

# The bioinformatic analysis of transcription factor SPL7 in *Arabidopsis thaliana*

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# Background

# CRR1 & SPL7

- ❑ The transcription factor **Copper response regulator1** (Crr1), containing a **SBP** (for SQUAMOSA promoter binding protein) domain, mediates this switching of photosynthesis machinery in response to **copper deficiency** and is also hypothesized to be somehow involved in copper sensing.
- ❑ **SPL7** is homologous to Copper response regulator1, the transcription factor that is required for switching between plastocyanin and cytochrome c6 in response to copper deficiency in *Chlamydomonas reinhardtii*.
- ❑ Squamosa promoter-binding-like protein 7 is a trans-acting factor that binds specifically to the consensus nucleotide sequence 5'-TNCGTACAA-3'.

# Structure of CRR1 and SPL7

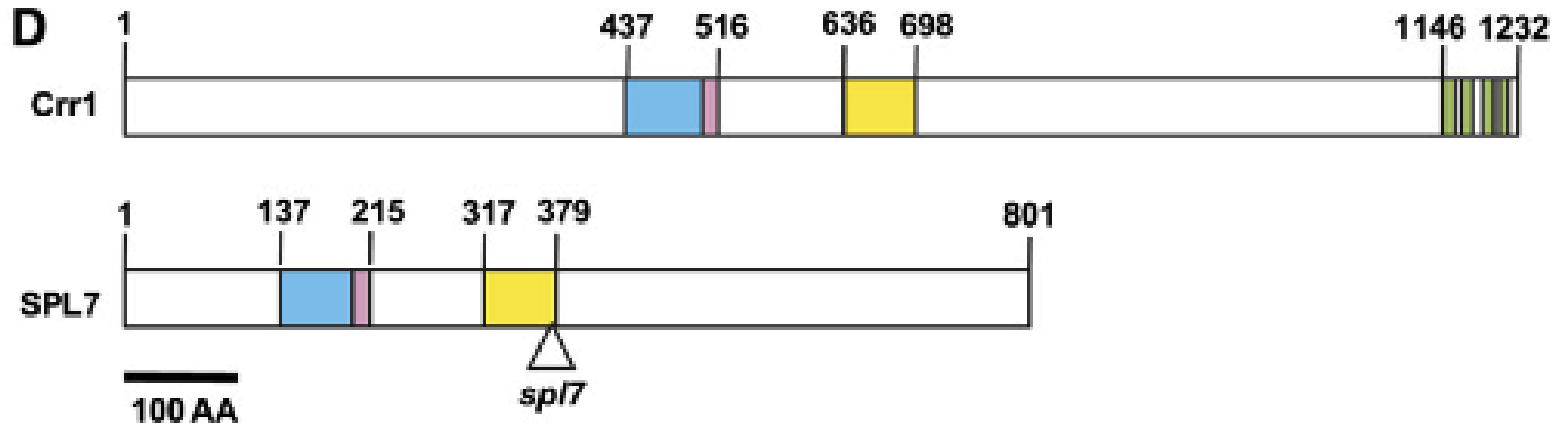
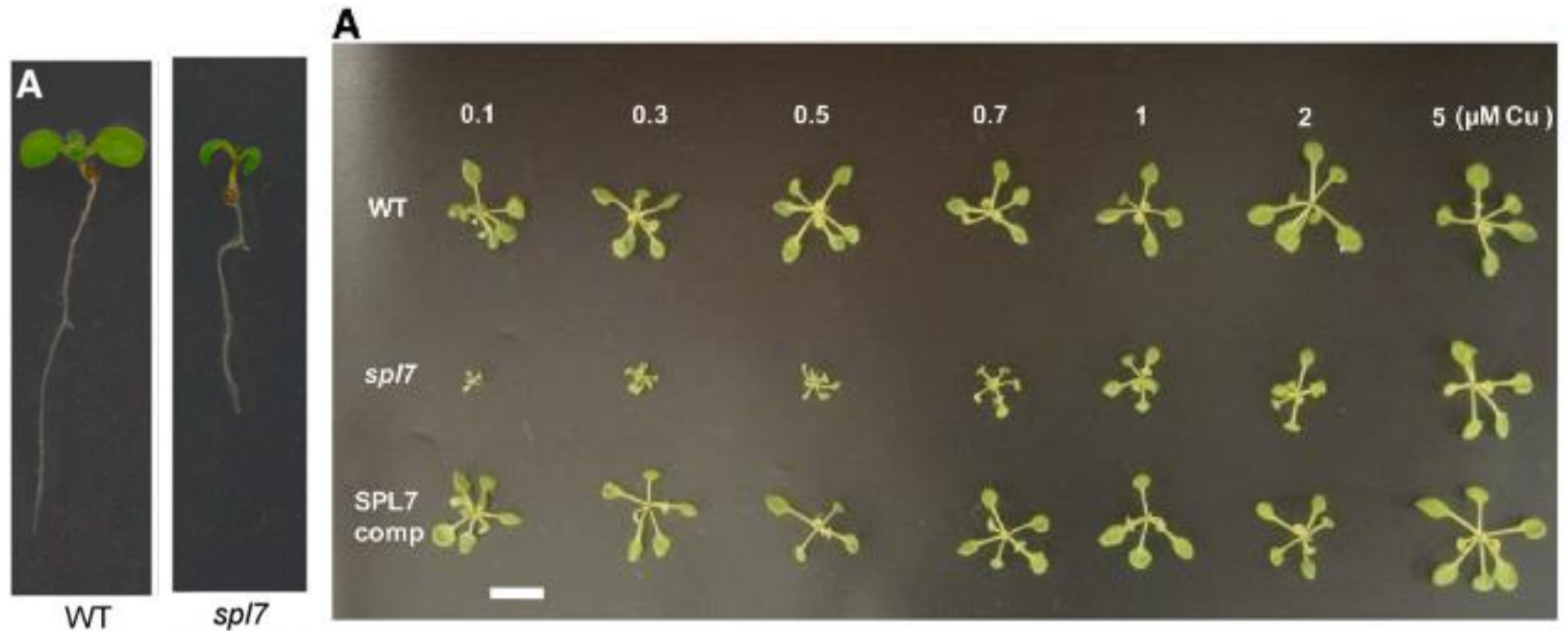


