



Prediction of nectary developmental genes (proteins) in two types of Male Sterile Lines of Broccoli

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2013.12.2



Content

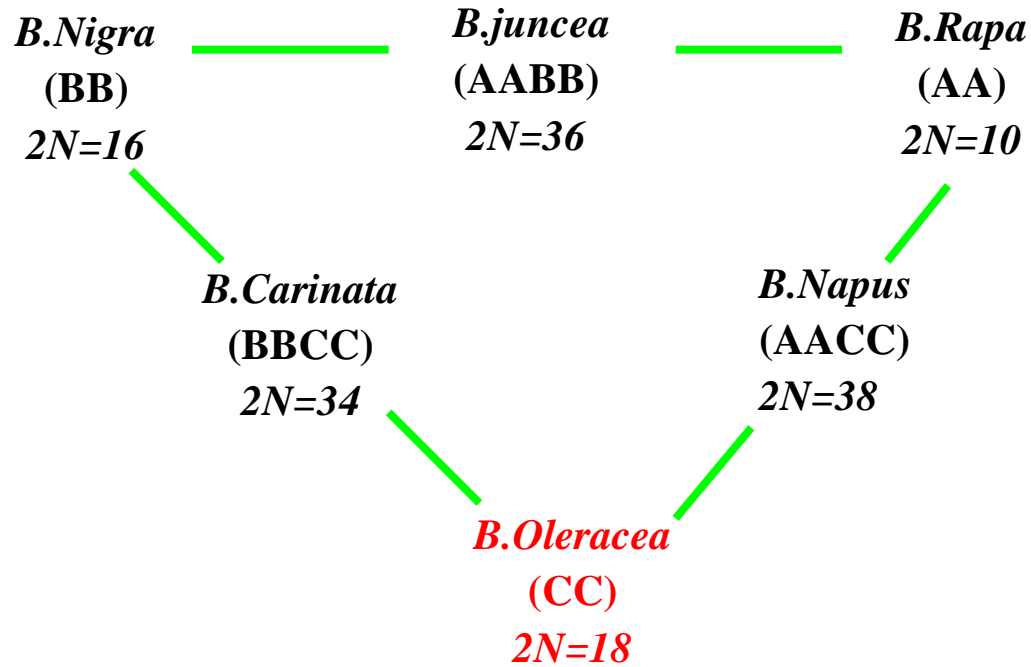


- **Background**
- **Purpose and Significance**
- **Materials and Methods**
- **Prediction and Analysis**
- **Conclusion and Programme**

Background






Morphological diversity among varieties of *Brassica*

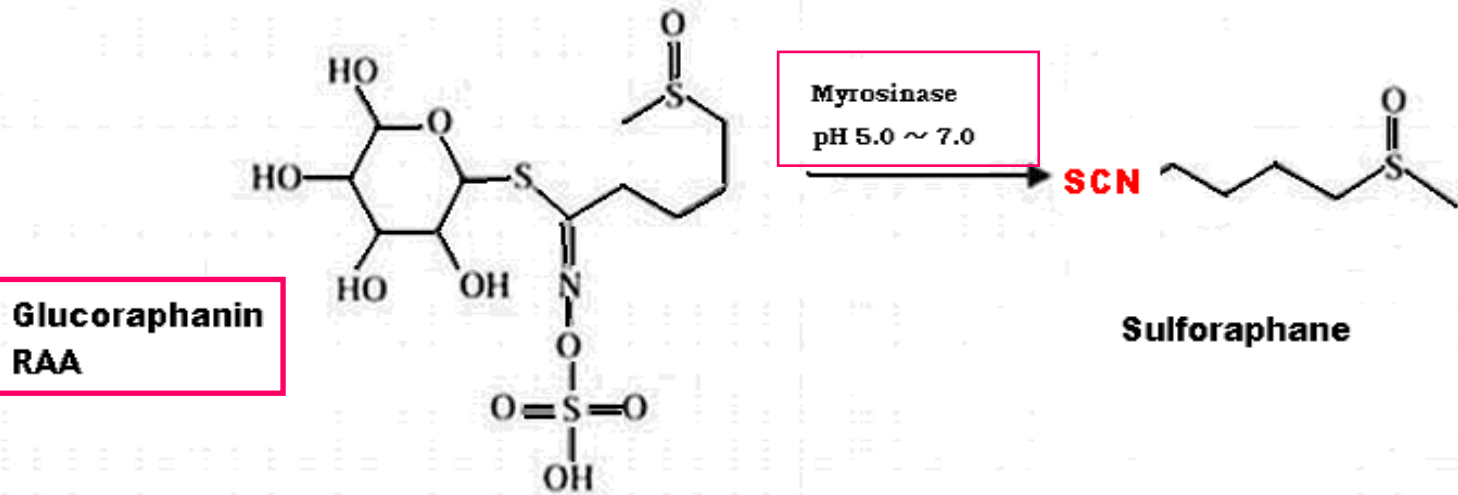


Background



Position of Broccoli in vegetable

-  An important vegetable crop in Crucifers
-  Anti-cancer effect sulforaphane, SF, $C_6H_{11}NOS_2$
(Isothiocyanate Salts mainly exists in Broccoli and other Cruciferous vegetables)
-  Secondary metabolic reactions



Background



Produce hybrid



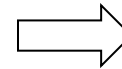
B. Oleracea L. var. *italica* Planch



Inbred



DGMS



CMS



Application of Broccoli Male Sterile Lines

Background



Types of Male Sterile Lines in Broccoli



Dominant Genic Male Sterility (DGMS) lines



***Ogura* Cytoplasmic Male Sterility (*Ogura* CMS) lines**

Background



Visiting bees between two lines in the field



DGMS 8554



Ogura CMS8554

Seed yield was significantly different between DGMS and Ogura CMS

Background



Why Seed Yield has Significant Difference between DGMS and Ogura CMS?

- Buds Death Degree
- Flower Organs Size
- Numbers of Visiting Bees
(Pollination -Nectar-Nectary)

Background



Buds Death Degree



93219



DGMS93219



OguCMS93219

Background



Flower Organs Size



93219

DGMS93219

0guCMS93219

Background



Nectary

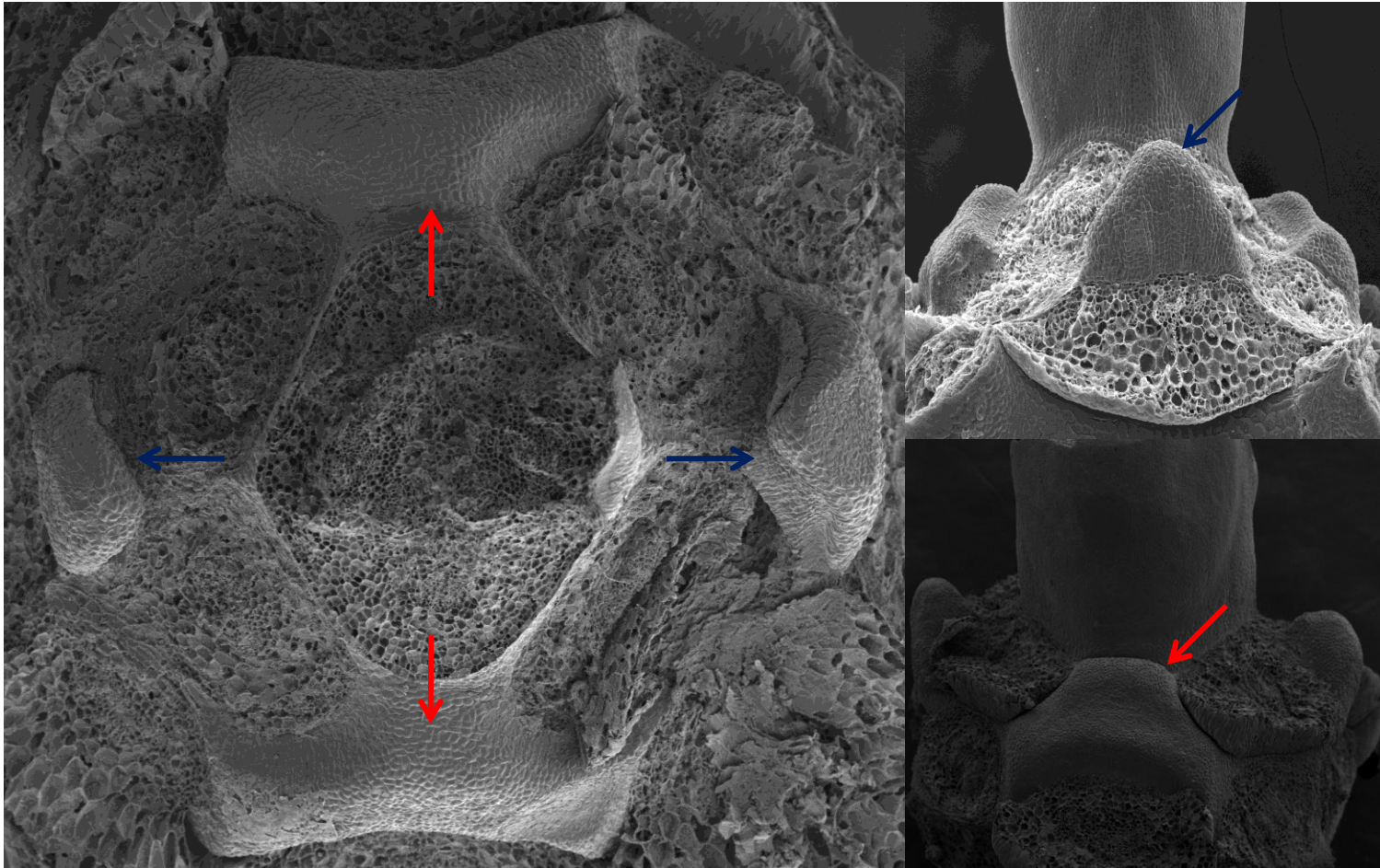
What Is Nectary ?

- Nectary is the unique gland to **secret nectar** in Crucifers, its development level determines the plant's ability to secrete nectar, affects the insect **pollination** and **seed production**.

