大丽轮枝菌蛋白激发子PevD1结构预测及其基因克隆

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1 背景及意义

2 技术路线

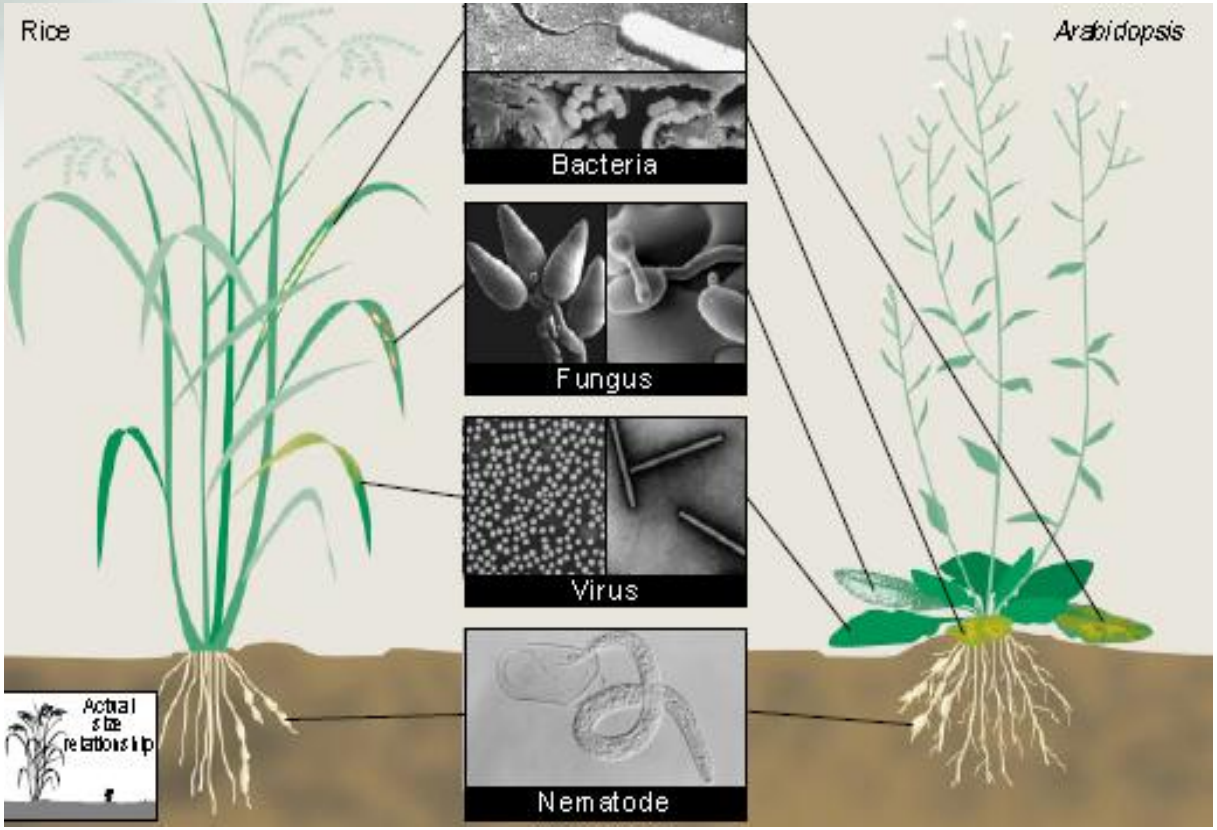
3 内容和结果

4 结论

一、研究背景及意义

何为激发子?

