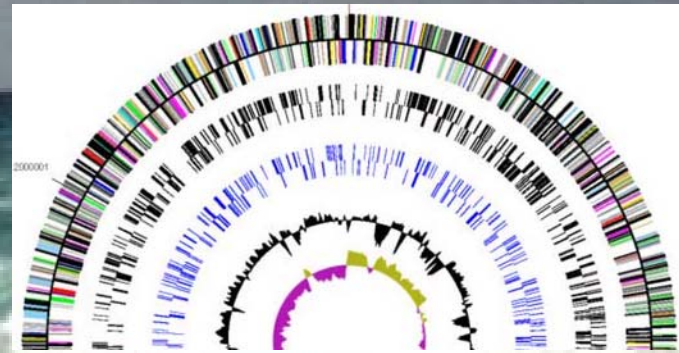
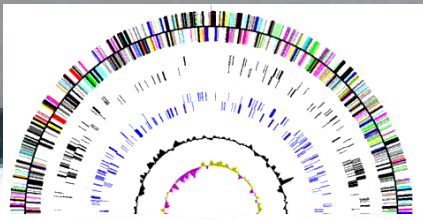


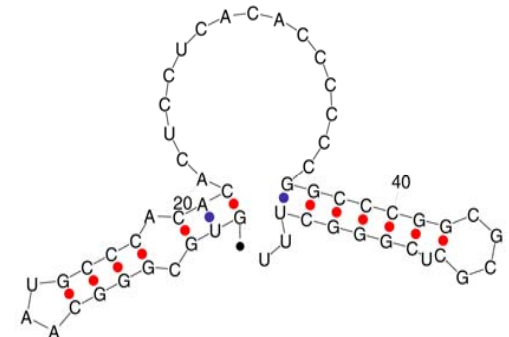
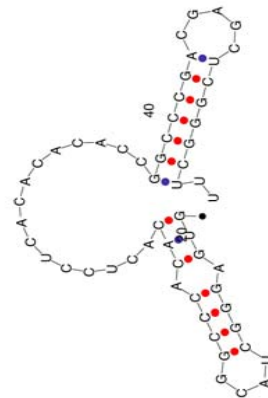
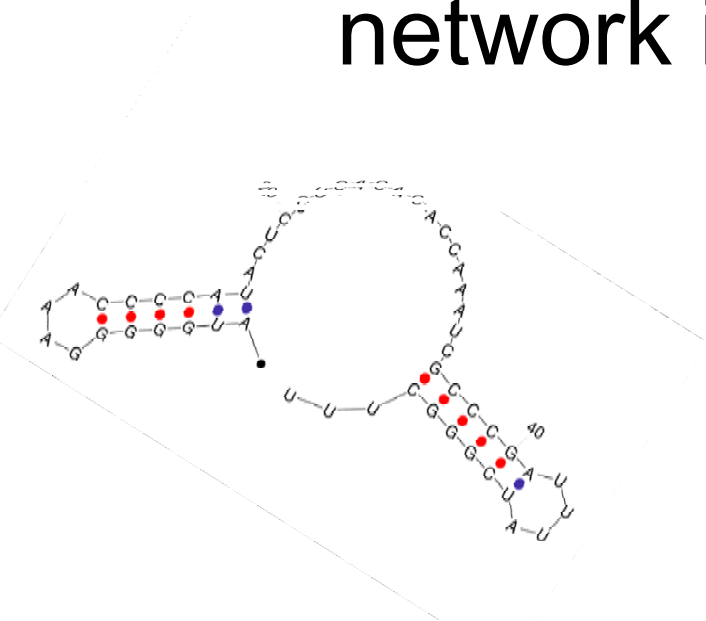
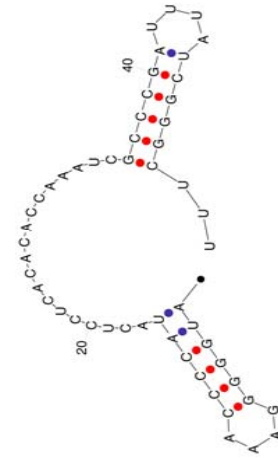
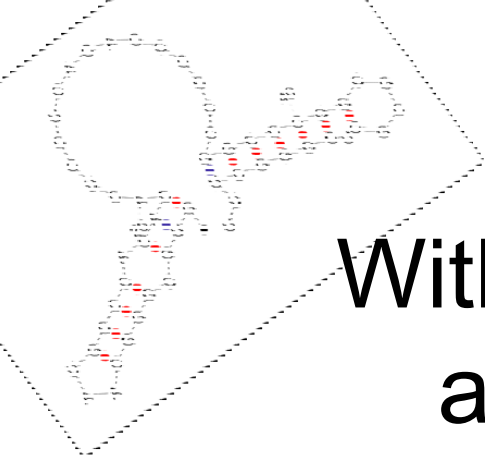
*Analyses of regulatory RNA in cyanobacteria for a systems biology level of understanding*