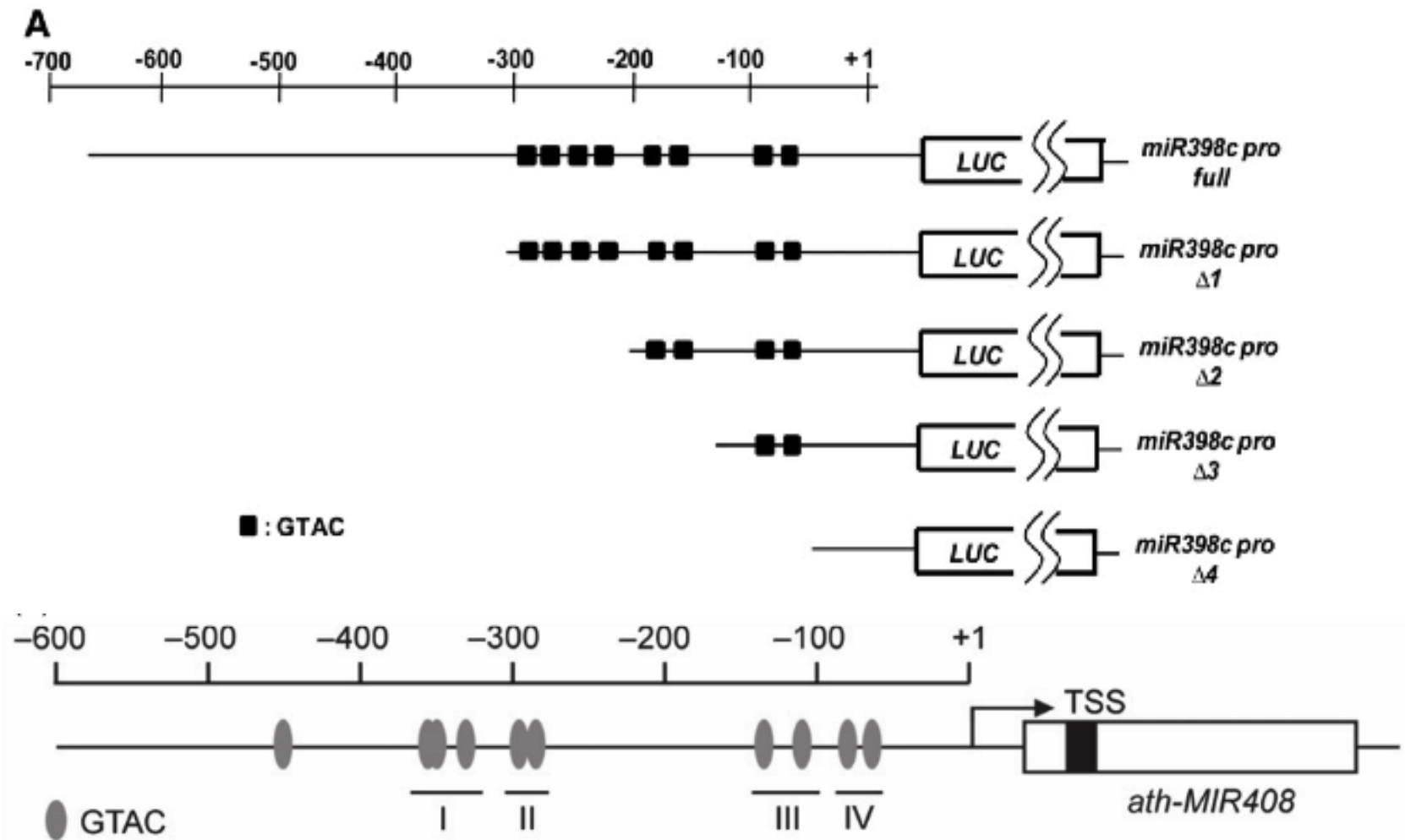
Diagram of the motif structures of Crr1 and SPL7. A triangle indicates the position from which the C-terminal part is truncated by the T-DNA insertion in *spl7*. The **blue boxes** indicate the SBP domain, and the **pink boxes** describe a putative nuclear localization signal. The **yellow boxes** show an unknown motif that is conserved between Crr1 and SPL7, and the **green boxes** indicate putative metal binding motifs.