对植物而言，所有能够激发植物发生防御反应的物质都可以称为激发子



一、研究背景及意义

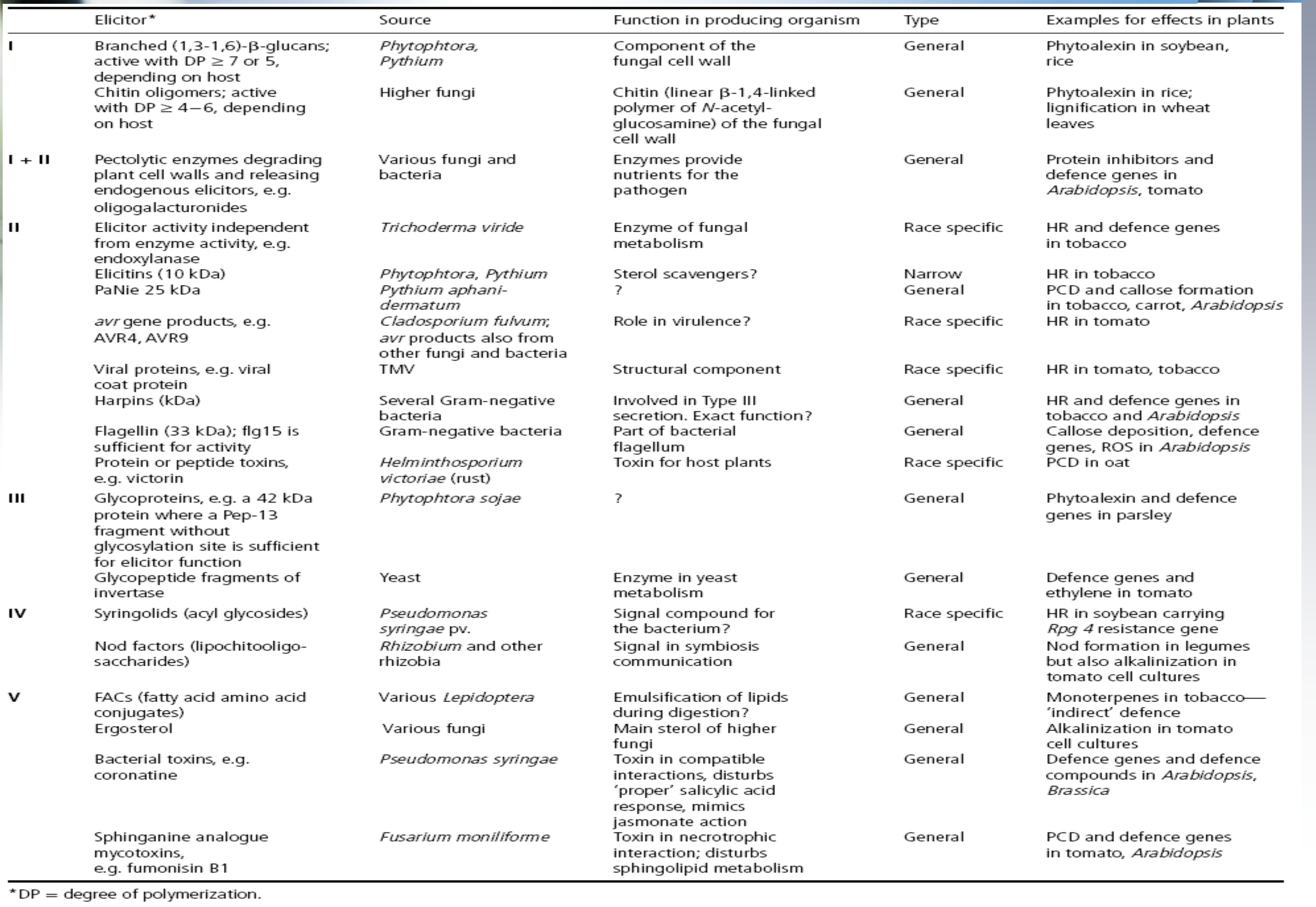
激发子类型

生物源激发子
- 蛋白质
- 糖蛋白
- 脂类及脂多糖类
- 寡聚糖

非生物激发子

人工合成的化学物质如乙酰水杨酸，多聚腺苷酸，2-氯乙烯磷酸等

已报道的激发子类型

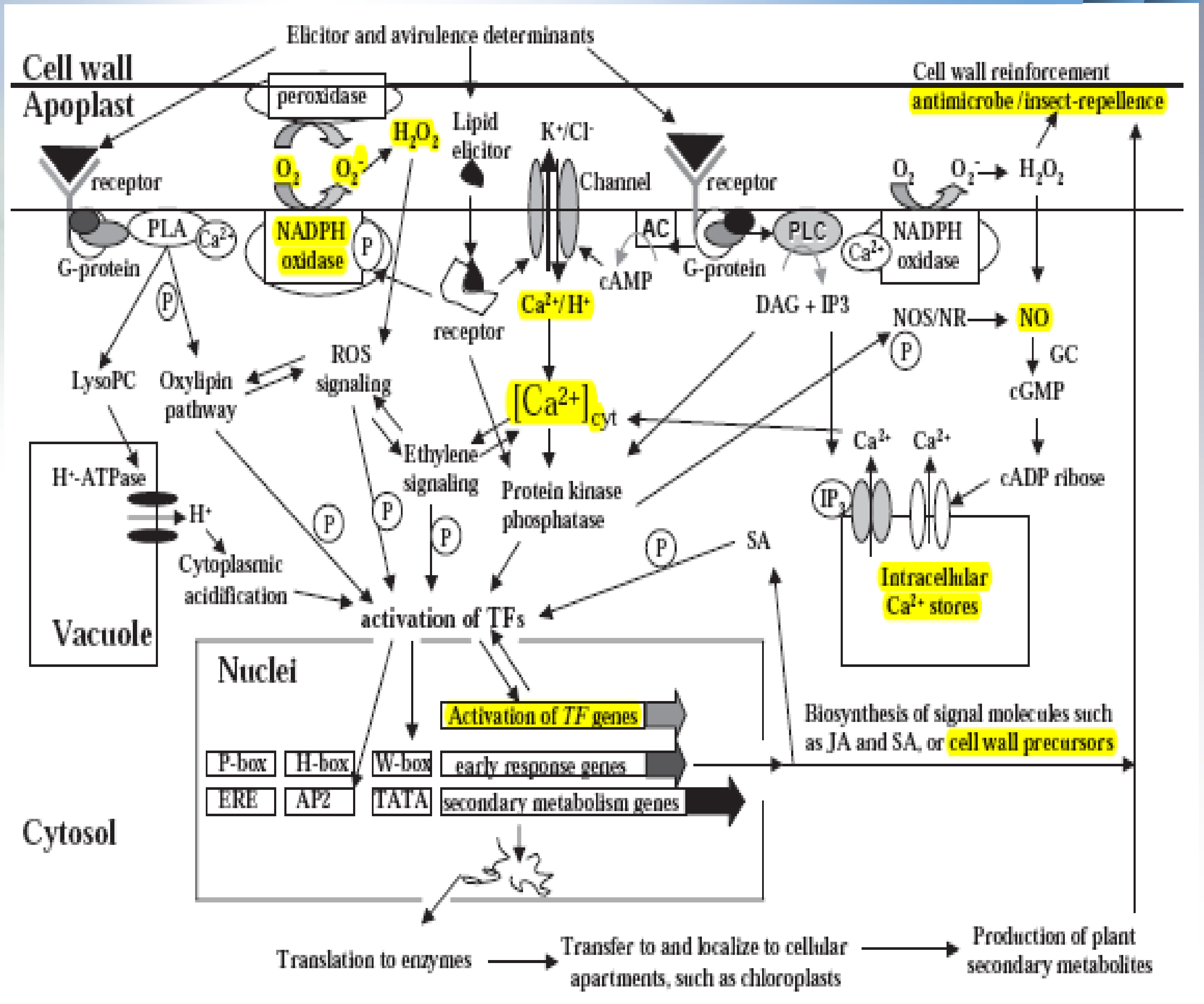


	Elicitor*	Source	Function in producing organism	Type	Examples for effects in plants
I	Branched (1,3-1,6)-β-glucans; active with DP ≥ 7 or 5, depending on host	*Phytophtora*, *Pythium*	Component of the fungal cell wall	General	Phytoalexin in soybean, rice
	Chitin oligomers; active with DP $\geq 4-6$, depending on host	Higher fungi	Chitin (linear β-1,4-linked polymer of *N*-acetyl-glucosamine) of the fungal cell wall	General	Phytoalexin in rice; lignification in wheat leaves
I + II	Pectolytic enzymes degrading plant cell walls and releasing endogenous elicitors, e.g. oligogalacturonides	Various fungi and bacteria	Enzymes provide nutrients for the pathogen	General	Protein inhibitors and defence genes in *Arabidopsis*, tomato
II	Elicitor activity independent from enzyme activity, e.g. endoxylanase	*Trichoderma viride*	Enzyme of fungal metabolism	Race specific	HR and defence genes in tobacco
	Elicitins (10 kDa)	*Phytophtora*, *Pythium*	Sterol scavengers?	Narrow	HR in tobacco
	PaNie 25 kDa	*Pythium aphanidermatum*	?	General	PCD and callose formation in tobacco, carrot, *Arabidopsis*
	avr gene products, e.g. AVR4, AVR9	*Cladosporium fulvum*; *avr* products also from other fungi and bacteria	Role in virulence?	Race specific	HR in tomato
	Viral proteins, e.g. viral coat protein	TMV	Structural component	Race specific	HR in tomato, tobacco
	Harpins (kDa)	Several Gram-negative bacteria	Involved in Type III secretion. Exact function?	General	HR and defence genes in tobacco and *Arabidopsis*
	Flagellin (33 kDa); flg15 is sufficient for activity	Gram-negative bacteria	Part of bacterial flagellum	General	Callose deposition, defence genes, ROS in *Arabidopsis*
	Protein or peptide toxins, e.g. victorin	*Helminthosporium victoriae* (rust)	Toxin for host plants	Race specific	PCD in oat
III	Glycoproteins, e.g. a 42 kDa protein where a Pep-13 fragment without glycosylation site is sufficient for elicitor function	*Phytophtora sojae*	?	General	Phytoalexin and defence genes in parsley
	Glycopeptide fragments of invertase	Yeast	Enzyme in yeast metabolism	General	Defence genes and ethylene in tomato
IV	Syringolids (acyl glycosides)	*Pseudomonas syringae* pv.	Signal compound for the bacterium?	Race specific	HR in soybean carrying *Rpg 4* resistance gene
	Nod factors (lipochitooligosaccharides)	*Rhizobium* and other rhizobia	Signal in symbiosis communication	General	Nod formation in legumes but also alkalinization in tomato cell cultures
V	FACs (fatty acid amino acid conjugates)	Various *Lepidoptera*	Emulsification of lipids during digestion?	General	Monoterpenes in tobacco—'indirect' defence
	Ergosterol	Various fungi	Main sterol of higher fungi	General	Alkalinization in tomato cell cultures
	Bacterial toxins, e.g. coronatine	*Pseudomonas syringae*	Toxin in compatible interactions, disturbs 'proper' salicylic acid response, mimics jasmonate action	General	Defence genes and defence compounds in *Arabidopsis*, *Brassica*
	Sphinganine analogue mycotoxins, e.g. fumonisin B1	*Fusarium moniliforme*	Toxin in necrotrophic interaction; disturbs sphingolipid metabolism	General	PCD and defence genes in tomato, *Arabidopsis*

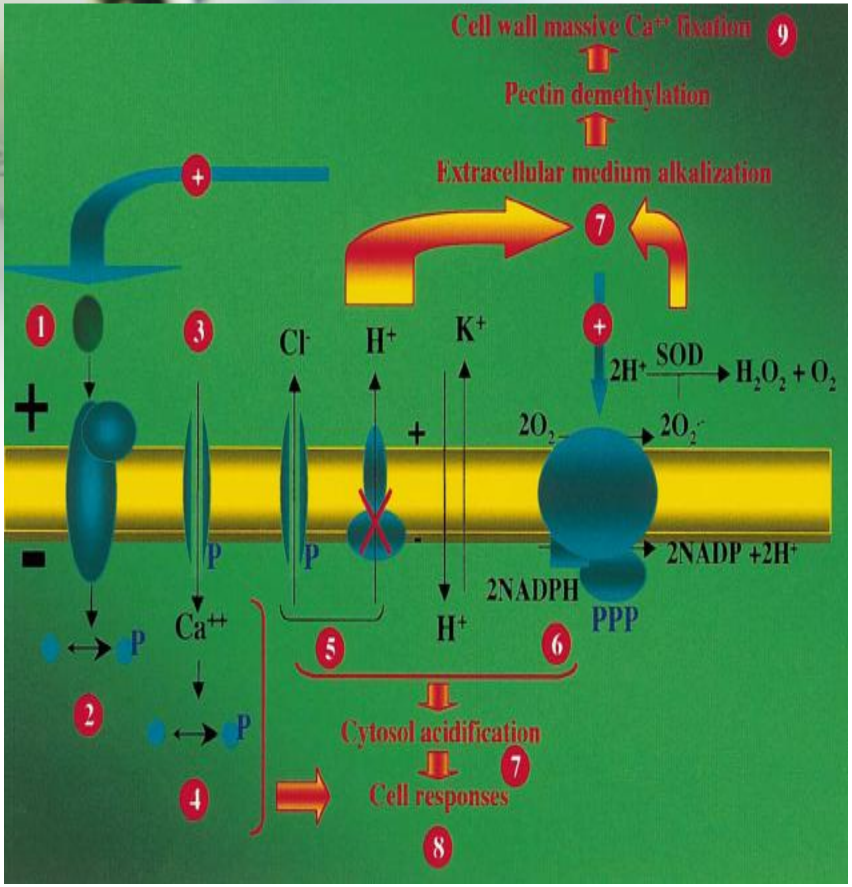
*DP = degree of polymerization.

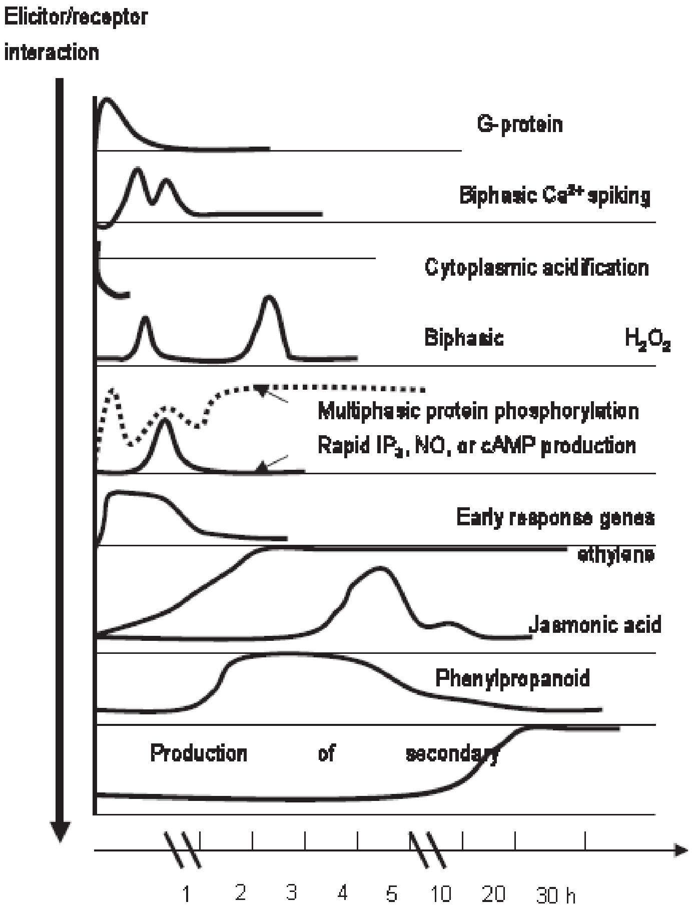
激发子诱导植物抗性途径

- 结合受体
- 激活第二信使
- 细胞膜去极化
- H_2O_2 NO生成
- 抗性基因激活
- SA、JA、ET等信号途径活化产生系统抗性



激发子诱导植物抗性早期信号事件





研究意义

- 分离纯化大丽轮枝菌胞外蛋白激发子，对已纯化的蛋白进行质谱分析，获得有关的肽段，通过生物信息学方法，得到其基因序列，根据已知基因序列设计特异性引物，扩增全长编码序列，构建表达载体，获到重组表达蛋白。为体外获得大量PevD1蛋白奠定基础。
- 对PevD1进行二级和三级结构预测，预测其作用位点，对蛋白互作及植物抗性机理研究有重要意义。