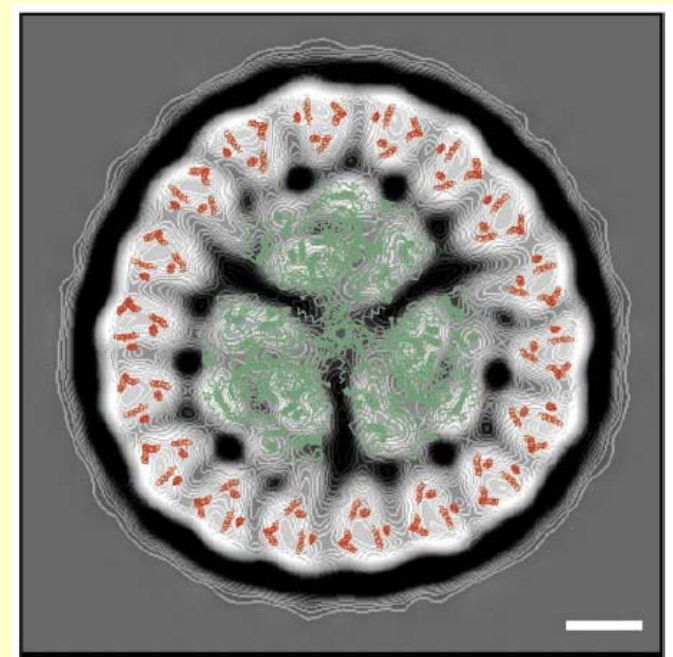
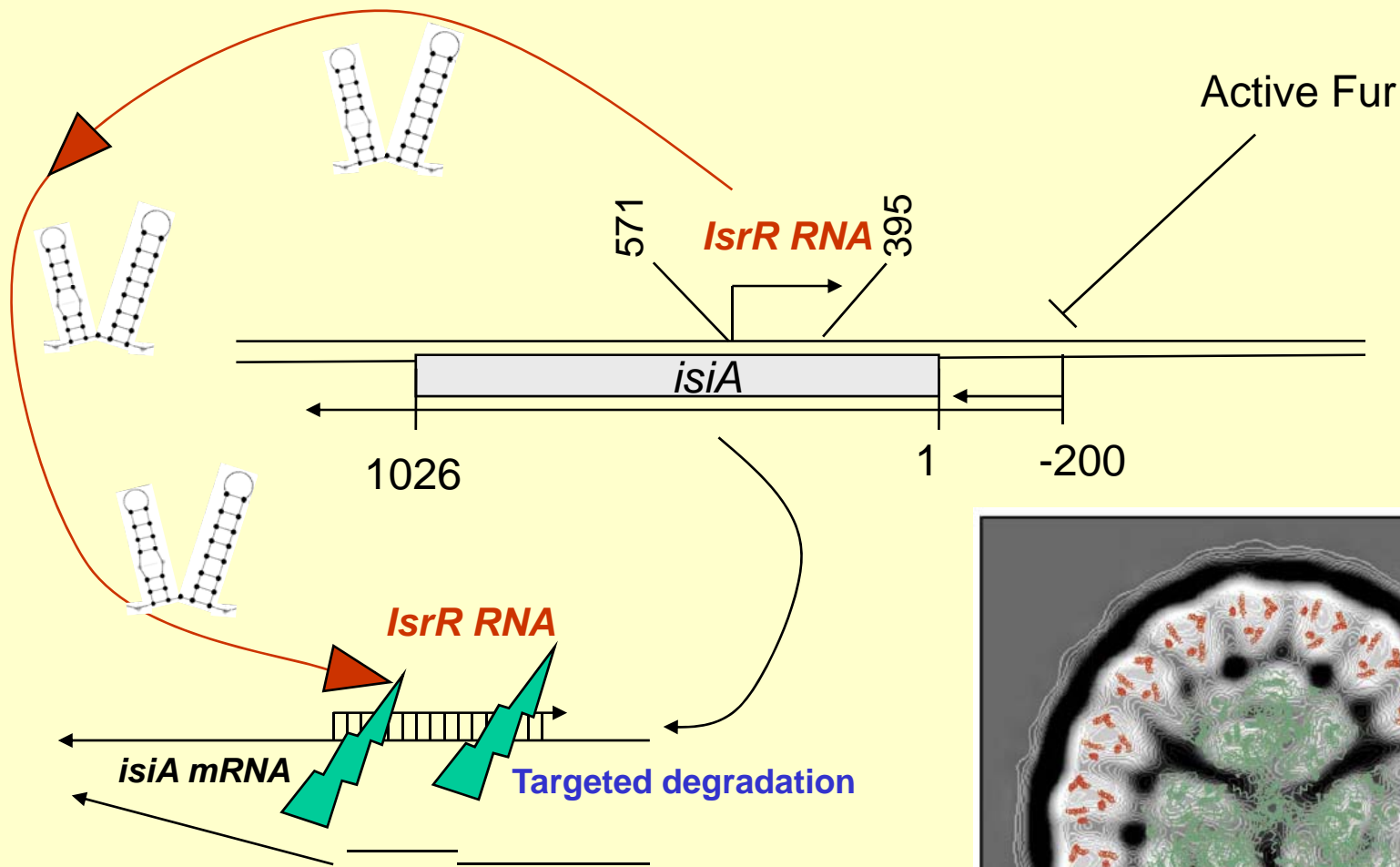






Without detailed information about the numbers and functions of these regulators it is not possible to understand the intracellular regulatory network in all its complexity.





Bibby et al.  
 NATURE | VOL 412 | 16 AUGUST 2001 |

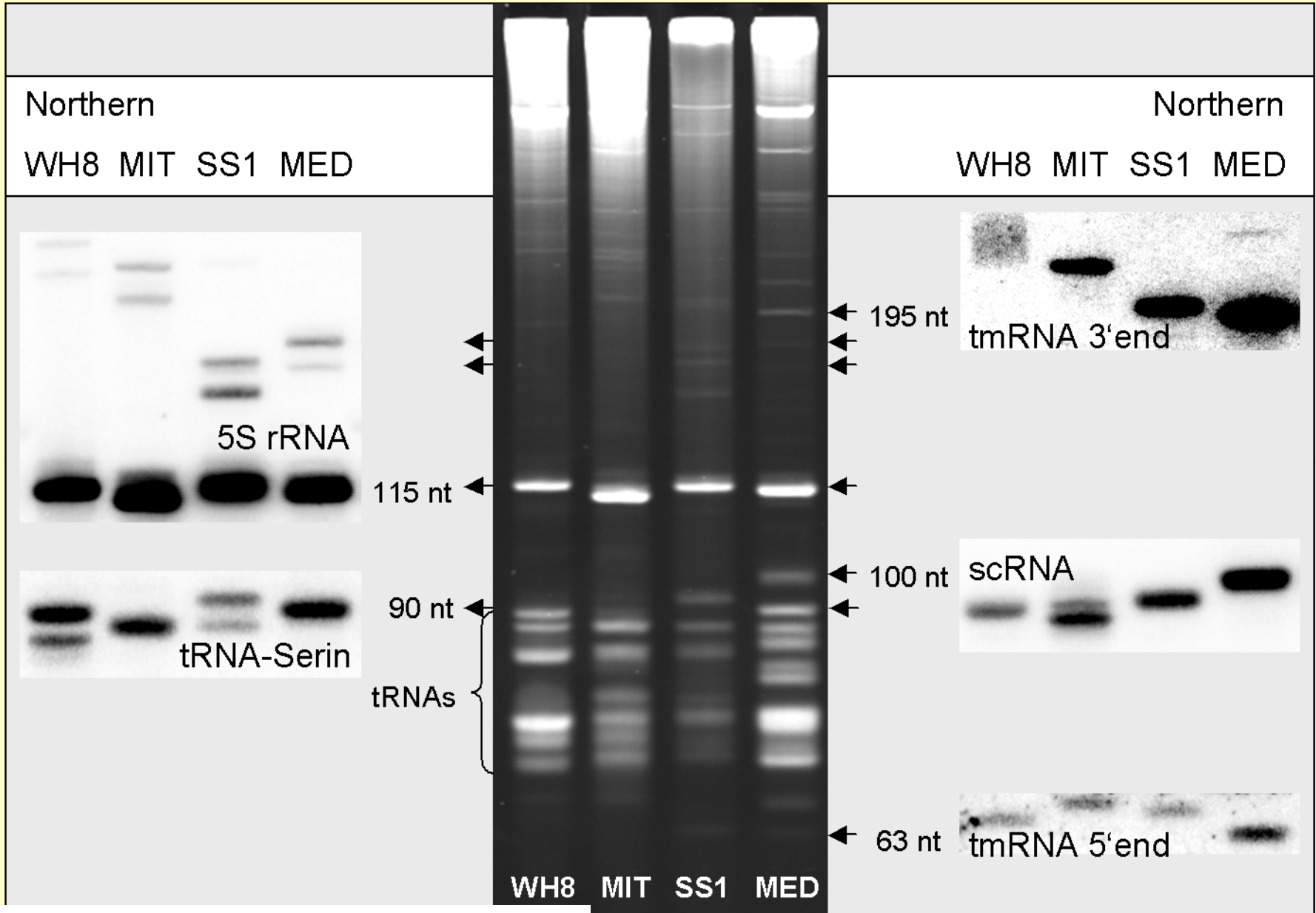
antisense RNA IsrR  
 in *Synechocystis* PCC 6803

## Other examples for naturally occurring antisense RNAs in cyanobacteria:

Csiszàr K.: Transcriptional analysis of the cyanobacterial *gvpABC* operon in differentiated cells: occurrence of an antisense RNA complementary to three overlapping transcripts. *Gene* (1987) 60, 29-37.