# The *spl7* Phenotype in Various Concentrations of Copper

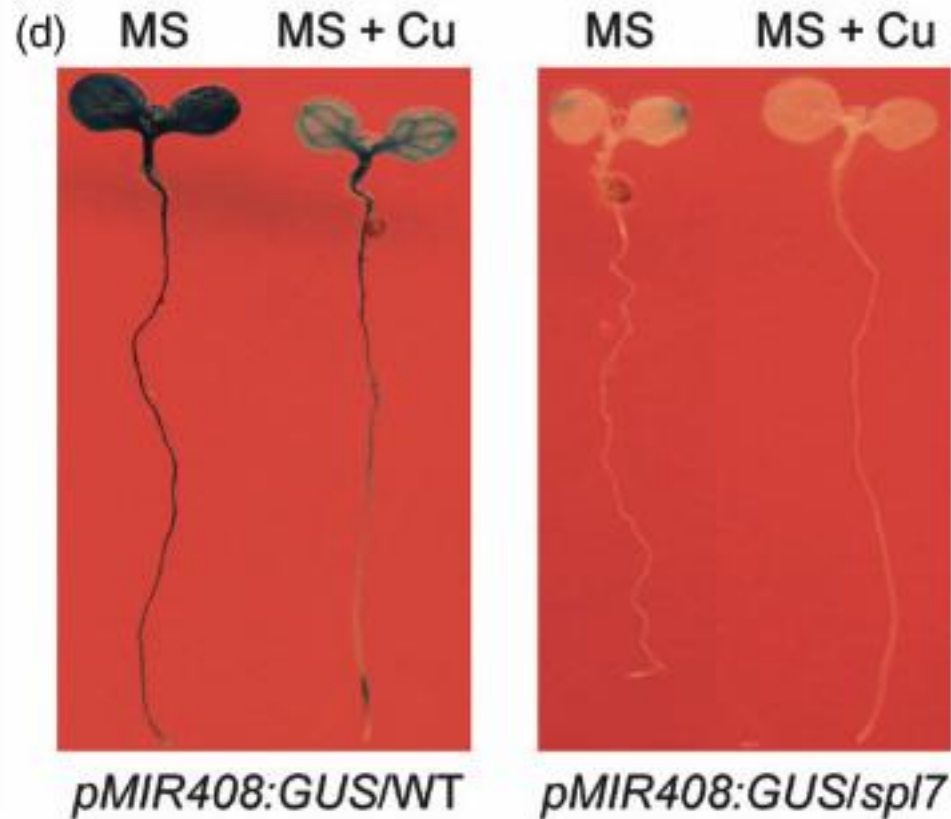


Growth phenotypes of the wild type, *spl7*, and SPL7 comp lines grown for 3 weeks on MS medium containing various concentrations of copper

SPL7 interacts directly with the miR398 promoter and miR408 promoter via its GTAC motifs.



# MIR408 is regulated by SPL7 at the transcription level



GUS staining of transgenic seedlings expressing the pMIR408:GUS reporter gene in the wild type (pMIR408:GUS/WT) or the *spl7* (pMIR408:GUS/*spl7*) background. Seedlings were grown under different copper regimes, as indicated.