Background



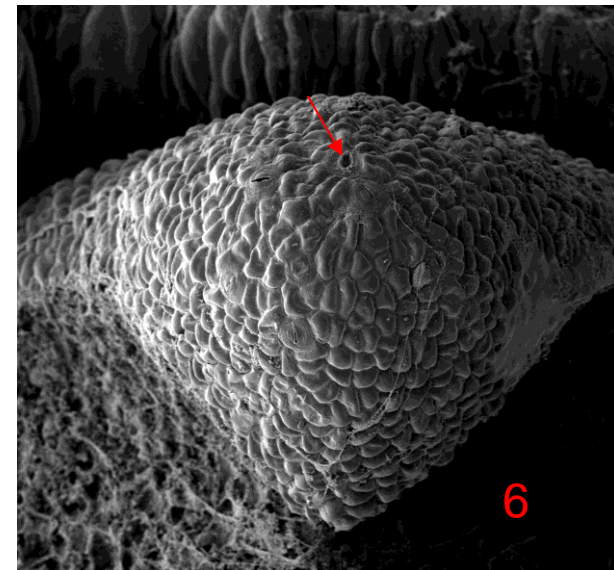
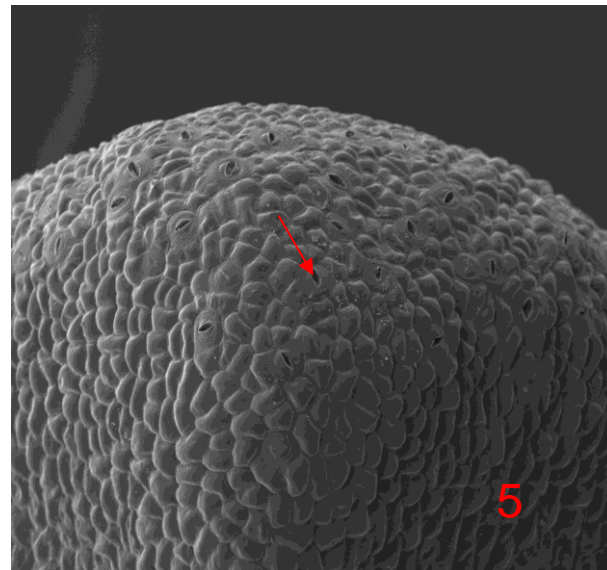
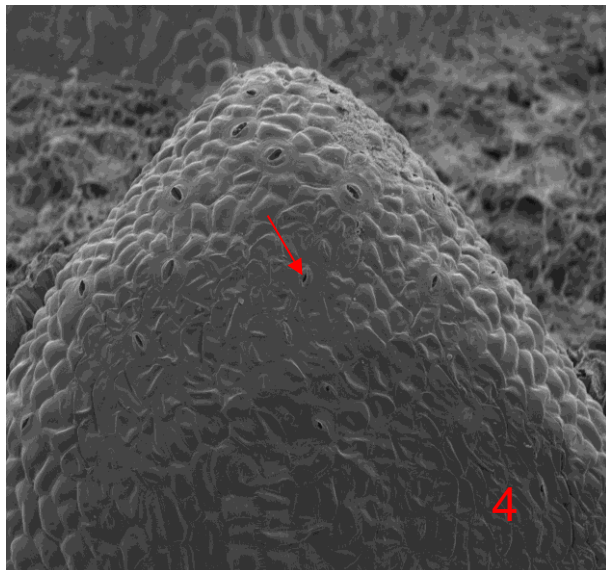
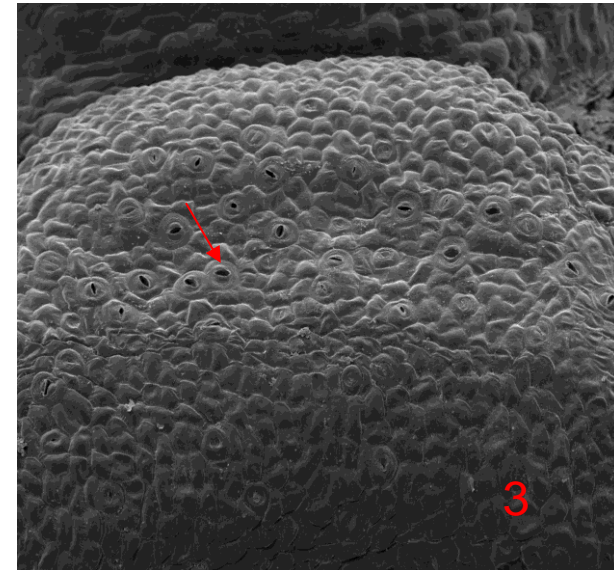
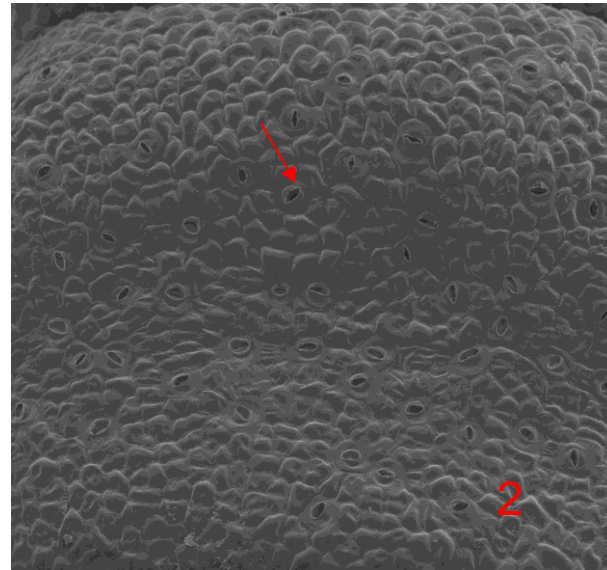
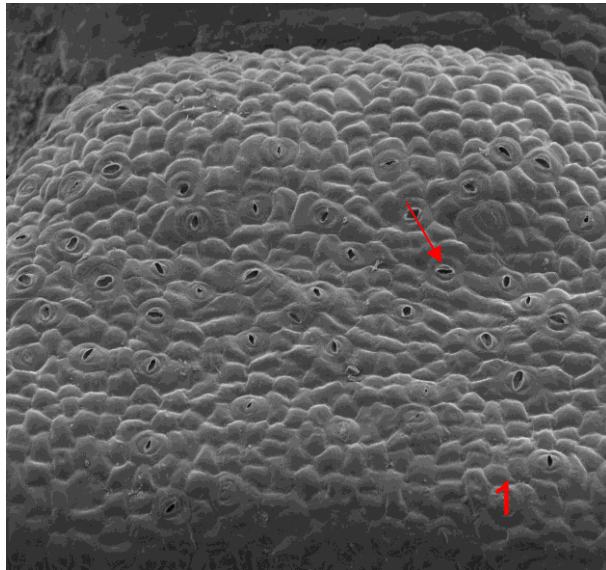
Morphology of Nectaries in Broccoli



**Median
Nectary**

**Lateral
Nectary**

Background



Questions



Why?

What?

How?

Final Objective

Seed Yield ↑

Purpose and Significance



- **Purpose** To Study the molecular regulation of nectary development.
- **Significance** Supply theories for improving the function of nectary in Broccoli.

Materials and Methods



- **Materials:** Inbred, DGMS and Ogura CMS of 8554 and 93219.
- **Methods:** Transcriptome Sequencing, Bioinformatics prediction of nectary development references genes (proteins) and experimental analysis.

Prediction and Analysis



1. Uniport search and database query (8/95)

Entry	Organism	Protein names	Gene names	Length
Q9SBK6	Brassica rapa subsp. pekinensis	Jasmonate O-methyltransferase	JMT NTR1	392AA
Q8L925	Arabidopsis thaliana	Protein CRABS CLAW	CRC At1g69180 F23O10.23 F4N2.14	181AA
Q9ZVC2	Arabidopsis thaliana	Regulatory protein NPR5	NPR5 BOP2 At2g41370 F13H10.8	491AA
Q9M1I7	Arabidopsis thaliana	Regulatory protein NPR6	NPR6 BOP1 At3g57130 F24I3.210	467AA
Q9FMY1	Arabidopsis thaliana	Cytochrome P450 86B1	CYP86B1 At5g23190 MKD15.5	559AA
Q94EG3	Nicotiana langsdorffii x Nicotiana sanderae	Nectarin-1	NEC1	229AA
Q84UV8	Nicotiana langsdorffii x Nicotiana sanderae	Bifunctional monodehydroascorbate reductase a...	NEC3	274AA
Q9FPN0	Petunia hybrida	Bidirectional sugar transporter NEC1	NEC1	265AA

Prediction and Analysis



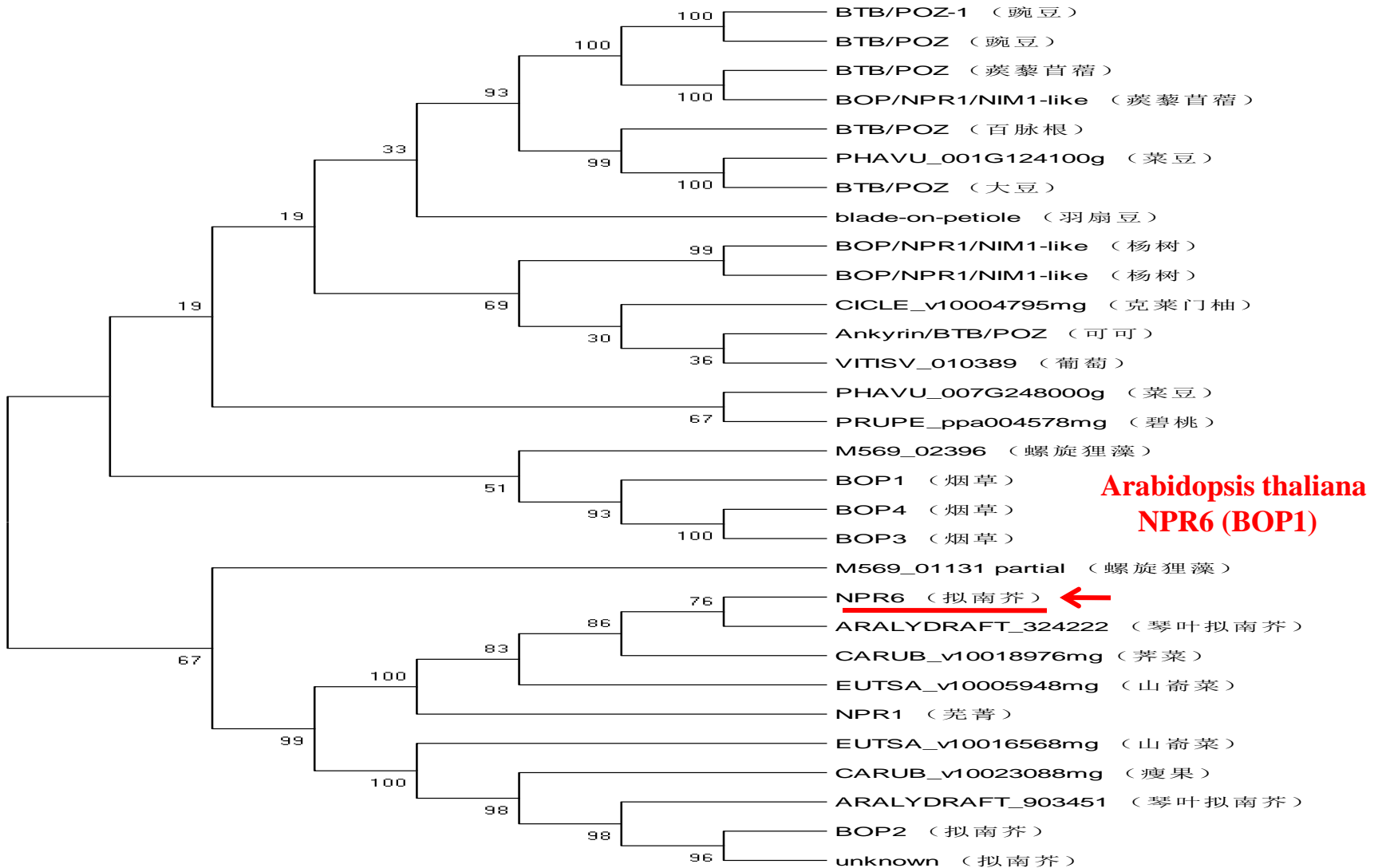
2. Sequence analysis

Identical positions	0
Identity	0%
Similar positions	1

Prediction and Analysis



3. Molecular phylogeny analysis



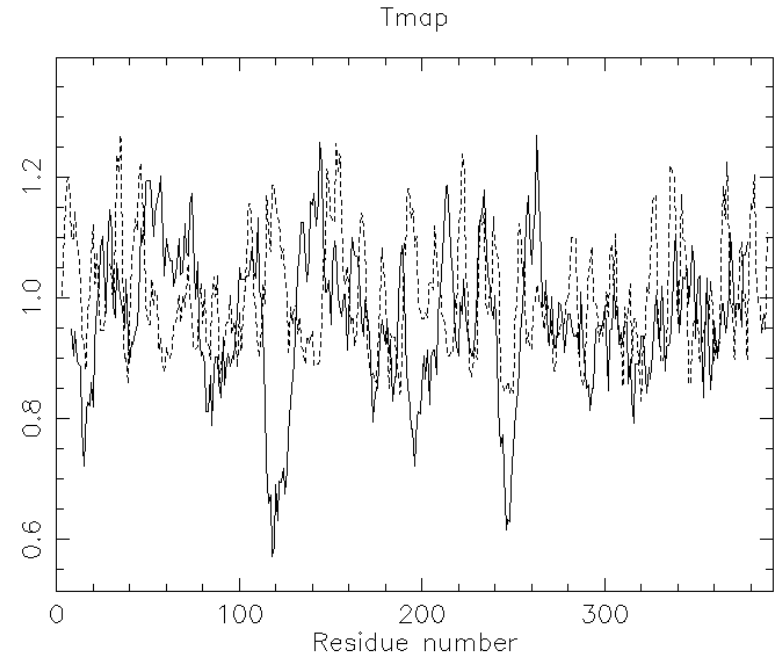
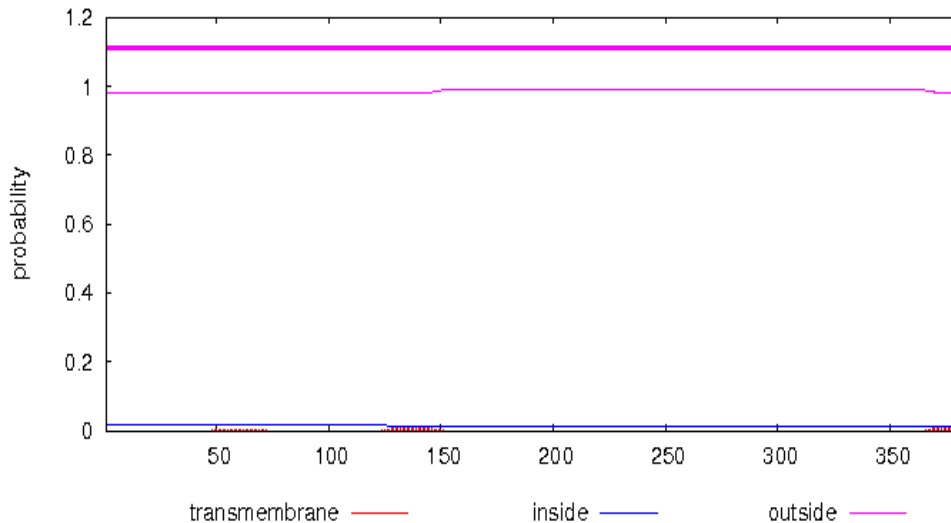
Prediction and Analysis



