



## Soybean *phyB* Gene Cloning



Wu Faqiang, Peng Bo, Chen Lirei,  
Yu Xiudao, Chen Dan

Jan. 13, 2008



# Outline

1. Introduction of phytochrome
2. Cloning Materials & Methods involved
3. Analyzing sequences with Bioinformatics methods



## I Introduction of Phytochrome (phy)



# Soybean phyB Gene Cloning

“**Photomorphogenesis**” in Higher Plants

Plants detect light

and respond to

- direction
- wavelength
- Duration



● **de-etiolation**

● germination

● shade avoidance

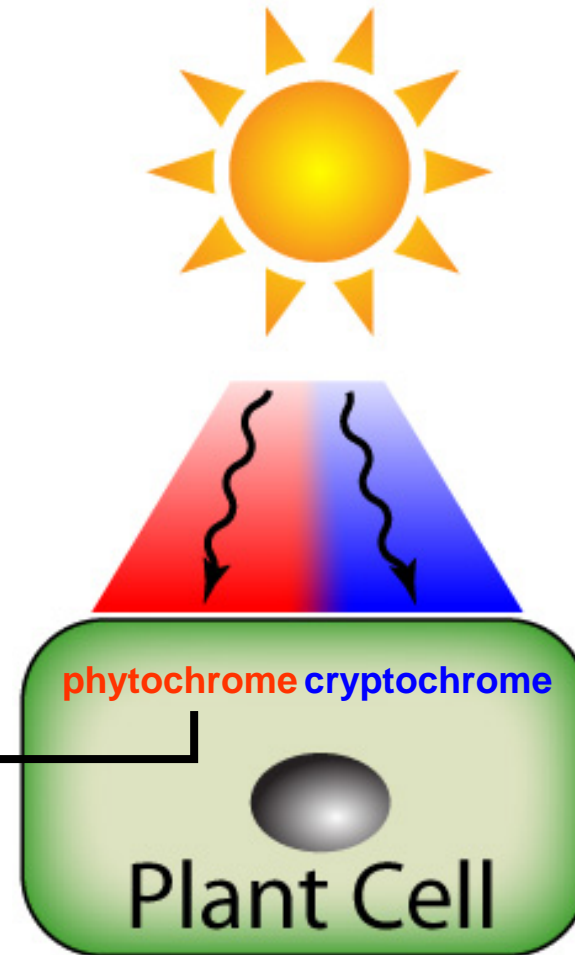
● flowering

● bending



# Soybean phyB Gene Cloning

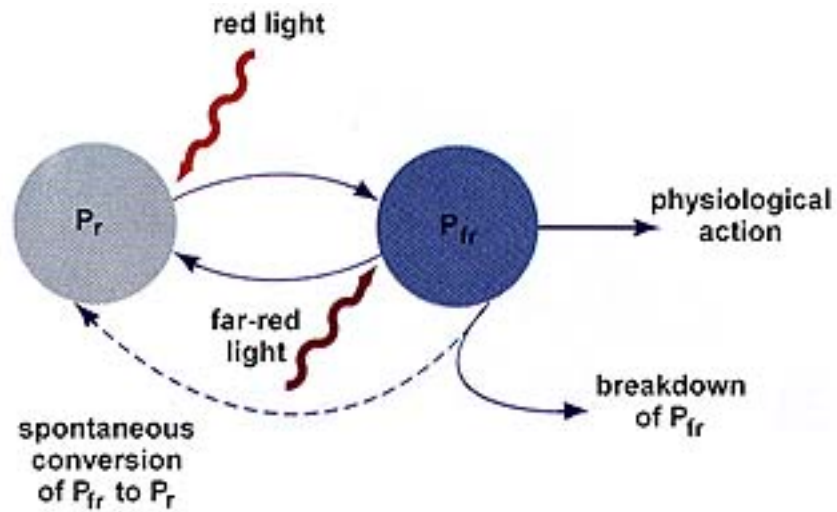
Five  
**Phytochromes** in  
*Arabidopsis*:  
**PhyA~PhyE**





# Soybean phyB Gene Cloning

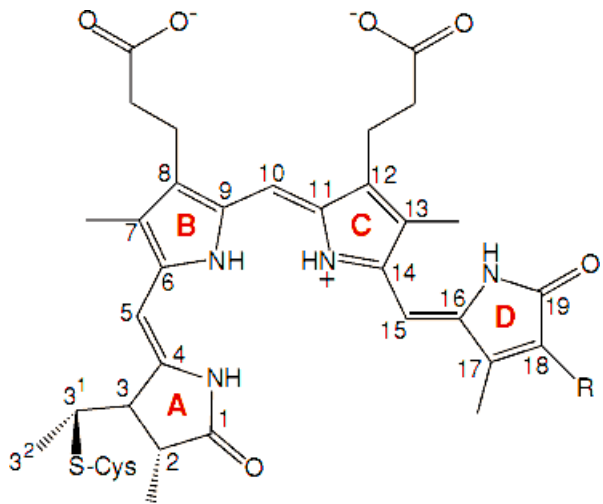
Phytochrome exists in two forms, **Pr** and **Pfr**