# Gene analysis



# Gene information

RefSeq<sup>i</sup> NP\_197384.1. NM\_121888.2. [Q8S9G8-1]  
 NP\_850850.1. NM\_180519.1. [Q8S9G8-2]

## Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
2406	11591.5	2328/2406 (96.8%)	2328/2406 (96.8%)	78/2406 ( 3.2%)

```

AJ011613.1_cd 2001 GCATCAAAGCCCTATAGAGTCAAAGGTGAATCCTCCTTCGTCAGGTTGCT 2050
                |||
NM_180519.1_c 2001 GCATCAAAGCCCTATAGAGTCA----- 2022

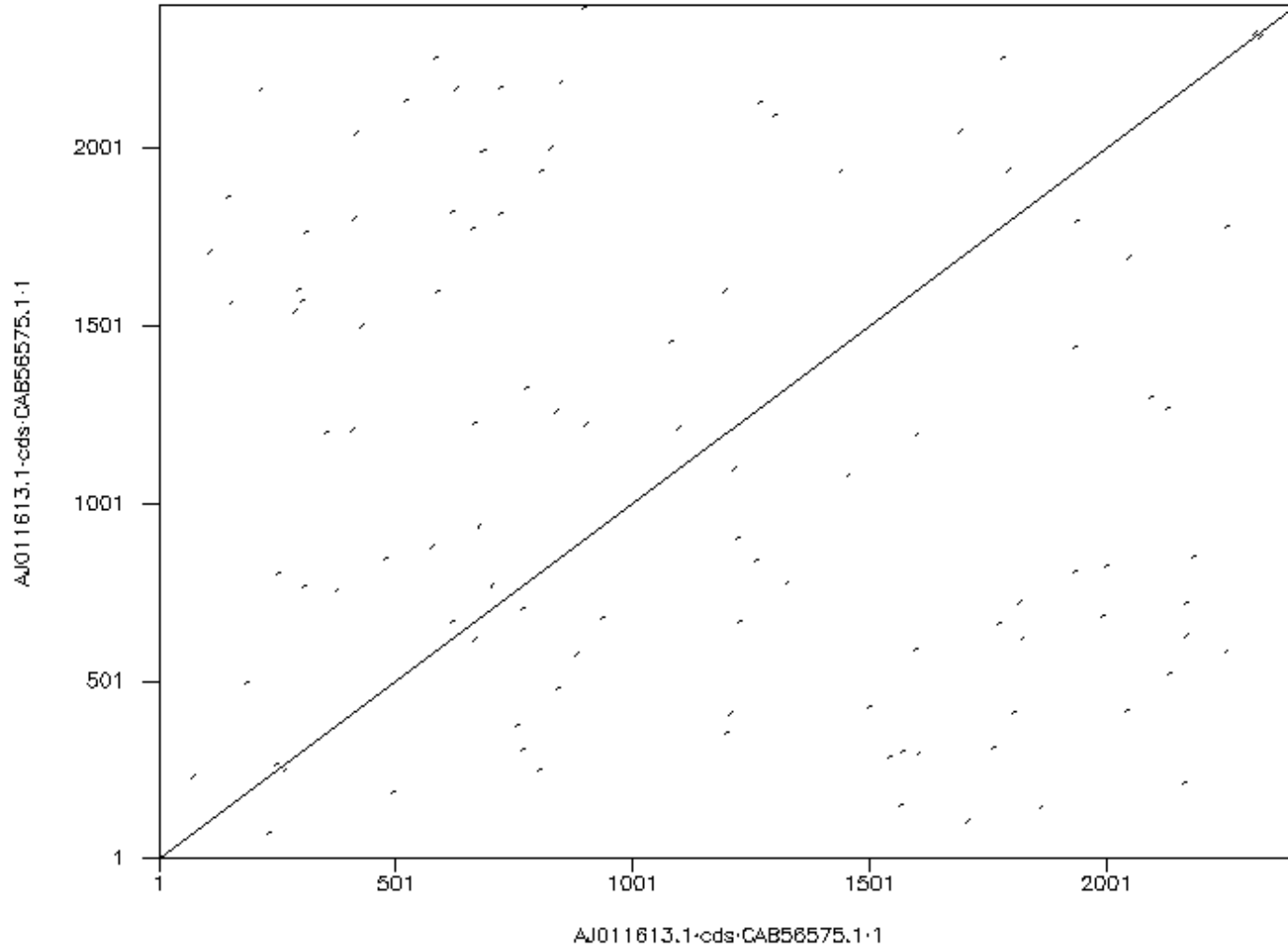
AJ011613.1_cd 2051 GTTGTGTGAGTAGTCAGAAGGACATACCATCAAGAATATTAAACTTCAAT 2100
NM_180519.1_c 2022 ----- 2022

AJ011613.1_cd 2101 AAGGATCCTGAAGCAGGATTAGATTGTAAAGAGAGAATACAGGCAGACTG 2150
                |||
NM_180519.1_c 2023 AAGGATCCTGAAGCAGGATTAGATTGTAAAGAGAGAATACAGGCAGACTG 2072
  