4. Transmembrane structure prediction (tmap THMHMM)

Jasmonate O-methyltransferase (*Brassica rapa* subsp. *Pekinensis*)

TMHMM posterior probabilities for sp|Q9SBK6|JMT_BRARP

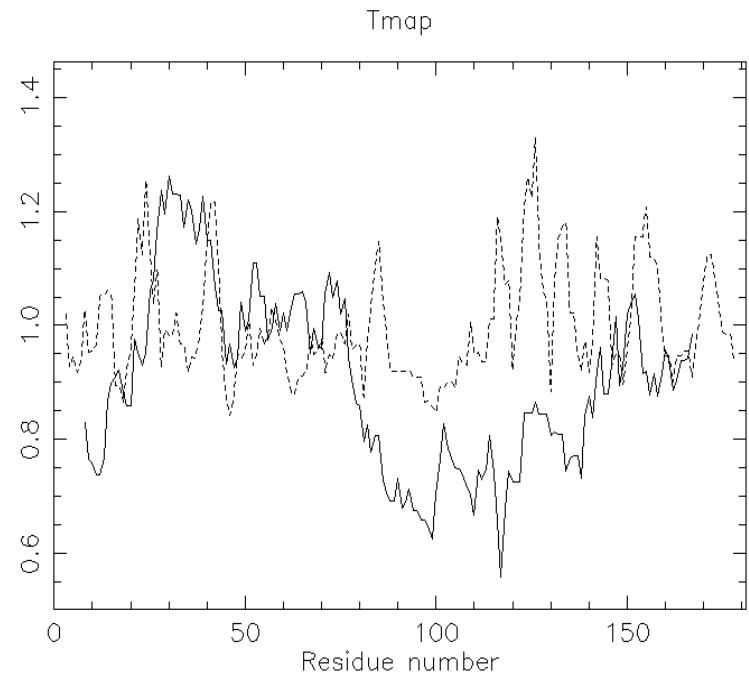
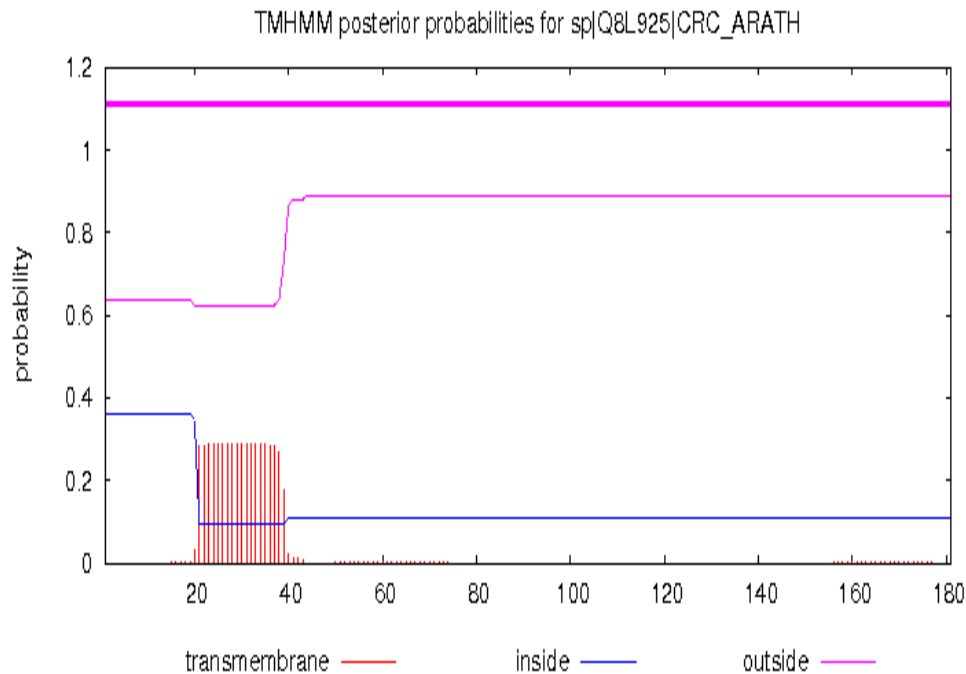


Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Protein CRABS CLAW (Arabidopsis thaliana **transcription factor**)



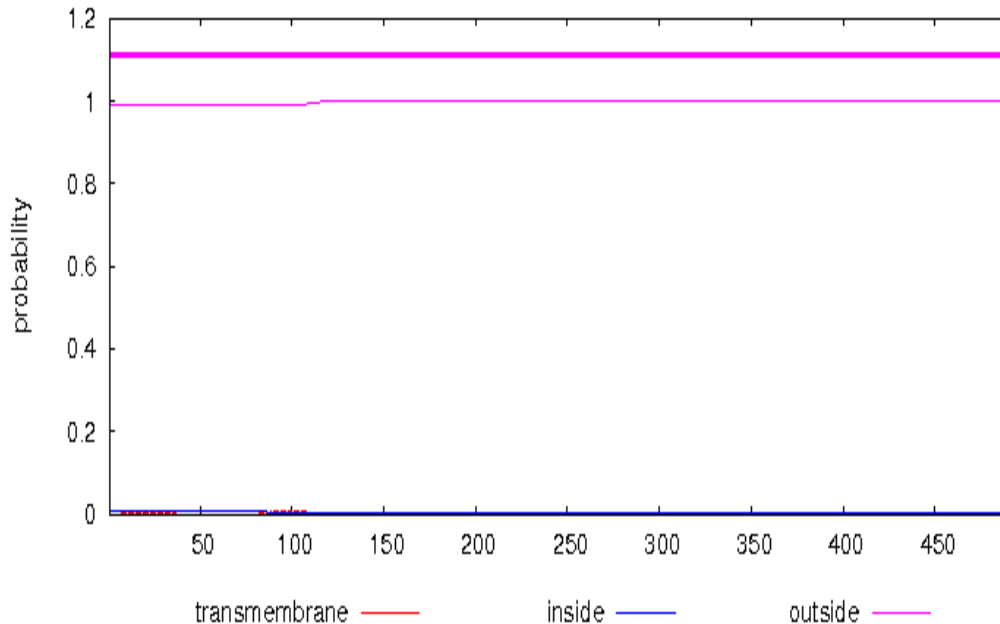
Prediction and Analysis



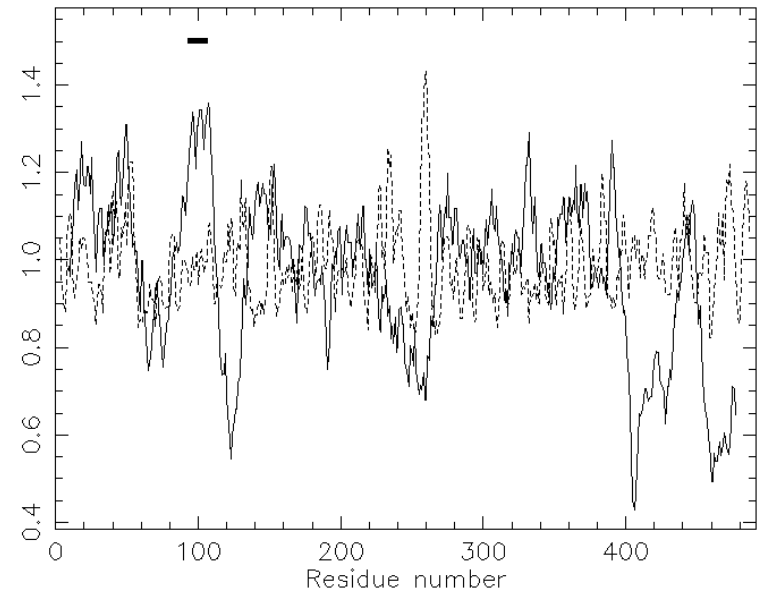
Transmembrane structure prediction (tmap THMHMM)

Regulatory protein NPR5 (*Arabidopsis thaliana*)

TMHMM posterior probabilities for sp|Q9ZVC2|NPR5_ARATH



Tmap

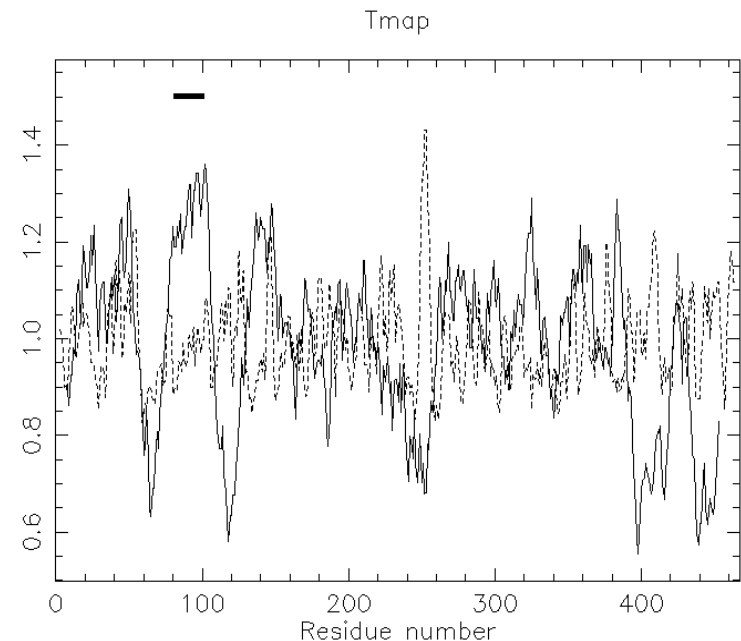
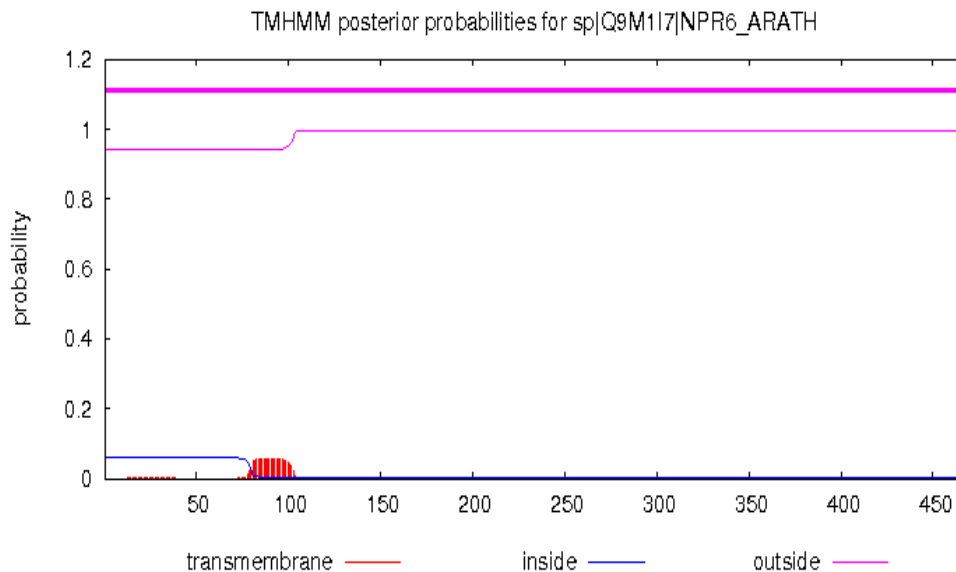


Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Regulatory protein NPR6 (*Arabidopsis thaliana*)



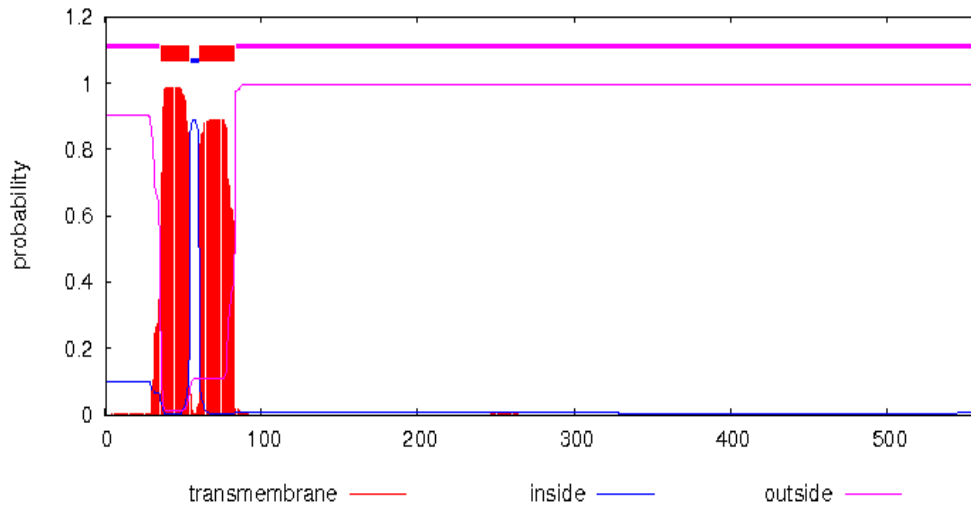
Prediction and Analysis



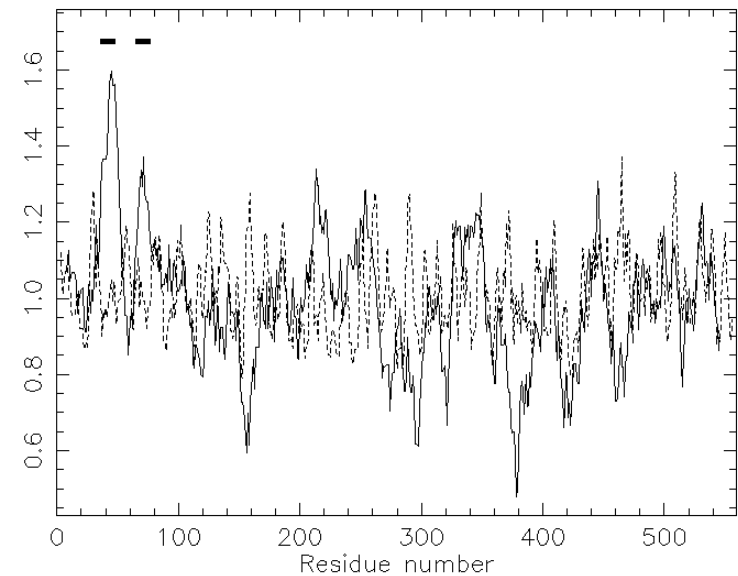
Transmembrane structure prediction (tmap THMHMM)

Cytochrome P450 86B1 (*Arabidopsis thaliana*)

TMHMM posterior probabilities for sp|Q9FMY1|C86B1_ARATH



Tmap



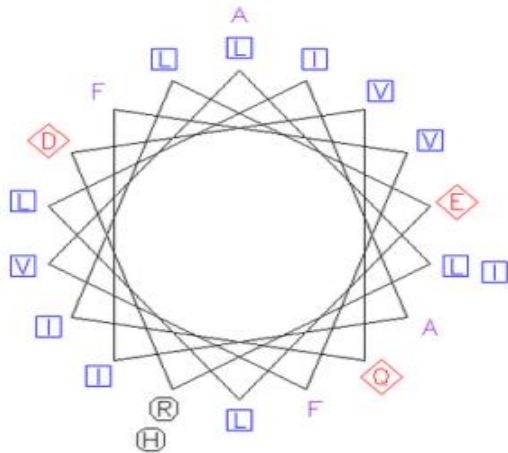
Prediction and Analysis



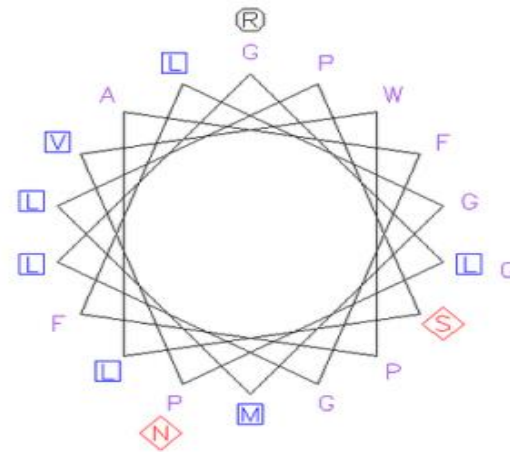
Cytochrome P450 86B1 (*Arabidopsis thaliana*)

Start	End	TransMem	Sequence
32	52	1	LLRDVQILELLIAIFVFVAIH
61	81	2	GLPVWPFLGMLPSLAFGLRGN

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Helical wheel of row::682729
Sat 30 Nov 2013 15:24:06

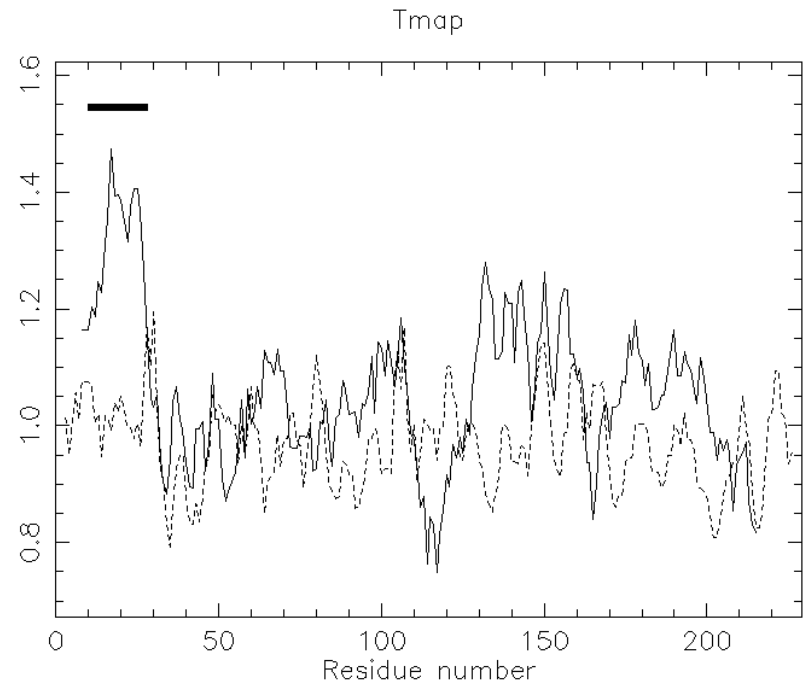
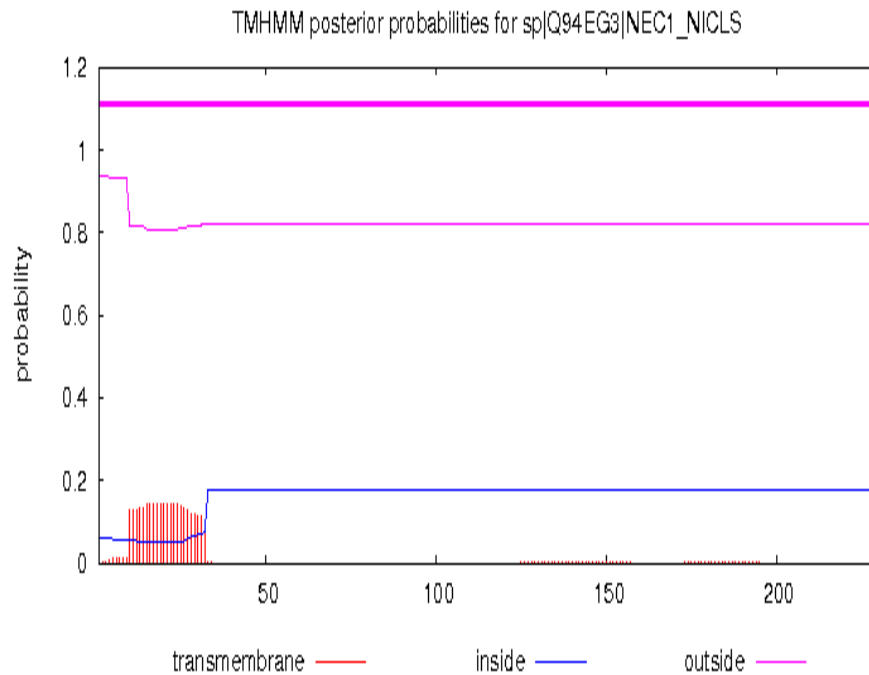


Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Nectarin-1 (*Nicotiana langsdorffii* x *Nicotiana sanderae*)



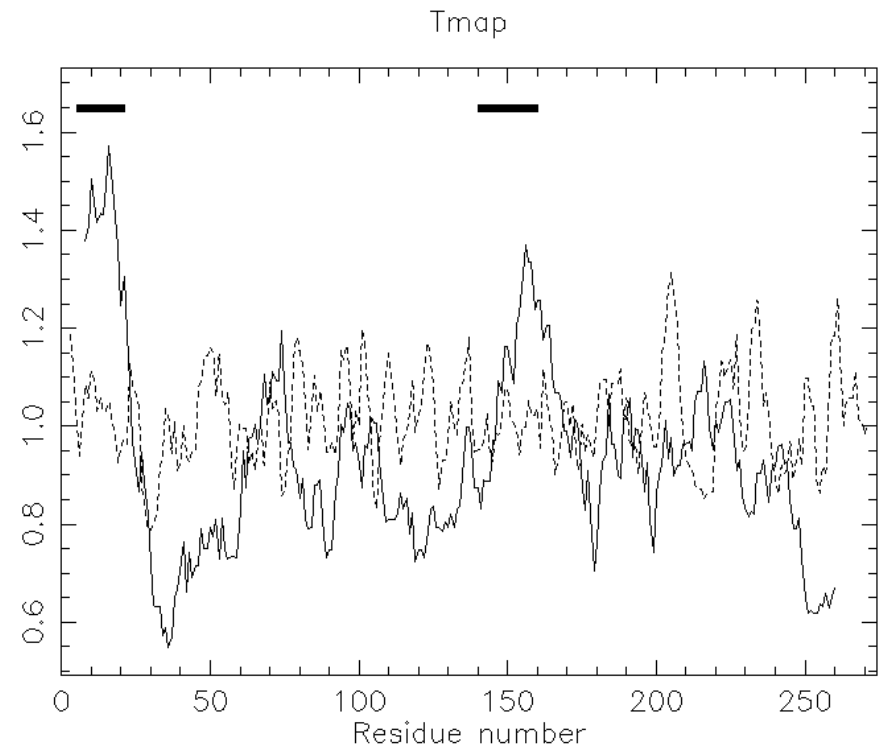
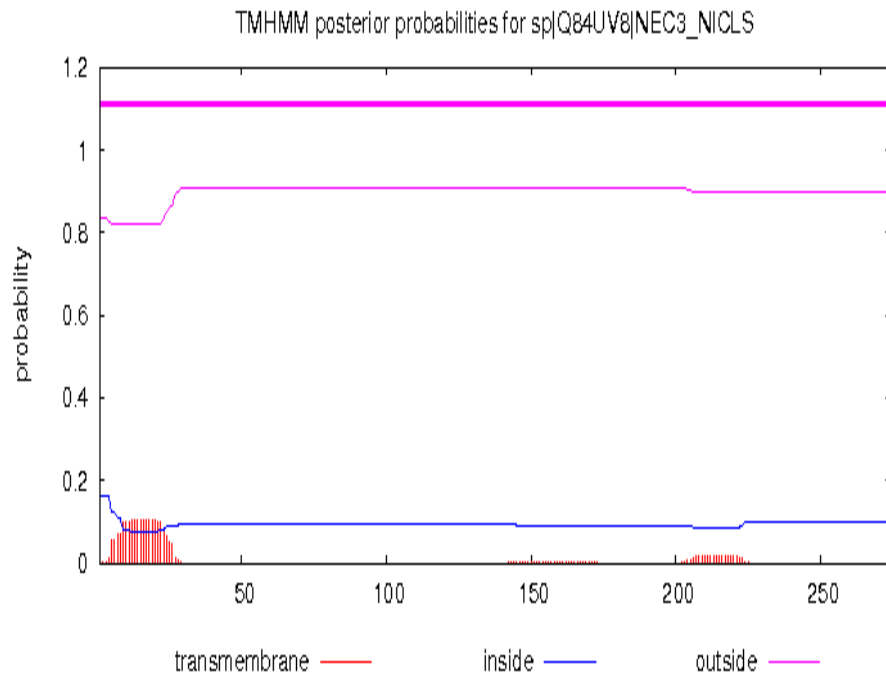
Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Bifunctional monodehydroascorbate reductase a...

