



# 棉铃虫对Bt毒蛋白抗性基因的分析

## The analysis of resistance to Bt toxic protein in *Helicoverpa armigera*

汇报人：张丹丹

组员：李静 任小霞

李志敏 张丹丹

时间:2014年1月12日

# 汇报提纲

- 研究背景
- 核酸序列分析
- 蛋白序列分析
- 研究展望

# 研究背景



- 棉铃虫
- 俗名钻心虫，属鳞翅目夜蛾科。是棉花蕾龄期的大害虫。广泛分布在中国及世界各地，黄河流域棉区、长江流域棉区和新疆棉区发生较严重。**1992年**发生特大暴发，历史罕见，造成超过百亿的经济损失。

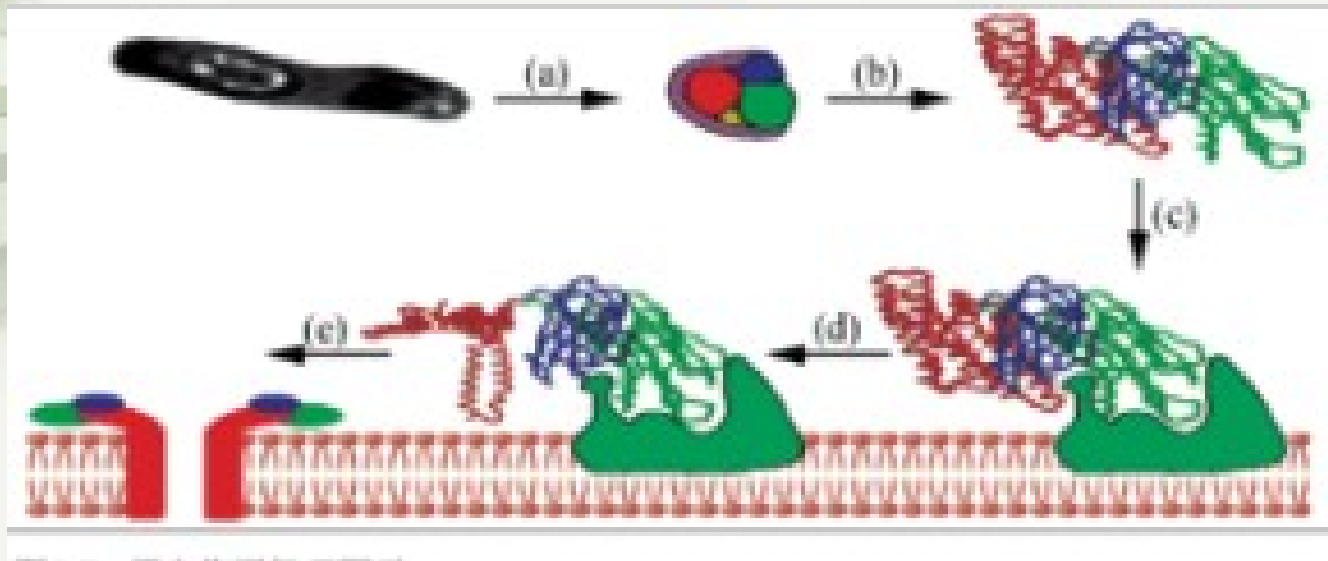
# 研究背景

- 1996年转基因作物开始商业化种植，Bt棉有效控制了棉铃虫的为害。但是，随着转基因作物的长期持续种植，害虫开始对Bt毒蛋白产生不同程度的抗性。




# 研究背景

- Bt毒蛋白作用机制



# 研究背景

- 害虫产生抗性主要与**Bt**毒蛋白与中肠细胞膜上的特异性受体的亲和力降低有关。但是，对其在遗传方面的研究尚未清楚。目前，发现**ABC**转运体**ABCC2**的突变与**Bt**抗性的产生有关。已从实验室筛选的高抗棉铃虫品系中提取出该抗性基因。

The background features a hand holding a pen over papers on the left and wireframe cubes on the right, all in a light green color scheme.

# 核酸序列分析

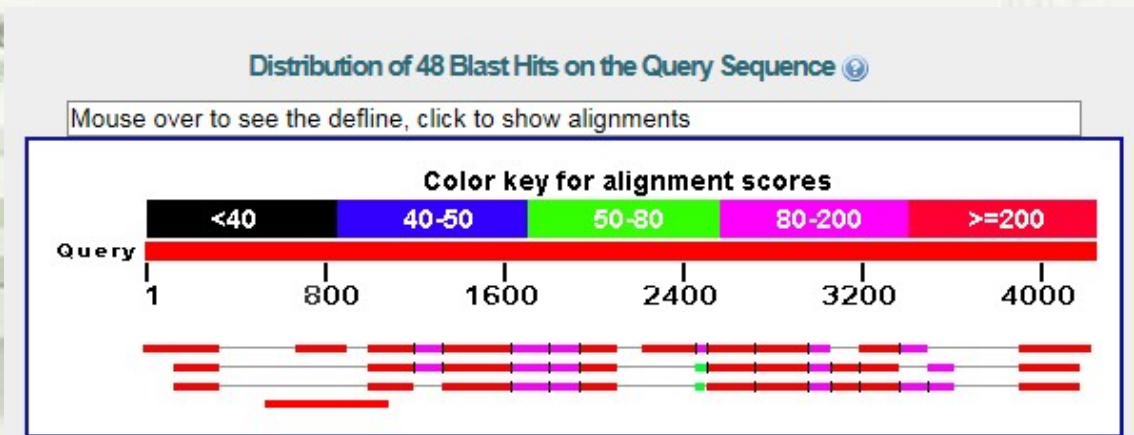
# 基因序列

- GTATTTAATNTTTAGTCTTCAGGAA  
TTGCTCACATTAGGCCAACGTCAT  
GTTGAGAATACAGTAGTAAAAGT...  
.....GATAGTGATAATAA  
GGAGGCTTAAGATAATTATTGTTTT  
ATTTTATTTTTTAATACTAATTTATTG  
TTTACATAAAGAAAAAAAAAAAAA