José A. Hernández: Identification of a *furA cis* Antisense RNA in the Cyanobacterium *Anabaena* sp. PCC 7120. *J. Mol. Biol.* (2006) 355, 325–334.

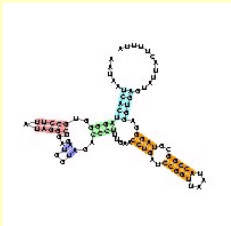
# Small RNAs in 4 strains of marine cyanobacteria



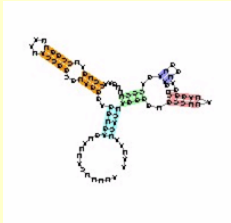
.....if it goes so well,  
how many more are there ?



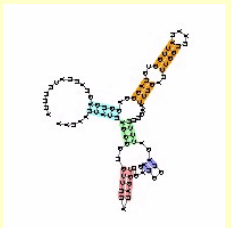
# How to find regulatory RNAs?



Prediction using bioinformatic algorithms and validation in experiments

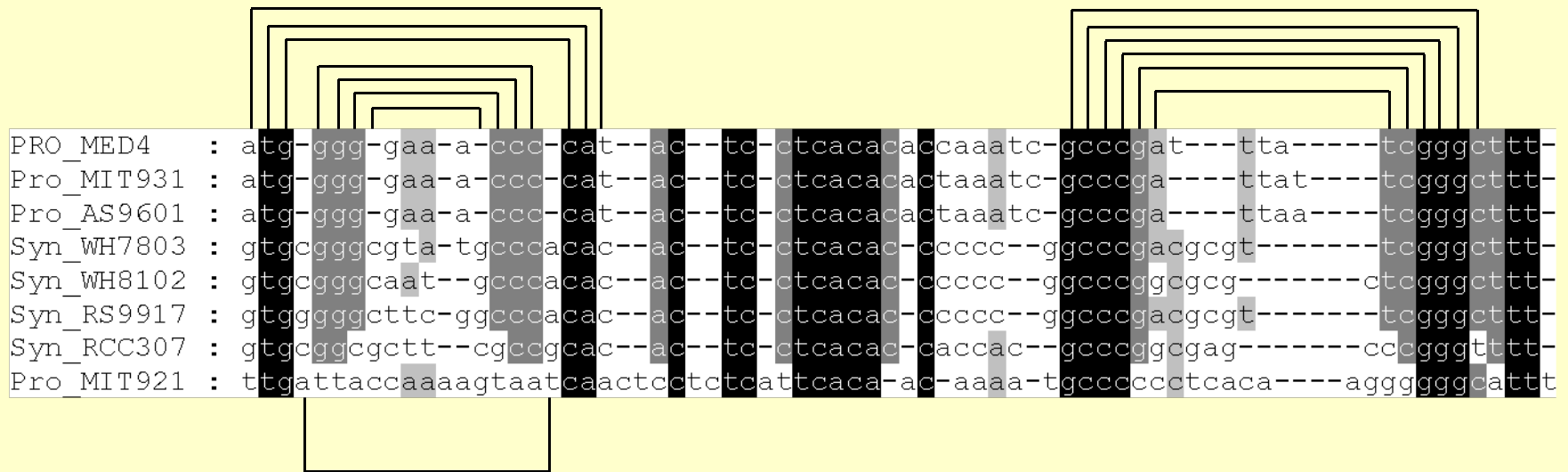


Identification via microarrays



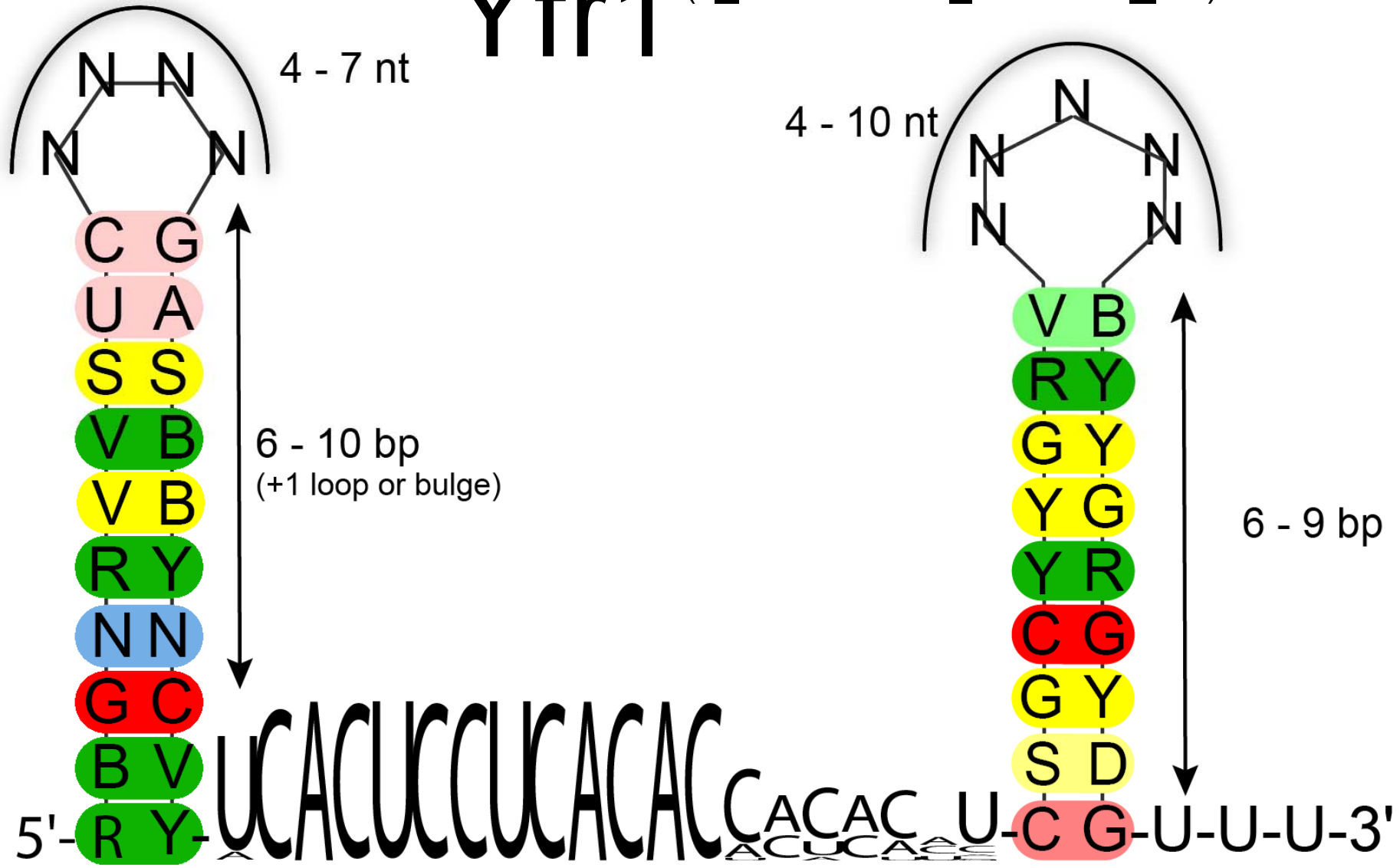
Isolation and sequence analysis of all small RNAs („RNomics“, 454)

