



Institute of Crop Science, CAAS

## Soybean *phyB* Gene Cloning



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# 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

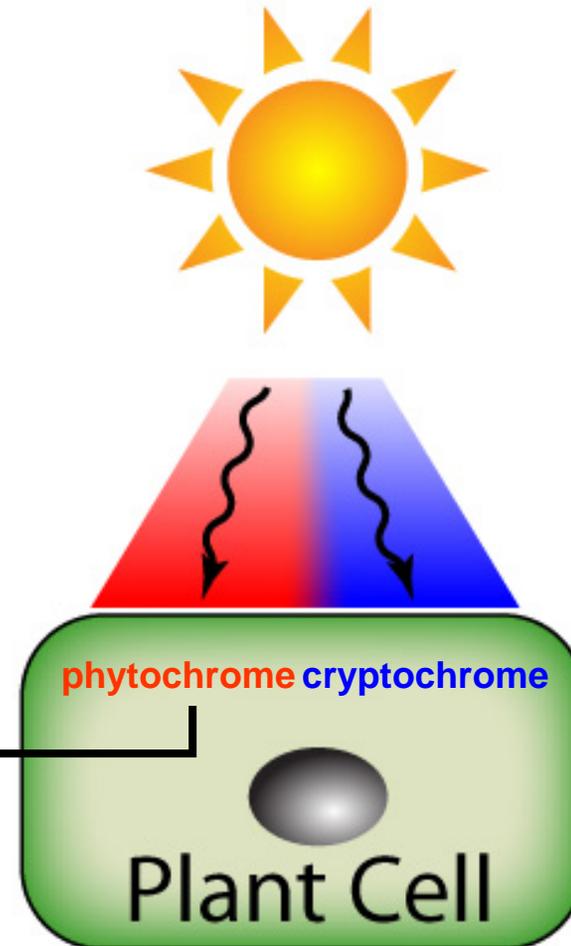
- flowering

- bending



# Soybean phyB Gene Cloning

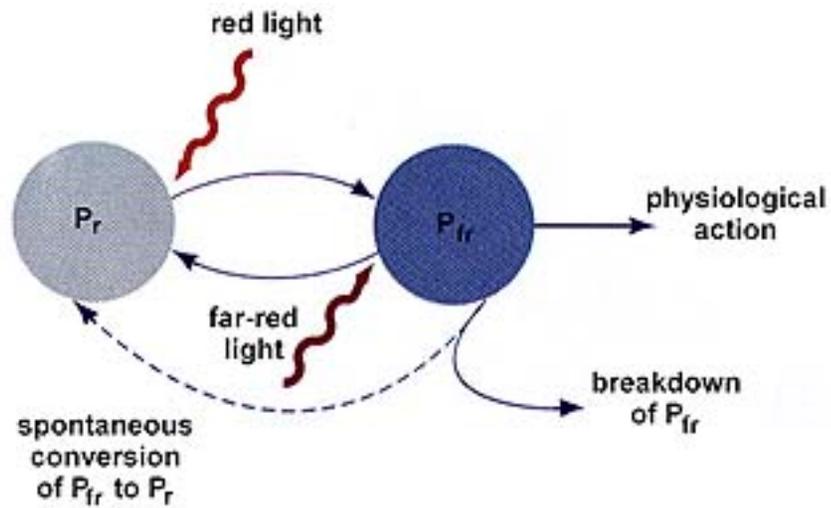
Five  
**Phytochromes** in  
*Arabidopsis*:  
**PhyA~PhyE**





# Soybean phyB Gene Cloning

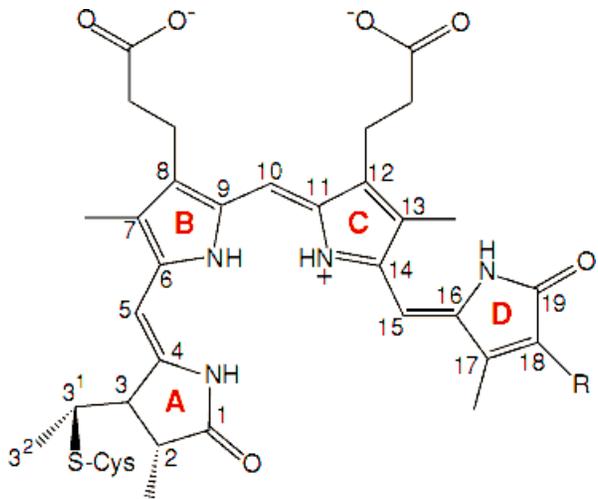
Phytochrome exists in two forms, **Pr** and **Pfr**