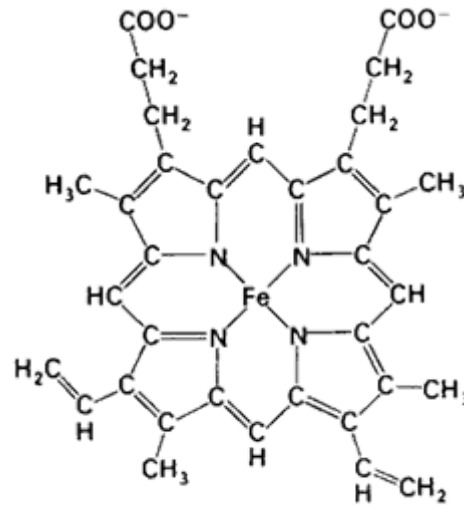


# Soybean phyB Gene Cloning

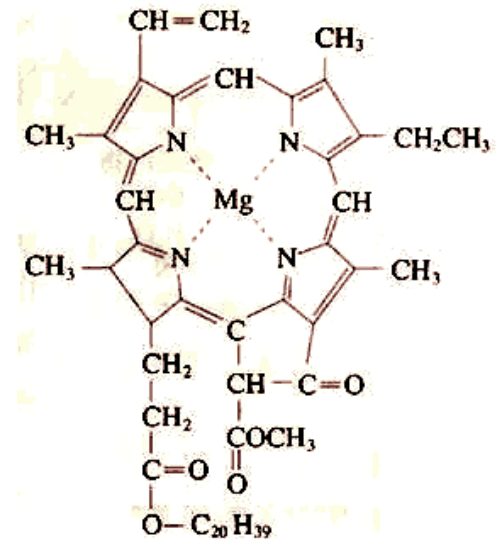
Phytochrome is a family of proteins with a small covalently-bound pigment molecule



chromophore



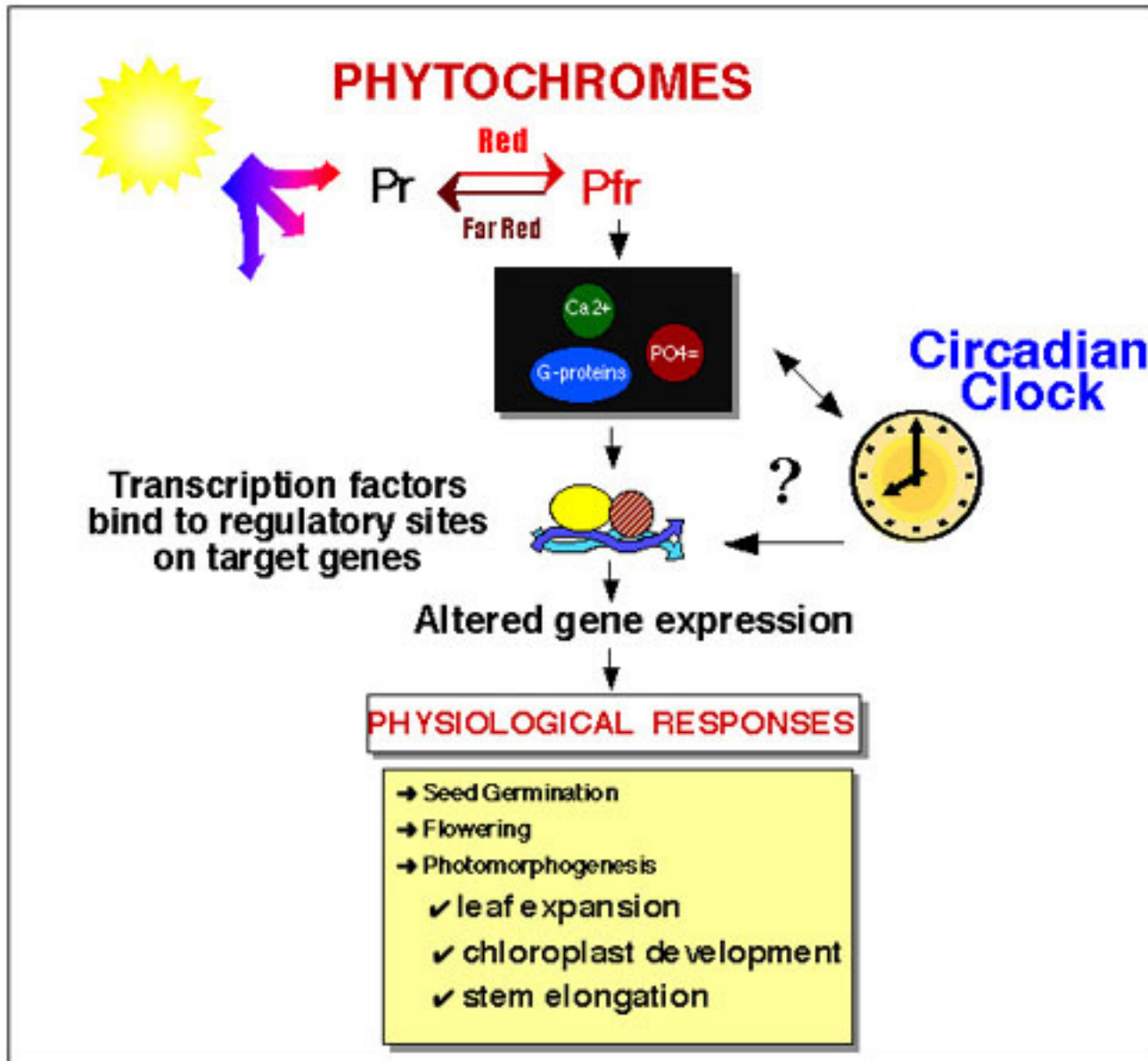
Heme  
(Fe-protoporphyrin IX)