# blast搜索

- nucleotide collection (nr/nt) 数据库:



Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<a href="#">Heliobacterium rubrum clone 89F08 ABC transporter family C protein ABCC3 (ABCC3) gene, partial cds; ABC transporter family C protein ABCC2 (ABCC2) and SM</a>	477	4473	74%	4e-130	92%	<a href="#">GQ332573.1</a>
<input type="checkbox"/>	<a href="#">Heliobacterium virescens strain YHD3 truncated ABC transporter family C protein ABCC2 (ABCC2) gene, ABCC2-r1 allele, complete cds</a>	411	3682	61%	4e-110	94%	<a href="#">GQ332572.1</a>
<input type="checkbox"/>	<a href="#">Heliobacterium virescens strain YFO ABC transporter family C protein ABCC2 (ABCC2) gene, ABCC2-s1 allele, complete cds</a>	405	3686	61%	2e-108	94%	<a href="#">GQ332571.1</a>
<input type="checkbox"/>	<a href="#">Ostrinia scapulalis ABC transporter C2 mRNA, partial cds</a>	243	243	13%	2e-59	75%	<a href="#">KC758896.1</a>

# 基因预测

CDSf: 起始外显子; CDSi: 居间外显子; CDSi: 末端外显子; **CDSo: 单外显子**; PoIA: 末端 polyA 区; TSS: 转录起始位点。 CDSf: 起始外显子; CDSi: 居间外显子; CDSi: 末端外显子; **CDSo: 单外显子**; PoIA: 末端 polyA 区; TSS: 转录起始位点。

FGENESH 2.6 Prediction of potential genes in *Populus\_trichocarpa* genomic DNA

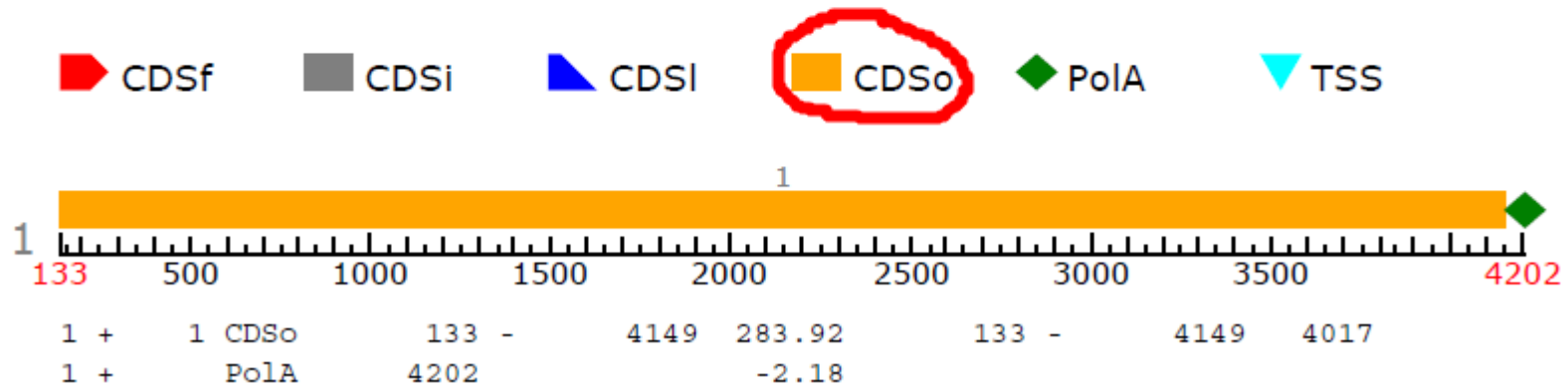
Seq name: test sequence

Length of sequence: 4214

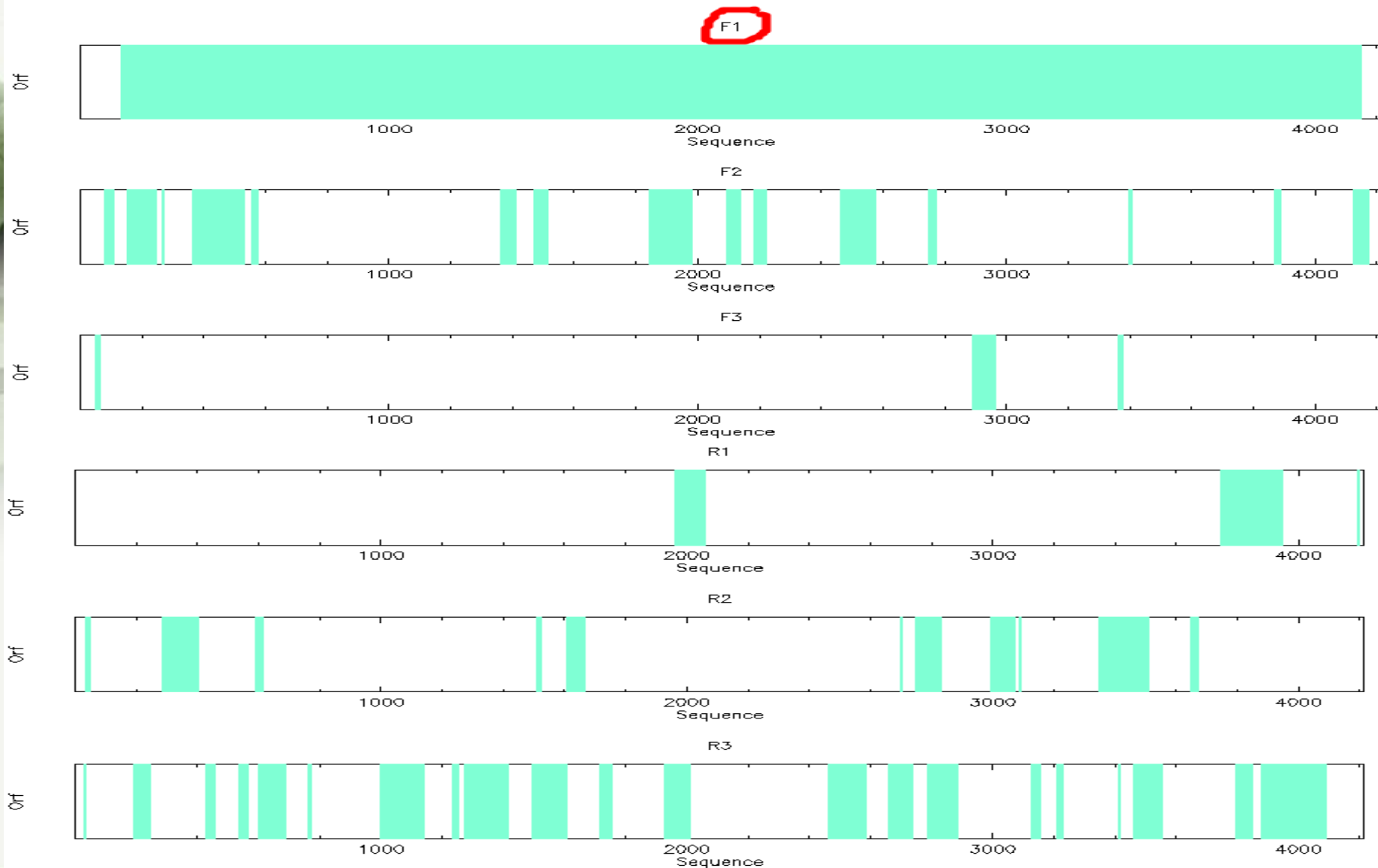
Number of predicted genes 1: in +chain 1, in -chain 0.