(*Nicotiana langsdorffii* x *Nicotiana sanderae*)



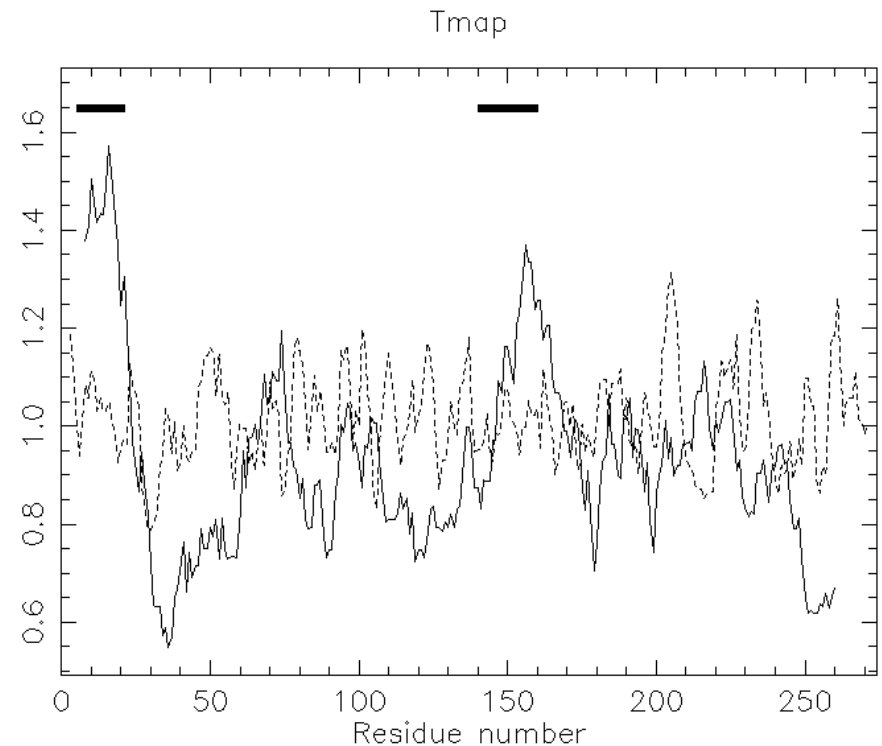
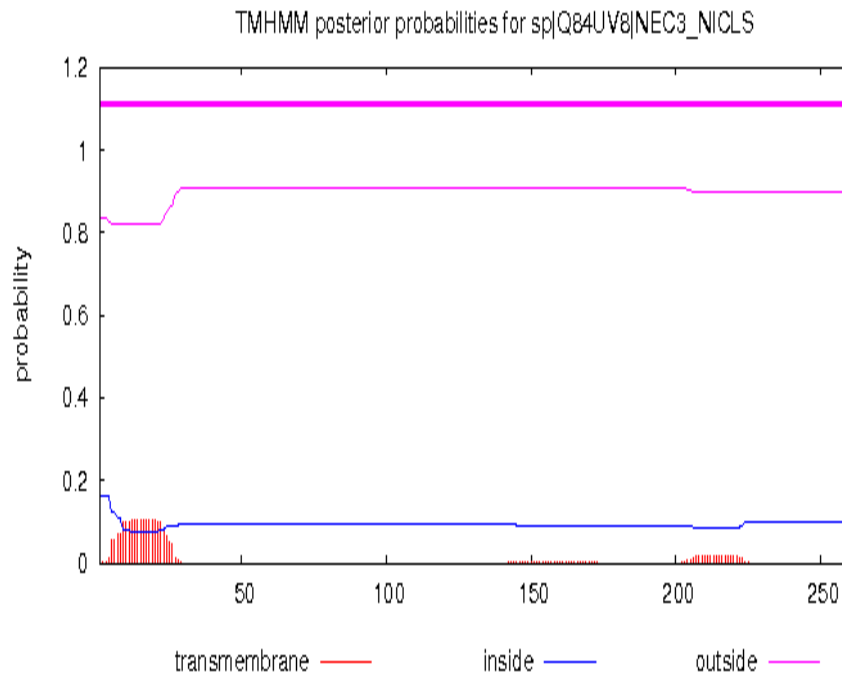
Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Bifunctional monodehydroascorbate reductase a...

(*Nicotiana langsdorffii* x *Nicotiana sanderae*)

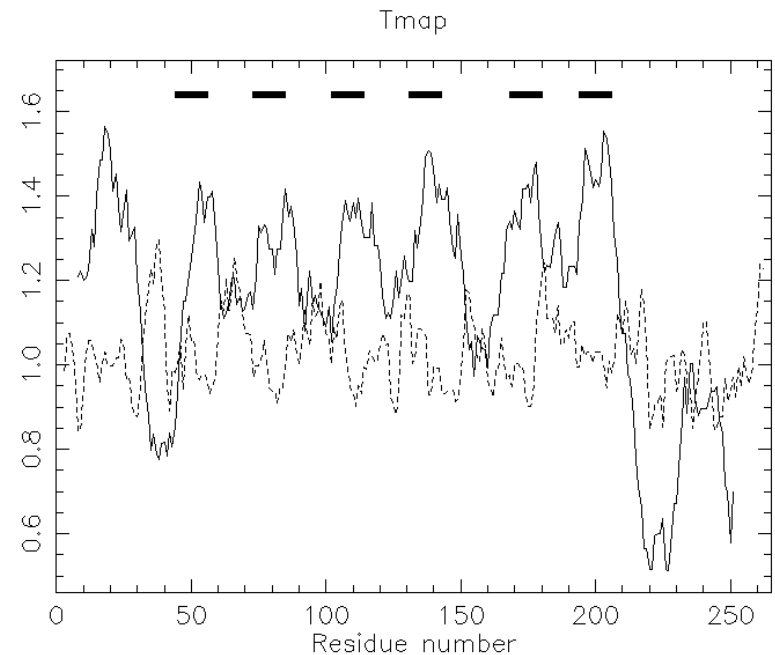
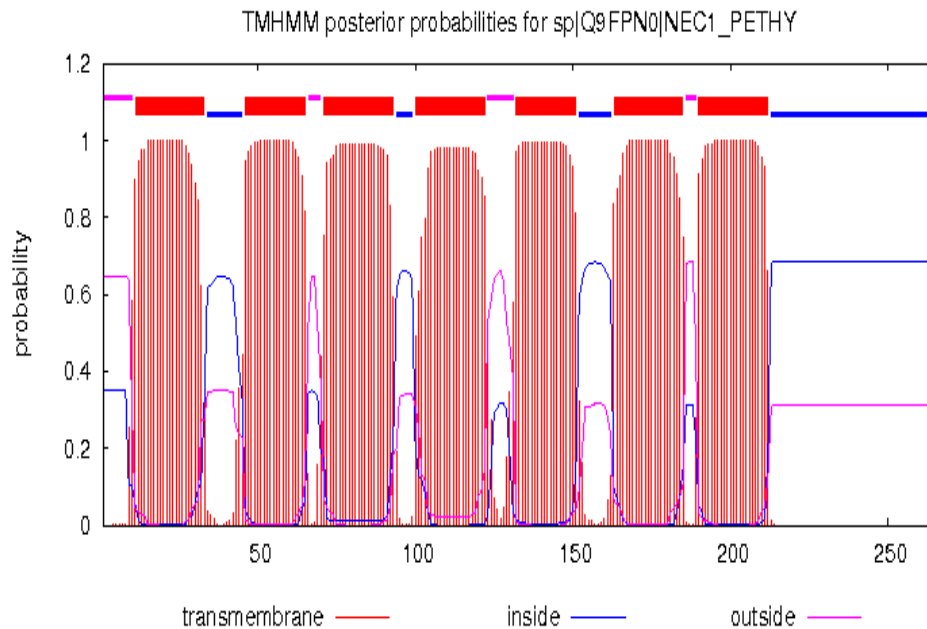


Prediction and Analysis



Transmembrane structure prediction (tmap THMHMM)

Bidirectional sugar transporter NEC1 (*Petunia hybrida*)



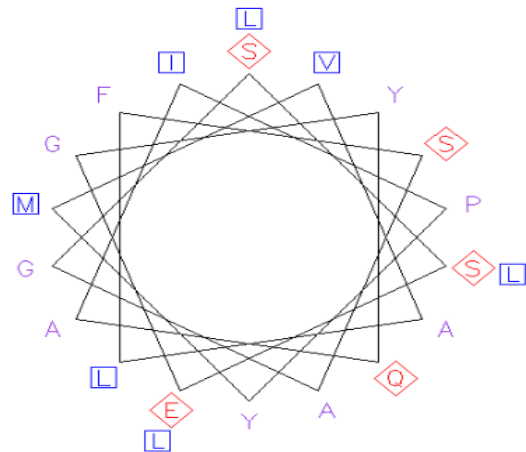
Prediction and Analysis



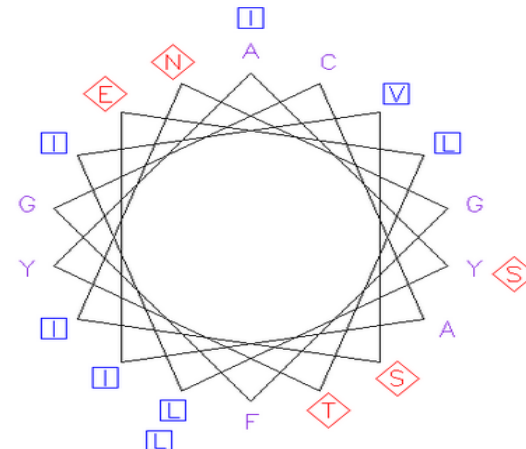
Bidirectional sugar transporter NEC1 (*Petunia hybrida*)

Start	End	TransMem	Sequence
40	60	1	SSEGYQAIPLYMVALFSAGLLL
69	89	2	AYLIVSINGFGCAIELTYISL
98	118	3	SKIFTGWLMLLELGALGMVMP
127	147	4	SHRVMIVGWICAAINVAVFAA
164	184	5	MPFTLSLFLTLCATMWFFYGF
190	210	6	YIAFPNILGFLFGIVQMLLYF

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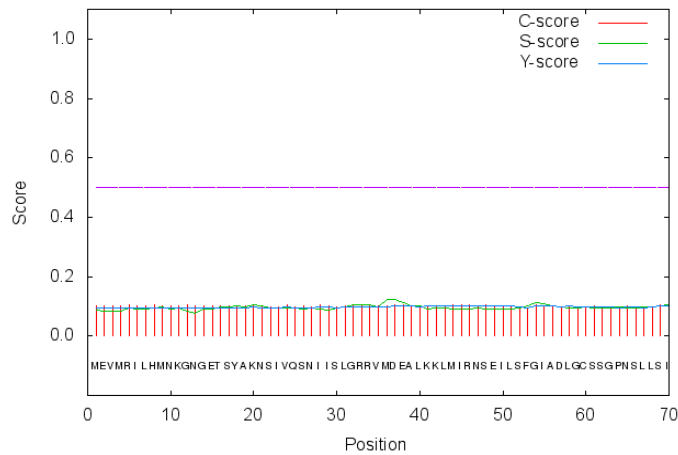


