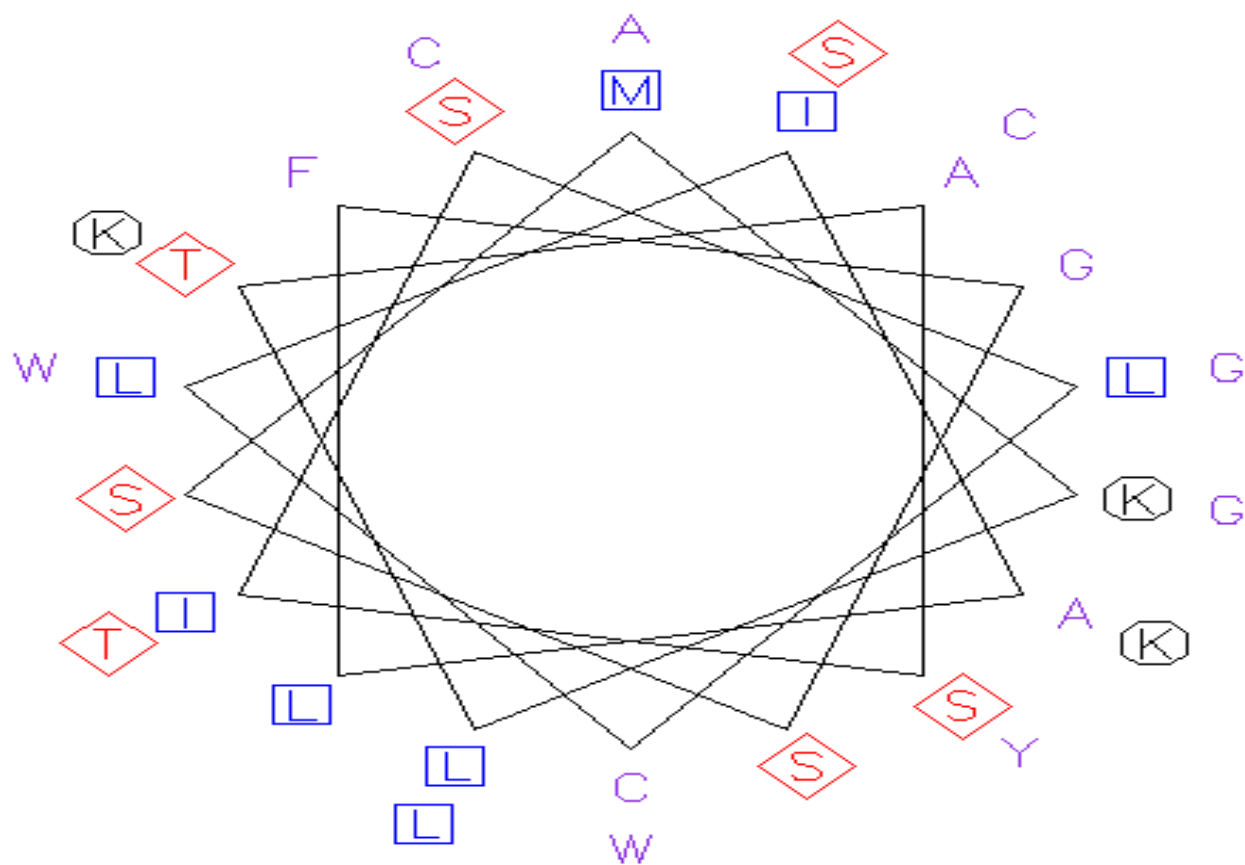
Helical wheel of fasta::586837:Hydrophob