Number of predicted exons 1: in +chain 1, in -chain 0.

Positions of predicted genes and exons: Variant 1 from 1, Score:273.272803



# 用weblab中的plotorf对其阅读框进行分析： F1为其可能的开放阅读框



# 用weblab中cusp分析其密码子使用特征：

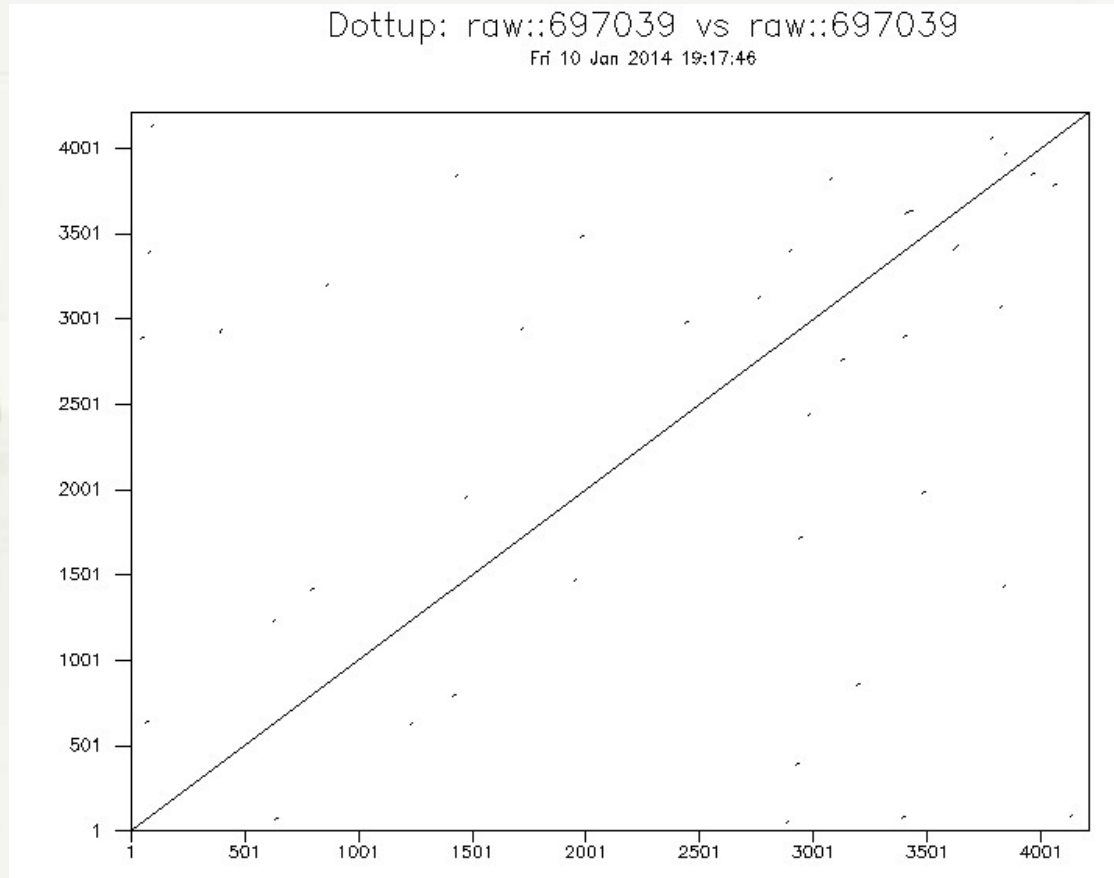
- cusp

氨基酸	丙氨酸 A	半胱氨酸 C	天冬氨酸 D	谷氨酸 E	苯丙氨酸 F	甘氨酸 G	组氨酸 H	异亮氨酸 I	赖氨酸 K	亮氨酸 L	天冬酰胺 N	脯氨酸 P	谷氨酰胺 Q	精氨酸 R	丝氨酸 S	苏氨酸 T	缬氨酸 V	酪氨酸 Y
偏好性	G C T	T G T	G A C	G A A	T T C	G G A	C A C	A T T	A A A	T T G	A A T	C C A	C A A/G	A G A	T C C	A C T	G T G	T A C

