



UNIVERSITY OF LEEDS

# The Regulons of AtrA



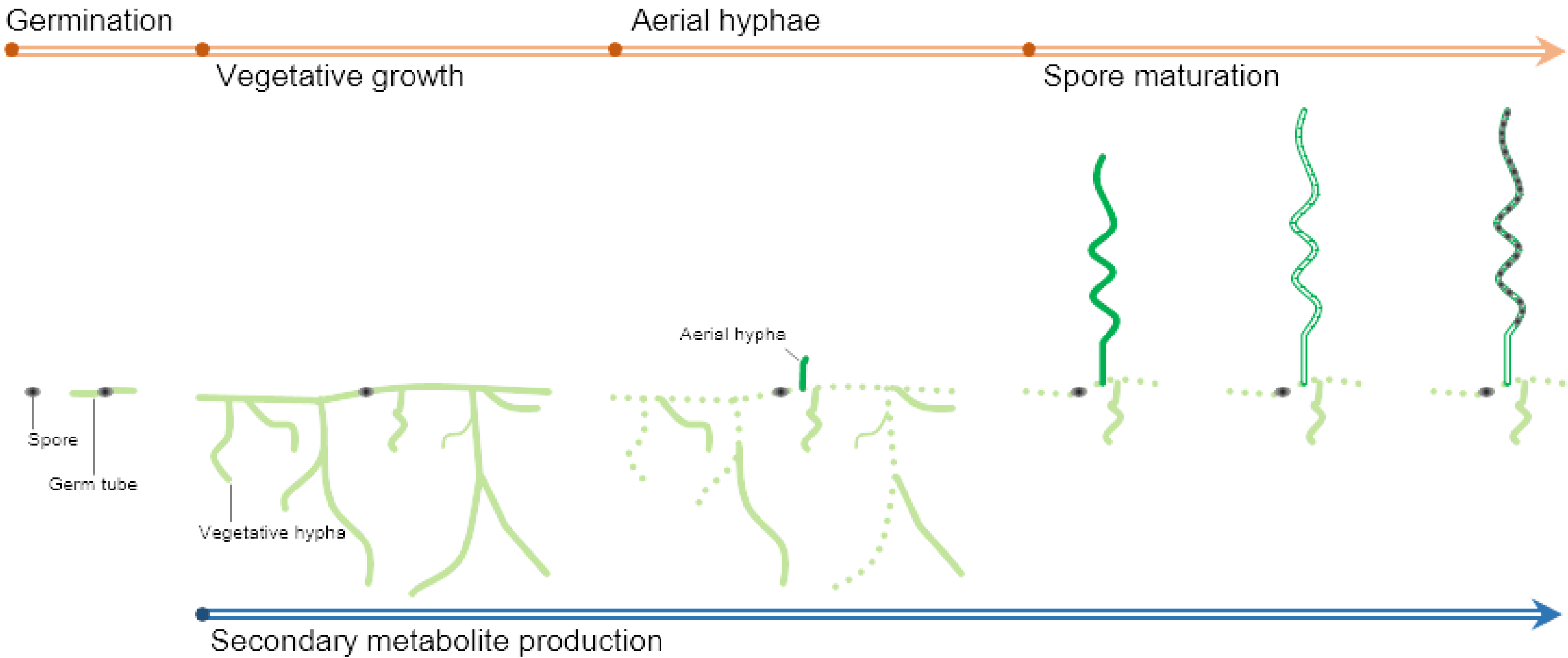
## ➤ Background information

- ✓ Streptomyces
- ✓ Directed evolution and in silico predictions
- ✓ Regulation of regulators other than *actII-ORF4*