chlorophyll



# Soybean phyB Gene Cloning





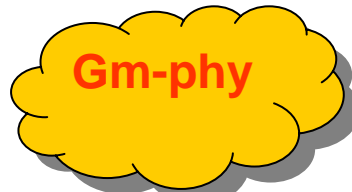


# Soybean phyB Gene Cloning

## Study of phytochrome (*Phy*) of soybean



Soybean  
(*Glycine max*)



*Arabidopsis*



# Soybean phyB Gene Cloning

## II Cloning Materials & Methods involved



# Soybean phyB Gene Cloning

Etiolated seedlings of soybean cv. Kenlong 18



RNA extraction, cDNA

Retrieving cDNA sequence of Gm-phyB from the internet



Primer designing



PCR amplification



Extraction, ligation with vector and transformation into *E. coli*



Screening & Selection of positive clones



Sequencing & analysis



## III Analyzing Sequences with Bioinformatics methods

- Are these clones Gm-phyB we want?
- What's the difference between the clones?
- Do the consensus disagreement loci **really exist** or **PCR error**?
- How come these consensus disagreement?



# Soybean phyB Gene Cloning

## Assembling sequences & locating SNP with SeqMan

Translate ▶ Consensus

170 180 190 200 210 220 230 240

ACCTC**ACGAACAAAG**ACTGAGCCACCAC-**AGCAG**-----CAACAACAACAACAACATCGACTCCAT**TGAGCAAG**

*GmphyB-TC227575.seq* (1>4195) → ACCTCACGAACAAAGACTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb12.seq (1>3858) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb8.seq (1>3851) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb13.seq (1>3859) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb11.seq (1>3867) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb5.seq (1>3856) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb4.seq (1>3856) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

tb1.seq (1>3803) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb6.seq (1>3766) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb3.seq (1>3856) → ACCTCACGAACAAAG-CTGAGCCACCAC-AGCAG-----CAACAACAACAACAACATCGACTCCATGAGCAAG

eb1.seq (1>3986) → ACCTC**GCGGACGAAG**-CTGAGCCACCAC**CACCACAACAAACAA**CAACAACAACAACAACATCGACTCCAC**GAGCAAG**

eb14.seq (1>3990) → ACCTC**GCGGACGAAG**-CTGAGCCACCAC**CACCACAACAAACAA**CAACAACAACAACAACATCGACTCCAC**GAGCAAG**

eb9.seq (1>4084) → ACCTC**GCGGACGAAG**-CTGAGCCACCAC**CACCACAACAAACAA**CAACAACAACAACAACATCGACTCCAC**GAGCAAG**

eb7.seq (1>3987) → ACCTC**GCGGACGAAG**-CTGAGCCACCAC**CACCACAACAAACAA**CAACAACAACAACAACATCGACTCCAC**GAGCAAG**

These clones can be classified into 2 main groups

Multi-copy in soybean?



# Soybean phyB Gene Cloning

## Frame shifting

insertion

```
GraphyB-TC227575.seq(1>4195) → CCACCCTTAGGGCTCCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb12.seq(1>3858) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb8.seq(1>3851) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb13.seq(1>3859) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb11.seq(1>3867) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb5.seq(1>3856) ← CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb4.seq(1>3856) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
tb1.seq(1>3803) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb6.seq(1>3766) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb3.seq(1>3856) → CCACCCTTAGGGCACCTCACGGTTGTCATGCTCAGTATATGGCTAA
eb1.seq(1>3986) → CCACGCTCAGGGCGCCTCACGGTTGCCATGCTCAGTATATGGCTAA
eb14.seq(1>3990) → CCACGCTCAGGGCGCCTTCGGTTGCCATGCTCAGTATATGGCTAA
eb9.seq(1>4084) → CCACGCTCAGGGCG PCR error TTGCCATGCTCAGTATATGGCTAA
eb7.seq(1>3987) → CCACGCTCAGGGCGCCTCACGGTTGCCATGCTCAGTATATGGCTAA
```