#Codon	AA	Fraction	Frequency	Number
GCA	A	0.215	14.245	20
GCC	A	0.247	16.382	23
GCG	A	0.118	7.835	11
GCT	A	0.419	27.778	39
TGC	C	0.294	3.561	5
TGT	C	0.706	8.547	12
GAC	D	0.574	24.929	35
GAT	D	0.426	18.519	26
GAA	E	0.622	32.764	46
GAG	E	0.378	19.943	28
TTC	F	0.638	31.339	44
TTT	F	0.362	17.806	25
GGA	G	0.346	19.943	28
GGC	G	0.296	17.094	24
GGG	G	0.037	2.137	3
GGT	G	0.321	18.519	26
CAC	H	0.692	6.410	9
CAT	H	0.308	2.849	4
ATA	I	0.198	12.821	18
ATC	I	0.352	22.792	32
ATT	I	0.451	29.202	41
AAA	K	0.541	28.490	40
AAG	K	0.459	24.217	34
CTA	L	0.075	8.547	12
CTC	L	0.151	17.094	24
CTG	L	0.245	27.778	39
CTT	L	0.101	11.396	16
TTA	L	0.119	13.533	19
TTG	L	0.308	34.900	49
ATG	M	1.000	40.598	57
AAC	N	0.373	15.670	22
AAT	N	0.627	26.353	37
CCA	P	0.333	12.108	17
CCC	P	0.176	6.410	9
CCG	P	0.216	7.835	11
CCT	P	0.275	9.972	14
CAA	Q	0.500	19.943	28
CAG	Q	0.500	19.943	28
AGA	R	0.348	16.382	23
AGG	R	0.182	8.547	12

# 用weblab中dottup分析是否存在重复序列:

- dottup





A hand holding a pen over a notepad, with wireframe cubes in the background.