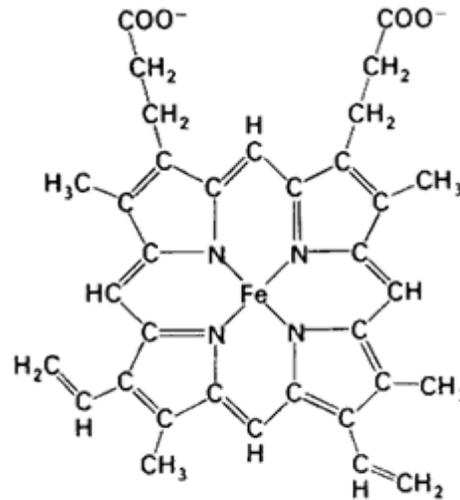


# Soybean phyB Gene Cloning

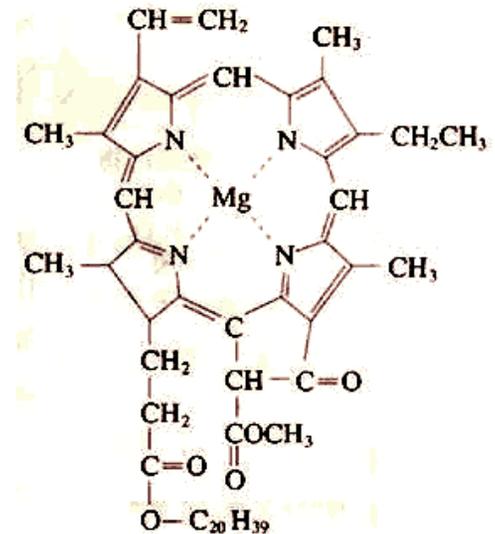
Phytochrome is a family of proteins with a small covalently-bound pigment molecule



chromophore



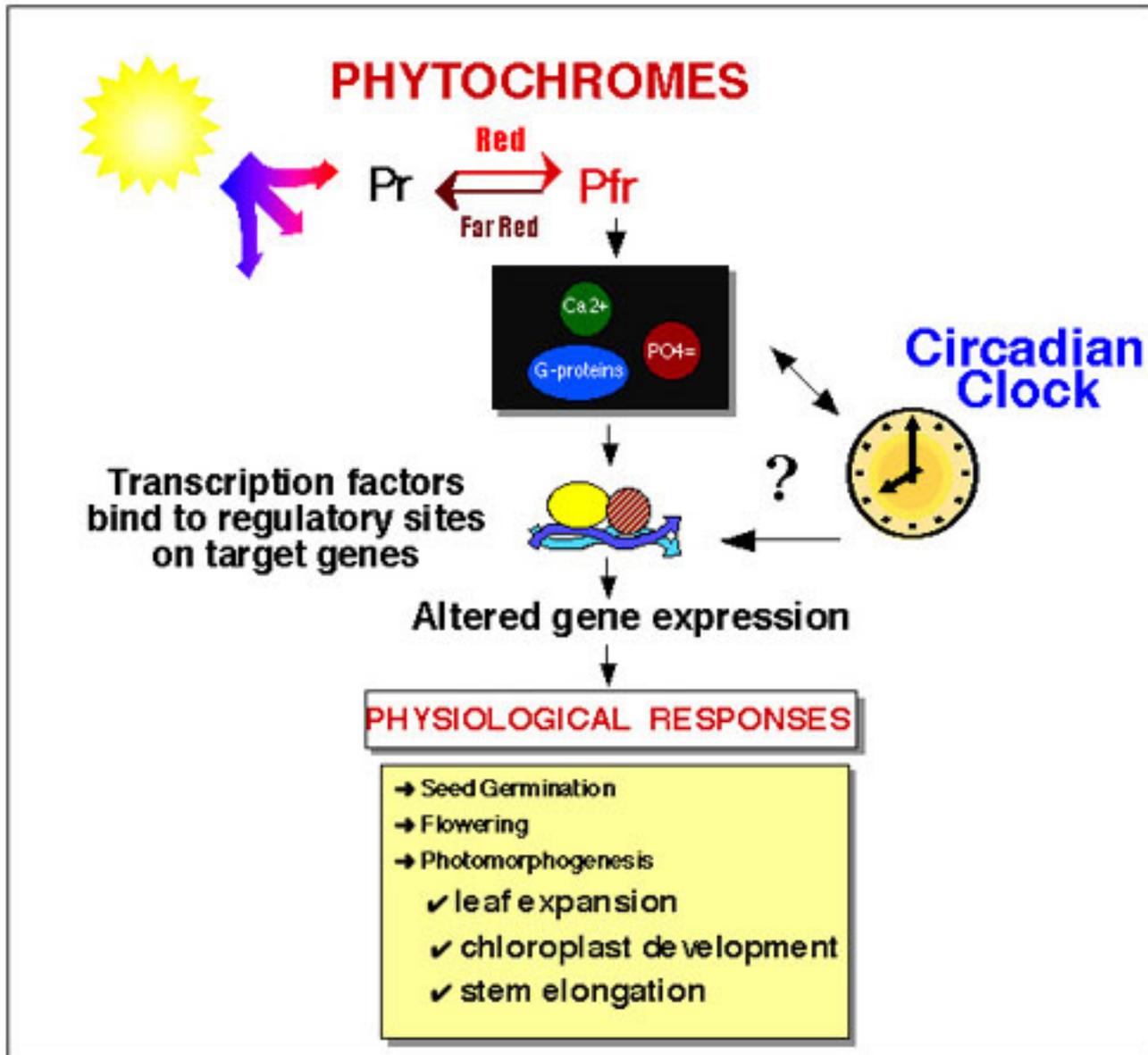
Heme  
(Fe-protoporphyrin IX)