二、技术路线

PevD1分离纯化及序列测定

培养液制备

↓

粗蛋白获得

↓

蛋白激发子纯化

↓

质谱鉴定及测序

PevD1基因的获得及体外重组

在NCBI上blast比对找到同源蛋白

↓

利用该同源蛋白编码序列设计引物克隆PevD1基因

↓

与载体连接体外重组获得重组蛋白

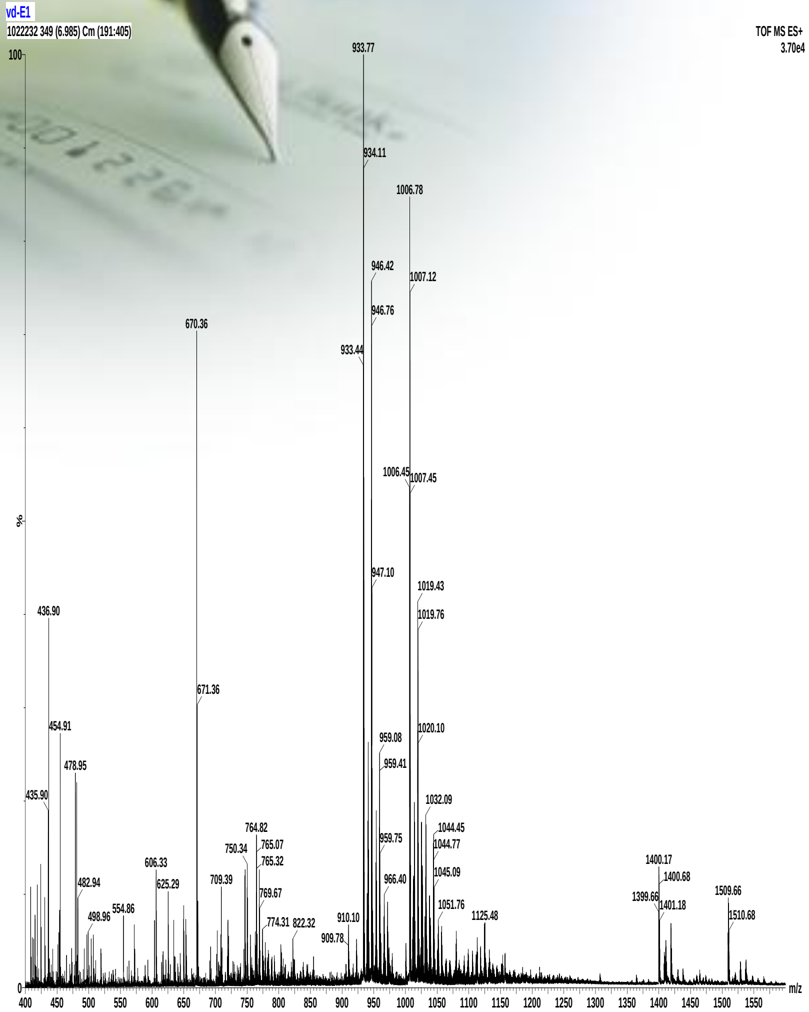
PevD1二级及三级结构预测

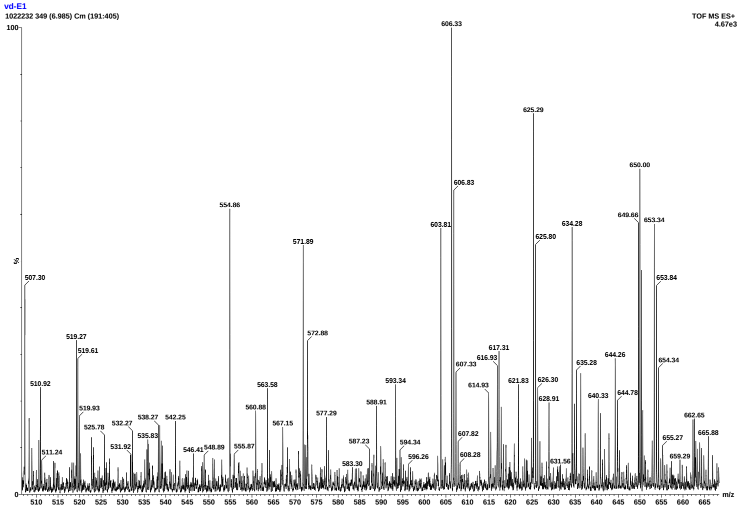
对PevD1进行二级结构预测

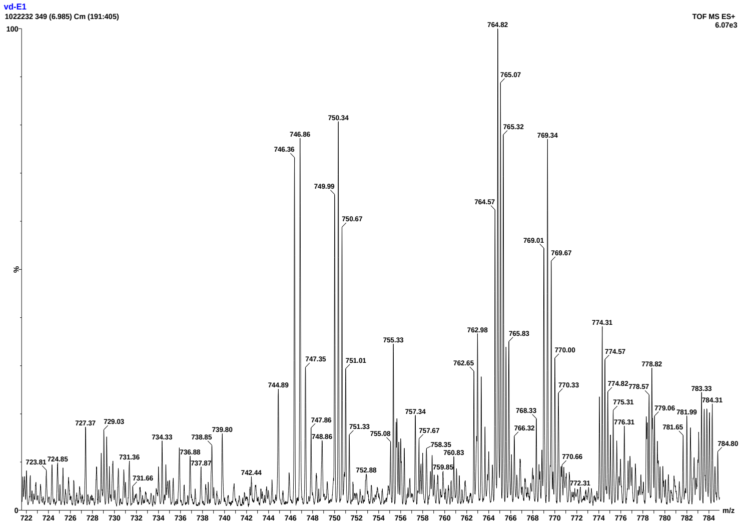
对PevD1进行三级结构预测

以此预测蛋白互作的作用位点

蛋白激发子PevD1基因克隆







蛋白激发子PevD1基因克隆

通过denovo测序，得到三段氨基酸序列。依次为:

1、qppdpnmyenldladfnvr

2、kgedgtlgkyvnfk

3、vdasdadgllcgeaqnpglpsnvltgcgesk

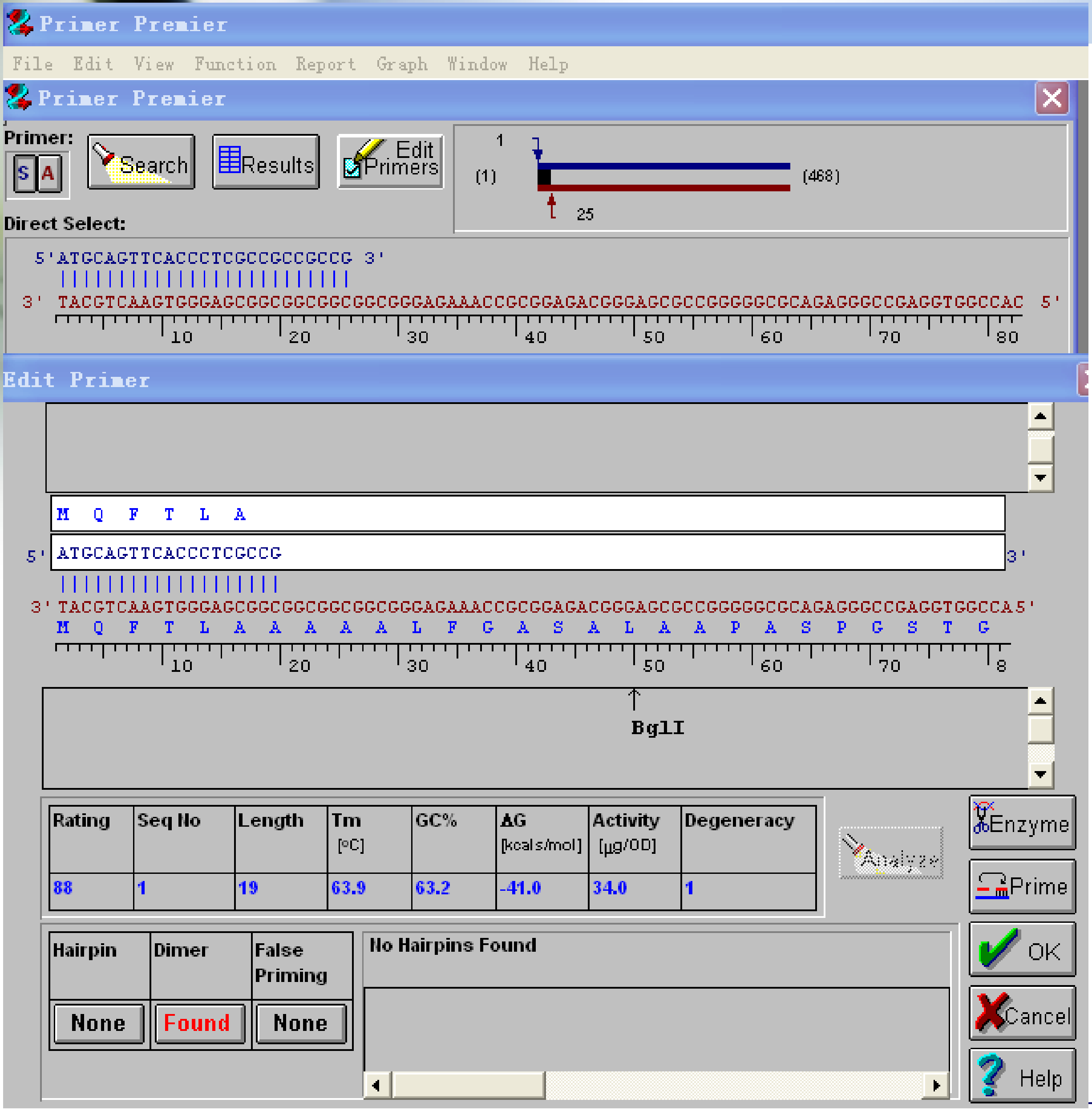
在NCBI上BLAST均比对上为同一蛋白，此蛋白GeneBank登陆号为: XP_003008076。该蛋白为黑白轮枝菌的一个155 aa 的保守假设蛋白。