Sat 19 Jan 2013 20:28:57

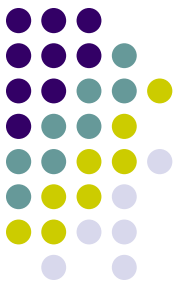


Helical wheel of fasta::586837:Hydrophob

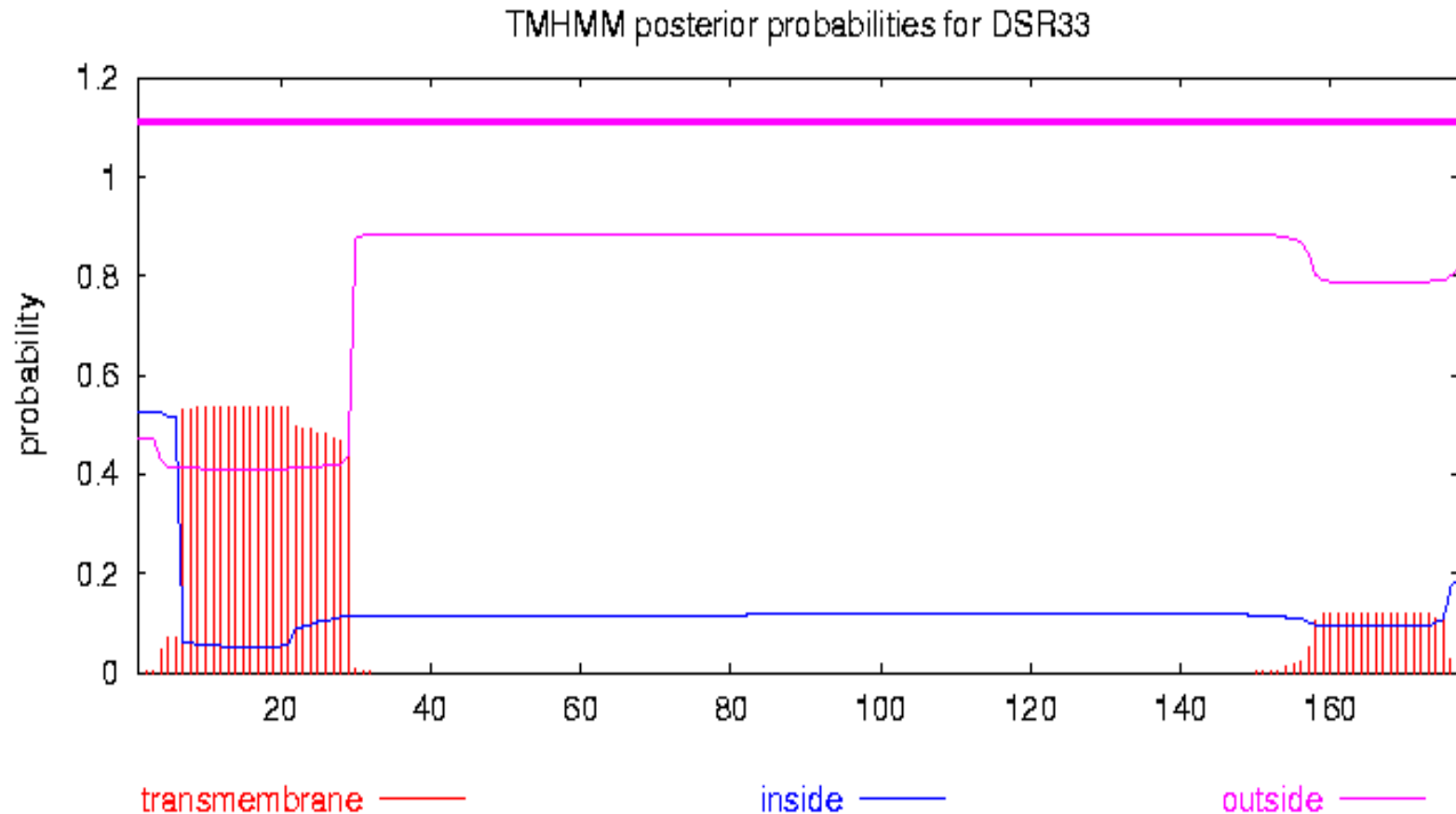
Sat 19 Jan 2013 20:08:18

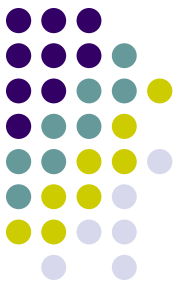


分析肽链前**30**个氨基酸残基

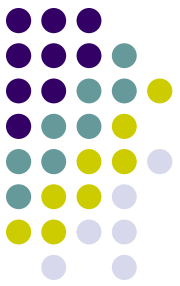


9. TMHMM Server v. 2.0 (Prediction of transmembrane helices in proteins)





- # DSR33 Length: 177
- # **DSR33 Number of predicted TMHs: 0** ? ?
- # DSR33 Exp number of AAs in TMHs:
11.82865
- # DSR33 Exp number, first 60 AAs: 10.10426 #
DSR33 Total prob of N-in: 0.48341
- # DSR33 POSSIBLE N-term signal sequence
DSR33 TMHMM1.0 outside 1 177