Prediction and Analysis

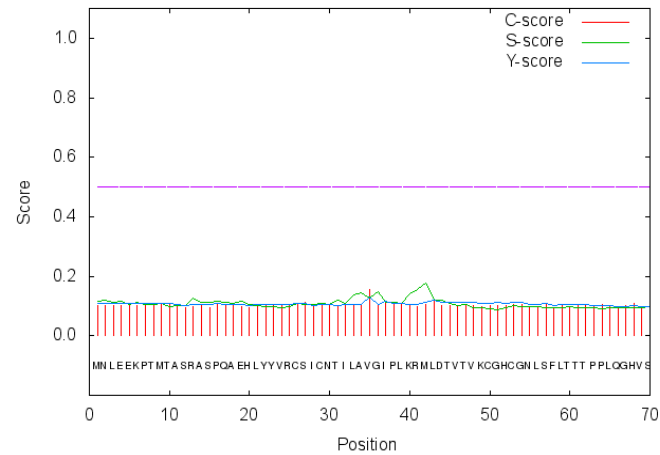


5. Signal peptide (SingleIP)

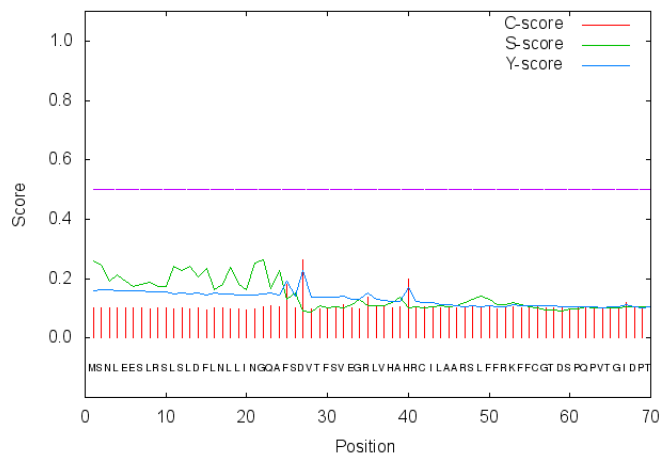
SignalP-4.1 prediction (euk networks): sp_Q9SBK6_JMT_BRARP



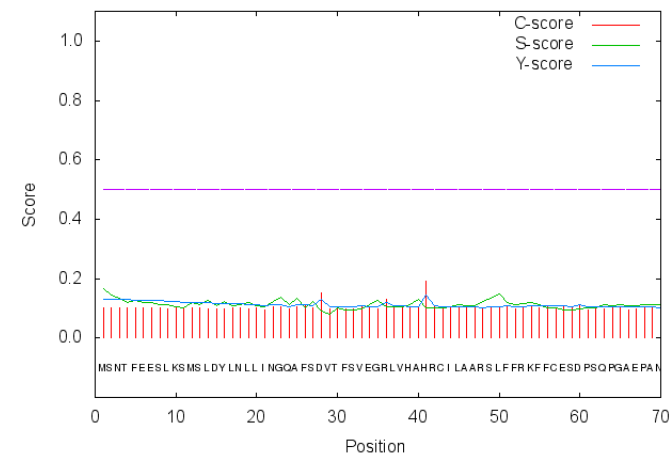
SignalP-4.1 prediction (euk networks): sp_Q8L925_CRC_ARATH



SignalP-4.1 prediction (euk networks): sp_Q9ZVC2_NPR5_ARATH



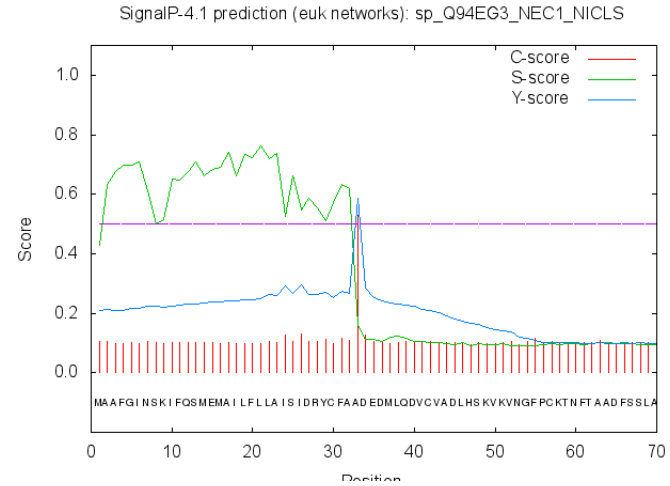
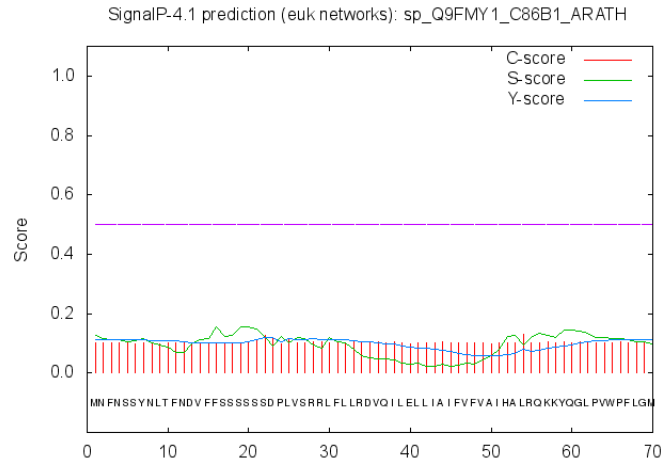
SignalP-4.1 prediction (euk networks): sp_Q9M117_NPR6_ARATH



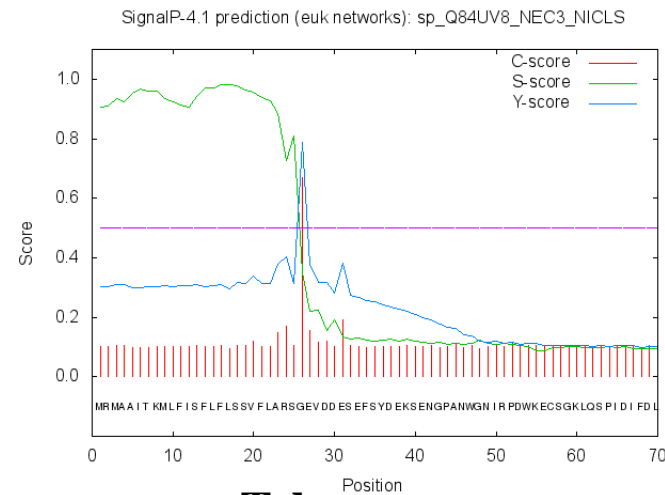
Prediction and Analysis



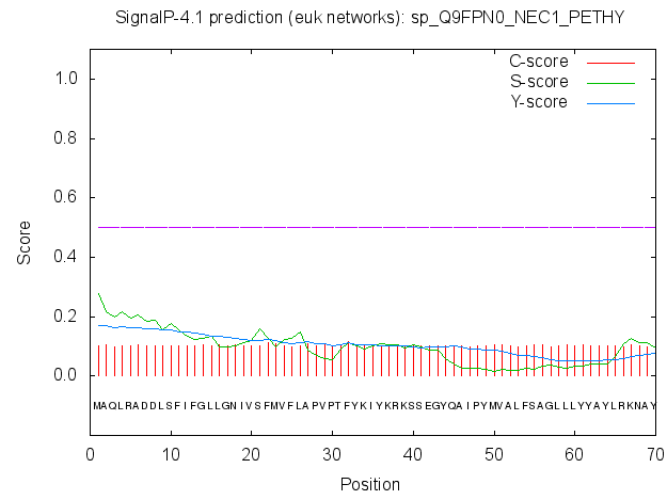
Signal peptide (SingleIP)



Tobacco



Tobacco



Prediction and Analysis



6. Subcellular localization (TargetP) 1-2

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q9SBK6_JMT_BRARP	392	0.133	0.131	0.029	0.735	_	2
cutoff		0.000	0.000	0.000	0.000		

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q8L925_CRC_ARATH	181	0.035	0.186	0.010	0.882	_	2
cutoff		0.000	0.000	0.000	0.000		

Prediction and Analysis



Subcellular localization (TargetP) 3-4

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q9ZVC2_NPR5_ARATH	491	0.110	0.032	0.297	0.396	_	5

cutoff		0.000	0.000	0.000	0.000		

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q9M1I7_NPR6_ARATH	467	0.046	0.056	0.380	0.602	_	4

cutoff		0.000	0.000	0.000	0.000		

Prediction and Analysis



Subcellular localization (TargetP) 5-6

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q9FMY1_C86B1_ARAT	559	0.194	0.007	0.184	0.641	_	3
cutoff		0.000	0.000	0.000	0.000		

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q94EG3_NEC1_NICLS	229	0.001	0.030	0.968	0.134	S	1
cutoff		0.000	0.000	0.000	0.000		

Tobacco

Prediction and Analysis



Subcellular localization (TargetP) 7-8

Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q84UV8_NEC3_NICLS	274	0.003	0.036	0.992	0.038	S	1
cutoff		0.000	0.000	0.000	0.000		

Tobacco

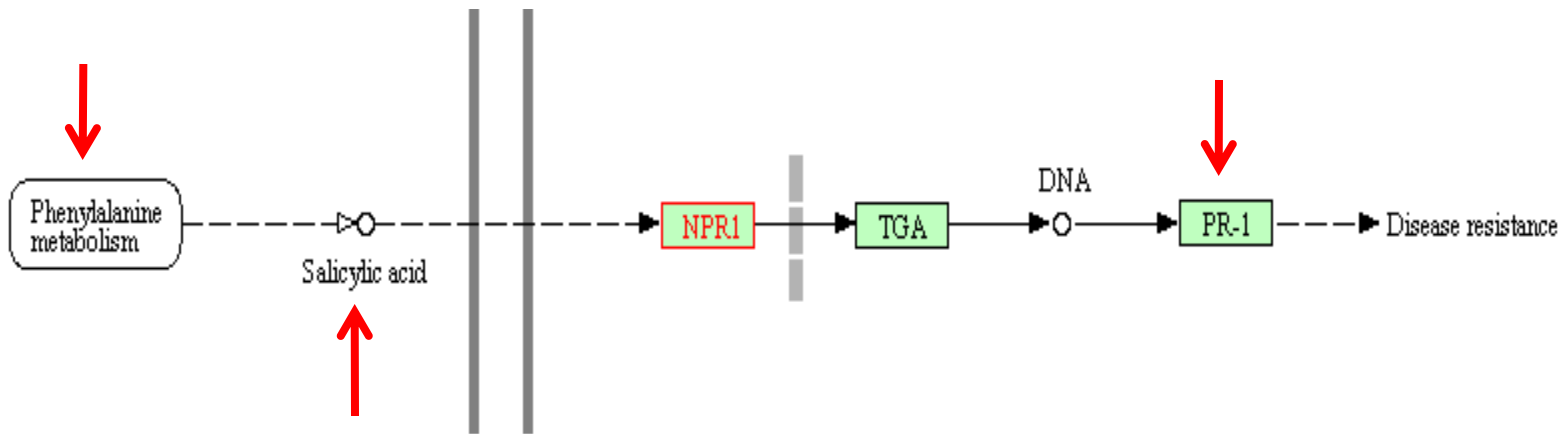
Name	Len	cTP	mTP	SP	other	Loc	RC
sp_Q9FPNO_NEC1_PETHY	265	0.005	0.025	0.884	0.555	S	4
cutoff		0.000	0.000	0.000	0.000		

Petunia

Prediction and Analysis



Pathway of NPR1 (KEGG)



Prediction and Analysis



8. Homology modeling

3D Structure prediction

Petunia hybrida Bidirectional sugar transporter NEC1



Model (left) based on template [c3b9yA](#)

Top template information

PDB header: transport protein
Chain: A: **PDB Molecule:** ammonium transporter family rh-like protein;
PDBTitle: crystal structure of the nitrosomonas europaea rh protein