蛋白激发子PevD1基因克隆

ATGCAGTTCACCCTCGCCGCCGCCGCCGCCCTCTTTGGC
GCCTCTGCCCTCGCGGCCCCCGCGTCTCCCGGCTCCACC
GGTGCTCCTCCCGACCCCAACATGTACGAGAACATTGAC
ATTGCCGACTTCAACGTCCGCAAGGGCGAGGACGGCAC
CATCAAGTACGTCAACTTCAAGCTGAGCGGCGACGACGC
CGACGGCCTCCTCTGCGAGGCCCAGAACCCCGGCCTGCC
TTCCAACGTCATCACCTGCGGCGAGTCCAAGTACCGCTT
CGCCCTGTCCTCTGGTAAGCAGTATGAGTTCGCTCTGAGC
CTGTACCACGAGCTCGGCCTTGCCGTCGGCTTCTACGGC
ACTGGCGAGATCTTCACCCACTGCCGTGCTGGCGGCCTT
GGTGACTTTATCTGCCAGCAGCAGAACCCCACCACCATT
GTCATCGACAGCCTGCCCGACGCTCCCGCCGAGGCTTAA

468bp–黑白轮枝菌 VaMs.102

蛋白激发子PevD1基因克隆



```
 5'ATGCAGTTCACCCTCGCCGCCGCCG 3'
   |||||||||||||||||||||||||
3' TACGTCAAGTGGGAGCGGCGGCGGCGGCGGGAGAAACCGCGGAGACGGGAGCGCCGGGGGCGCAGAGGGCCGAGGTGGCCAC 5'
           10        20        30        40        50        60        70        80
```

```
   M  Q  F  T  L  A
5' ATGCAGTTCACCCTCGCCG                                                          3'
   |||||||||||||||||||
3' TACGTCAAGTGGGAGCGGCGGCGGCGGCGGGAGAAACCGCGGAGACGGGAGCGCCGGGGGCGCAGAGGGCCGAGGTGGCCA5'
   M  Q  F  T  L  A  A  A  A  A  L  F  G  A  S  A  L  A  A  P  A  S  P  G  S  T  G
           10        20        30        40        50        60        70        8
                                              BglI
```

Rating	Seq No	Length	Tm [°C]	GC%	ΔG [kcals/mol]	Activity [μg/OD]	Degeneracy
88	1	19	63.9	63.2	-41.0	34.0	1

Hairpin	Dimer	False Priming
None	Found	None

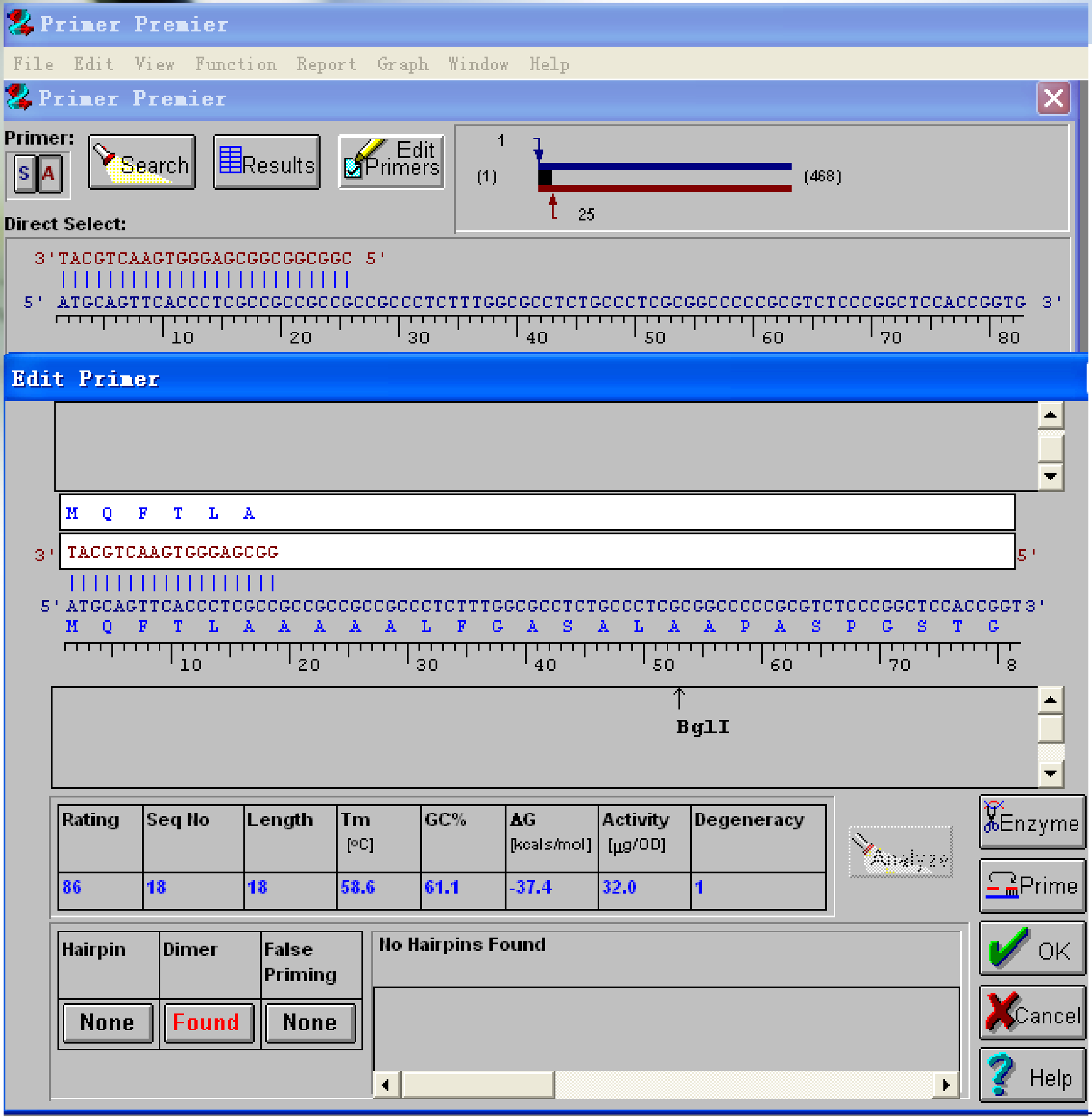
No Hairpins Found

使用Primer Premier软件进行引物设计，得到其特异引物为

pevd1-F:5'-ATGCAGTTCACCCTCGCCG-3'

pevd1-R:5'-TTAAGCCTCGGCGGGAGC-3’

蛋白激发子PevD1基因克隆



```
 3'TACGTCAAGTGGGAGCGGCGGCGGC 5'
   |||||||||||||||||||||||||
5' ATGCAGTTCACCCTCGCCGCCGCCGCCCTCTTTGGCGCCTCTGCCCTCGCGGCCCCCGCGTCTCCCGGCTCCACCGGTG 3'
            10        20        30        40        50        60        70        80
```

```
   M  Q  F  T  L  A
3' TACGTCAAGTGGGAGCGG 5'
   ||||||||||||||||||
5'ATGCAGTTCACCCTCGCCGCCGCCGCCCTCTTTGGCGCCTCTGCCCTCGCGGCCCCCGCGTCTCCCGGCTCCACCGGT3'
  M  Q  F  T  L  A  A  A  A  A  L  F  G  A  S  A  L  A  A  P  A  S  P  G  S  T  G
           10        20        30        40        50        60        70       8
                                                  ↑
                                                 BglI
```