```

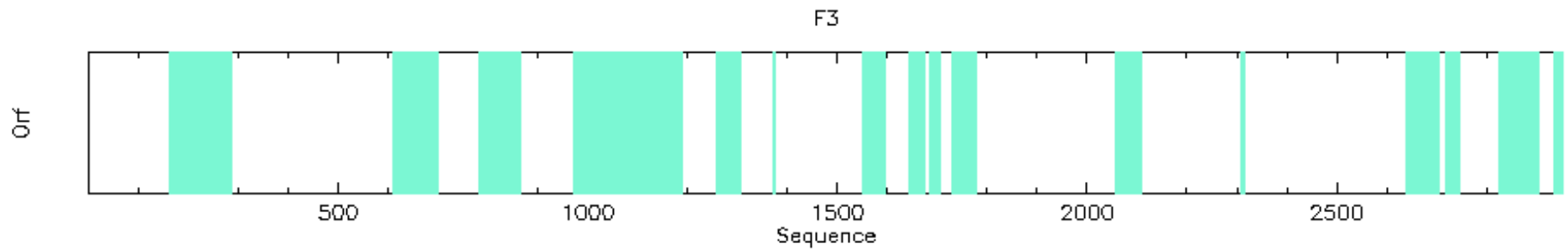
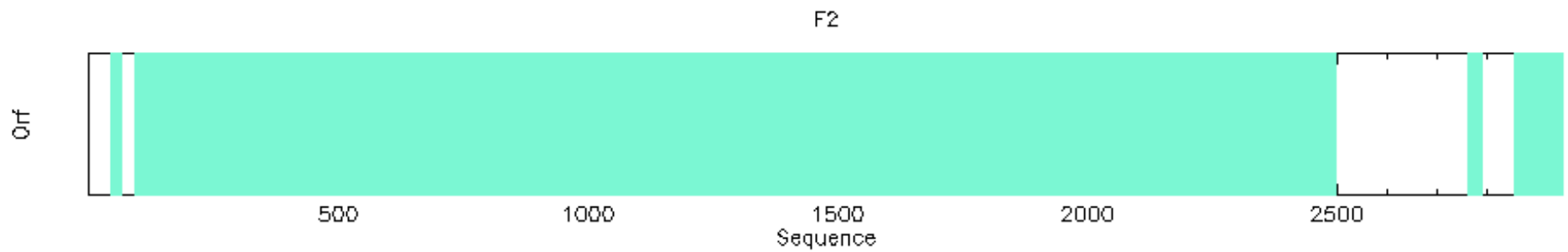
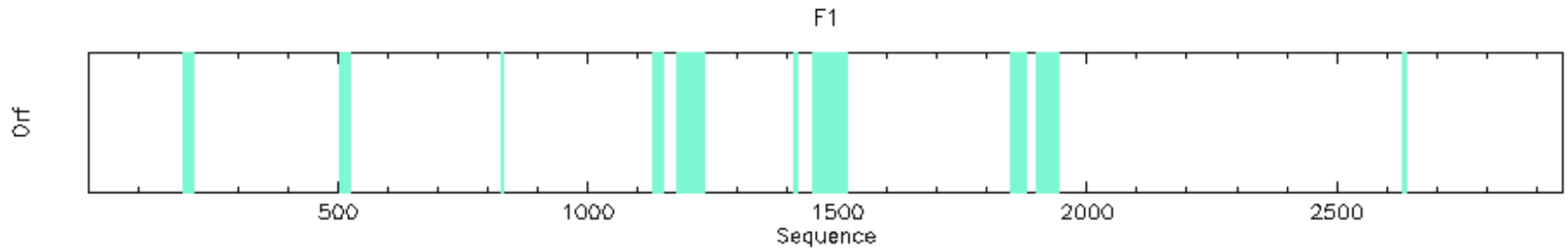
AT5G18830.1  
 AT5G18830.2