Confidence and coverage

Confidence: **94.1%** Coverage: **92%**

245 residues (92% of your sequence) have been modelled with 94.1% 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](#)

Prediction and Analysis



3D Structure prediction

Brassica rapa subsp. Pekinensis Jasmonate O-methyltransferase



Prediction and Analysis



Brassica rapa subsp. Pekinensis Jasmonate O-methyltransferase



Confidence Key
High(9) ██████████ Low (0)

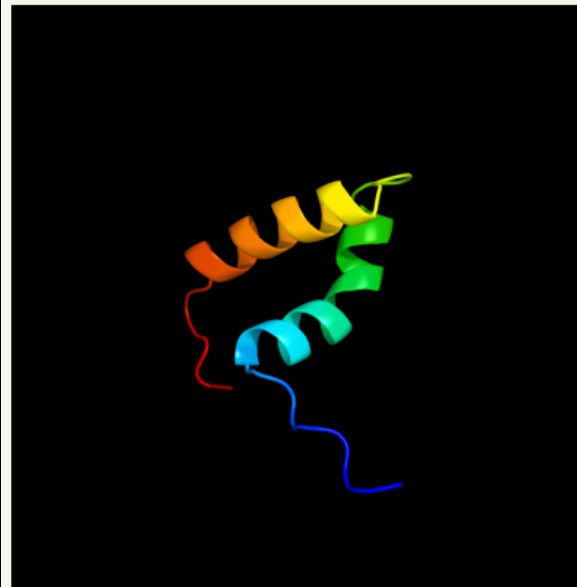
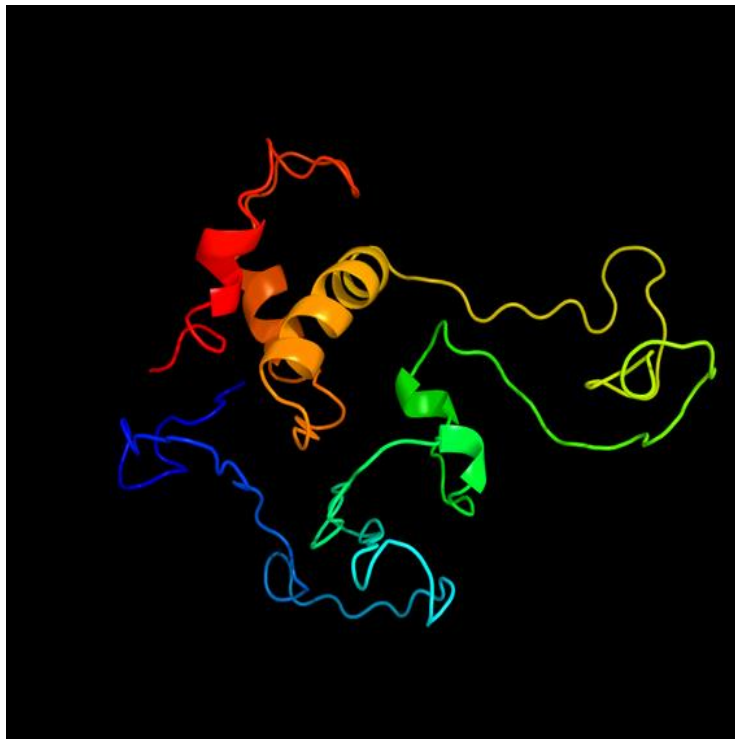
? Disordered (20%)
 Alpha helix (47%)
 Beta strand (14%)

Prediction and Analysis



3D Structure prediction

Arabidopsis thaliana CRABS CLAW (**transcription factor**)



Model dimensions (Å): **X**:25.095 **Y**:31.113 **Z**:40.314

Model (left) based on template [d1qrva](#)

Top template information

Fold:HMG-box
Superfamily:HMG-box
Family:HMG-box

Confidence and coverage

Confidence: **98.5%** Coverage: **27%**

49 residues (27% of your sequence) have been modelled with 98.5% confidence by the single highest scoring template.



You may wish to submit your sequence to [Phyrealarm](#). This will automatically scan your sequence every week for new potential templates as they appear in the Phyre2 library.

Warning: 60% of your sequence is predicted disordered. Disordered regions cannot be meaningfully predicted.

3D viewing

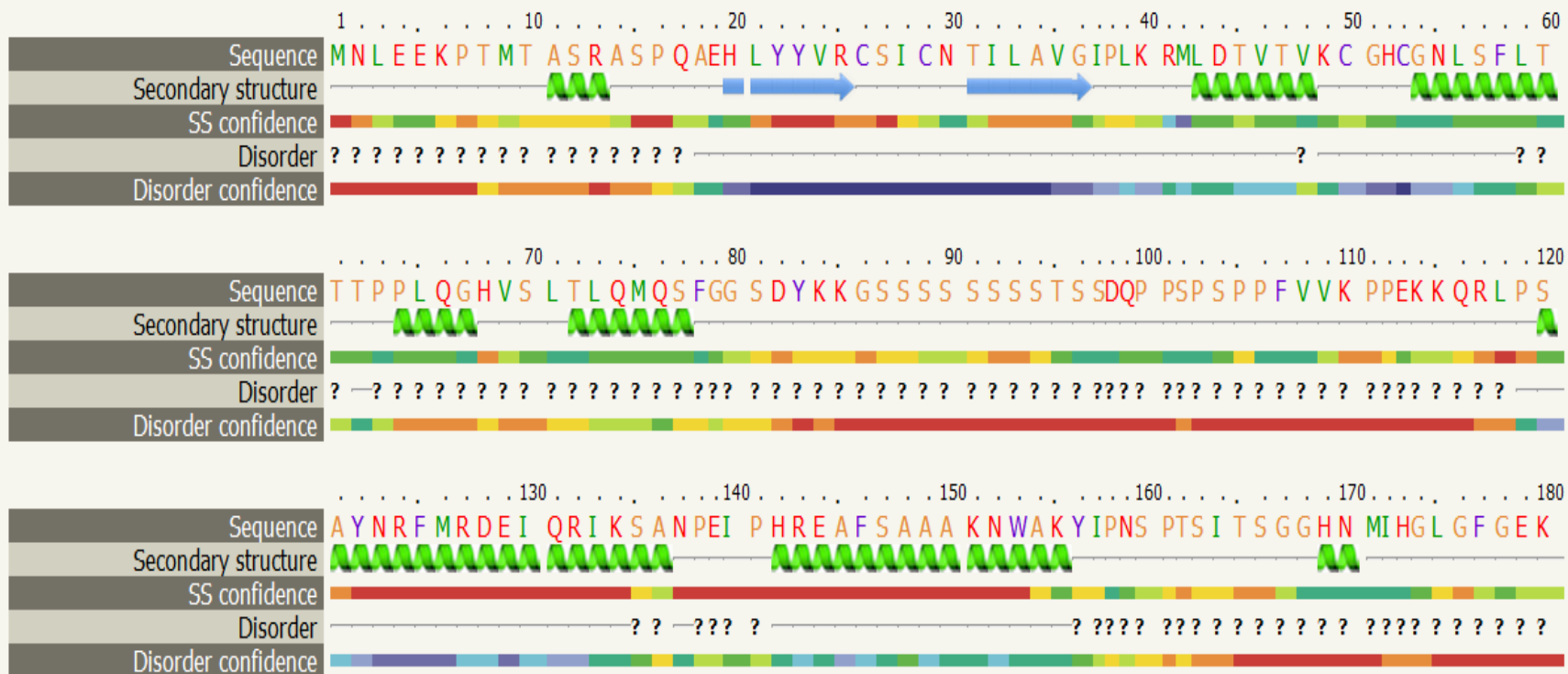
[Interactive 3D view in JSmol](#)

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

Prediction and Analysis



Arabidopsis thaliana CRABS CLAW (transcription factor)



Confidence Key
High(9) Low (0)

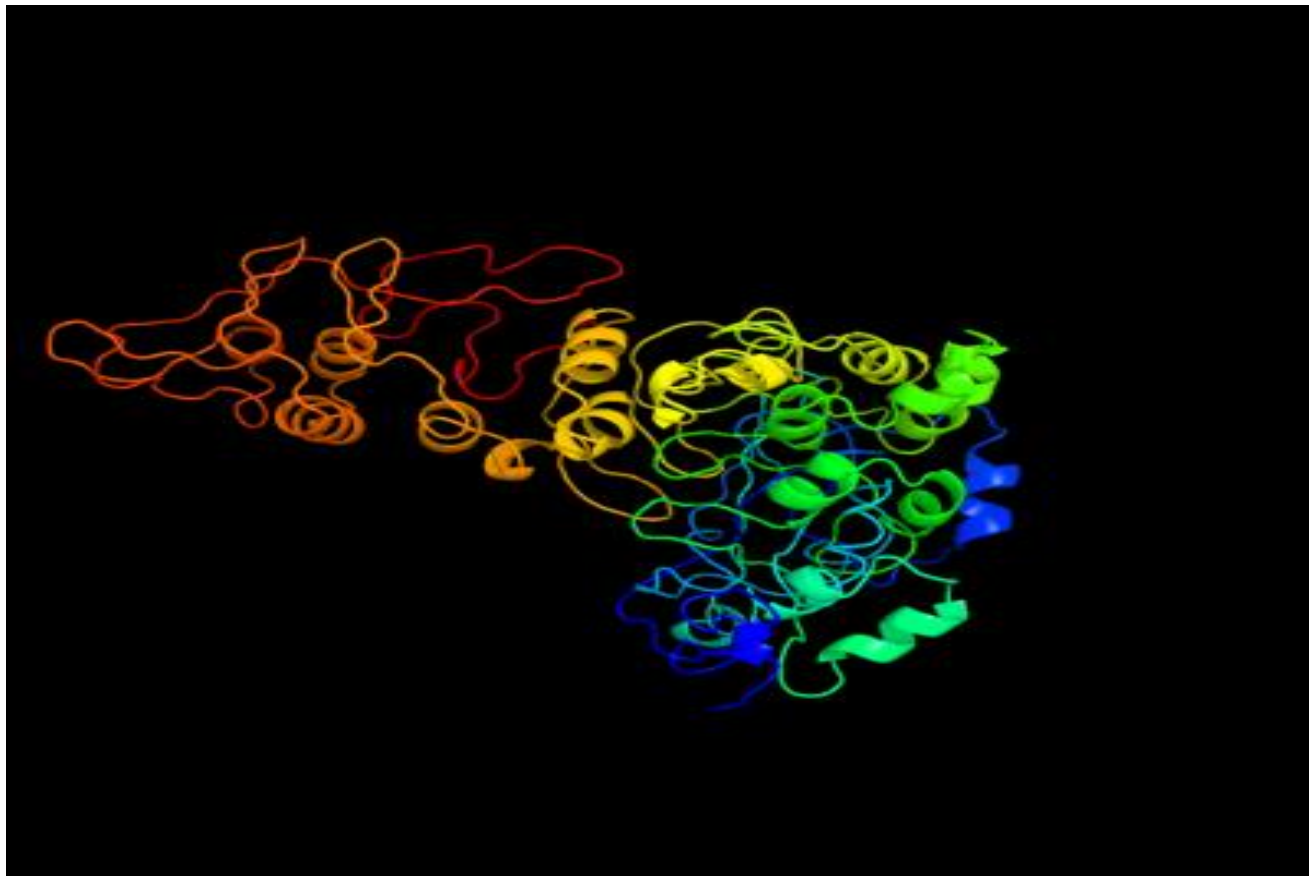
? Disordered (60%)
Alpha helix (33%)
Beta strand (7%)