## ➤ Work in Progress

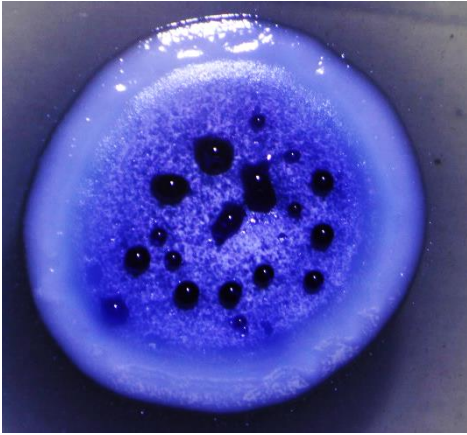
- ✓ Chip on Chip

# *Streptomyces coelicolor* life cycle

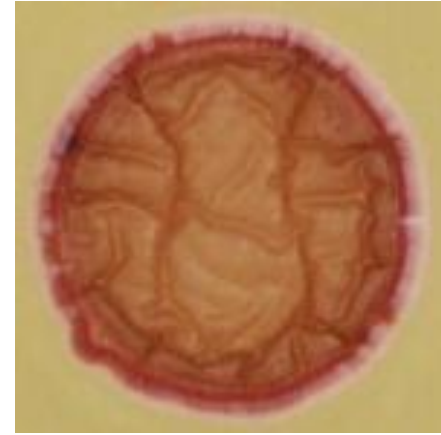


## *Streptomyces coelicolor* Secondary Metabolites

1. Actinorhodin (ACT) is blue



2. Undecylprodigiosin (RED) is red

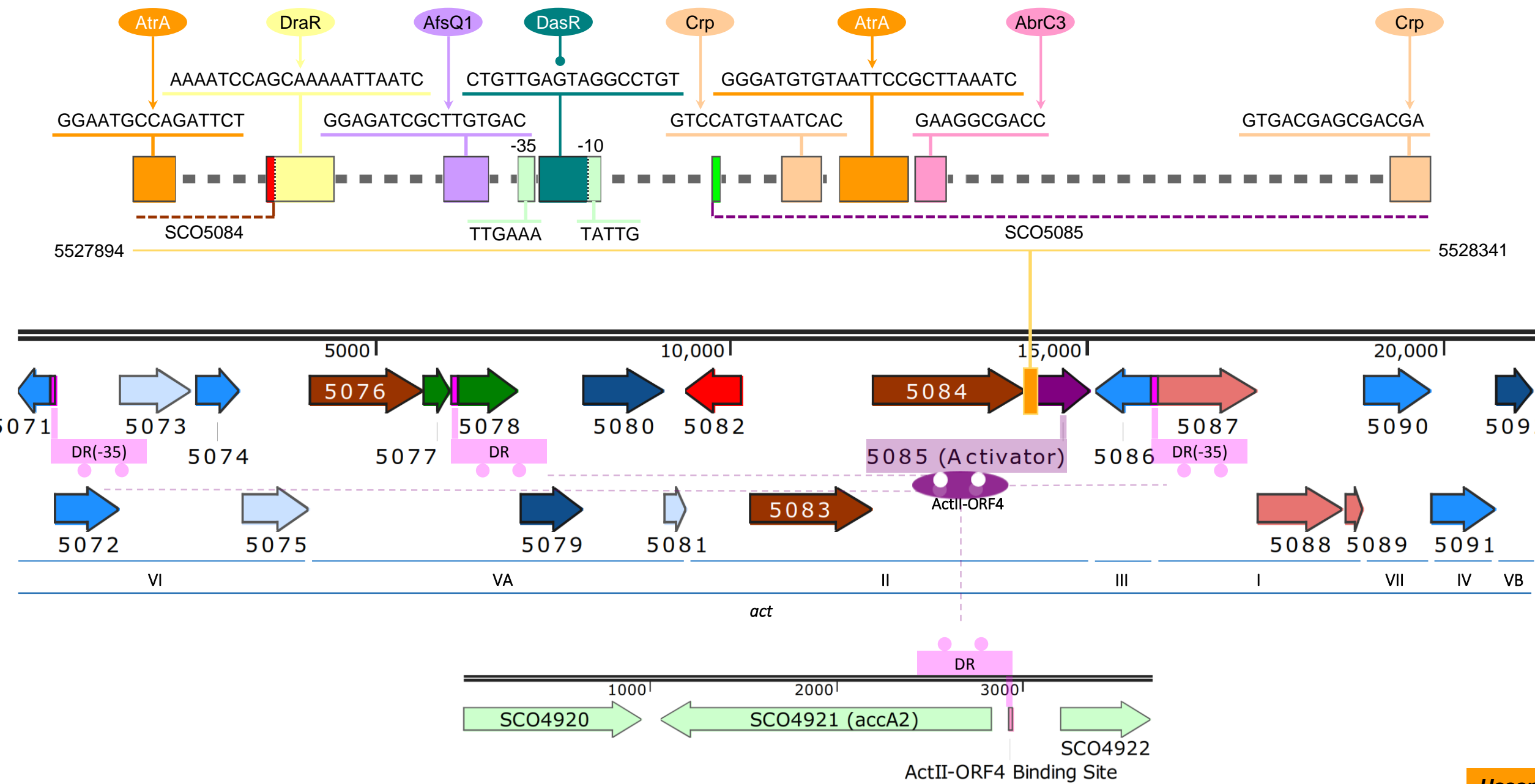


3. Calcium dependent antibiotics

4. Cryptic polyketide

5. Methylenomycins

Background information/ *Streptomyces actinorhodin* gene cluster



Phenotype on TSB (96 hrs)