10. TargetP 1.1 Server (predicts the subcellular location of eukaryotic proteins)

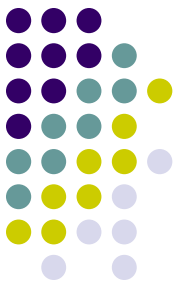
```
### targetp v1.1 prediction results #####  
Number of query sequences: 1  
Cleavage site predictions not included.  
Using NON-PLANT networks.
```

Name	Len	mTP	SP	other	Loc	RC
DSR33	177	0.018	0.965	0.066	S	1
cutoff		0.000	0.000	0.000		

N-terminal presequences: chloroplast transit peptide (**cTP**),
mitochondrial targeting peptide (**mTP**)
or secretory pathway signal peptide (**SP**).

11. GPI Modification Site Prediction

(http://mendel.imp.ac.at/gpi/cgi-bin/gpi_pred.cgi)



Output of the prediction tool:

~~~~~

Query sequence DSR33 (length 177 amino acids):  
MKLTASISLC LIALFGSSAG LKCYTCGWWS KSCGDPFLKD DYLLVECNR AINDFNHELG  
NTLNTASNAL QNFANQVGFN INQNNFNLP TISEDVSGCT KVLTHGEDI VRVARGCVYN  
KADLCKGMQR LDDELKTLKY CGSCDDDG**CN** GSRSLKSSAV ATILTTMATC LFYRLQH

The site with the best score is shown in black.

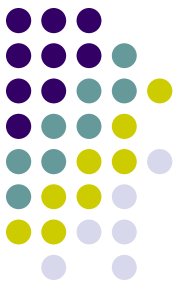
~~~~~

Prediction of potential C-terminal GPI-Modification Sites

Use of the prediction function for METAZOA

None potential GPI-modification site was found.
Among all positions checked, sequence position 149 had the best score.

Typically, the existence of a **signal peptide leader** is sufficient.