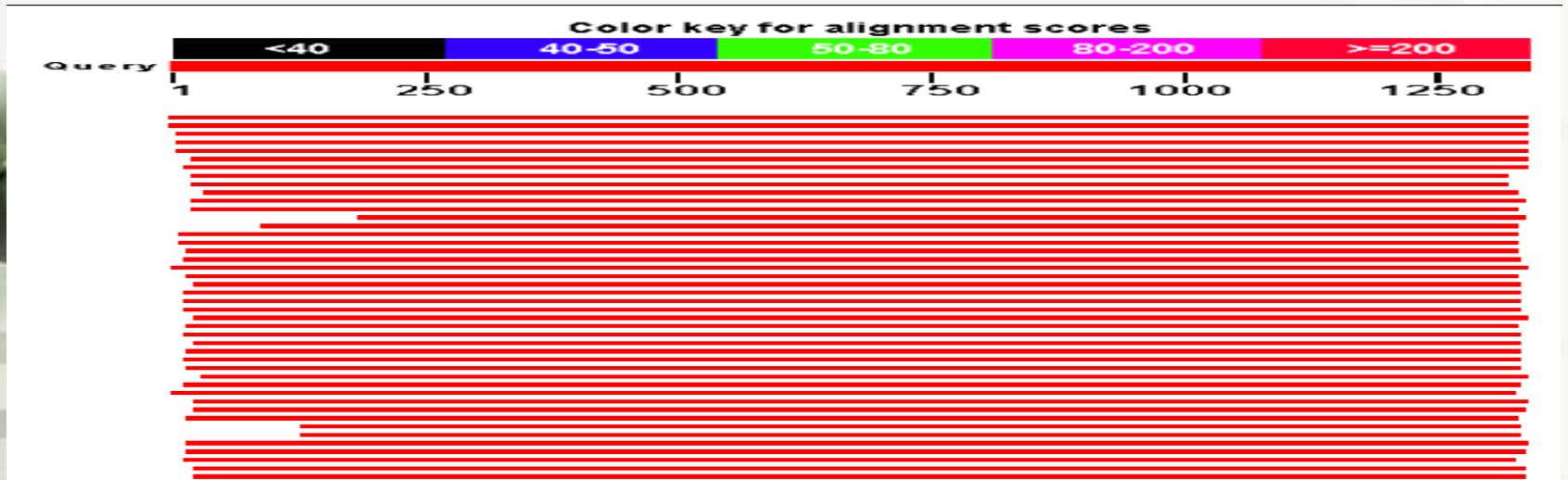
# 蛋白质序列分析

用weblab中getorf将基因序列翻译成氨基酸序列：

- MGVENKNNVQNAEGPARKTYK  
RPNILSRIFLWWMCPVLITGNKR  
NVEESDLIPPSNL.....LSNPNS  
KFFSMVRETGESMTRTLMEVAK  
AKYDSDNKEA



# 蛋白质序列blast搜索

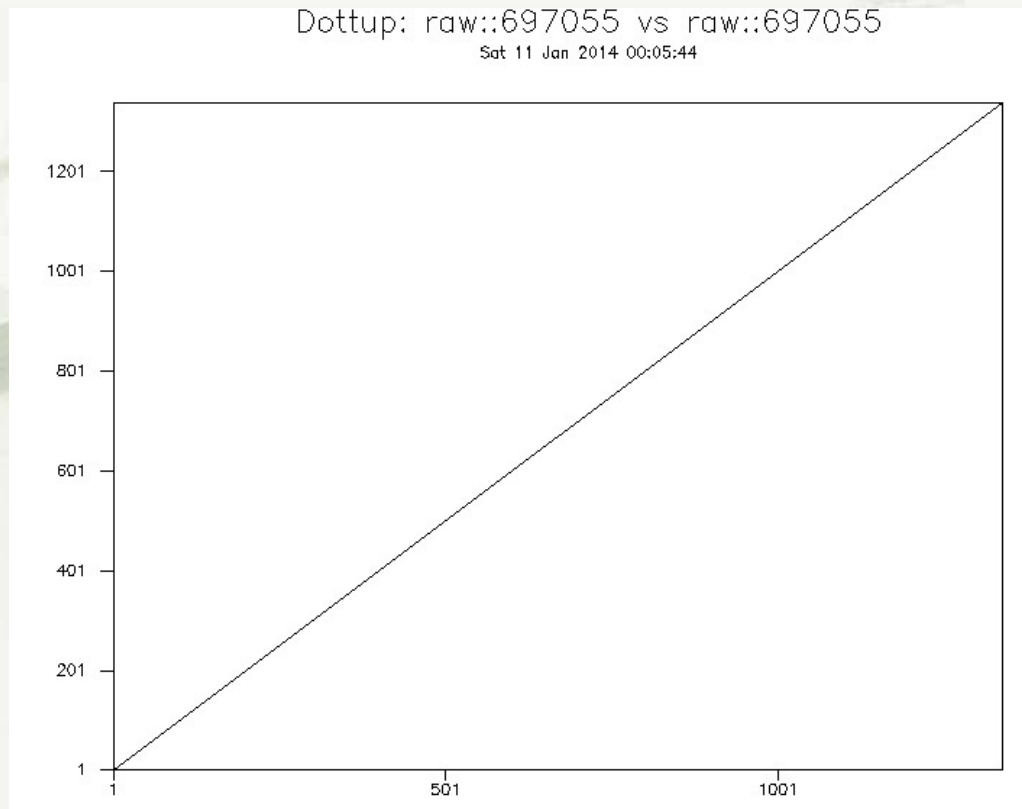


	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Heliothis virescens]</a>	2587	2587	100%	0.0	93%	<a href="#">ADH16740.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Heliothis subflexa]</a>	2583	2583	100%	0.0	93%	<a href="#">ADH16744.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter [Bombyx mandarina]</a>	1971	1971	99%	0.0	72%	<a href="#">AFI44049.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter [Bombyx mori]</a>	1963	1963	99%	0.0	72%	<a href="#">BAK82126.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter [Bombyx mori] &gt;dbj BAK82127.1 ABC transporter [Bombyx mori]</a>	1959	1959	99%	0.0	71%	<a href="#">NP_001243945.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Plutella xylostella]</a>	1853	1853	98%	0.0	66%	<a href="#">AEI27596.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter C2, partial [Ostrinia scapulalis]</a>	1848	1848	98%	0.0	68%	<a href="#">AGO01048.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2, partial [Plutella xylostella]</a>	1836	1836	96%	0.0	66%	<a href="#">AEI27592.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2, partial [Plutella xylostella]</a>	1813	1813	96%	0.0	66%	<a href="#">AEI27593.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Danaus plexippus]</a>	1653	1653	96%	0.0	61%	<a href="#">EHJ75446.1</a>
<input type="checkbox"/>	<a href="#">PREDICTED: multidrug resistance-associated protein 4 [Bombyx mori]</a>	1469	1469	98%	0.0	54%	<a href="#">XP_004928499.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Danaus plexippus]</a>	1461	1461	97%	0.0	53%	<a href="#">EHJ75445.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter, partial [Bombyx mori]</a>	1330	1330	85%	0.0	56%	<a href="#">BAK86406.1</a>
<input type="checkbox"/>	<a href="#">ABC transporter family C protein ABCC2 [Danaus plexippus]</a>	1241	1241	92%	0.0	48%	<a href="#">EHJ72514.1</a>
<input type="checkbox"/>	<a href="#">PREDICTED: similar to ATP-binding cassette transporter [Tribolium castaneum]</a>	1150	1150	98%	0.0	45%	<a href="#">XP_969849.1</a>



# 分析蛋白质序列中是否存在重叠序列:

- Dottup



# 用weblab中pepstats分析该蛋白质的氨基酸组成及特征：

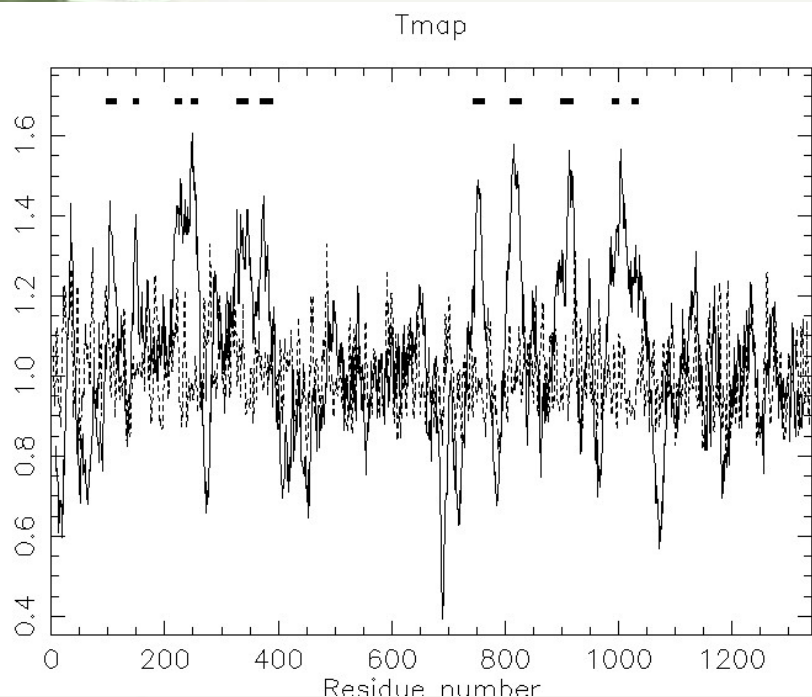
- pepstats

氨基酸	丙氨酸 A	半胱氨酸 C	天冬氨酸 D	谷氨酸 E	苯丙氨酸 F	甘氨酸 G	组氨酸 H	异亮氨酸 I	赖氨酸 K	亮氨酸 L	天冬酰胺 N	甲硫氨酸 M	脯氨酸 P	谷氨酰胺 Q	精氨酸 R	丝氨酸 S	苏氨酸 T	缬氨酸 V	酪氨酸 Y	色氨酸 W
数量	92	13	60	72	64	80	11	84	67	15 5	55	57	50	55	65	10 6	9 2	99	44	19