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



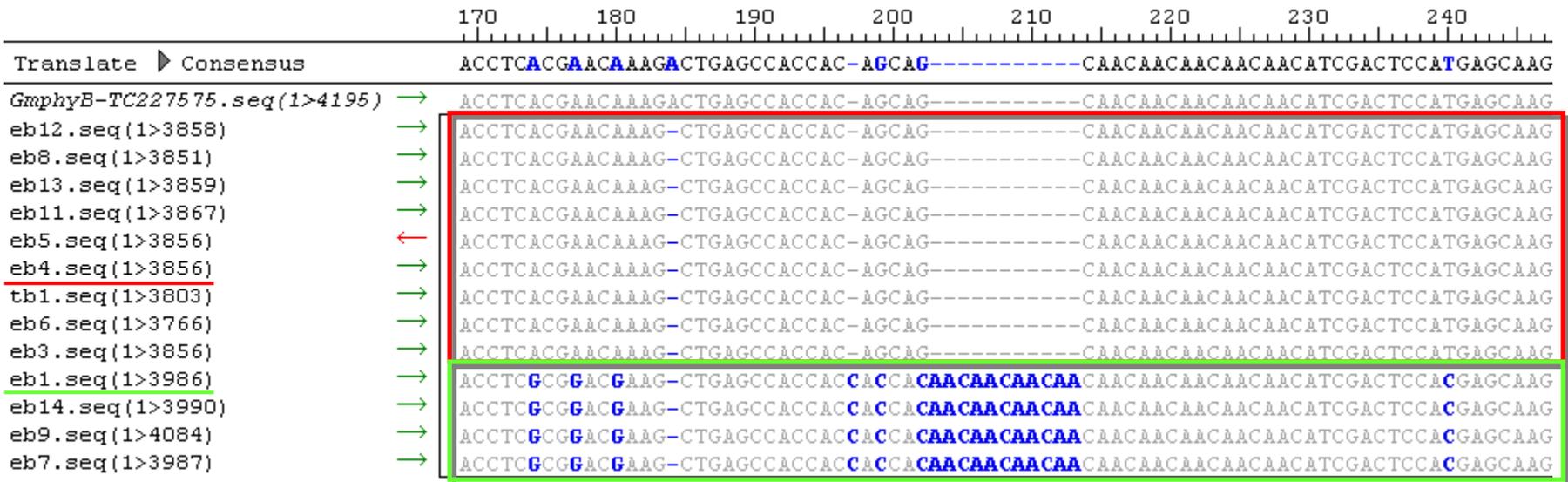
## III Analyzing Sequences with Bioinformatics methods