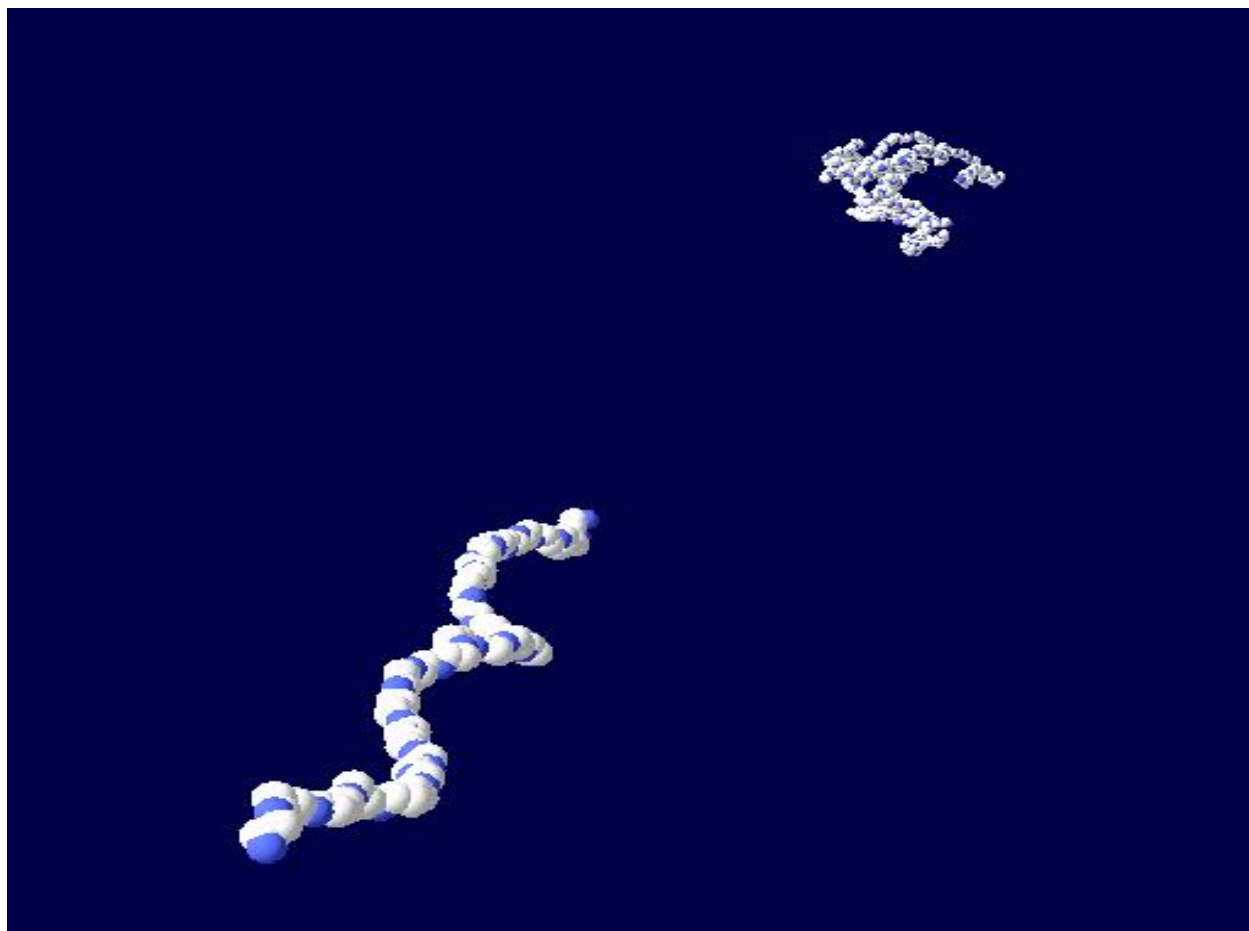


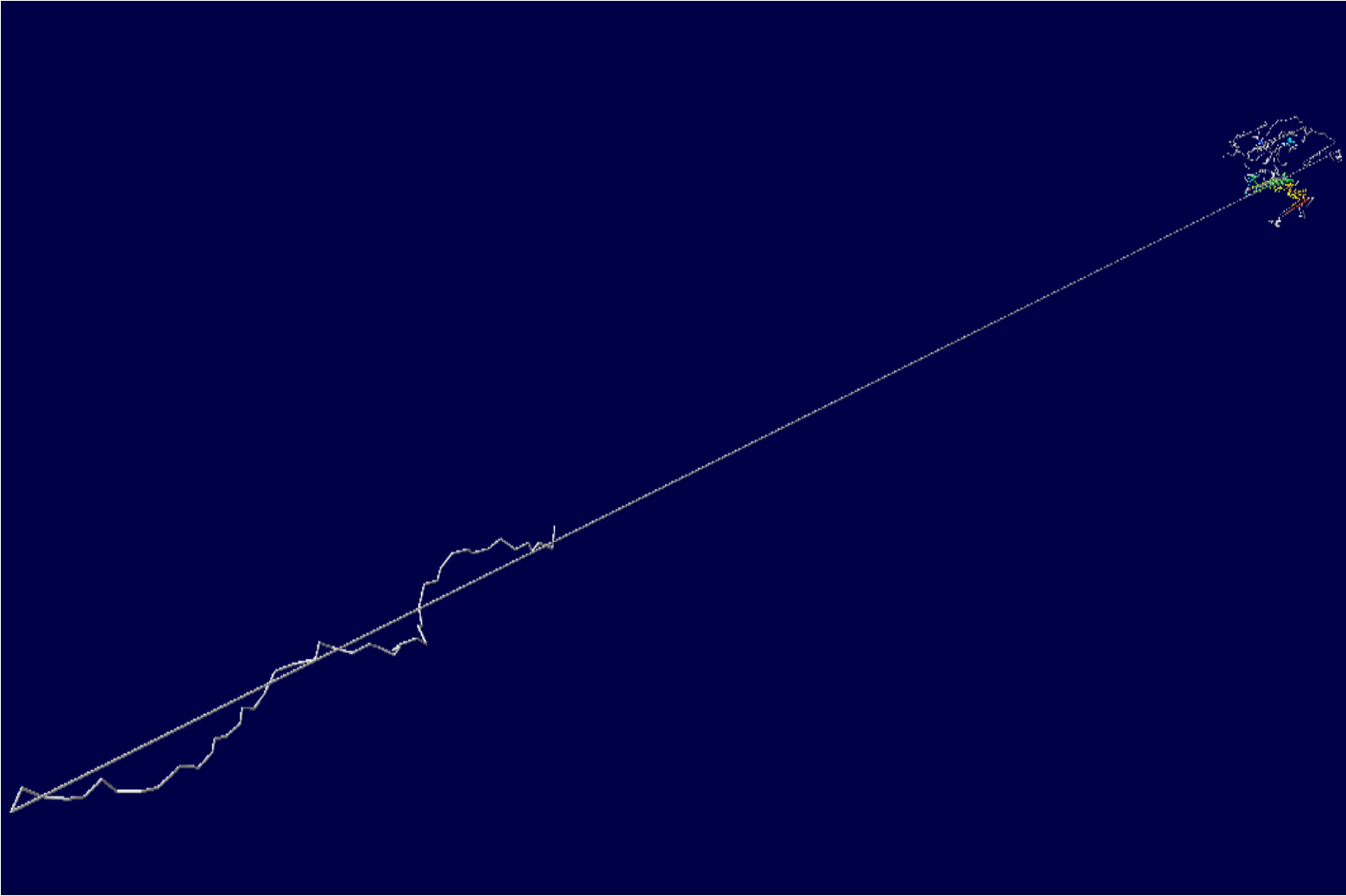
12. antigenic(v6.0.1) - Finds antigenic sites