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	383	28.582
Small	(A+B+C+D+G+N+P+S+T+V)	647	48.284
Aliphatic	(A+I+L+V)	430	32.090
Aromatic	(F+H+W+Y)	138	10.299
<u>Non-polar</u>	(A+C+F+G+I+L+M+P+V+W+Y)	757	<u>56.493</u>
Polar	(D+E+H+K+N+Q+R+S+T+Z)	583	43.507
Charged	(B+D+E+H+K+R+Z)	275	20.522
Basic	(H+K+R)	143	10.672
Acidic	(B+D+E+Z)	132	9.851

# 用weblab中的tmap对其跨膜区域进行分析:

- tmap



```
# Sequence:      from: 1   to: 1340
```

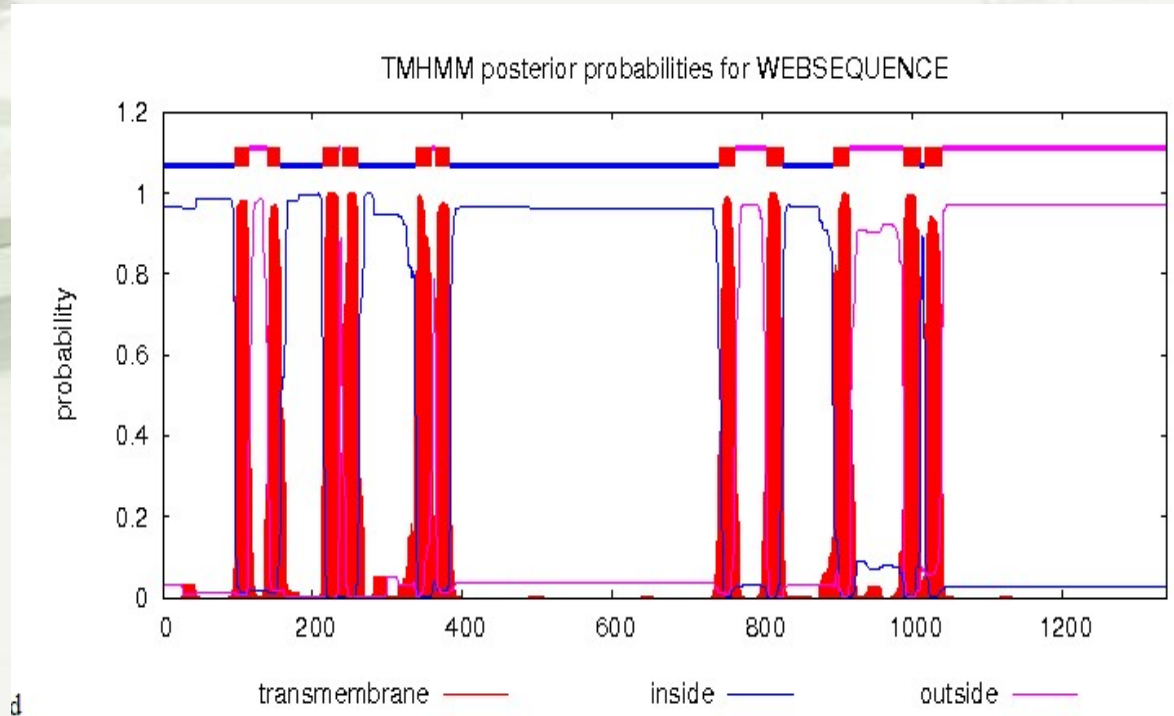
```
# HitCount: 11
```

```
#=====
```

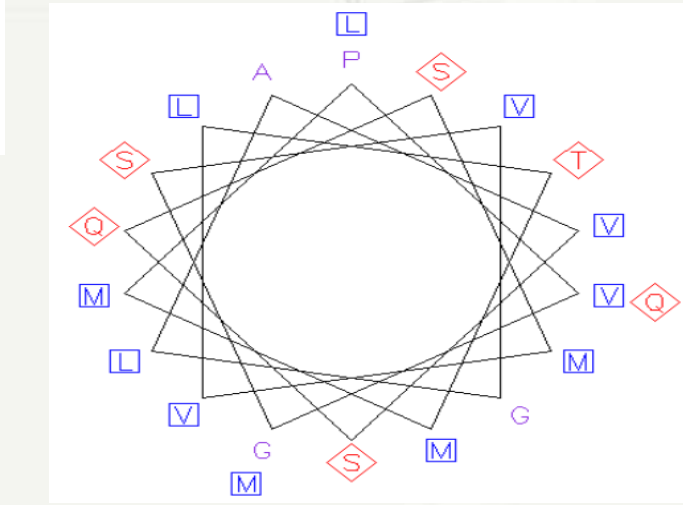
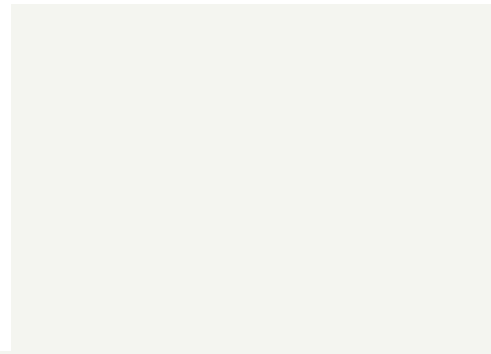