L646,  $\uparrow atrA^+$



L645,  $\Delta atrA$

M145

M145

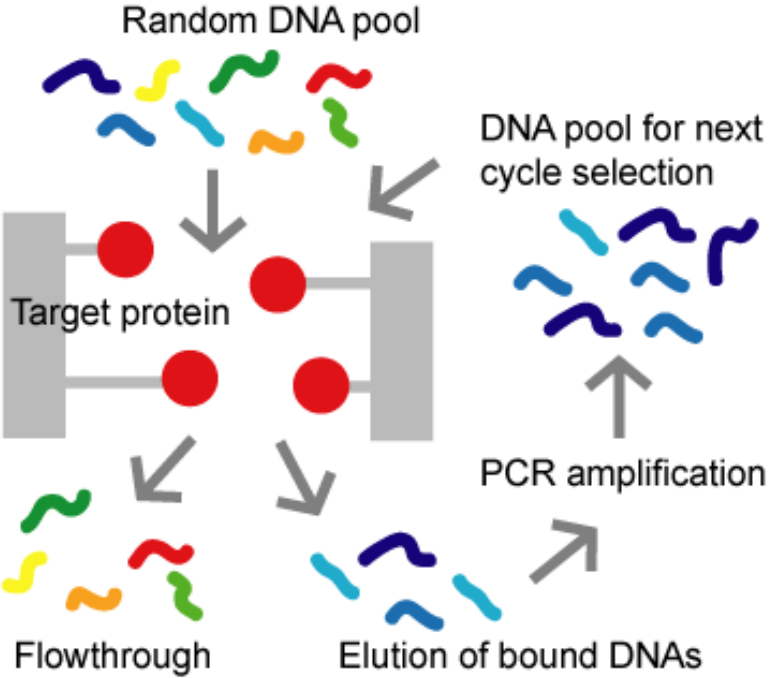


L645

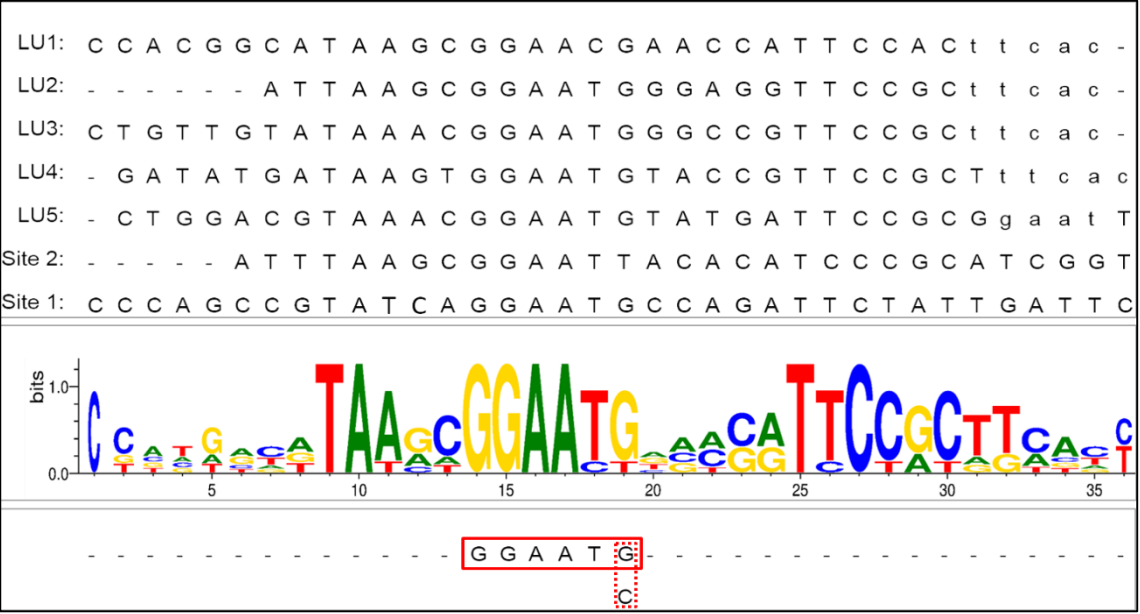


- Disruption of *atrA* (SCO4118) blocks or moderates actinorhodin production and reduces aerial hypha growth
- Associated with decrease in level of *actII*-ORF4 transcript
- Overexpression of *atrA* results in Act being produced earlier and too much higher levels
- Disruption of *atrA* reduces aerial growth and blocks gamma-butyrolactone-induced Act production