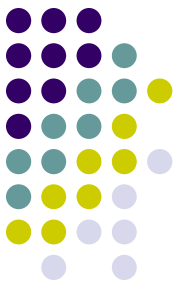
```
(1) Score 1.210 length 24 at residues 4->27
      *
Sequence: T A S I S L C L I A L F G S S A G L K C Y T C G
           |                                     |
           4                                     27
Max_score_pos: 12
(2) Score 1.182 length 35 at residues 94->128
      *
Sequence: E D S V G C T K V V L T H G E D I V R V A R G C V Y N K A D L C K G M
           |                                     |
           94                                     128
Max_score_pos: 100
(3) Score 1.168 length 11 at residues 39->49
      *
Sequence: K D D Y L L V E C N T
           |             |
           39             49
Max_score_pos: 44
(4) Score 1.150 length 20 at residues 155->174
      *
Sequence: L K S S A V A T I L T T M A T C L F Y R
           |                                     |
           155                                 174
Max_score_pos: 173
(5) Score 1.150 length 8 at residues 137->144
      *
Sequence: T L K Y C G S C
           |             |
           137           144
Max_score_pos: 141
```

发现五个可能的抗原位点

13. ESyPred3D 预测立体结构 (ESyPred3D Web Server 1.0)

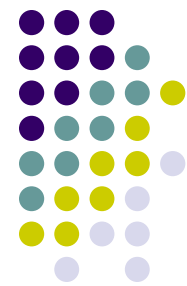






结论:

- 下一步构建的DSR33 RNAi表达载体不会对人或其他动物造成影响;
- DSR33可能是麦长管蚜唾液腺分泌蛋白（Salivary secretory protein），而非跨膜蛋白



下一步工作:

- 构建DSR33的RNAi表达载体
- 转化拟南芥及四倍体小麦
- 检测表达效率
- 进行蚜虫饲喂实验



致谢：

- 感谢罗老师这学期的辛勤劳动和对我的谆谆教导，是您让我体会到了什么是学无止境，诲人不倦！
- 感谢各位同学，尤其是几位组员的热心帮助，在此衷心的感谢你们！

Thank U for Ur attention!