# Complementary mutations indicate non-coding RNAs



Comparison of 4 *Prochlorococcus* and 4 *Synechococcus*

# Yfr1 (cYanobacterial Functional RNA)



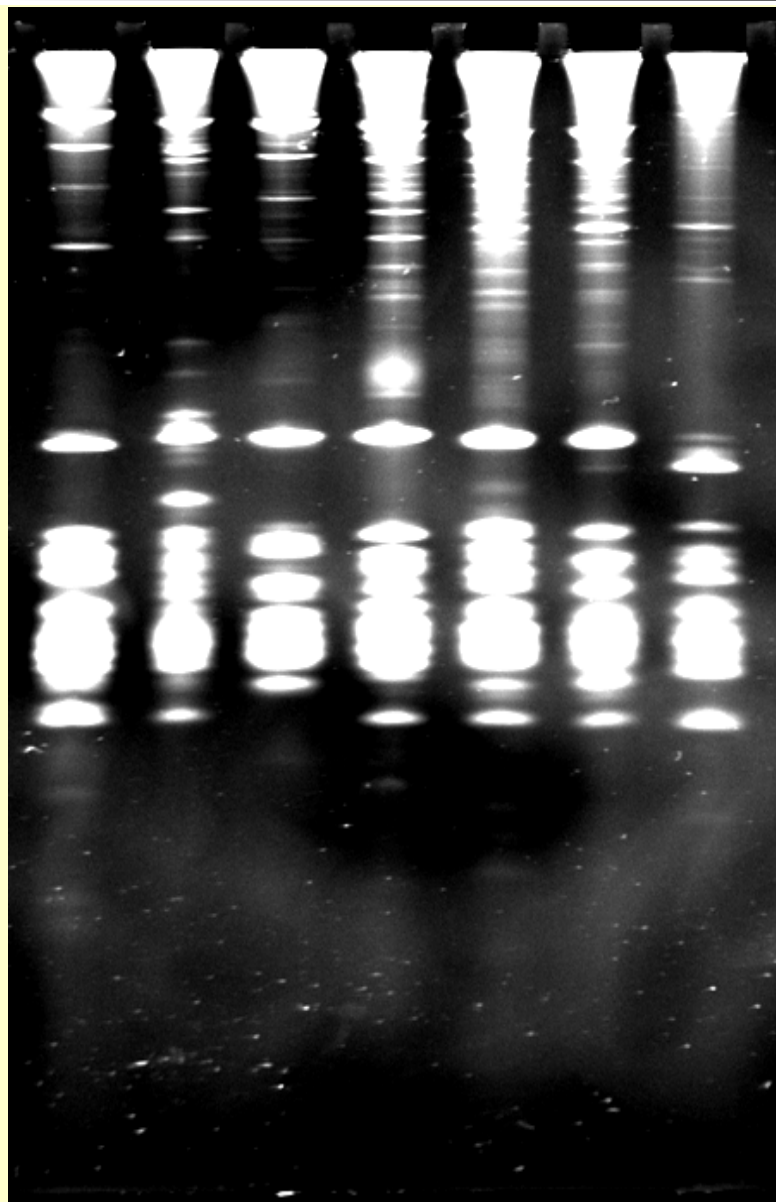
Sequence/structure model for Yfr1 of 31 cyanobacteria.

Voss et al., (2007) *BMC Genomics* 8:375,

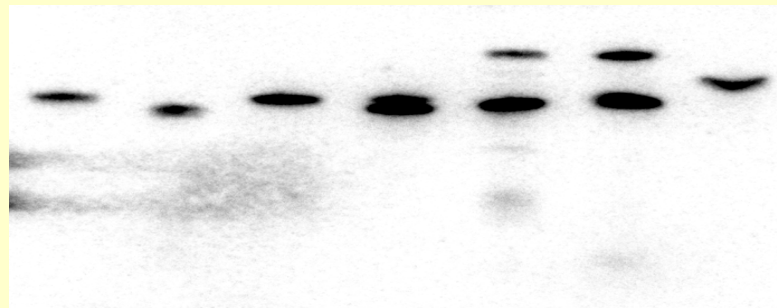
(R: A or G; Y: C or U; M: A or C; S: G or C; B: G, U or C; V: G, C or A; D: G, T or A).  
 Colors: number of different base pairs at this position (red = 1, yellow = 2, green = 3 and blue = 4 or more).  
 Shading: frequency of base pairing.

**A**

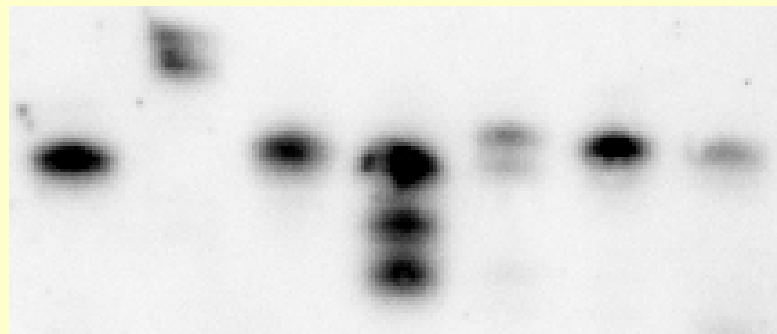
Syn.	Tsyn.	Sync.	Mic.	Nos.	Nos.	Gloe.
7942	elong.	6803	7806	7120	punct.	7421