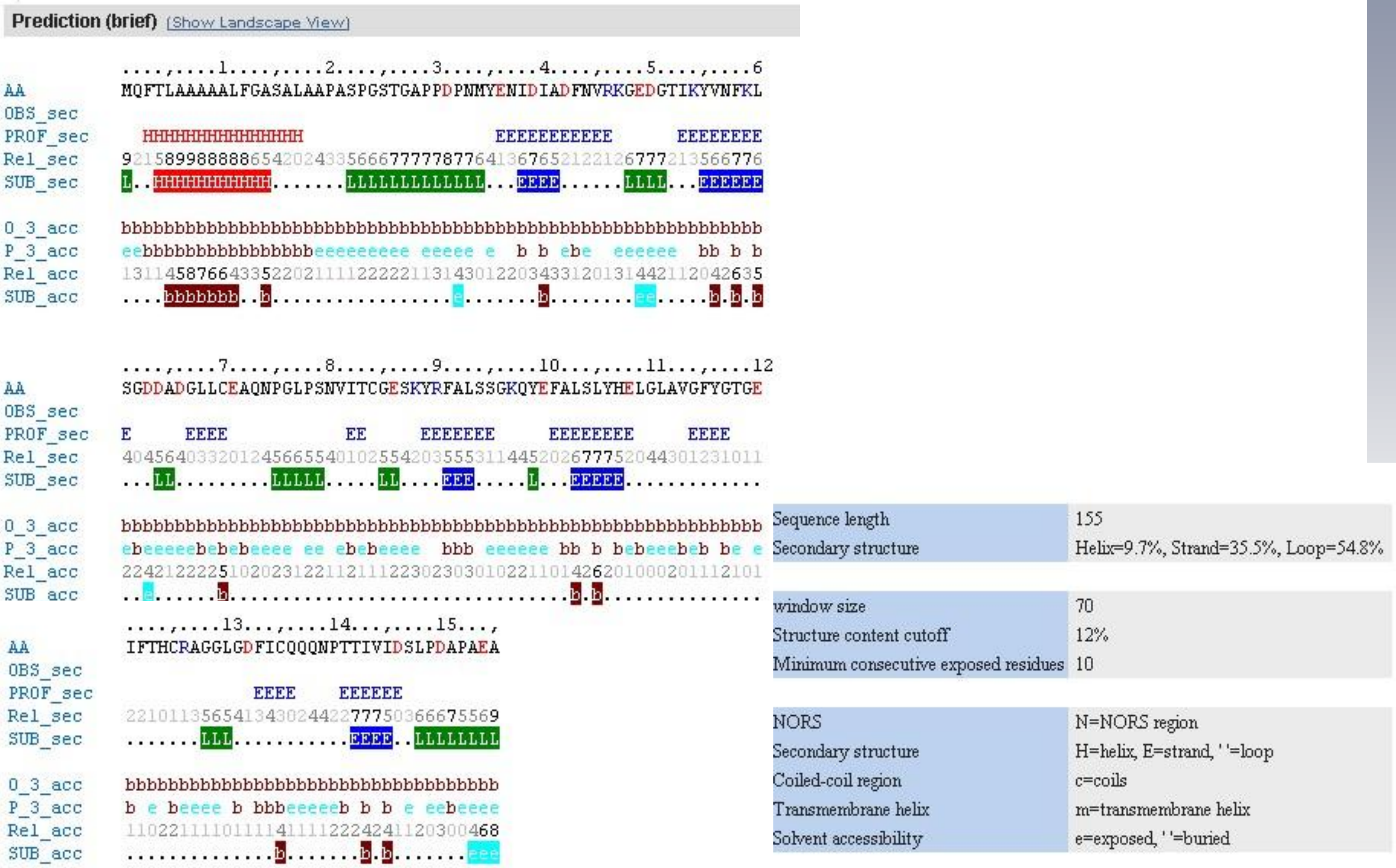
Rating	Seq No	Length	Tm [°C]	GC%	ΔG [kcals/mol]	Activity [µg/OD]	Degeneracy
86	18	18	58.6	61.1	-37.4	32.0	1

Hairpin	Dimer	False Priming
None	Found	None

No Hairpins Found

PCR扩增PevD1基因后利用双酶切在连接酶的作用下与载体连接，之后导入到感受态细胞中，进而进行重组蛋白诱导表达。

PevD1结构预测



Prediction (brief) [Show Landscape View]

```
               ....,....1....,....2....,....3....,....4....,....5....,....6
AA             MQFTLAAAAALFGASALAAPASPGSTGAPPDPNMYENIDIADFNVRKGEDGTIKYVNFKL
OBS_sec
PROF_sec         HHHHHHHHHHHHHHH                  EEEEEEEEEEE      EEEEEEEE
Rel_sec        921589988888654202433566677777877641367652122126777213566776
SUB_sec        L..HHHHHHHHHHH.......LLLLLLLLLLLLL...EEEE......LLLL...EEEEEE

O_3_acc        bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb
P_3_acc        eebbbbbbbbbbbbbbbbeeeeeeeee eeeee e  b b ebe  eeeeee  bb b b
Rel_acc        131145876643352202111122222113143012203433120131442112042635
SUB_acc        ....bbbbbbb..b.................e.......b........ee.....b.b.b


               ....,....7....,....8....,....9....,....10...,....11...,....12
AA             SGDDADGLLCEAQNPGLPSNVITCGESKYRFALSSGKQYEFALSLYHELGLAVGFYGTGE
OBS_sec
PROF_sec       E     EEEE           EE     EEEEEEE     EEEEEEEE     EEEE
Rel_sec        404564033201245665540102554203555311445202677752044301231011
SUB_sec        ...LL.........LLLLL.....LL....EEE.....L...EEEEE.............

O_3_acc        bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb
P_3_acc        ebeeeeebebebeeee ee ebebeeee  bbb eeeeee bb b bebeeebeb be e
Rel_acc        224212222510202312211211122302303010221101426201000201112101
SUB_acc        ..e......b................................b.b...............

               ....,....13...,....14...,....15...,
AA             IFTHCRAGGLGDFICQQQNPTTIVIDSLPDAPAEA
OBS_sec
PROF_sec                   EEEE    EEEEEE
Rel_sec        22101135654134302442277750366675569
SUB_sec        .......LLL...........EEEE..LLLLLLLL

O_3_acc        bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb
P_3_acc        b e beeee b bbbeeeeeb b b e eebeeee
Rel_acc        11022111101111411112224241120300468
SUB_acc        ..............b.......b.b.......eee
```

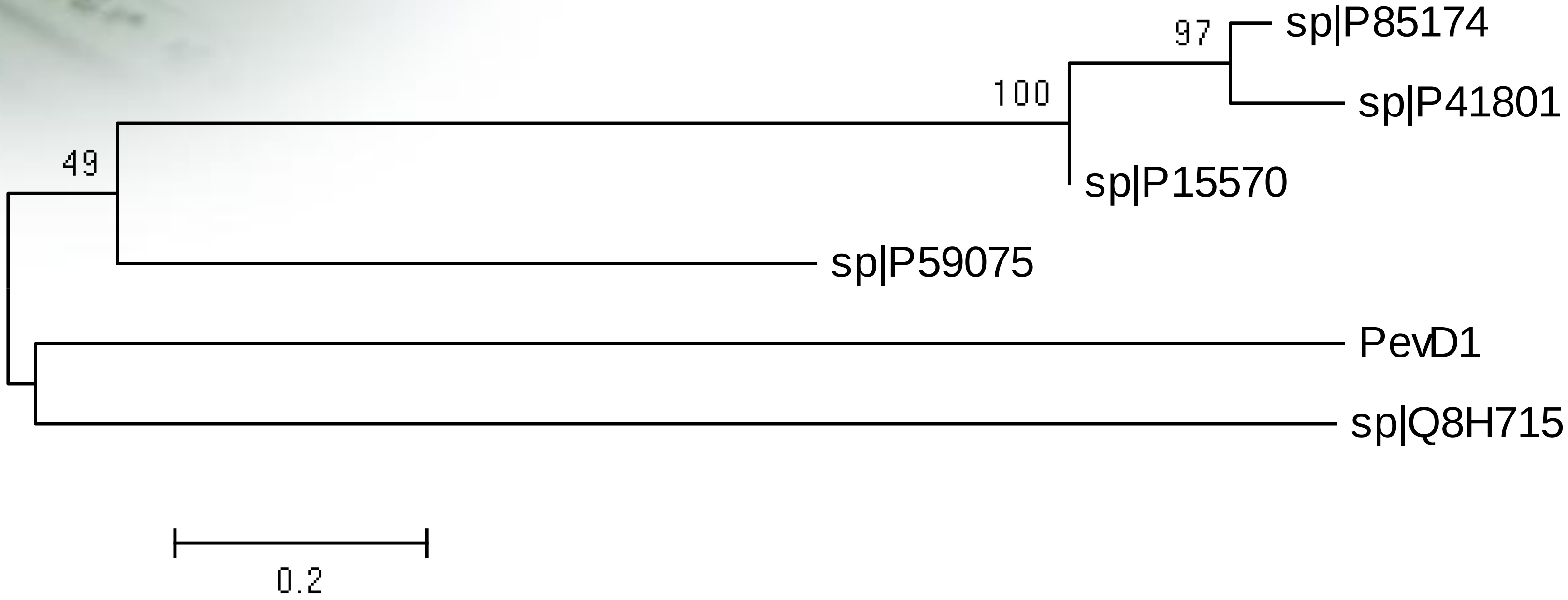
Sequence length	155
Secondary structure	Helix=9.7%, Strand=35.5%, Loop=54.8%

window size	70
Structure content cutoff	12%
Minimum consecutive exposed residues	10