# SELEX (Systematic Evolution of Ligands by Exponential Enrichment)

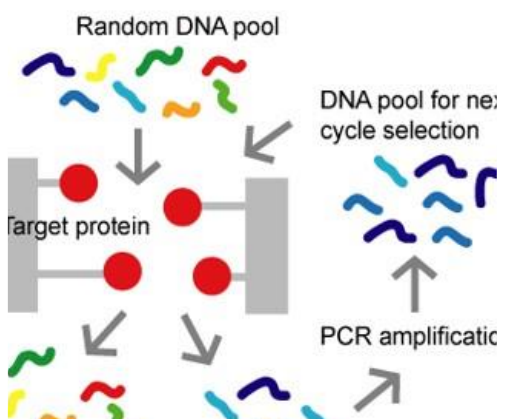


- Random region of 30 bp
- $10^{15}$  sequence variants in library
- 6 rounds of automated selection
- 20 “aptamers” cloned and sequences



- 5 unique sequences identified with similarity to sites in promoter region of *actII-ORF4*

# Combining similarity searches and EMSA



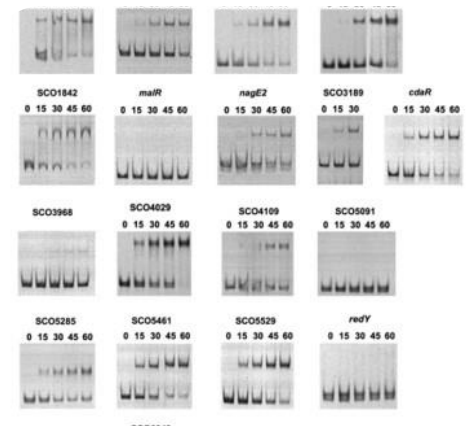
**SELES**

- Aptamers provide excellent starting point

4S-ELEMENT	TARGET	FUNCTION
122	CGCGGAACTCCCGTTCCTT	SCO695, upstream of murC
43	CGGGGAAACGCCACTCCGTT	SCO3189
122	ACCGTGGAAACCGPCTCACTCCGTT	SCO9529
135	GGTCGGAAACCGTCCACTCCGTT	SCO957c, SCO1658 (glycerol)
197	AGACCGGAAACCGCATTCCGTT	SCO1842c
14	AATCGGAAATGCTCTCTCCGTT	SCO5461
131	CCCGTGGAAACCGTCACTCCGTT	SCO987c, SCO5988
139	AAGACGGAAACCGGAGTCCGTT	SCO875
174	CCGGGAAATGATGCTTCCGTT	SCO5959 (glycerol)
137	GGTCGGAAACCGTCACTCCGTT	SCO9285, <i>lex</i>
183	GGACCGGAAATGCAACTCCGTT	SCO4829
161	GATCGGAAATGATCACTCCGTT	SCO5985, <i>actH</i>
137	CGTCGGAAACCGTCACTCCGTT	SCO1251
136	CAACCGAAATGATCACTCCGTT	SCO1842c
134	GATCGGAAATGCAACTCCGTT	SCO5984, <i>actB-3</i>
131	GGCGGAAATGCGTCACTCCGTT	SCO1247, <i>cdhR</i>
129	AGACCGGAAACCGCATTCCGTT	SCO1556c
12	CGGGGAAACCGCATTCCGTT	SCO747
134	ATCGGAAACCGTCACTCCGTT	SCO6946c
133	CGCGGAAACCGCATTCCGTT	SCO2286c
135	ATCGGAAATGCGTCACTCCGTT	SCO1803c
115	ATCGGAAACCGCATTCCGTT	SCO1977
133	CGCGGAAATGCGTCACTCCGTT	SCO1183
122	CCCGGAAATGCGTCACTCCGTT	SCO1429
112	ATCGGAAATGCGTCACTCCGTT	SCO1906c
131	TGCGGAAATGCGTCACTCCGTT	SCO697c