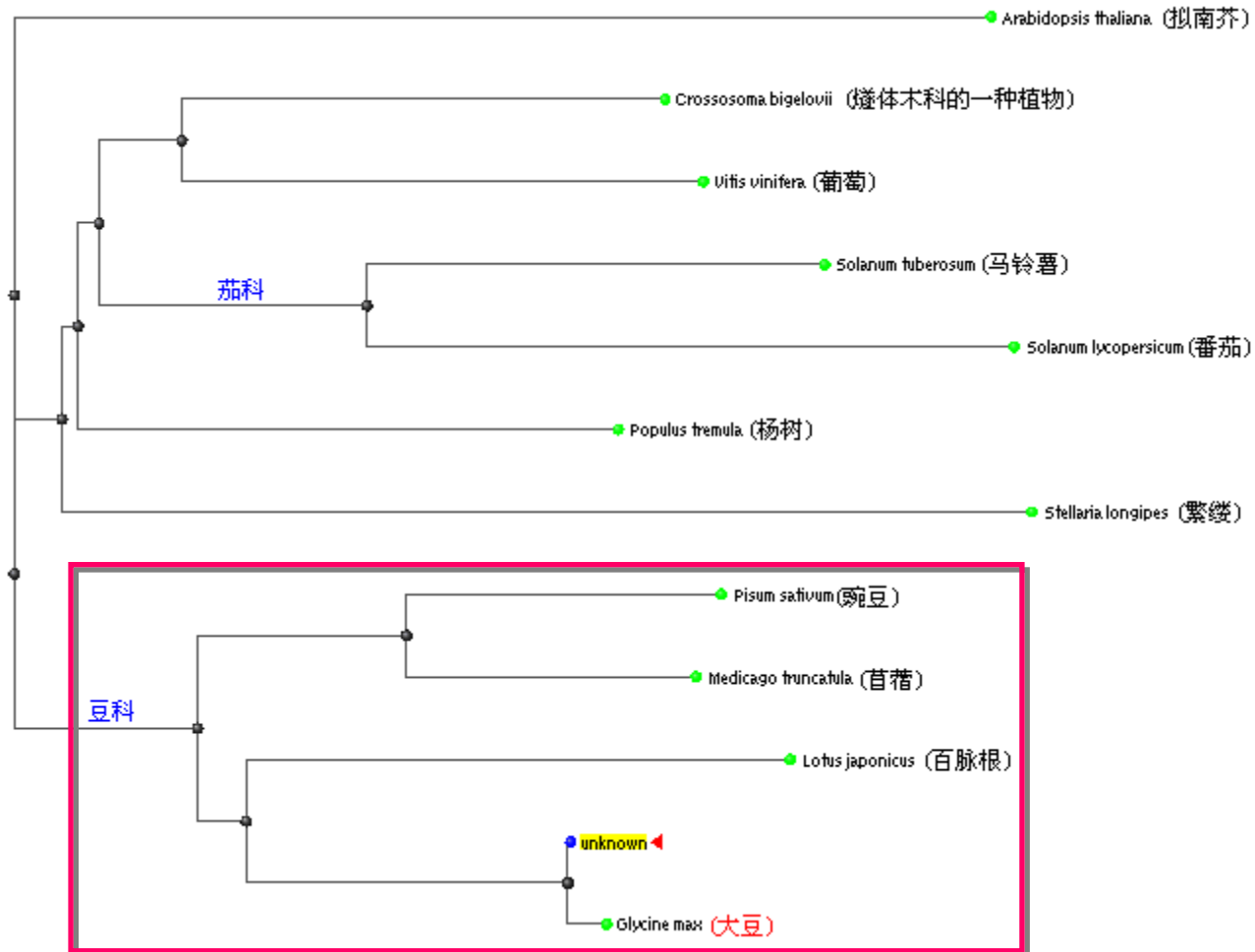
deletion

causing **only one** amino acid mutation (G → R)