**B**

Syn.	Tsyn.	Sync.	Mic.	Ana.	Nos.	Gloe.
7942	elong.	6803	7806	7120	punct.	7421

**6S RNA**

225

**Yfr1**

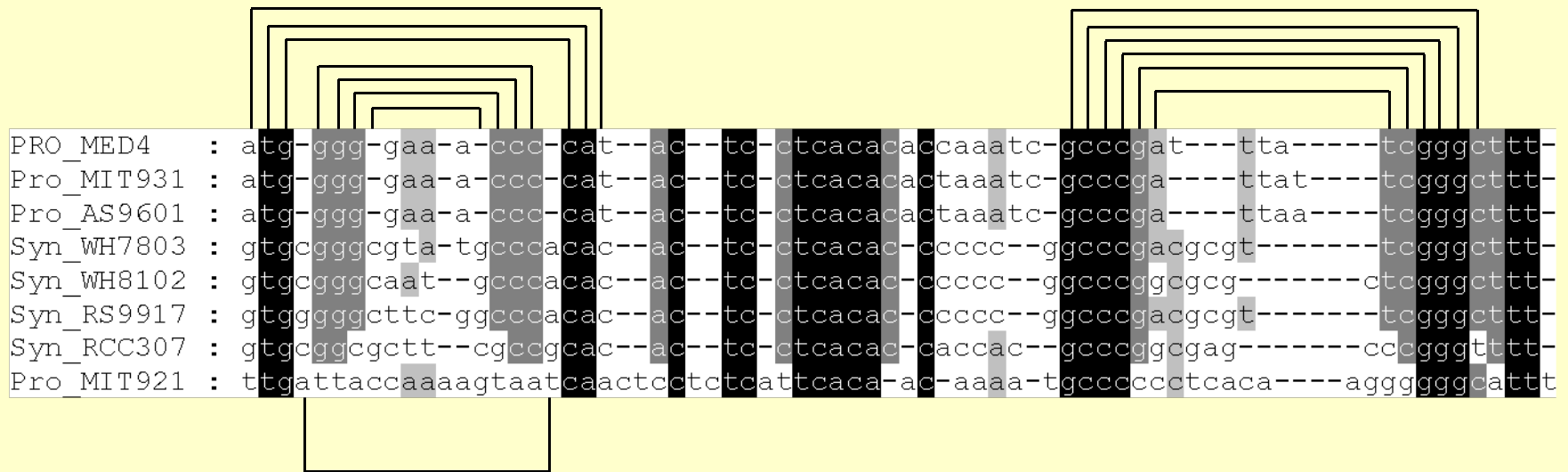
69

65

62

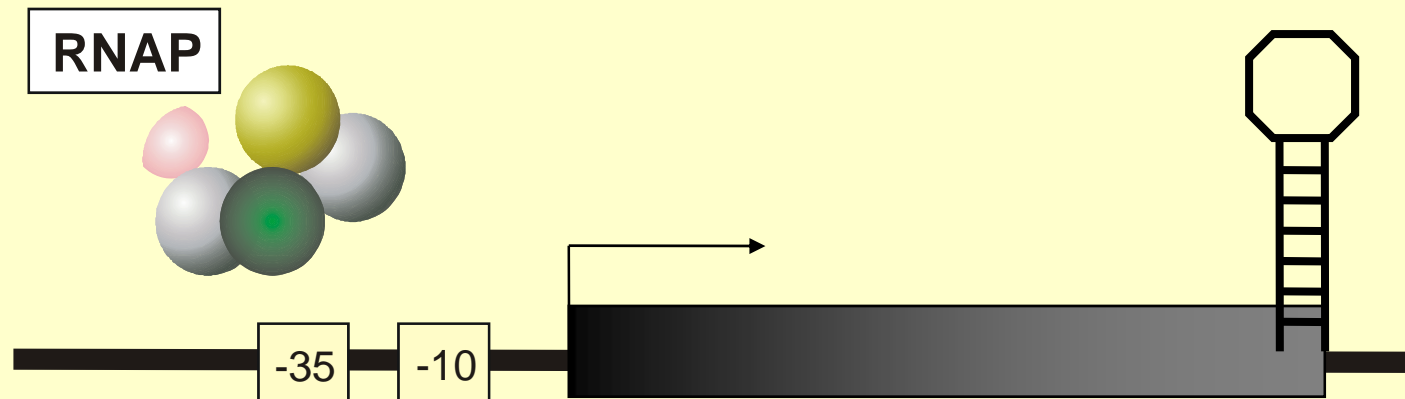
63

# Complementary mutations indicate non-coding RNAs

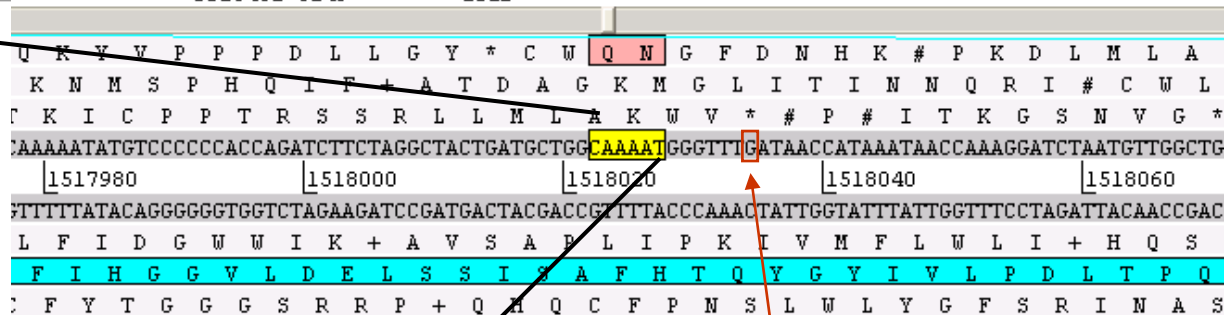
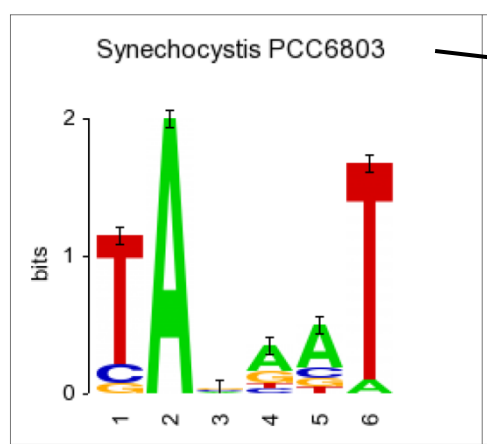