**Weighted Matrix**

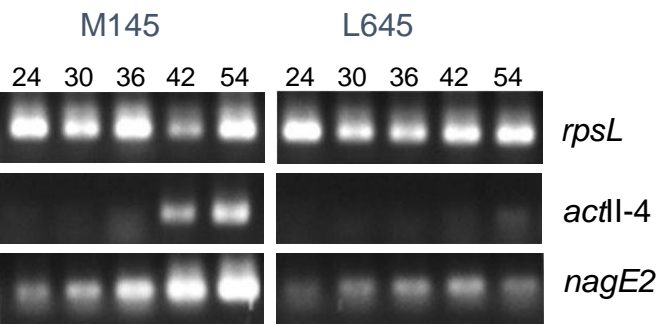
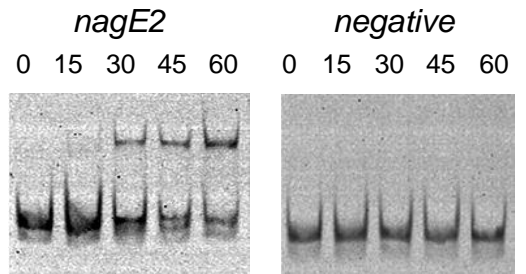
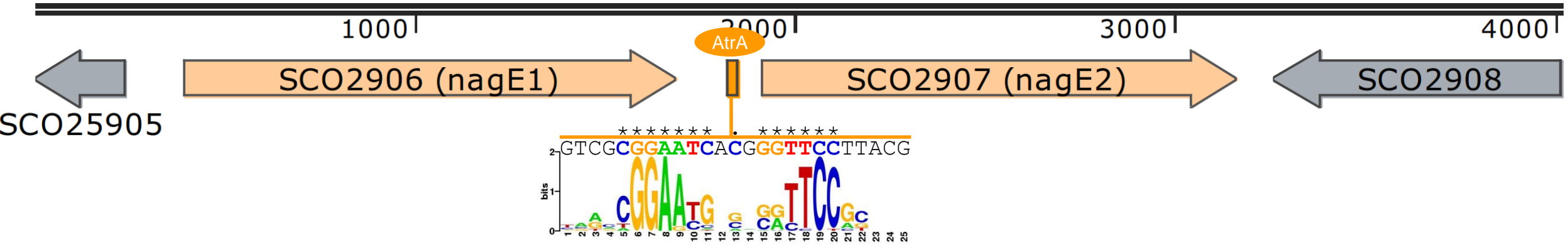
- PREDetector
- Sites in *actII-ORF4* towards top of initial "Hits"
- Binders rise to top of Hit List and non-binders drop out



**EMSA's**

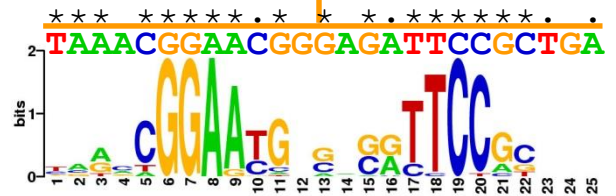
- Most of the binding sites have been proved

# A direct target of AtrA “SCO2907”

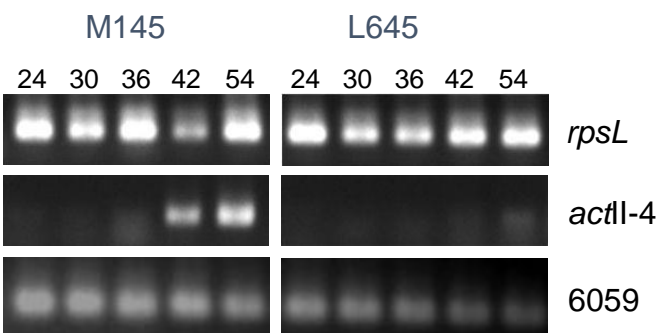
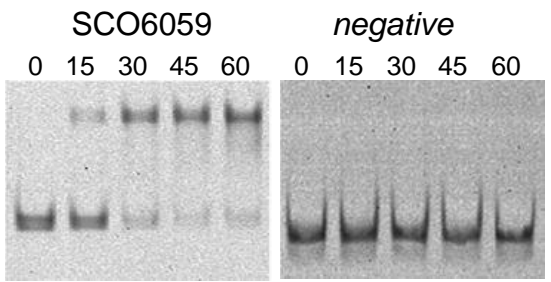


- AtrA binds in the intergenic region between *nagE1* and *nagE2*
- *nagE2* encodes the major high-affinity PTS permease for N-acetylglucosamine (GlcNAc)
- Disruption of *nagE2* shown to prevent growth on N-acetylglucosamine.
- In absence of functional AtrA, *nagE2* expression does not increase, at least to the same extent
- Thus, AtrA appears to be an activator of *nagE2* transcription