- 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



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
```

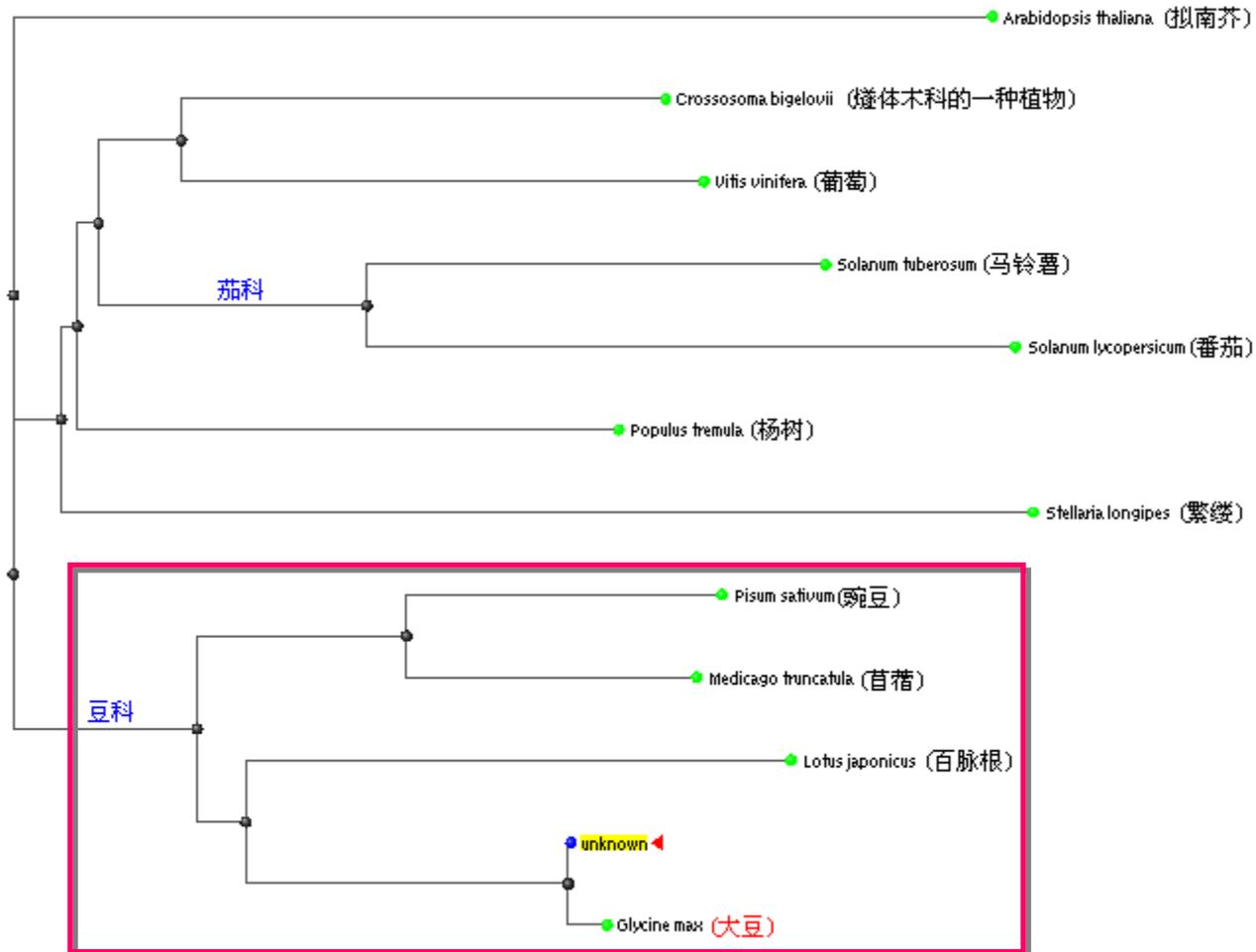
deletion

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



# Soybean phyB Gene Cloning

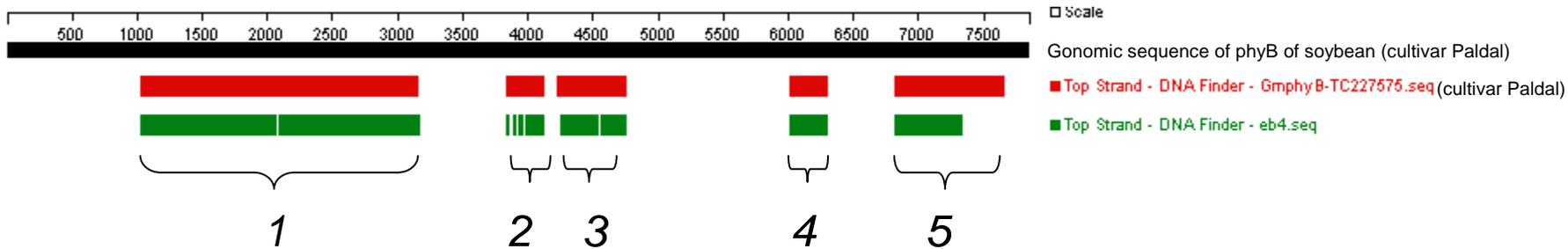
## Blast tree view (nucleotide) in NCBI





# Soybean phyB Gene Cloning

## intron & exon view in GeneQuest

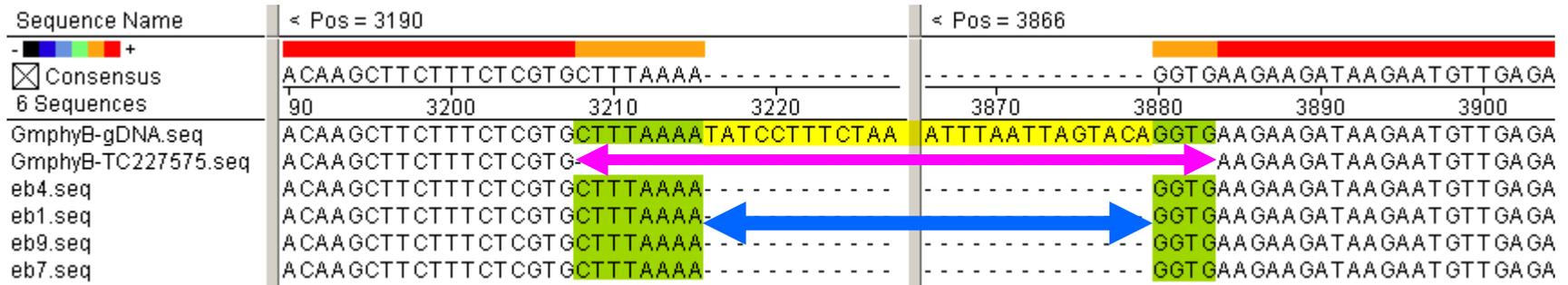