# Soybean phyB Gene Cloning

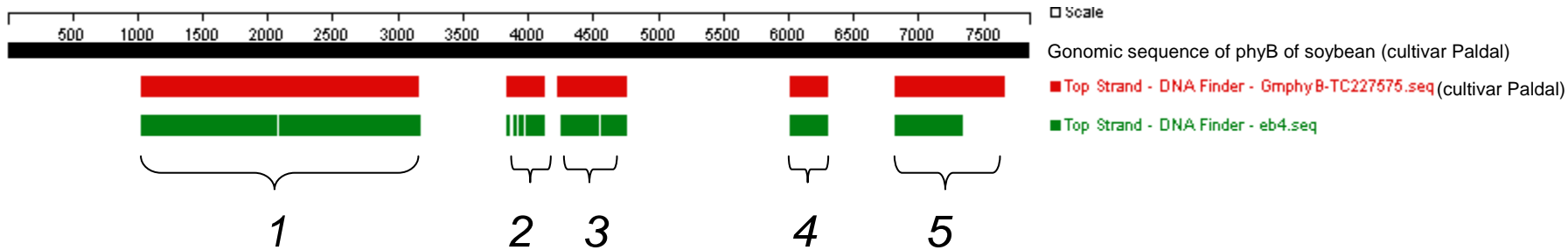
## Blast tree view (nucleotide) in NCBI





# Soybean phyB Gene Cloning

## intron & exon view in GeneQuest



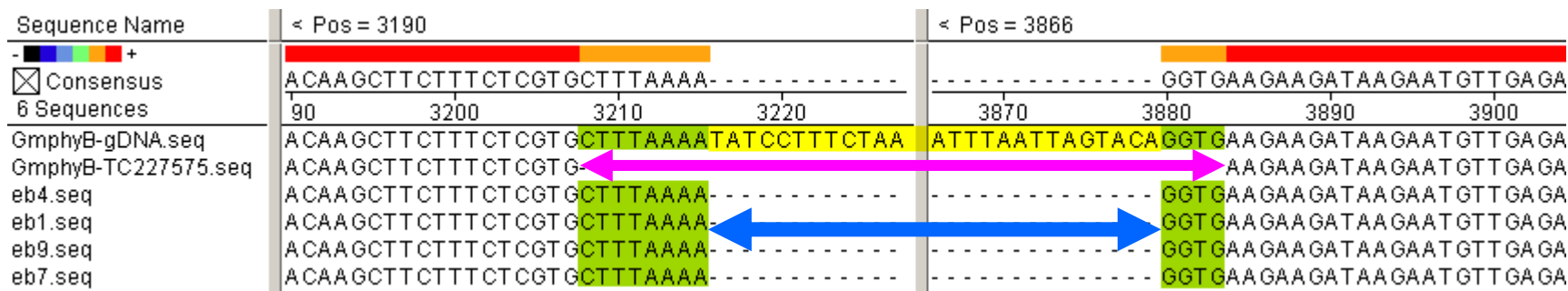
**Note:** gDNA, cDNA of Gm-phyB were retrieved from GmGI Gene Index