Prediction and Analysis



3D Structure prediction

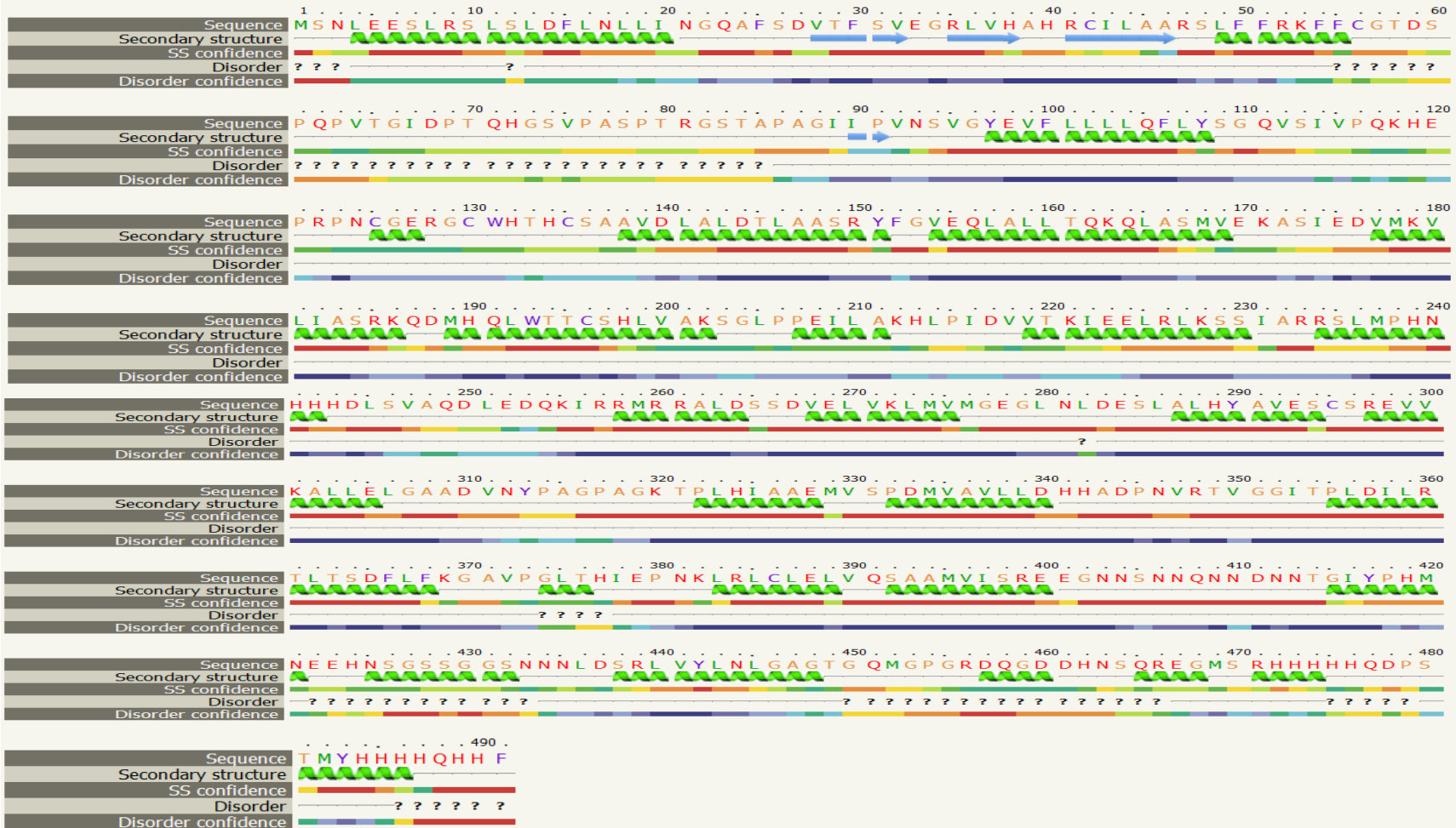
***Arabidopsis thaliana* Regulatory protein NPR5**



Prediction and Analysis



Arabidopsis thaliana Regulatory protein NPR5



Confidence Key
 High(9) [Color scale from red to blue] Low (0)

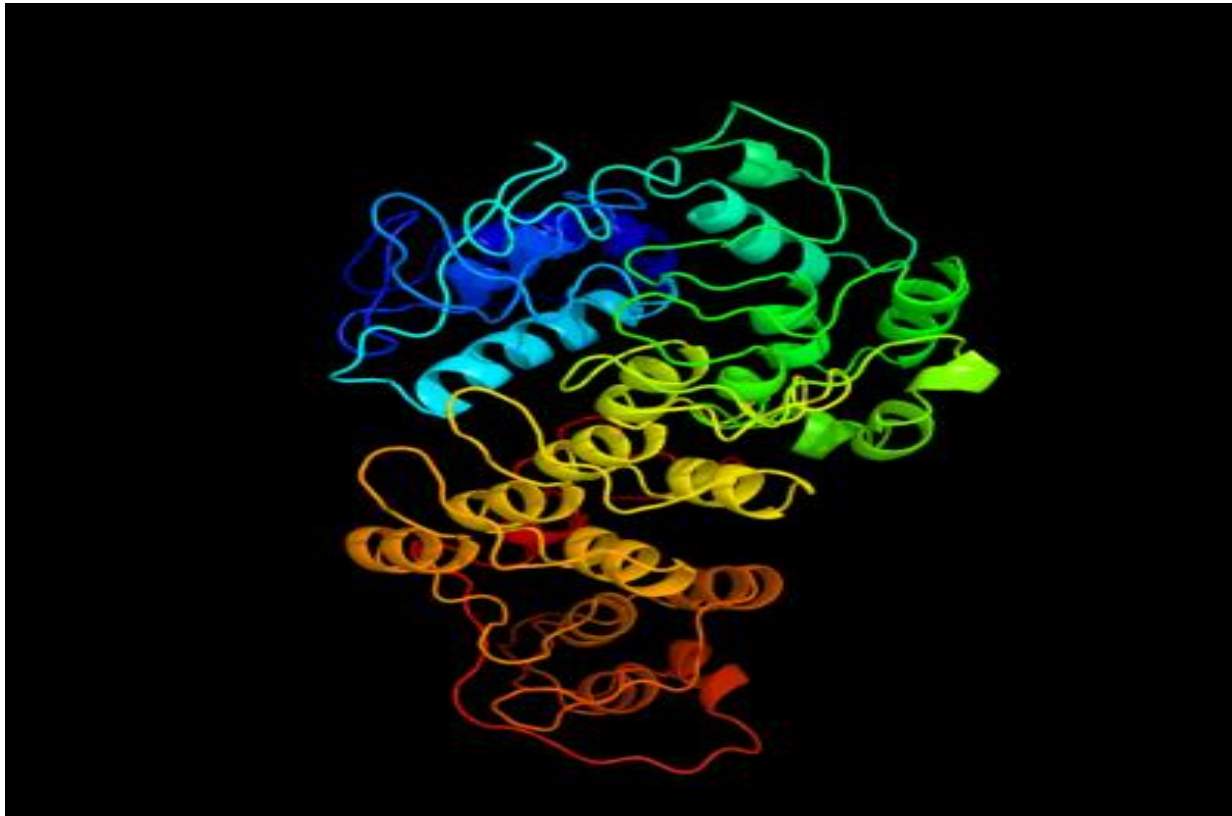
? Disordered (16%)
 Alpha helix (50%)
 Beta strand (3%)

Prediction and Analysis



3D Structure prediction

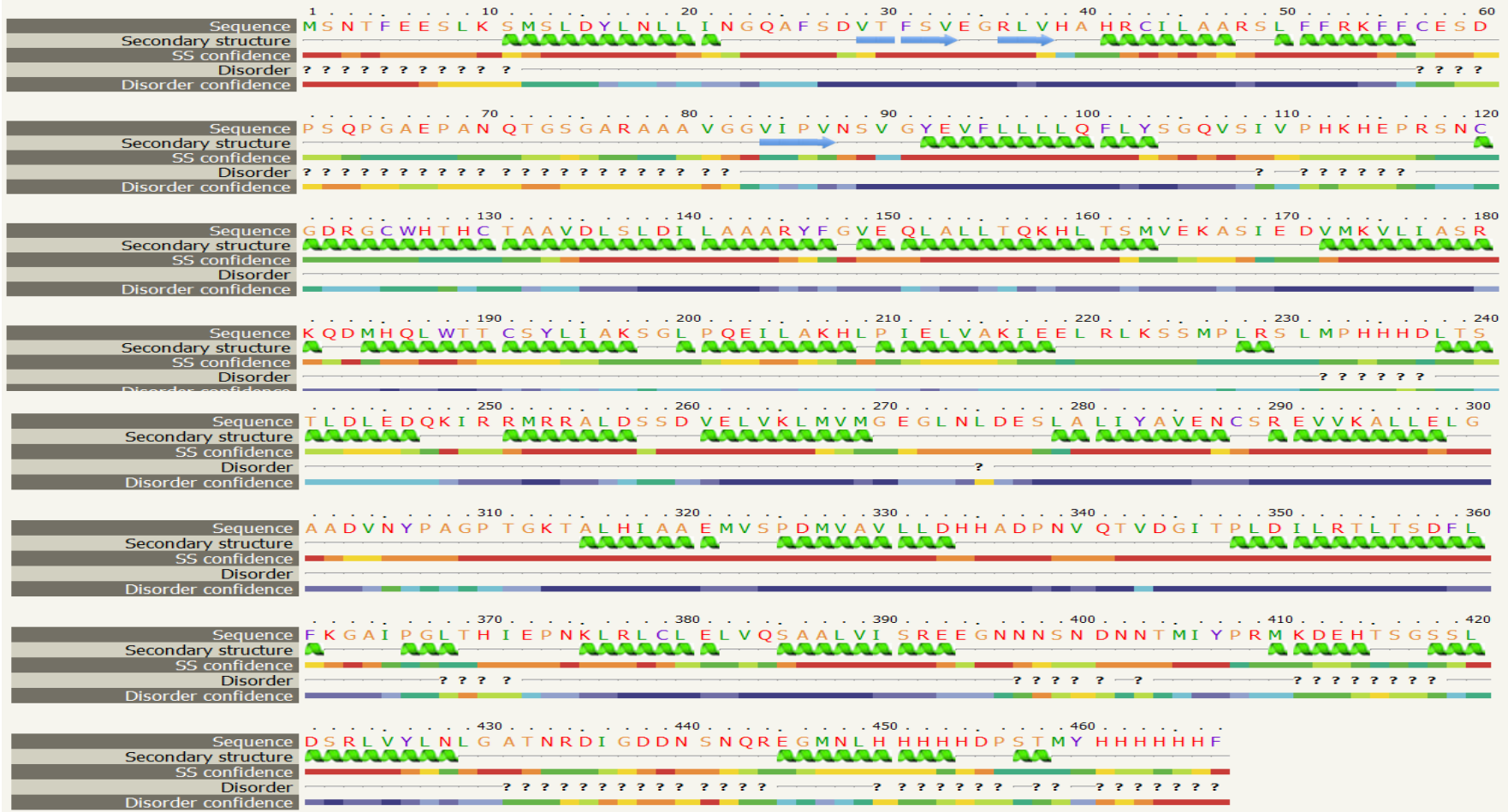
Arabidopsis thaliana Regulatory protein NPR6



Prediction and Analysis



Arabidopsis thaliana Regulatory protein NPR6



Confidence Key
 High(9) Low (0)

? Disordered (21%)

Alpha helix (52%)

Beta strand (3%)

Prediction and Analysis



3D Structure prediction

Conclusion:

The proportions of Alpha Helix are high among the nectary developmental proteins.

Conclusion and Programme



Conclusion: The nectary developmental related genes (proteins) are different in Sequence, Transmembrane structure, Signal peptide, Subcellular localization, 3D Structure among the existing sequences.

Programme: Analyze above genes or proteins (Jasmonate O-methyltransferase, transcription factor, Regulatory protein, Cytochrome P450, Sugar transporter, membrane protein, NEC3 - [alpha-carbonic anhydrase family](#)) and the new specific genes (protein) from the RNA-Seq Database.

References



NTR1 encodes a floral nectary-specific gene in *Brassica campestris* L. ssp. *pekinensis*. Song J.T., Seo H.S., Song S.I., Lee J.S., Choi Y.D. *Plant Mol. Biol.* 42(4):647-655(2000).

CRABS CLAW, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. Bowman J.L., Smyth D.R. *Development* 126(11):2387-2396(1999).

BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in *Arabidopsis*. Hepworth S.R., Zhang Y., McKim S., Li X., Haughn G.W. *Plant Cell* 17(5):1434-1448(2005).

Localization of CYP86B1 in the outer envelope of chloroplasts. Watson C.J., Froehlich J.E., Josefsson C.A., Chapple C., Durst F., Benveniste I., Coolbaugh R.C. *Plant Cell Physiol.* 42(8):873-878(2001). *Plant Cell Physiol.* 42(8): 873-878 (2001 Aug)

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