**Note:** gDNA, cDNA of Gm-phyB were retrieved from GmGI Gene Index



# Soybean phyB Gene Cloning

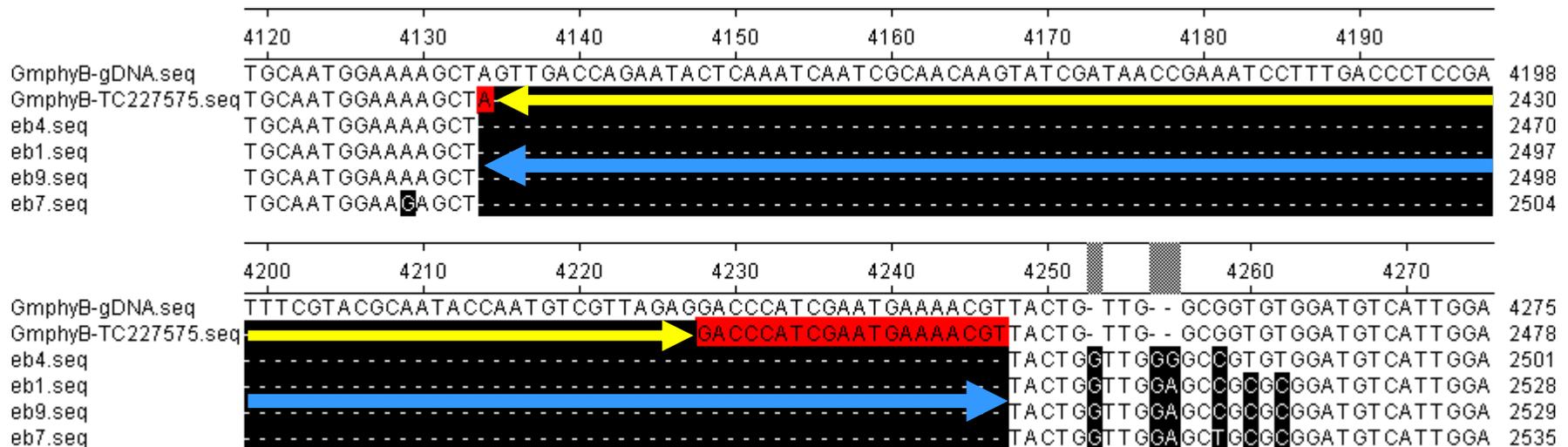
## Alternative splicing





# Soybean phyB Gene Cloning

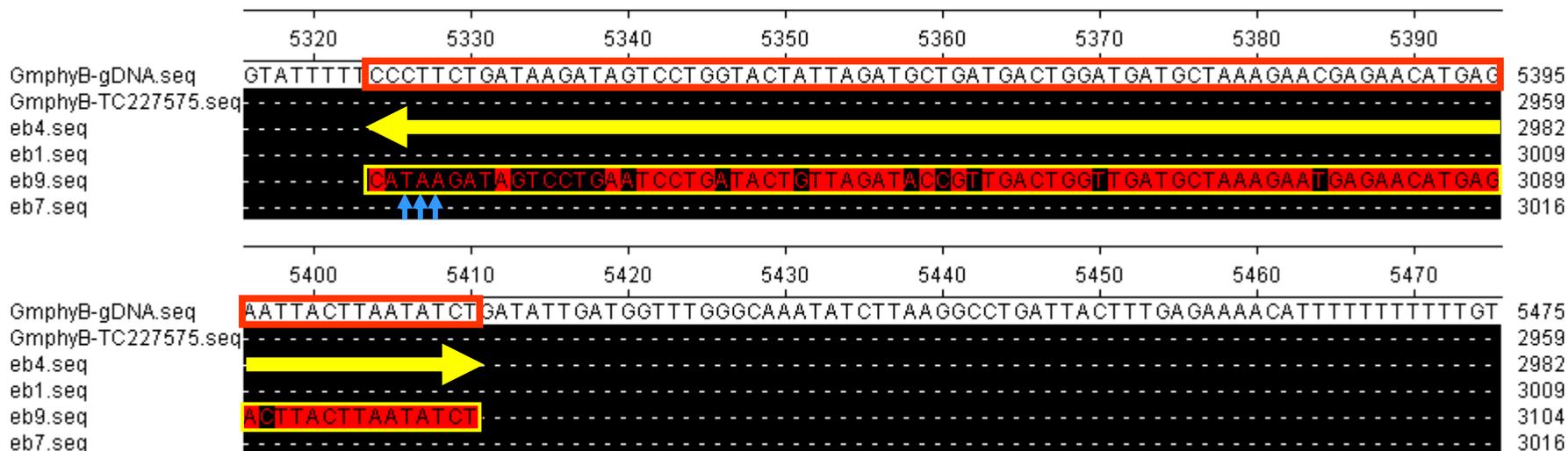
## Alternative splicing





# Soybean phyB Gene Cloning

eb9, a pseudogene(假基因)?





# Soybean phyB Gene Cloning

## Conserved domain search in NCBI Blast

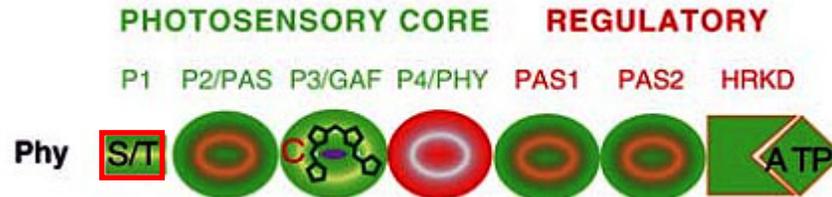


Fig. Domain architecture of phytochrome, Nathan C., 2006

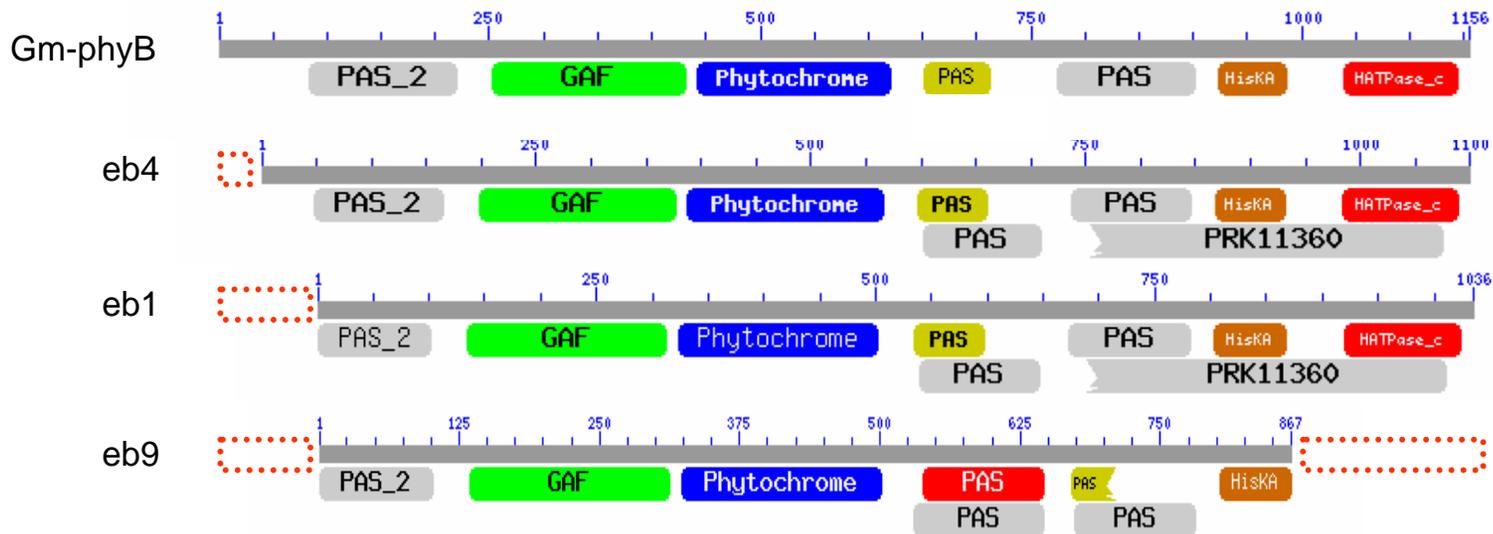