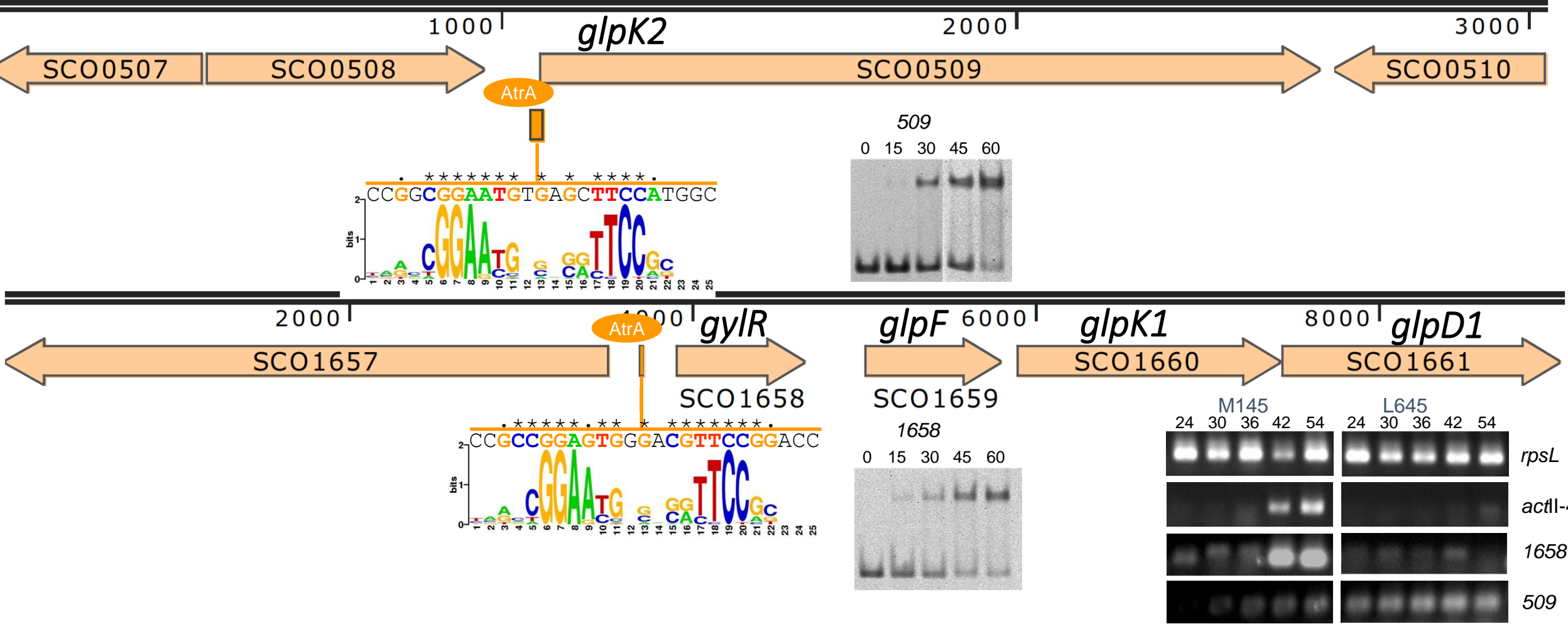
# A direct target of AtrA “SCO6059”



- AtrA binding site identified upstream of SCO6059 within intergenic region
- Binding confirmed *in vitro*
- AtrA appears to be required to maintain expression of SCO6059 (in Mannitol MM)
- Transcription from SCO6059 proceeds into SCO6060
- SCO6060 encodes UDP-N-acetylmuramoyl-L-alanine ligase (MurC)