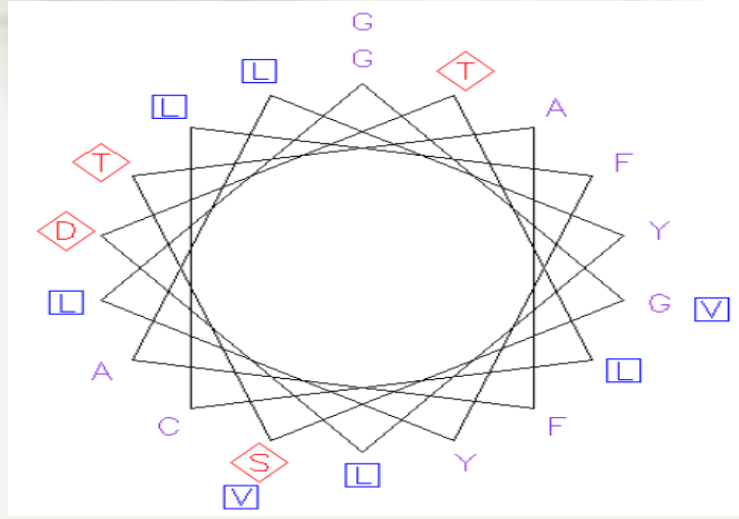
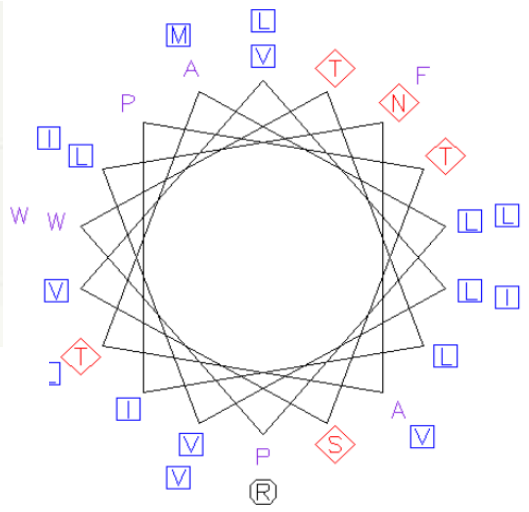
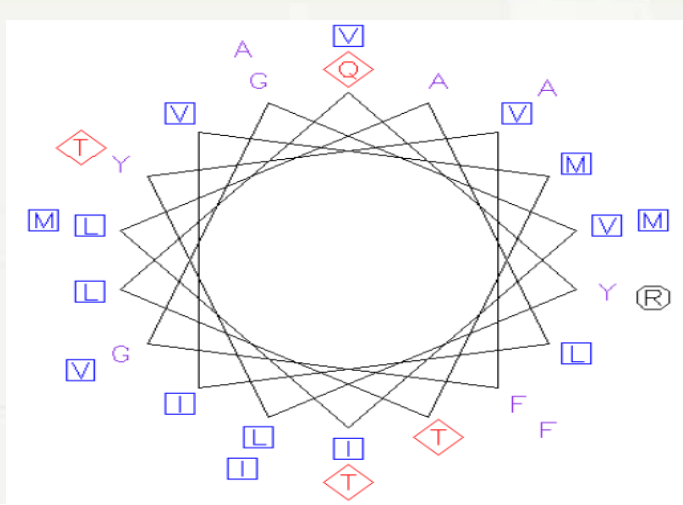
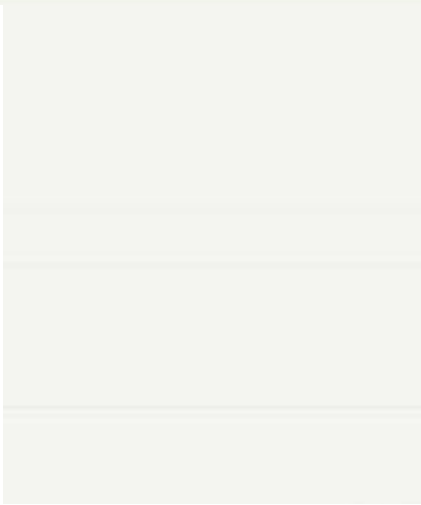
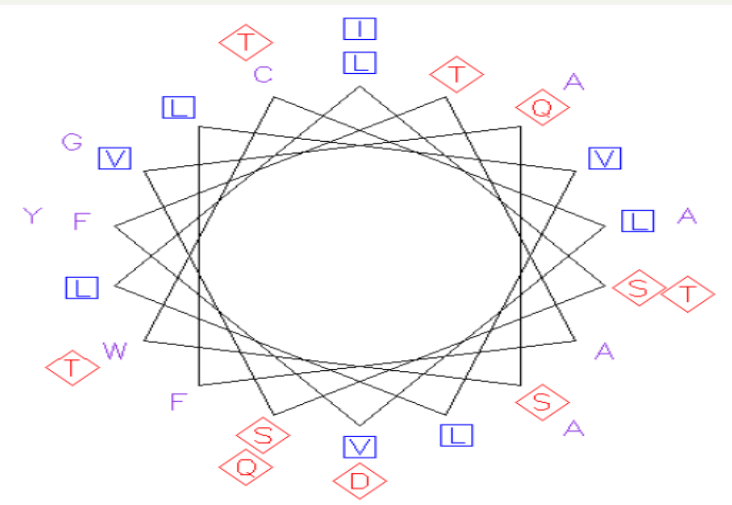
Start	End	TransMem	Sequence
94	119	1	RKAYWVSYMPGAIFIIIQSAARTYQP
141	157	2	GLYALAMLGLNFVSMCC
214	234	3	AFMFLHYLWIVPIQVAVVLYF
242	262	4	APFVGLFGVVILILPLQAGLT
323	351	5	IRSTFLGFMLPTERSIMFVTVLTALALTGT
365	393	6	FSIIQFNVTLIIPMAIASYSEMMVSIERI
740	768	7	LSSVQSWCLVFTAFLVLLITQGAATTADY
805	833	8	QYLYVFGGVILALIVMTLVKITAFVAMTM
895	923	9	VLVLNATALPWTLIPTSVLIVIFVLMMLRW
985	1005	10	GGSTAFALYLDLCLFYLGVV
1019	1039	11	PVGSVGLAVSQSMVLTMMMLQM