Comparison of 4 *Prochlorococcus* and 4 *Synechococcus*

# Prediction based on transcriptional signals



# Sequence and transcript start of IsrR



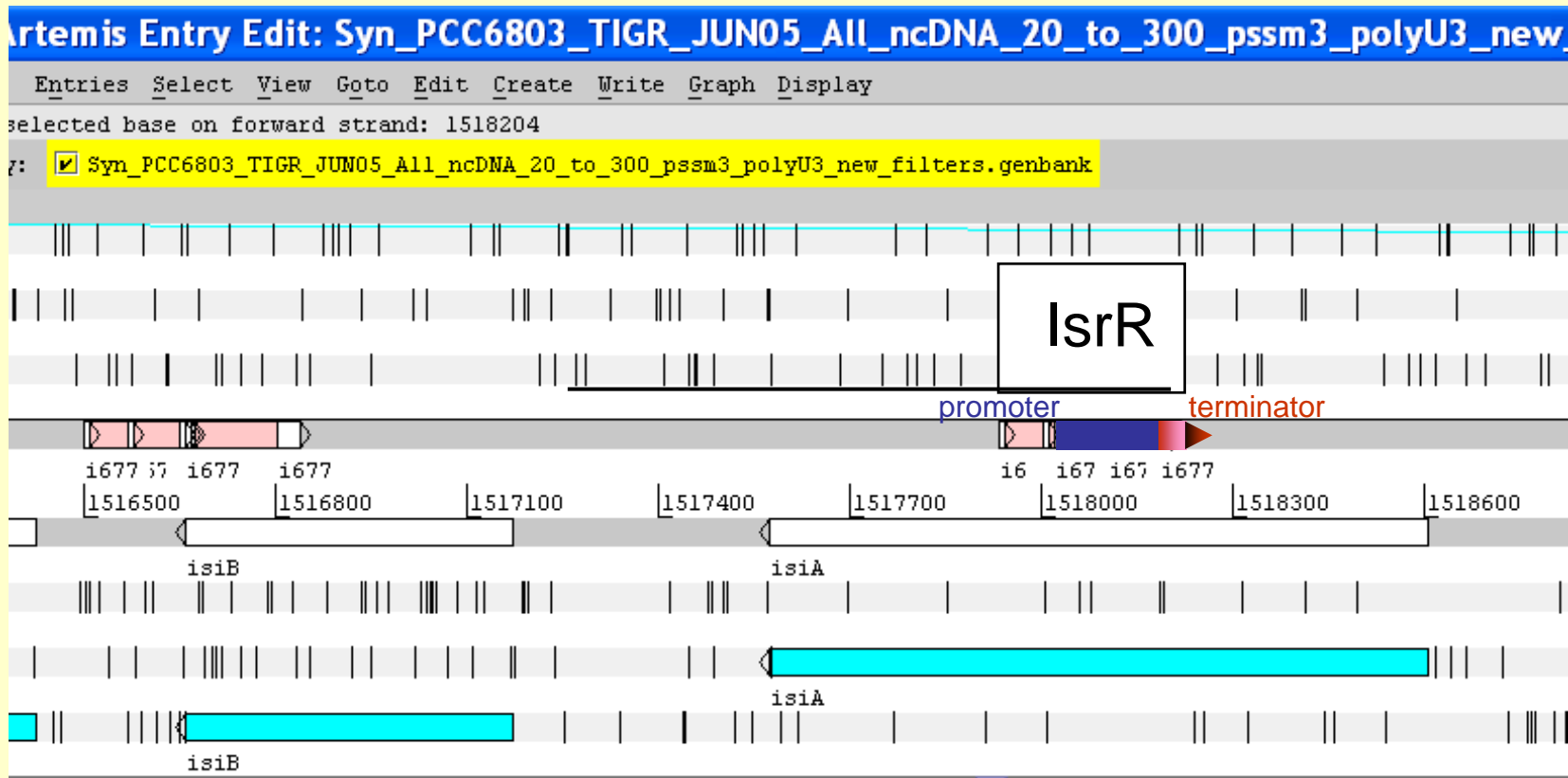
First transcribed nt:  
**G**AATAACCATAAATAACCAAAGGATCTAATGTTGGCTGA  
 CTGACTAGGCGCACCGTTTGGGTGGTGGCGTCGTACA  
 ATCCCCCAATACATGGCTTTGCCTGCCAACAGTAGA  
 GCTCCGGCCCCCAGGAACAGGAGATGATGACCCAAAA  
 TTATCCCTAGTTGCTGGGGGTTTTCCC

	-12	-11	-10	-9	-8	-7
A	-3.26	<b>1.82</b>	<b>-0.09</b>	<b>0.98</b>	<b>1.19</b>	-1.68
C	<b>-0.83</b>	-3.15	0.02	-0.83	-0.57	-3.15
G	-1.57	-3.15	0.43	0.02	-0.83	-3.15
T	1.54	-3.27	-0.46	-1.27	-1.27	<b>1.73</b>

PSSM values >2 indicate functional -10 box elements. Max. value: **+7.69** (Bantscheff et al., 2008 in prep.)