# Soybean phyB Gene Cloning

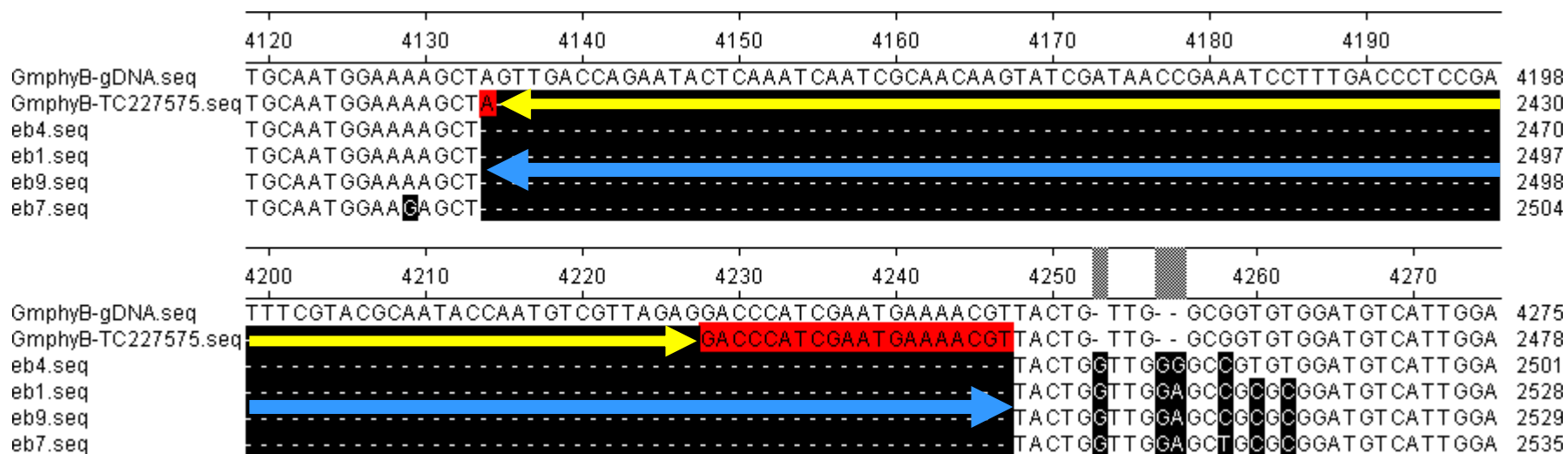
## Alternative splicing





# Soybean phyB Gene Cloning

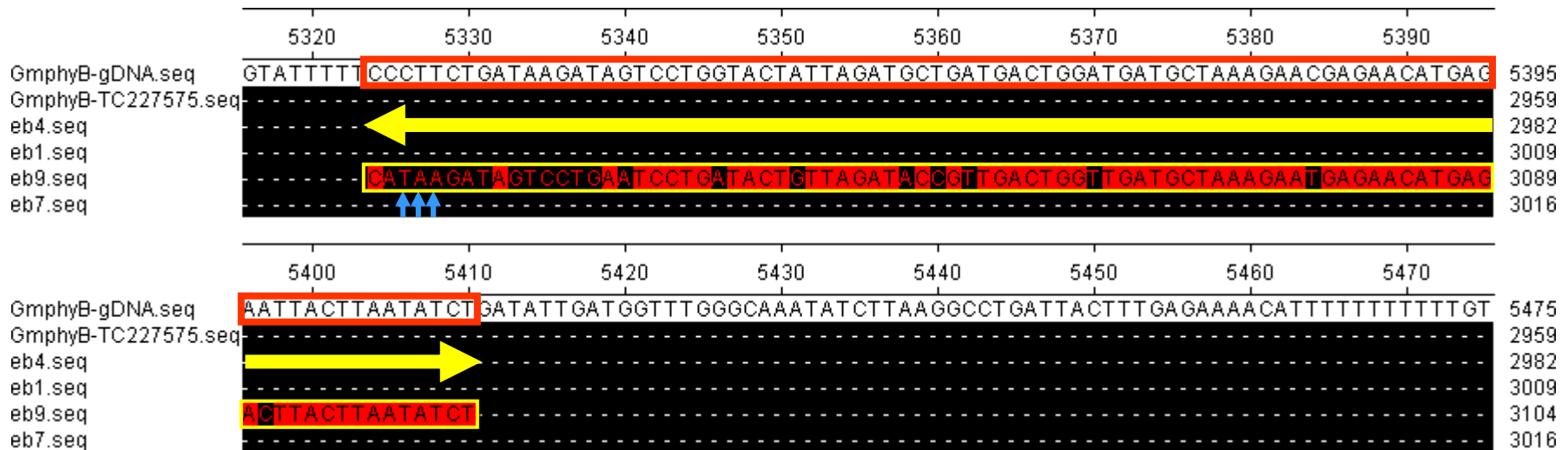
## Alternative splicing





# Soybean phyB Gene Cloning

eb9, a pseudogene(假基因)?