Fig. conserved domain search on NCBI



# Soybean phyB Gene Cloning

## Conserved domain search in SWISS MODEL WORKSPACE

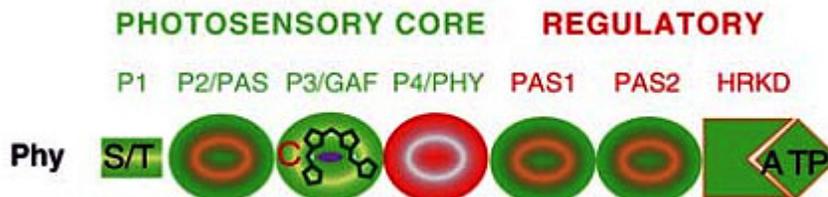


Fig. Domain architecture of phytochrome, Nathan C., 2006

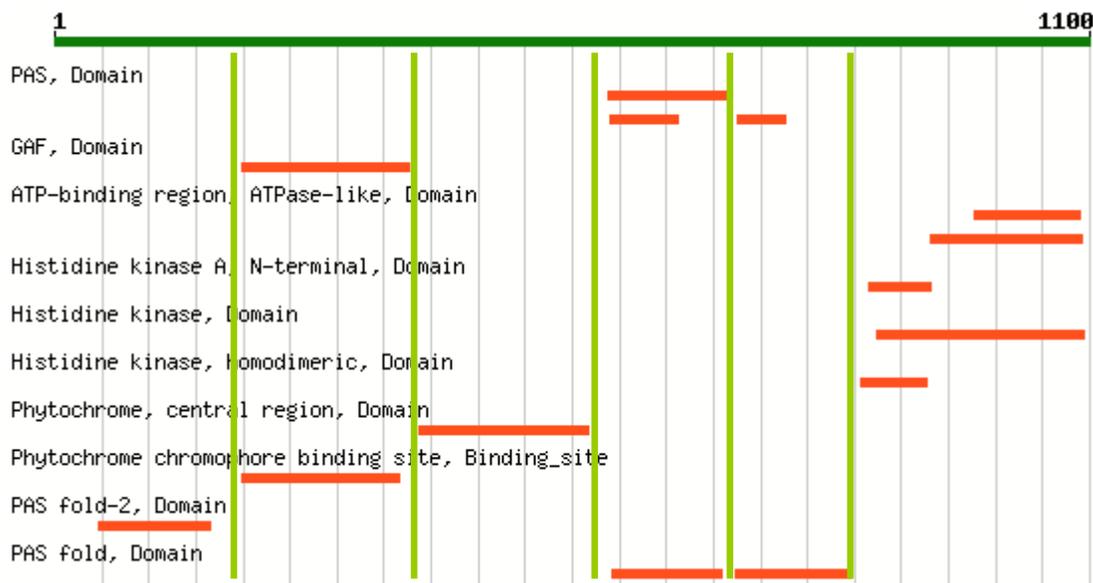
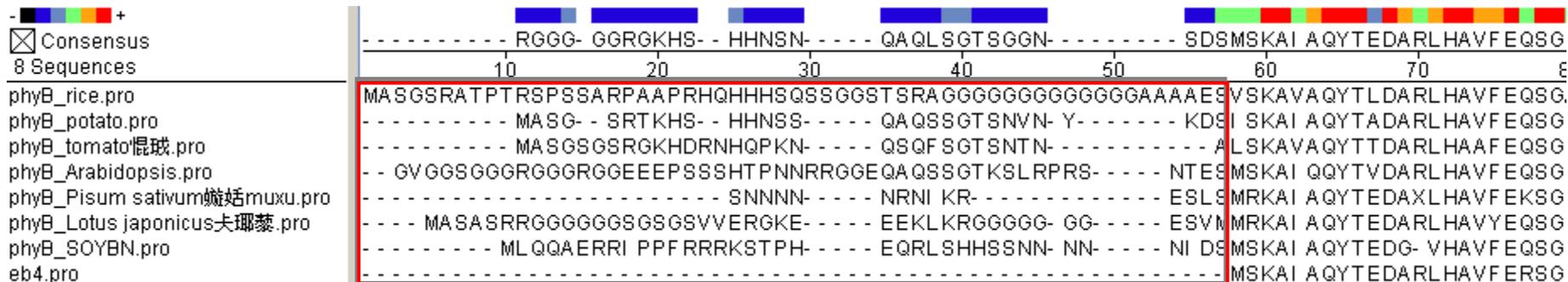


Fig. eb4 conserved domain search on NCBI



# Soybean phyB Gene Cloning

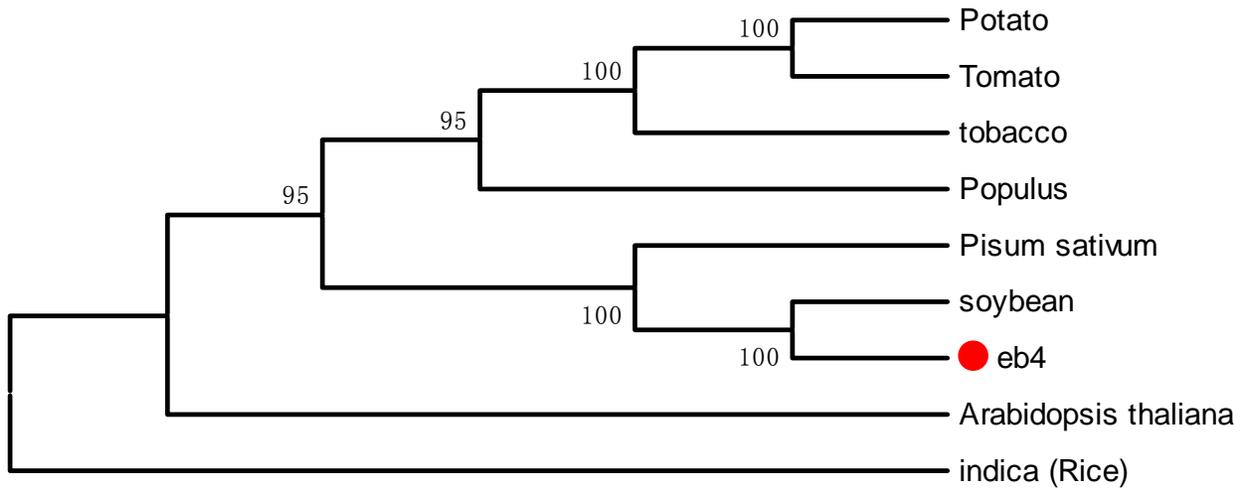
N-terminal, non-conserved domain?