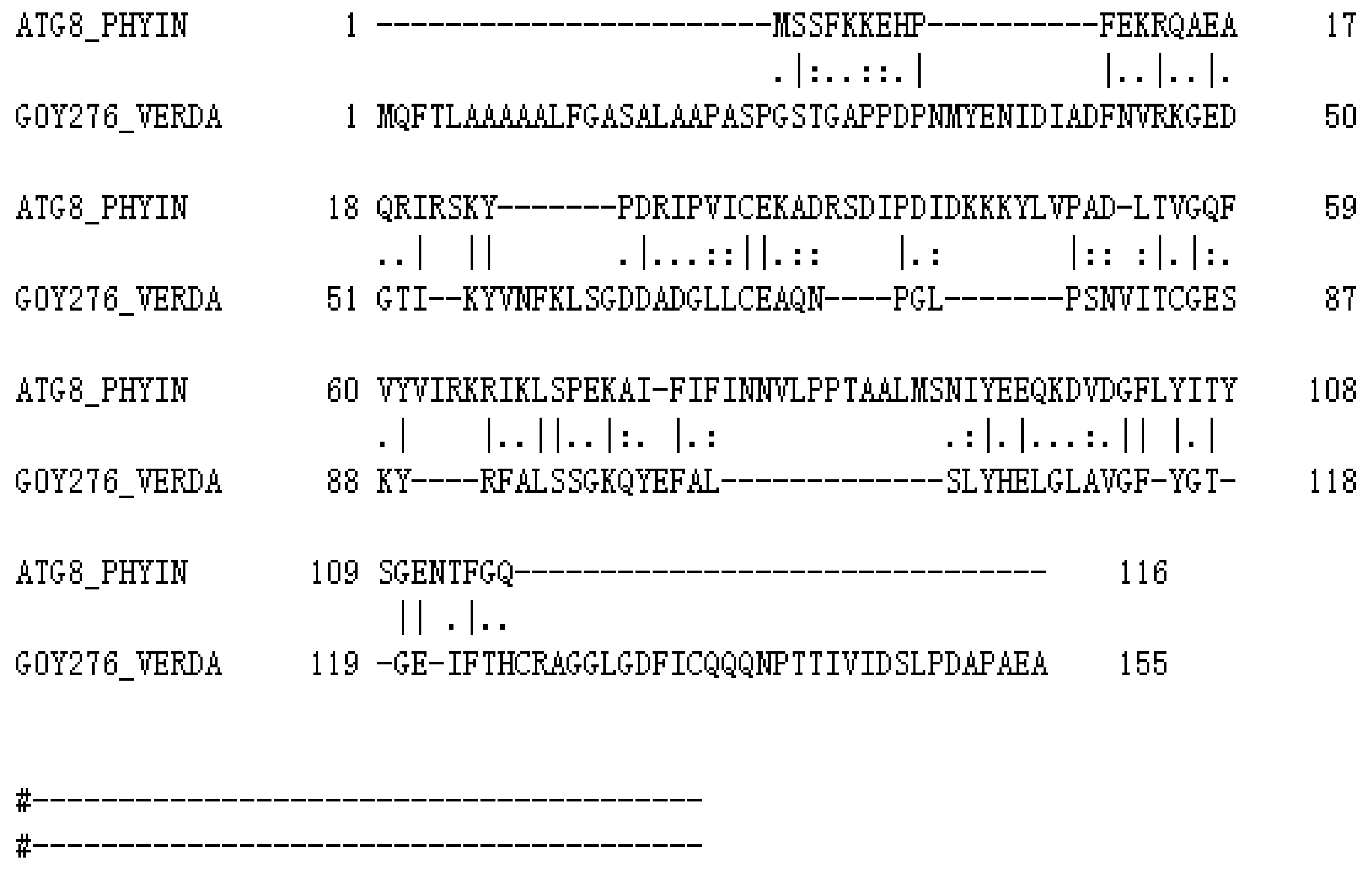
NORS	N=NORS region
Secondary structure	H=helix, E=strand, ' '=loop
Coiled-coil region	c=coils
Transmembrane helix	m=transmembrane helix
Solvent accessibility	e=exposed, ' '=buried

PevD1结构预测

构建系统发育树得到同源蛋白Q8H715



PevD1结构预测



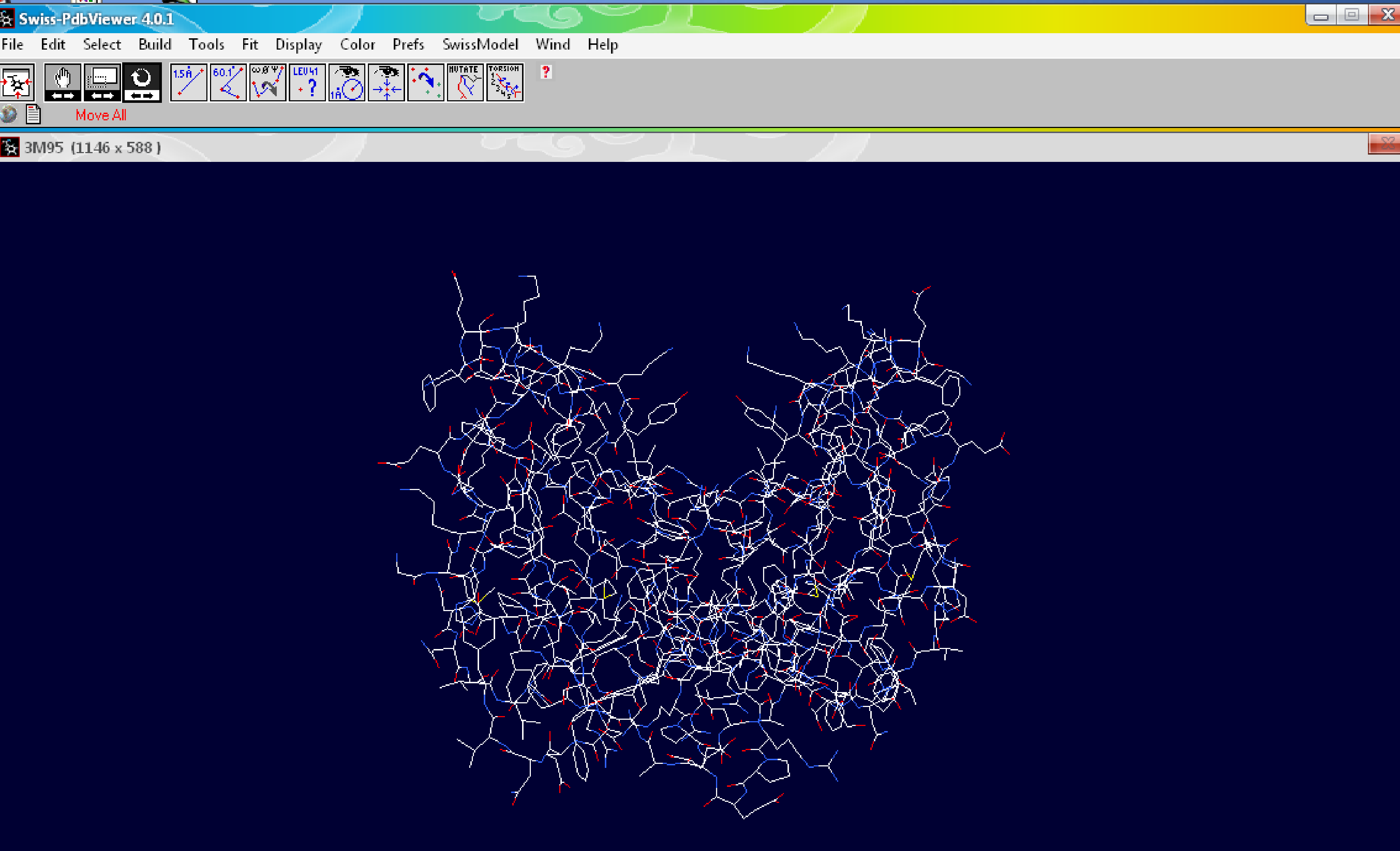
```
ATG8_PHYIN         1 -----------------------MSSFKKEHP----------FEKRQAEA     17
                                            .|:..::.|          |..|..|.
GOY276_VERDA       1 MQFTLAAAAALFGASALAAPASPGSTGAPPDPNMYENIDIADFNVRKGED     50

ATG8_PHYIN        18 QRIRSKY-------PDRIPVICEKADRSDIPDIDKKKYLVPAD-LTVGQF     59
                     ..|  ||       .|...::||.::    |.:        |:: :|.|:.
GOY276_VERDA      51 GTI--KYVNFKLSGDDADGLLCEAQN----PGL-------PSNVITCGES     87

ATG8_PHYIN        60 VYVIRKRIKLSPEKAI-FIFINNVLPPTAALMSNIYEEQKDVDGFLYITY    108
                     .|    |..||..|:. |.:             .:|.|...:.|| |.|
GOY276_VERDA      88 KY----RFALSSGKQYEFAL-------------SLYHELGLAVGF-YGT-    118

ATG8_PHYIN       109 SGENTFGQ-------------------------------    116
                      || .|..
GOY276_VERDA     119 -GE-IFTHCRAGGLGDFICQQQNPTTIVIDSLPDAPAEA    155


#---------------------------------------
#---------------------------------------
```

PevD1结构预测

- Length: 189
- Identity: 30/189 (15.9%)
- Similarity: 46/189 (24.3%)
- Gaps: 107/189 (56.6%)
- Score: 76.5