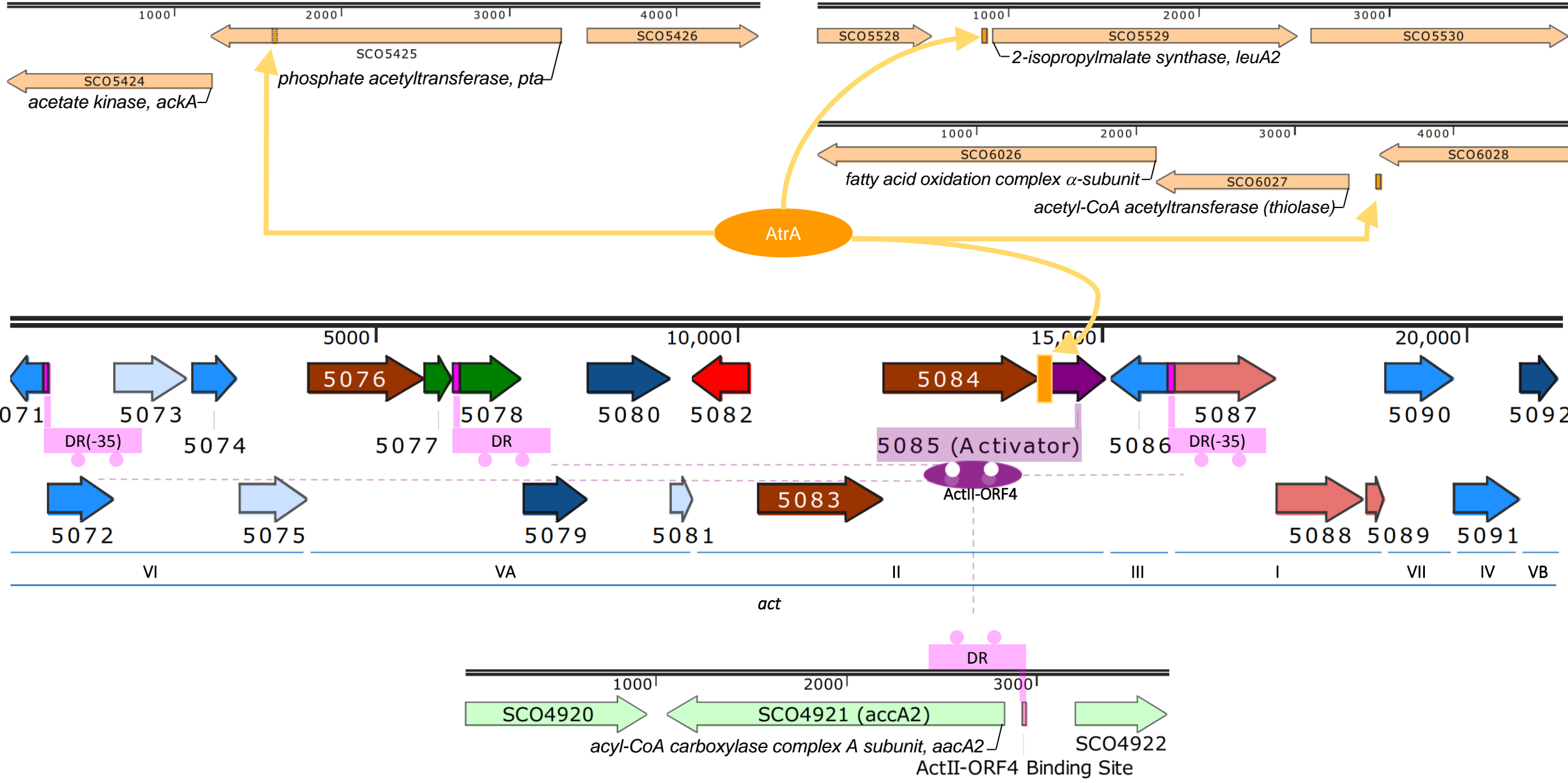


# A direct target of AtrA “SCO0509 & SCO1658”



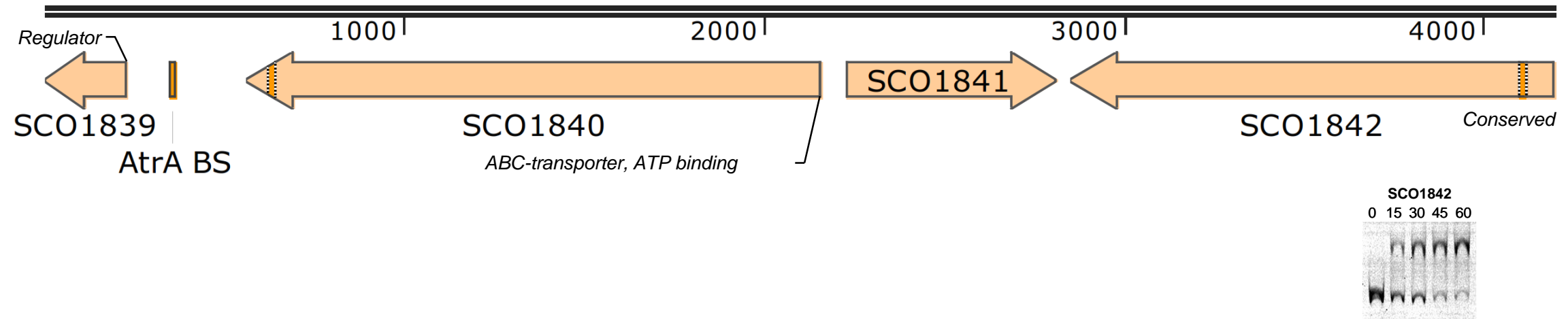
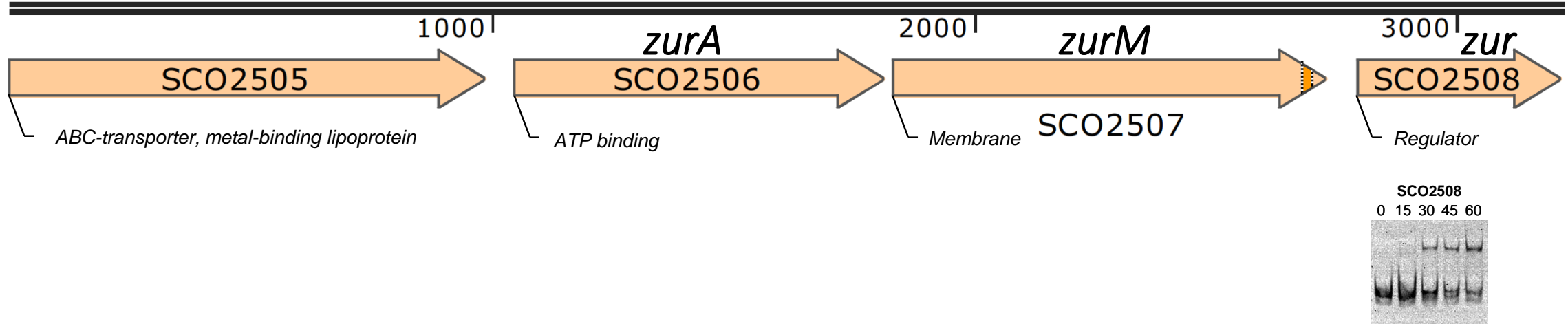
- AtrA appears to increase expression of the glycerol-inducible regulator (GylR) of the gene cluster for glycerol utilization.
- AtrA appears to also down regulated *glpK2*. Perhaps first example of AtrA functioning as a repressor
- Probable that regulation of glycerol metabolism is related to recycling and utilisation of vegetative lipids