# 用ExPASy中的软件对蛋白序列跨膜区域分析

## TMHMM



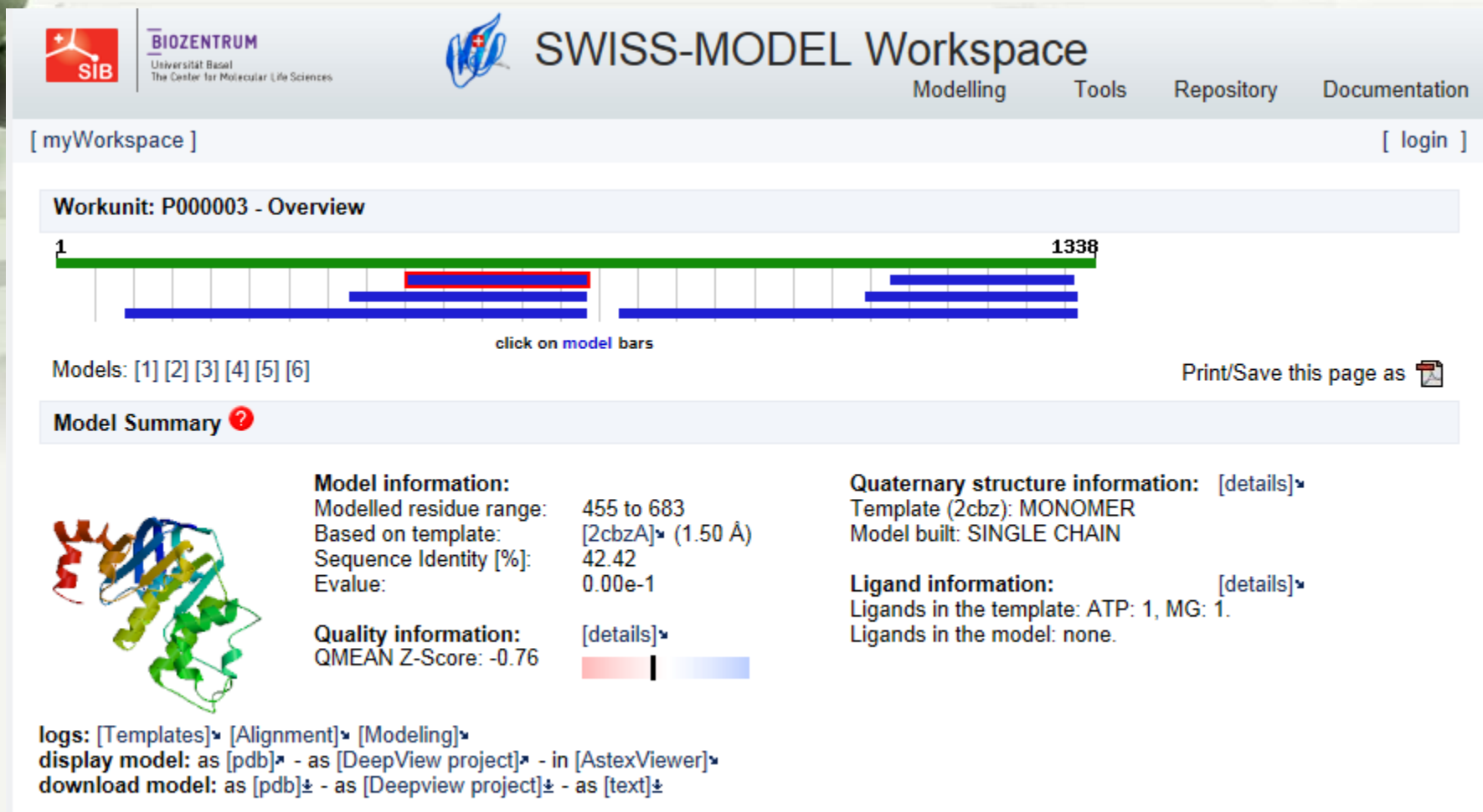






# 同源模建:得到6个模版序列

- 2CBA



**SIB** | **BIOZENTRUM**  
Universität Basel  
The Center for Molecular Life Sciences


**SWISS-MODEL Workspace**  
Modelling Tools Repository Documentation


[ myWorkspace ] [ login ]

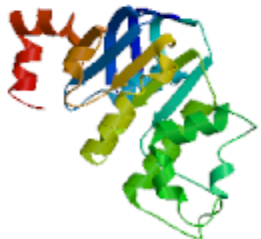
Workunit: P000003 - Overview

1 1338


click on model bars


Models: [1] [2] [3] [4] [5] [6] Print/Save this page as 


**Model Summary** 












**Model information:**  
Modelled residue range: 455 to 683  
Based on template: [2cbzA] (1.50 Å)  
Sequence Identity [%]: 42.42  
Evaluate: 0.00e-1

**Quality information:** [details]   
QMEAN Z-Score: -0.76

**Quaternary structure information:** [details]   
Template (2cbz): MONOMER  
Model built: SINGLE CHAIN

**Ligand information:** [details]   
Ligands in the template: ATP: 1, MG: 1.  
Ligands in the model: none.

logs: [Templates]  [Alignment]  [Modeling]   
display model: as [pdb]  - as [DeepView project]  - in [AstexViewer]   
download model: as [pdb]  - as [Deepview project]  - as [text] 

# MEME 预测 Motif

## MOTIF 1

[Next Top](#)

### Summary ?

E-value 3.9e-194  
Width 50  
Sites 6  
[show more](#)

### Sequence Logo ?



## MOTIF 2

[Previous](#) [Next Top](#)

### Summary ?

E-value 7.8e-190  
Width 50  
Sites 6  
[show more](#)

### Sequence Logo ?



## MOTIF 3

[Previous Top](#)

### Summary ?

E-value 6.2e-184  
Width 50  
Sites 6  
[show more](#)

### Sequence Logo ?



# 研究展望

- 我们可以通过该抗性基因的研究从基因和遗传方面揭示棉铃虫对**Bt**毒蛋白产生抗性的机制，有利于抑制棉铃虫**Bt**抗性的进一步演化，防止棉铃虫的再猖獗。

谢谢！

请大家批评指正！