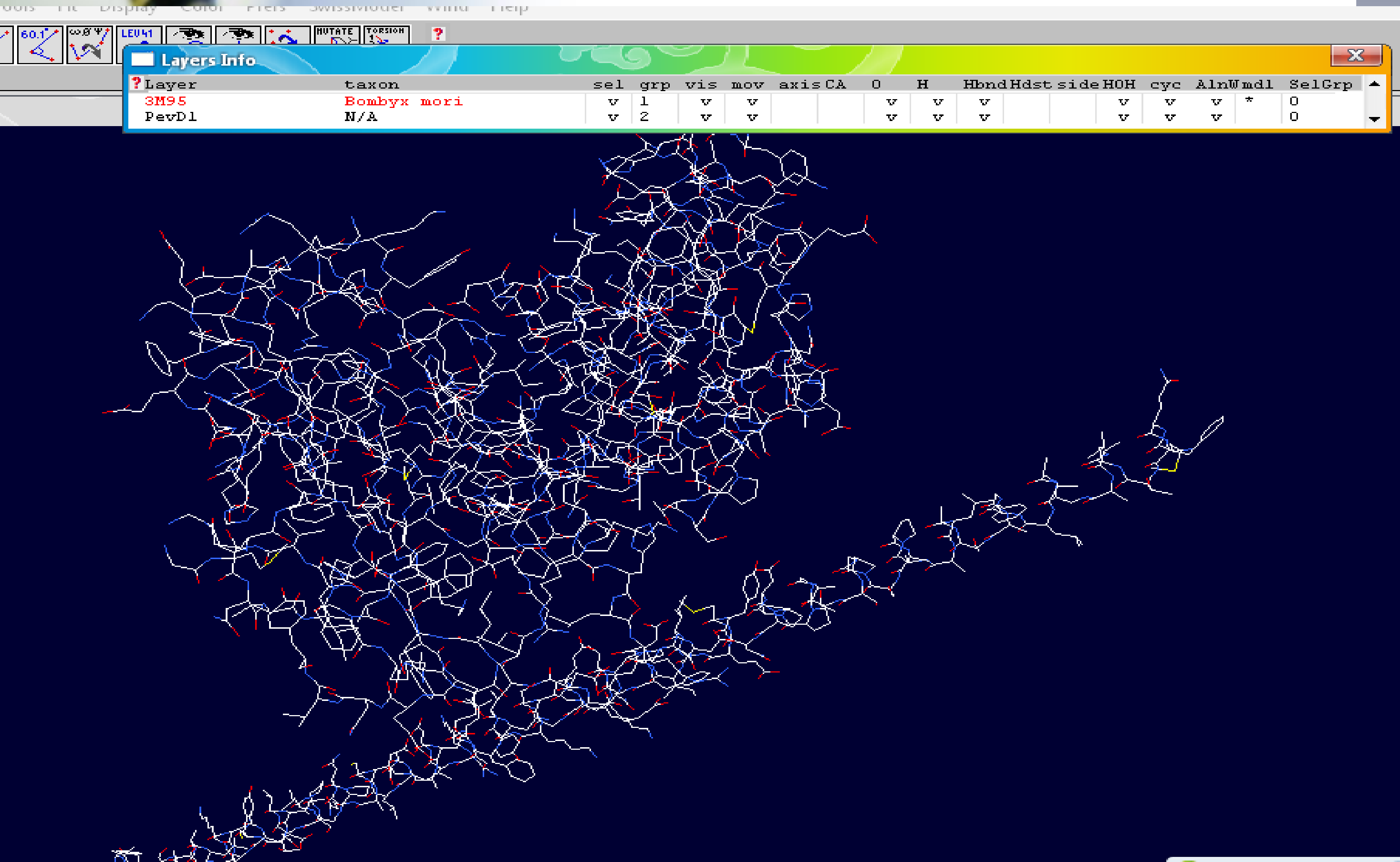
PevD1结构预测

将Q8H715这一蛋白氨基酸序列输入Swiss-Pdb Viewer



PevD1结构预测

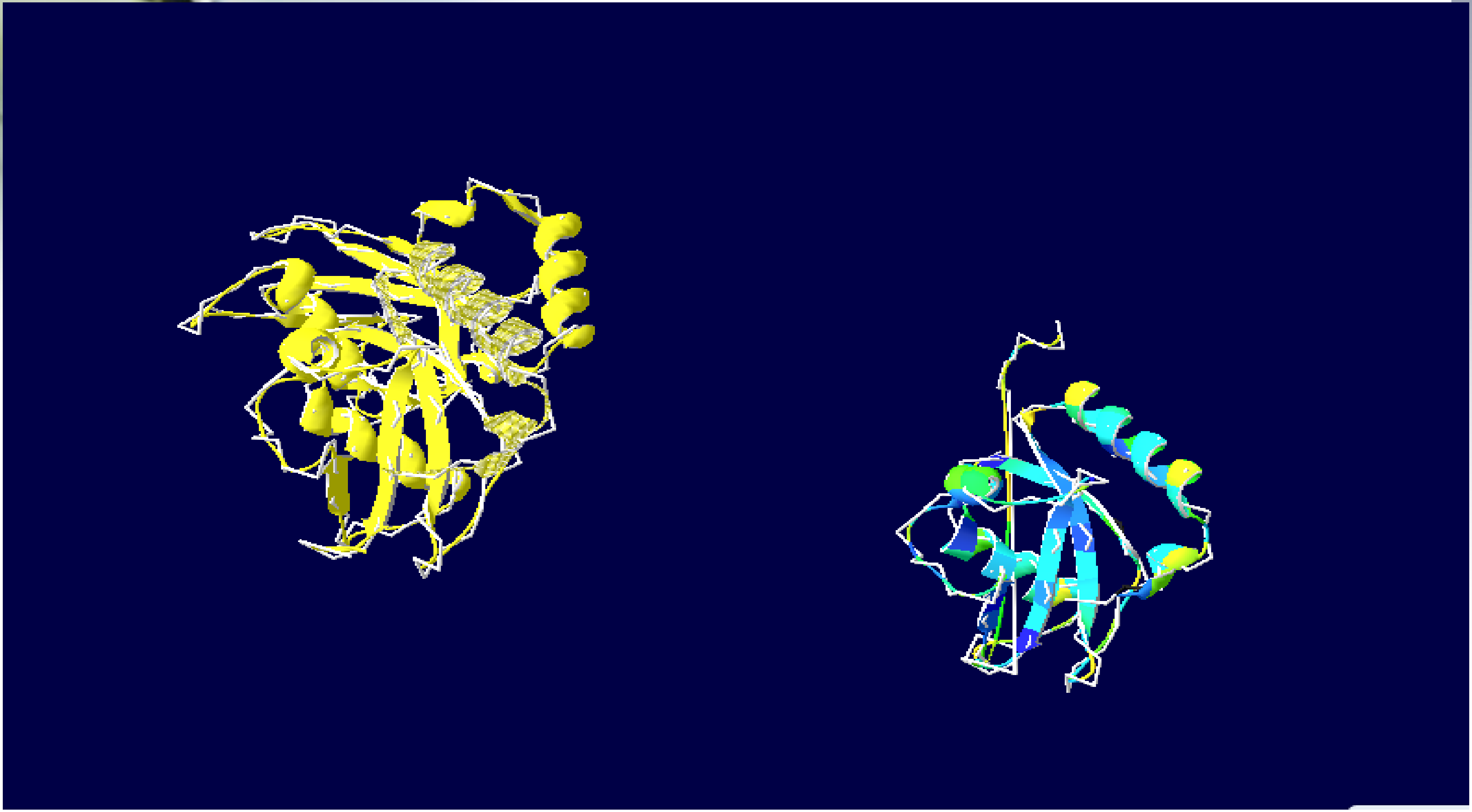
再将PevD1氨基酸序列输入Swiss-Pdb Viewer



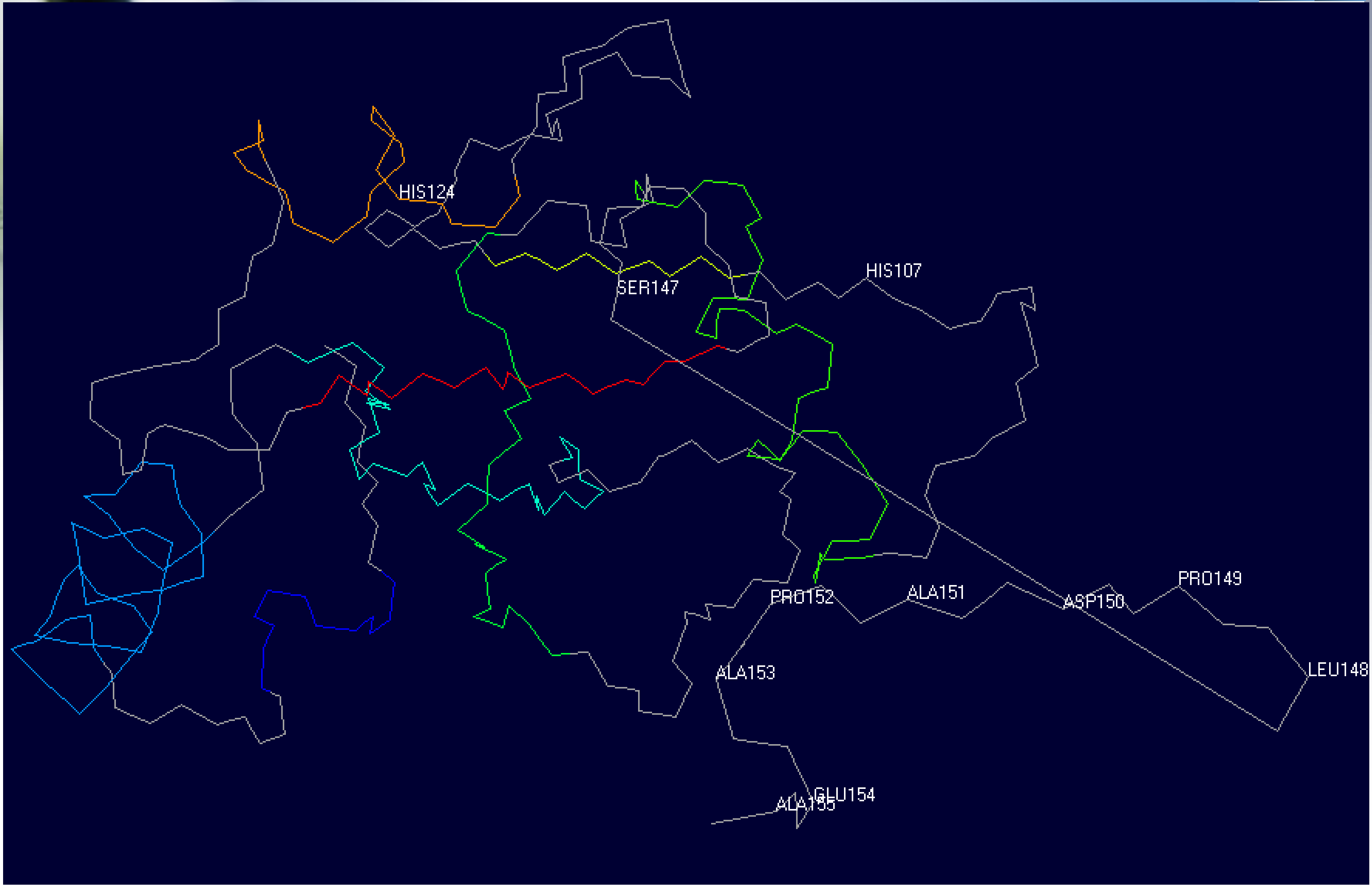
Layer	taxon	sel	grp	vis	mov	axis	CA	O	H	Hbnd	Hdst	side	HOH	cyc	AlnW	mdl	SelGrp
3M95	Bombyx mori	v	1	v	v			v	v	v			v	v	v	*	0
PevD1	N/A	v	2	v	v			v	v	v			v	v	v		0