# Soybean phyB Gene Cloning

## Conserved domain search in NCBI Blast

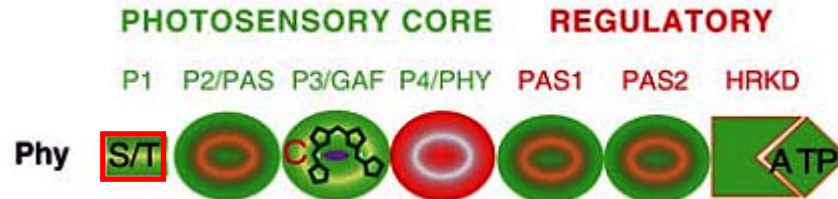


Fig. Domain architecture of phytochrome, Nathan C., 2006

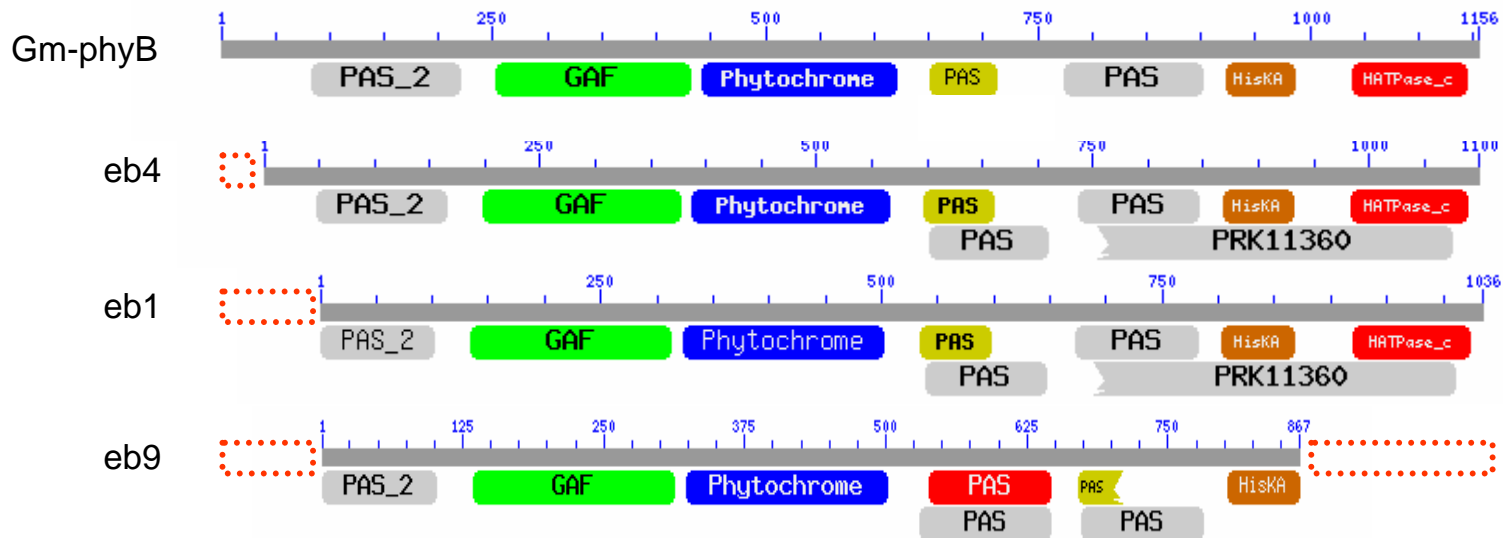


Fig. conserved domain search on NCBI



# Soybean phyB Gene Cloning

## Conserved domain search in SWISS MODEL WORKSPACE

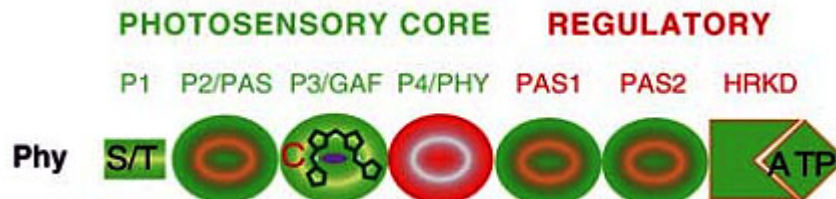


Fig. Domain architecture of phytochrome, Nathan C., 2006

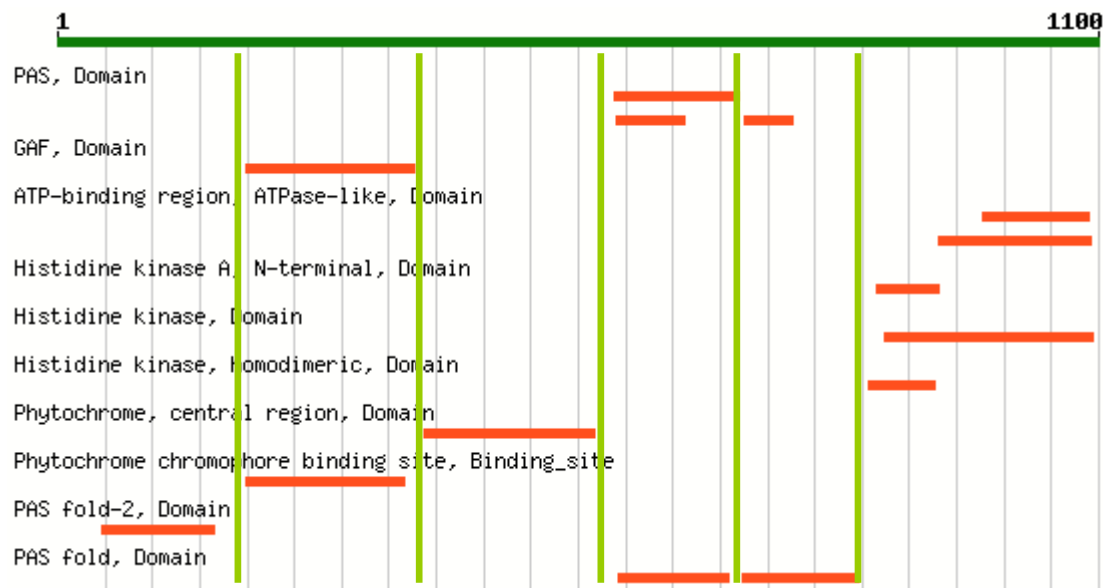
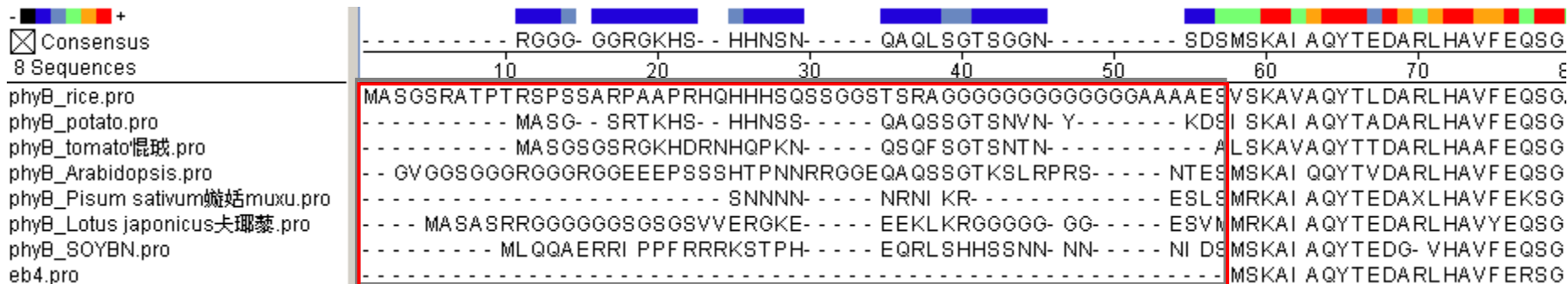