4.8 ! Almost perfect -10 box 15

# Genome-wide prediction based on transcriptional signals

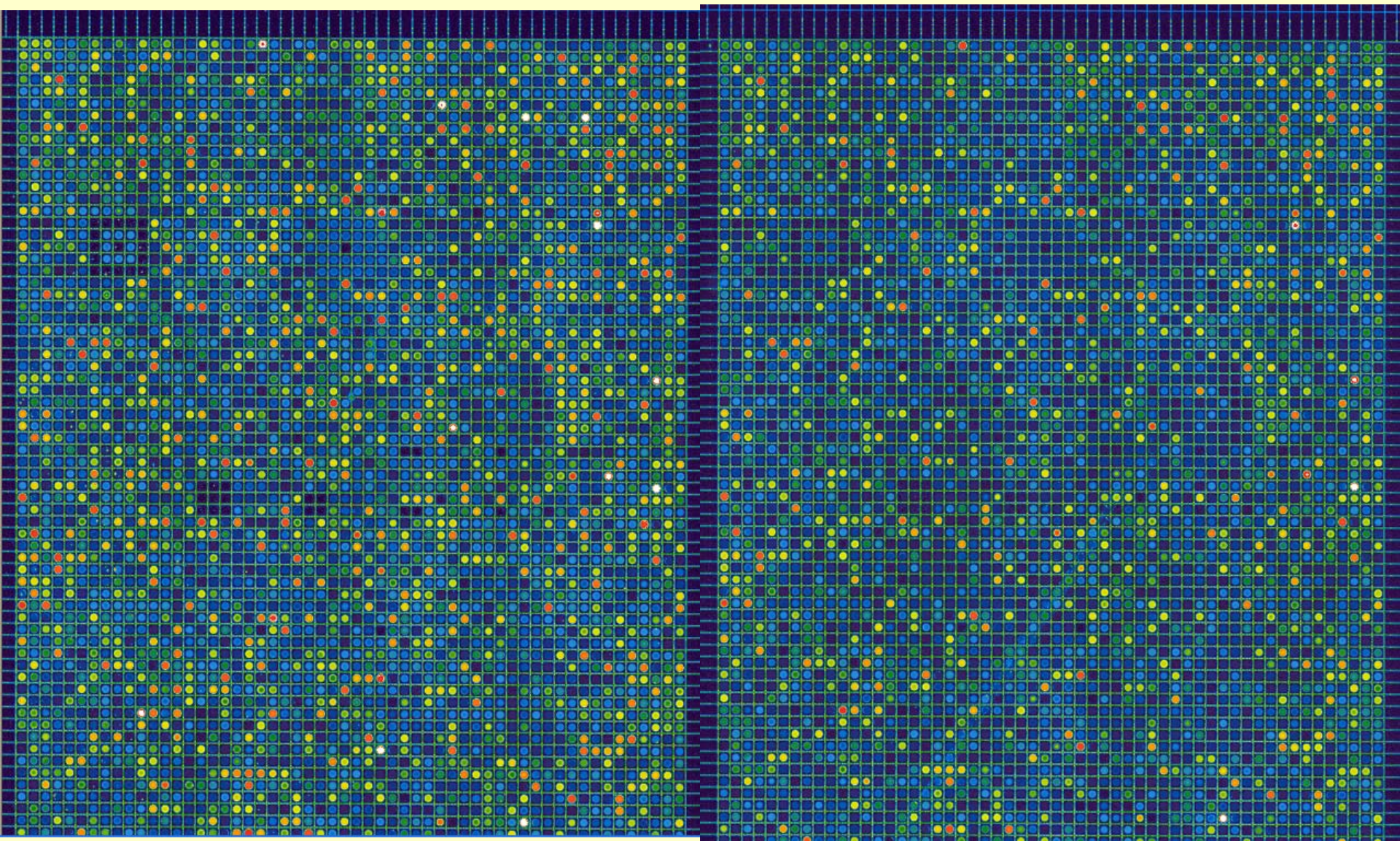


**369** predicted transcripts within all non-annotated sequences (intergenic and antisense). Only 42 have no TATA element (**327**); if -35 element is considered: **323**; very strong criteria for -35: **301**.  
**Weakening terminator rule by 1 mismatch or bulge: 714.**



„Intergenic spacer only“ array *Synechococcus* WH 7803

(tiling factor =11, sense + antisense); no protein-coding genes; Format: 12K

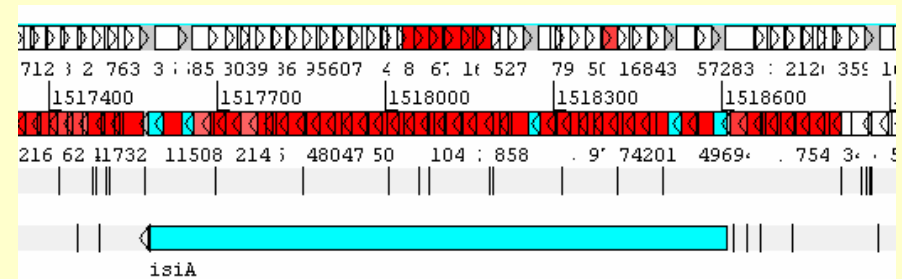
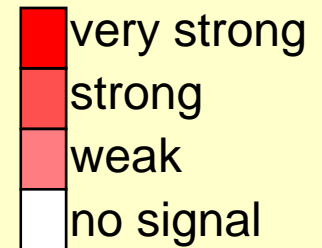
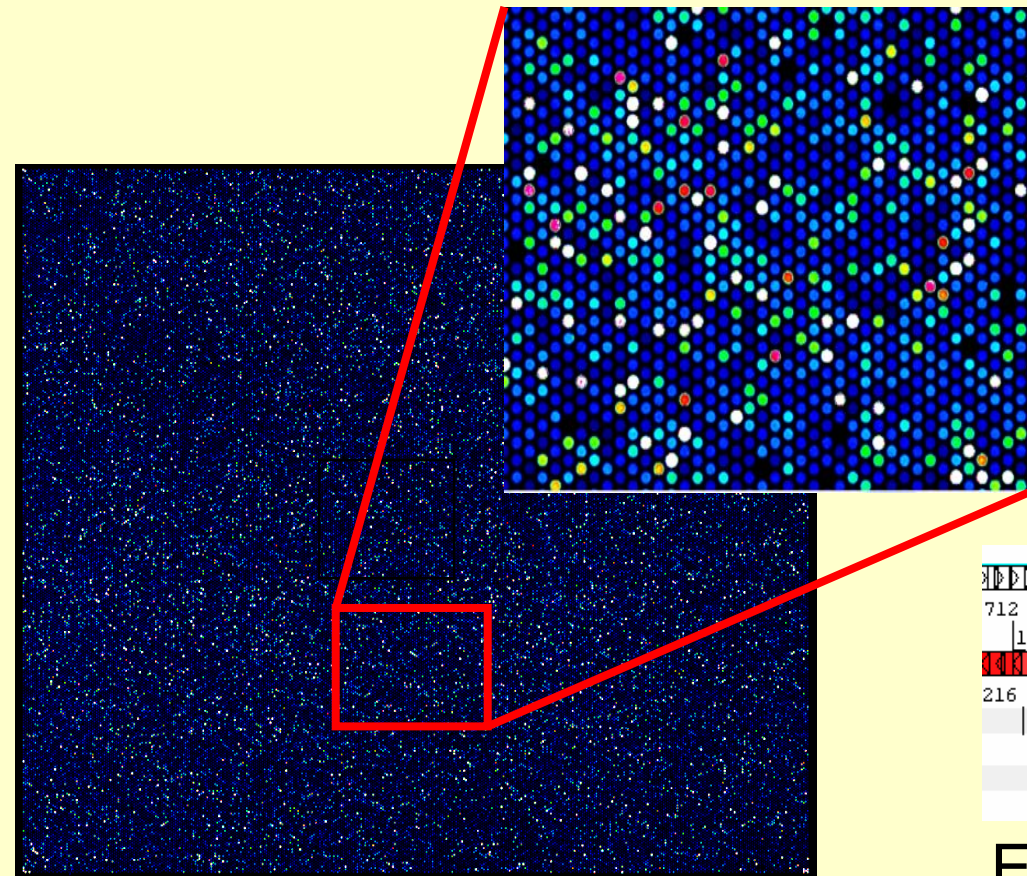