# 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... | AGYEGLERLGMQFVGNQVAYITERRDFLFWFRSHTAKEIK  | Soybean       |
| eb4                                      | AGYEGAAASLGDAVCGMAVAYITERRDFLFWFRSHTAKEIK | My clone      |
| Pisum_sativum-gi 6502525 gb AAF14344.... | AGYEGAAASLGDAVCGMAVAYITERRDFLFWFRSHTAKEIK | Pea           |
| Potato-P34094 PHYB_SOLTU_Phytochrome...  | AGYEGAAASLGDAVCGMAVAYITSKDFLFWFRSHTAKEIK  | Other species |
| Tomato-Q9ZS62 PHYB1_SOLLC_Phytochrome... | AGYEGAAASLGDAVCGMAVAYITSKDFLFWFRSHTAKEIK  |               |
| tobacco-gi 2370331 emb CAA74992.1 _ph... | AGYEGAAALLGDAVCGMAVAYITSKDFLFWFRSHTAKEIK  |               |
| Populus-gi 10954091 gb AAG25725.1 AF3... | AGYEGAAASLGNVAVCGMAVAYITRRDFLFWFRSHTAKEIK |               |
| Arabidopsis_thaliana-P14713 PHYB_ARAT... | AGYEGAAALGDAVCGMAVAYITRRDFLFWFRSHTAKEIK   |               |
| indica_(Rice)-A2XFW2 PHYB_ORYSI_Phyto... | AGYEGAAALGDAVSGMAVAYITPSDYLFWFRSHTAKEIK   |               |



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