Fig. eb4 conserved domain search on NCBI



# Soybean phyB Gene Cloning

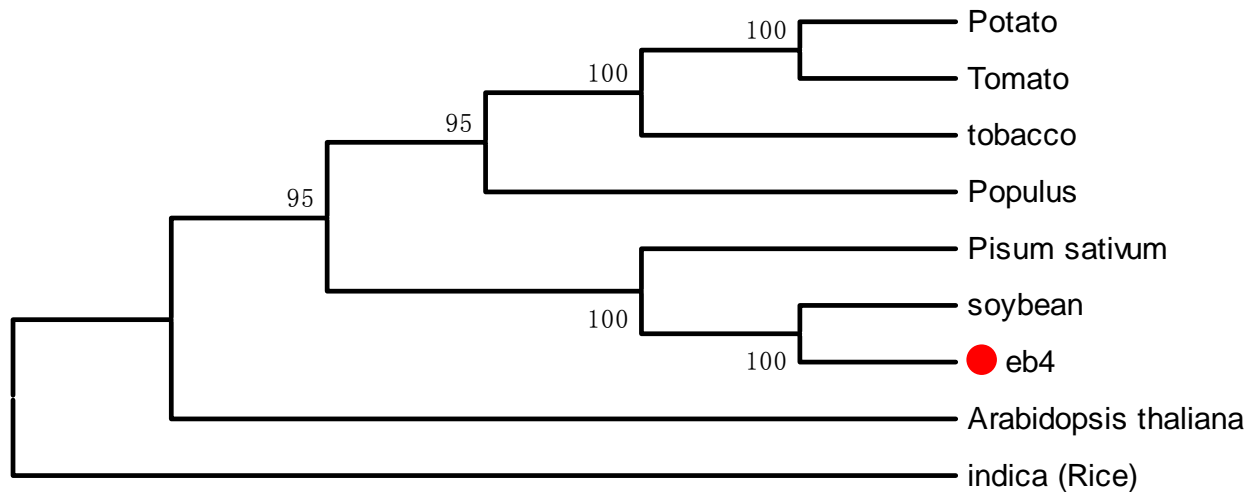
N-terminal, non-conserved domain?





# Soybean phyB Gene Cloning

Phylogenetic tree in Mega  
(protein)



Neighbor-joining (500 replicates)



# Soybean phyB Gene Cloning

A paradox,  
how come?

soybean-gi 1172496 sp P42499.1 PHYB_S...	AGYEGLERLGMQFVGVQVAYITERD	FLFWFRSHTAKEIR	Soybean
eb4	AGYEGAAASLGDAVCGMAVAYITERD	FLFWFRSHTAKEIR	My clone
Pisum_sativum-gi 6502525 gb AAF14344....	AGYEGAAASLGDAVCGMAVAYITERD	FLFWFRSHTAKEIR	Pea
Potato-P34094 PHYB_SOLTU_Phytochrome...	AGYEGAAASLGDAVCGMAVAYITSKD	FLFWFRSHTAKEIR	Other species
Tomato-Q9ZS62 PHYB1_SOLLC_Phytochrome...	AGYEGAAASLGDAVCGMAVAYITSKD	FLFWFRSHTAKEIR	
tobacco-gi 2370331 emb CAA74992.1 _ph...	AGYEGAAALLGDAVCGMAVAYITSKD	FLFWFRSHTAKEIR	
Populus-gi 10954091 gb AAG25725.1 AF3...	AGYEGAAASLGNAVCGMAVAYITRRD	FLFWFRSHTAKEIR	
Arabidopsis_thaliana-P14713 PHYB_ARAT...	AGYEGAAALGDAVCGMAVAYITRRD	FLFWFRSHTAKEIR	
indica_(Rice)-A2XFW2 PHYB_ORYSI_Phyto...	AGYEGAAALGDAVSGMAVAYITPSD	YLFWFRSHTAKEIR	





**Thanks &  
wish all of us a happy Holiday!**