cold stress RNA

control RNA

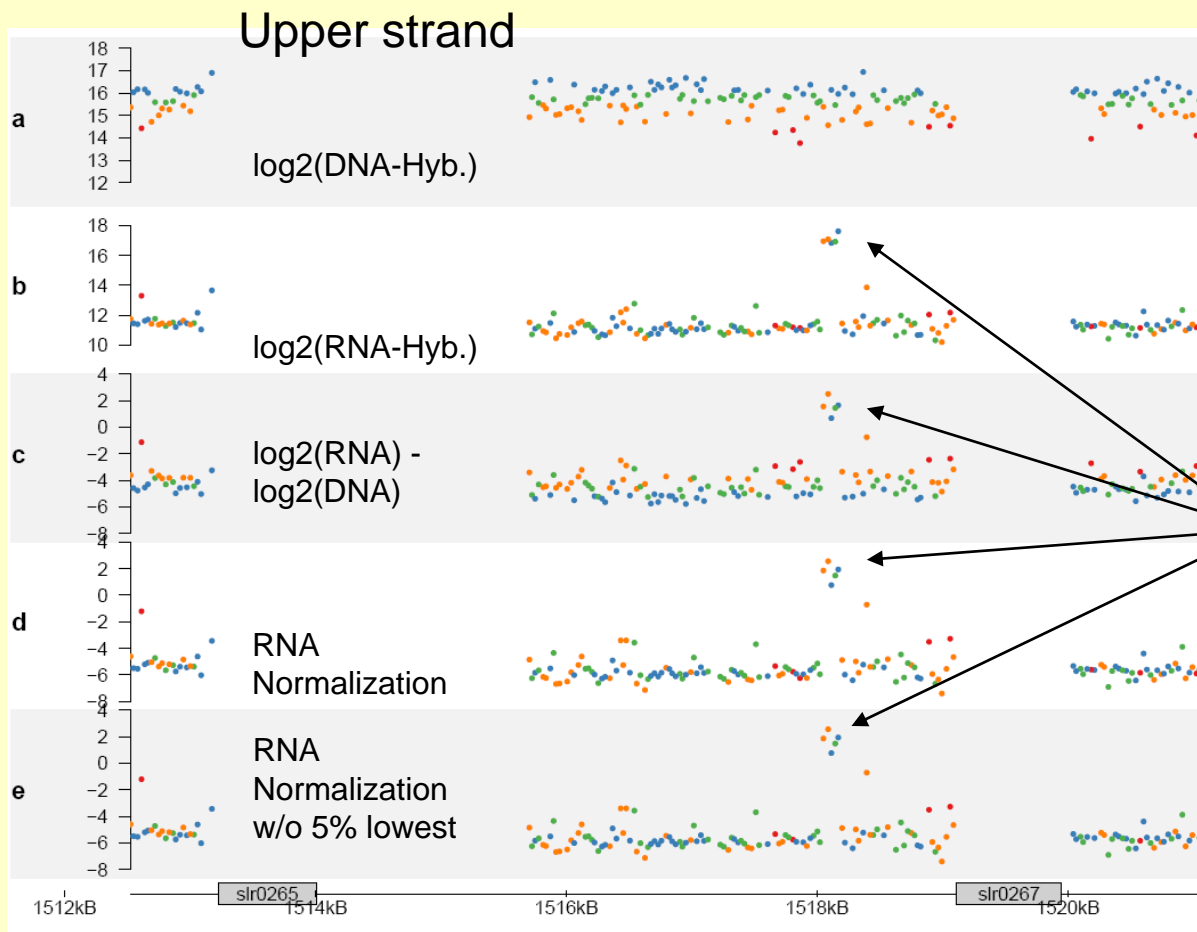


A 105K tiling array was designed covering 1/3 of the *Synechocystis* genome and with probes on both strands covering all 714 mRNAs with predicted *cis*-antisense RNAs, and an analog control set without predicted asRNAs.

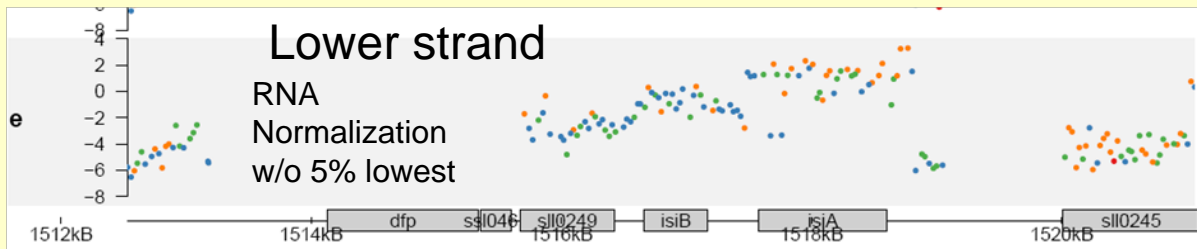


Example: *isiA* mRNA // IsrR  
antisense RNA

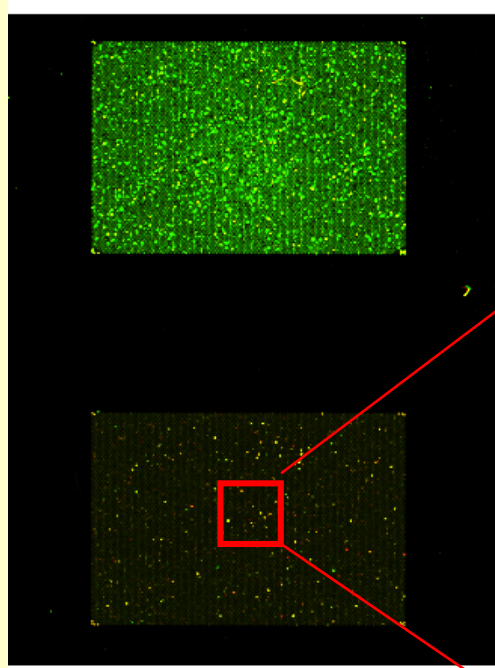
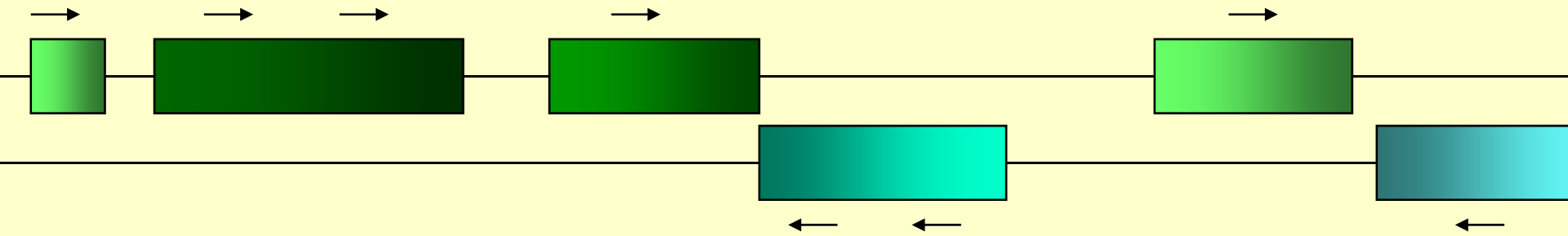
***Synechocystis* 6803 array**



IsrR



*Synechocystis* standard array (Eisenhut, et al. (2007) *Plant Physiol* 144:1946-59)



cDNA hybridization

direct RNA hybridization



# Acknowledgements

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