# Dot plot



# ORF prediction



# ORF verification

## Pairwise Alignment Result

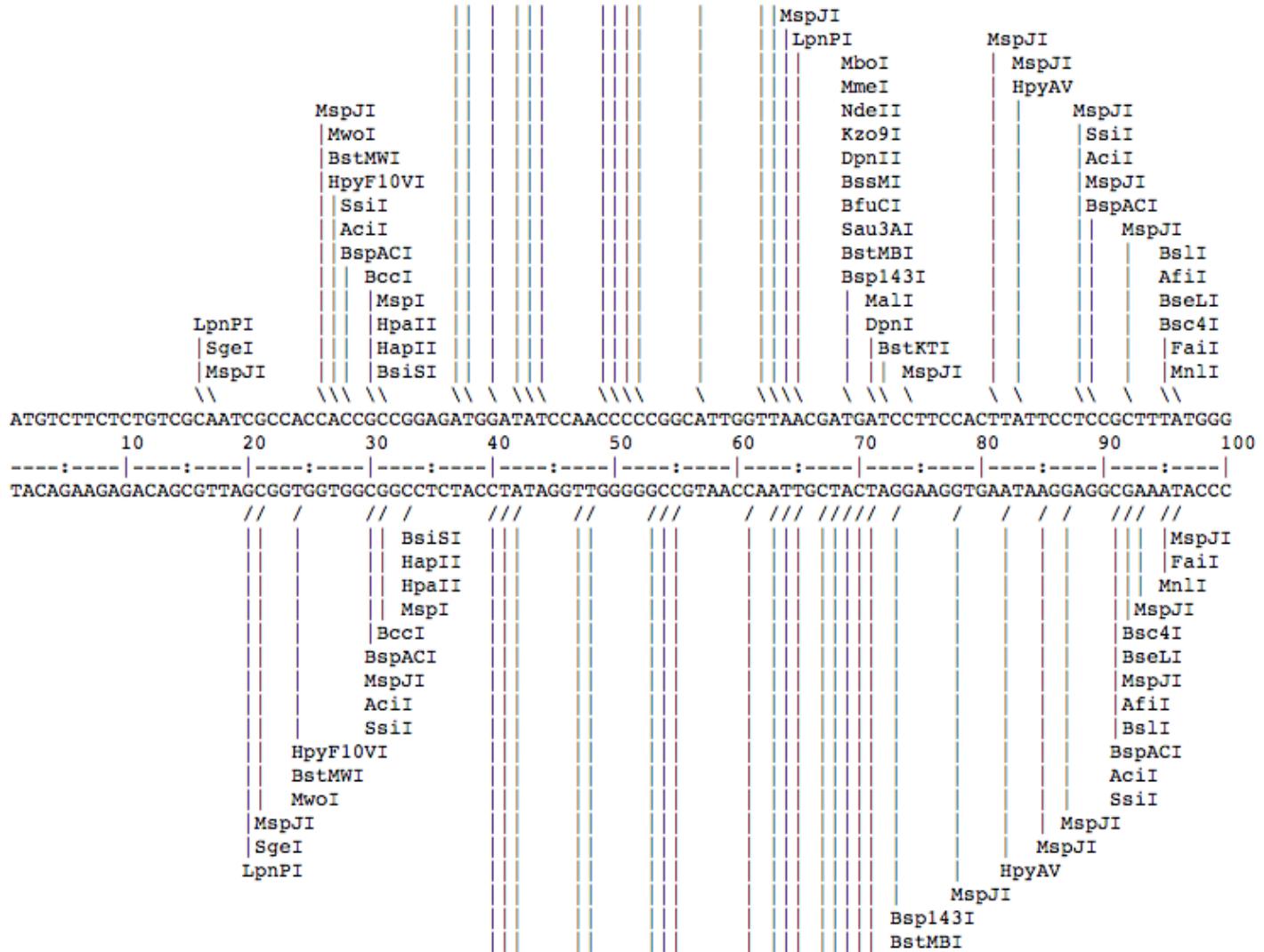
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
810	4272.0	801/810 (98.9%)	801/810 (98.9%)	9/810 ( 1.1%)

```

1 ILSEIEDSQMSSLSQSPPPEMDIQPPALVNDDPSTYSSALWDWGDLLDF      50
  |||
1 -----msslsqsppppemdiqppalvnndpstyssalwdwgdlldf      41

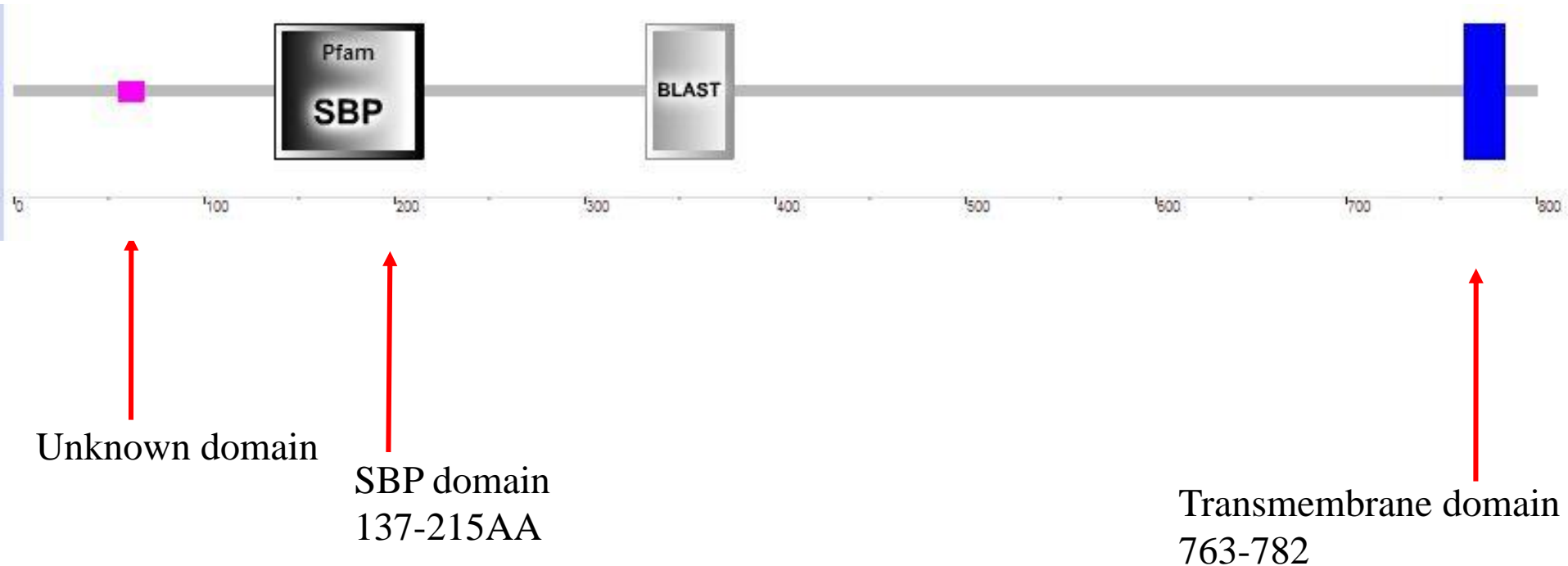
51 AADERLLVDQIHFPVLSPPPLPPLIPTQTPAESELDPSPEESGSGSDRVR    100
  |||
42 aaderllvdqihfppvlsppplppliptqtpaeseldpspeesgsgsdrv    91
  