PevD1结构预测

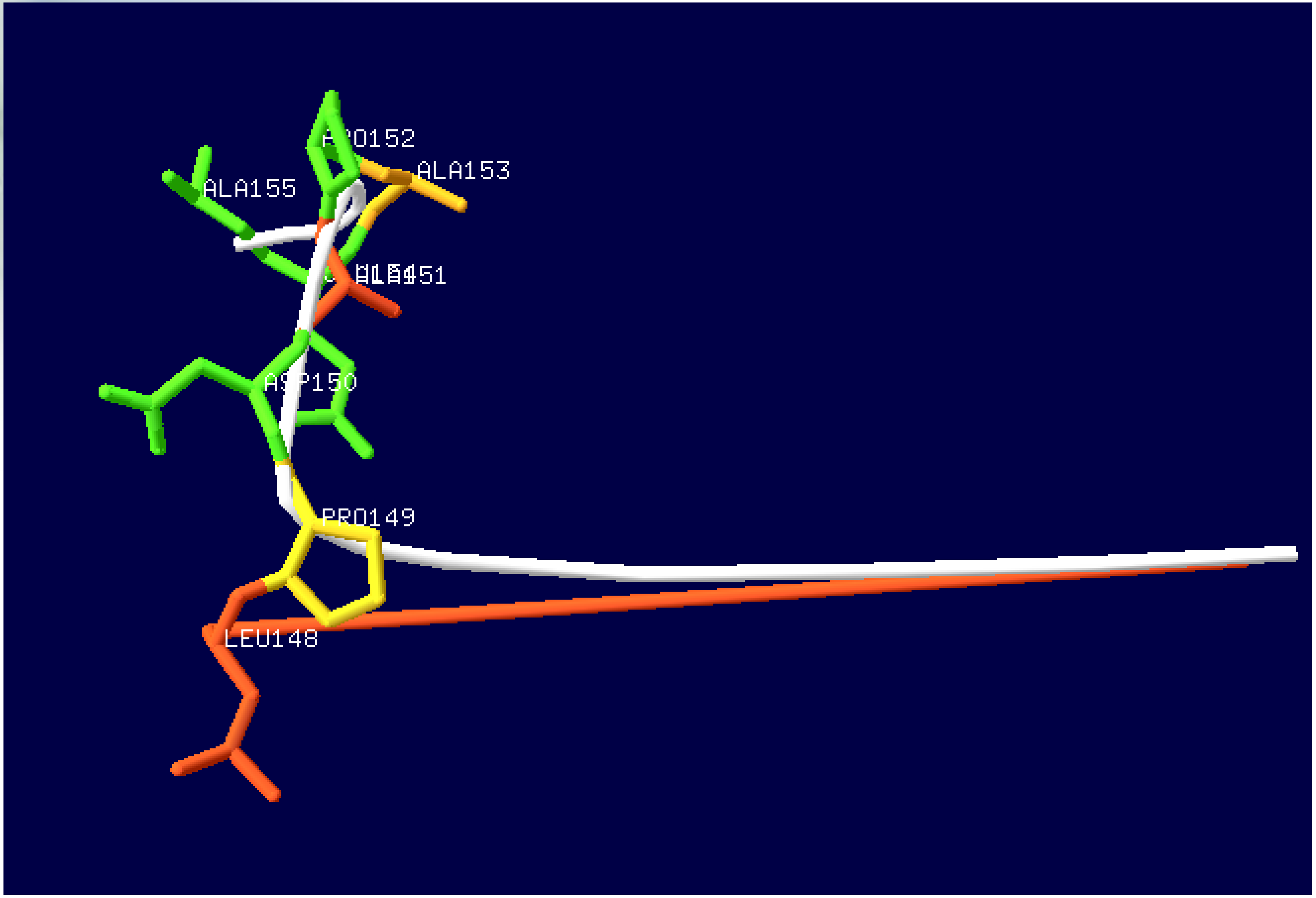
右图为Q8H715模型，左图为PevD1模型



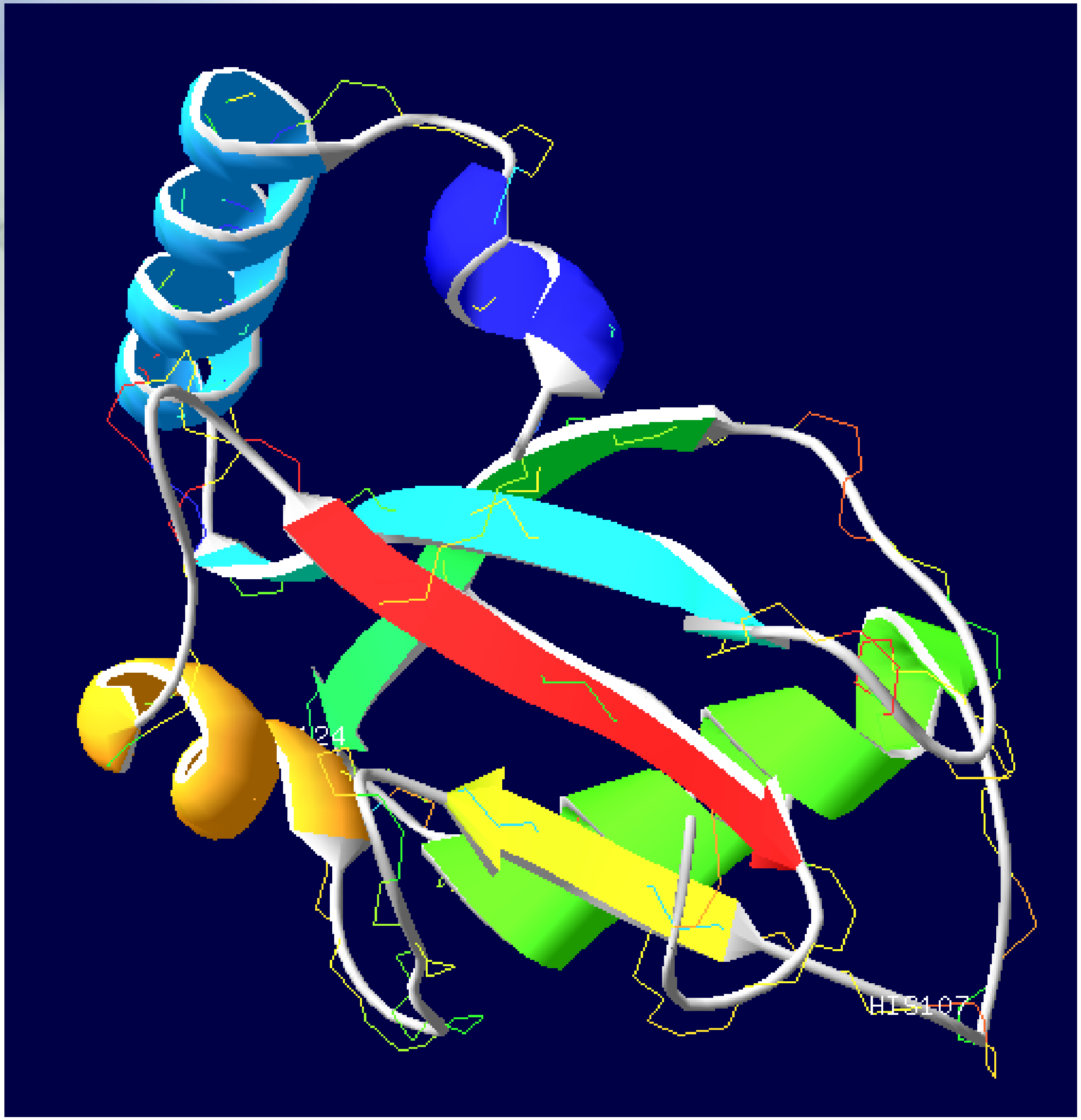
PevD1结构预测


HIS124
HIS107
SER147
PRO152
ALA151
ASP150
PRO149
LEU148
ALA153
ALA155
GLU154

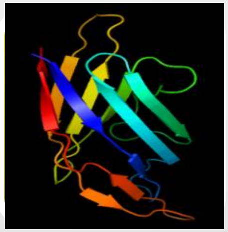
PevD1结构预测



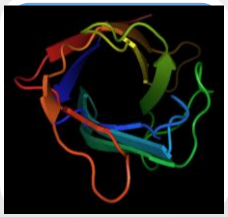
PevD1结构预测



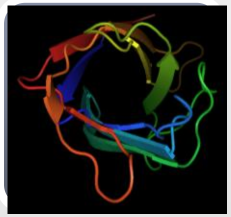
PevD1结构分析



PevD1三维结构类似于液氮勺147位氨基酸与148位氨基酸的连接很长



我们预测147-155这几个独立出来的几个氨基酸对诱导植物产生免疫抗性有关



147-155带有两个酸性氨基酸，猜测其对于植物去极化等信号传导有关

问题所在

? ? ?

为何147与148位氨基酸之间的连接如此之长?

到底是何处与受体结合?

三维结构预测结果是否可信?

Thank You !