```

# Digestion analysis

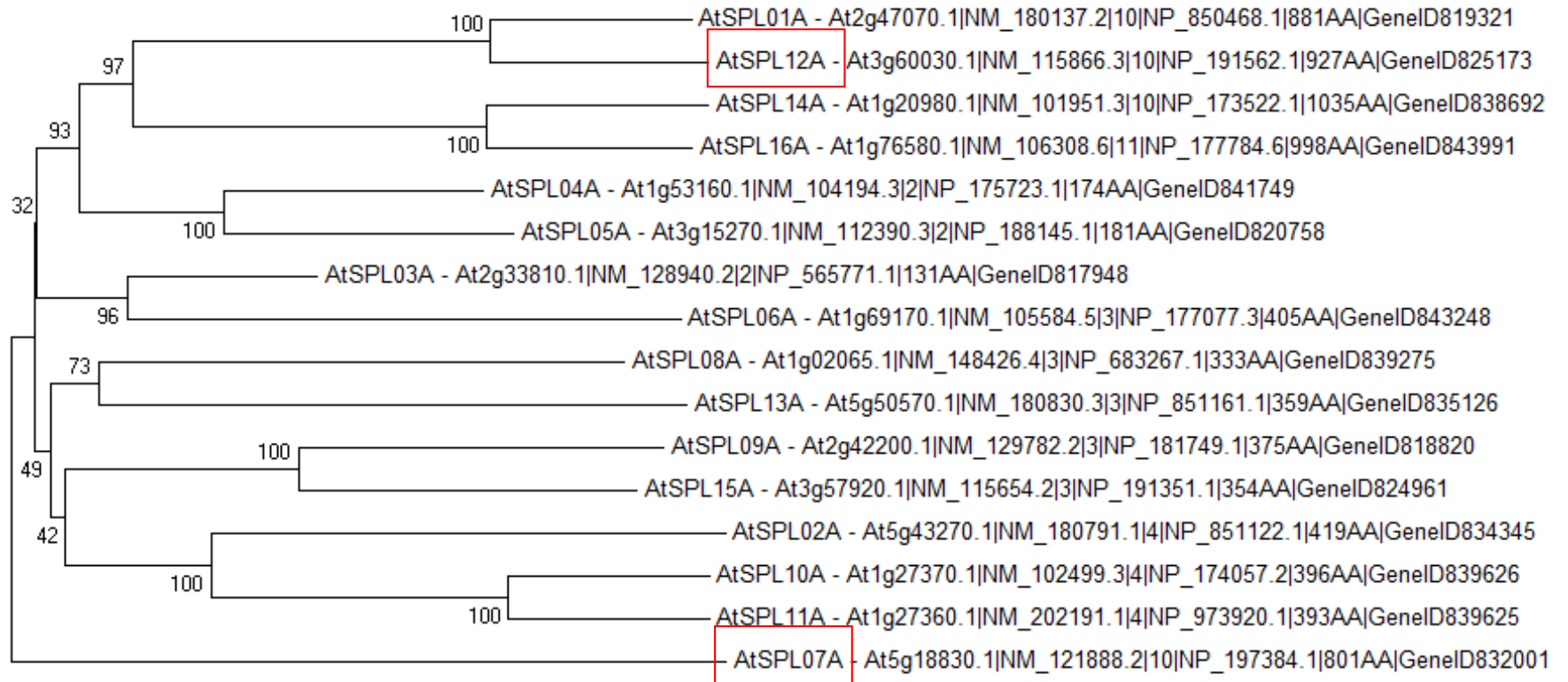


# Protein analysis

# SMART prediction



# Phylogenetic tree

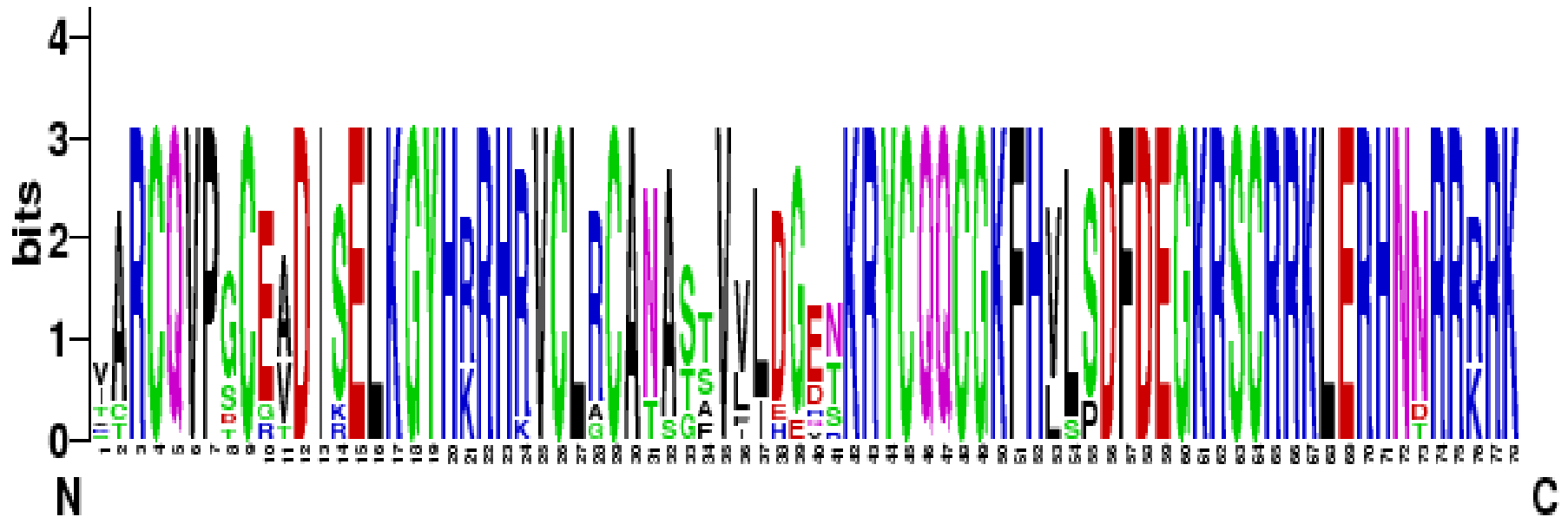


0.1

Mega6



# SBP/DNA-binding domain



135-212AA 锌指结构相当保守

# SBP/DNA binding domain



SPL7



SPL12

# DNA binding domain



SPL7与SPL12 DNA结合域的fit结果 :能够较好的fit上, 该结合域也相当保守

## Conclusion :

1. No metal binding domain ?
2. GTAC binding sequence in SBP domain?
3. Evolution analysis of SPL7?

# Main references

1. Yamasaki H et al. (2007) Regulation of copper homeostasis by microRNA in *Arabidopsis*. *J Biol Chem*. 282: 16369–16378.
2. Yamasaki H et al. (2009) SQUAMOSA promoter binding protein-like7 is a central regulator for copper homeostasis in *Arabidopsis*. *Plant Cell*. 21: 347–361.
3. Bernal M et al. (2012) Transcriptome sequencing identifies SPL7-regulated copper acquisition genes FRO4/FRO5 and the copper dependence of iron homeostasis in *Arabidopsis*. *Plant Cell*. 24: 738-761.
4. Zhang H, Li L. (2013) SQUAMOSA promoter binding protein-like7 regulated microRNA408 is required for vegetative development in *Arabidopsis*. *Plant J*. 74: 98–109.
5. Gayomba SR et al. (2013) The CTR/COPT-dependent copper uptake and SPL7-dependent copper deficiency responses are required for basal cadmium tolerance in *A. thaliana*. *Metallomics*. 5: 1262-1275.
6. Zhang H et al. (2014) MicroRNA408 Is Critical for the HY5-SPL7 Gene Network That Mediates the Coordinated Response to Light and Copper. *Plant Cell*. 5(9):1262-75.

# Acknowledgement