# AtrA exerts control over acetyl-CoA metabolism



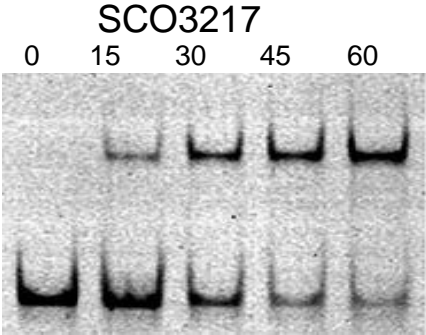
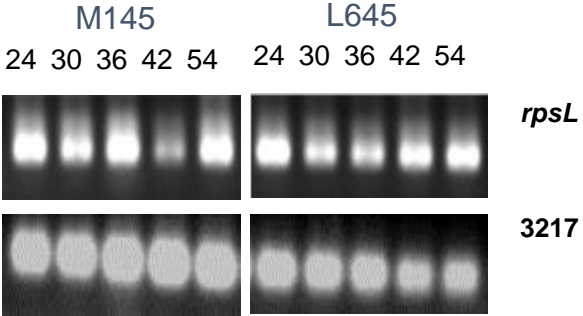
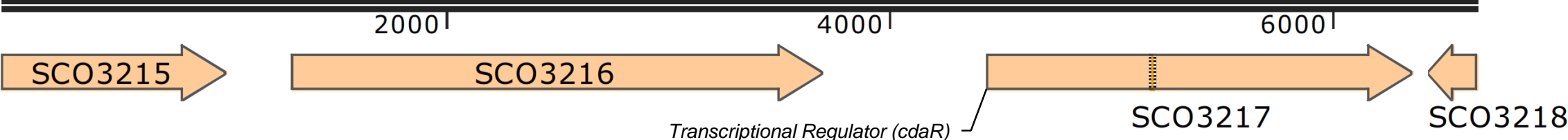
# Extension of AtrA influence

## A regulator of regulators



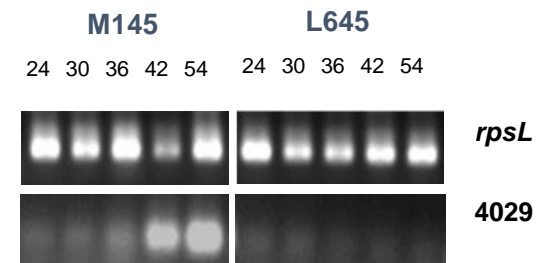
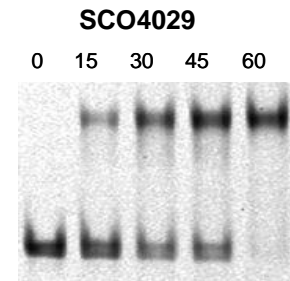
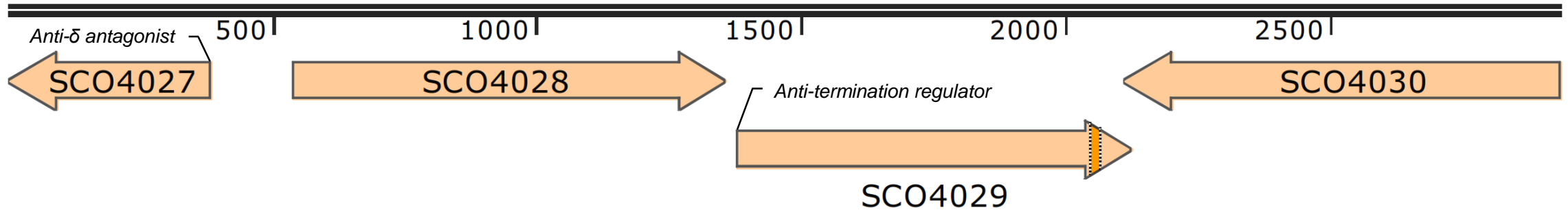
# Extension of AtrA influence

## A regulator of regulators



# Extension of AtrA influence

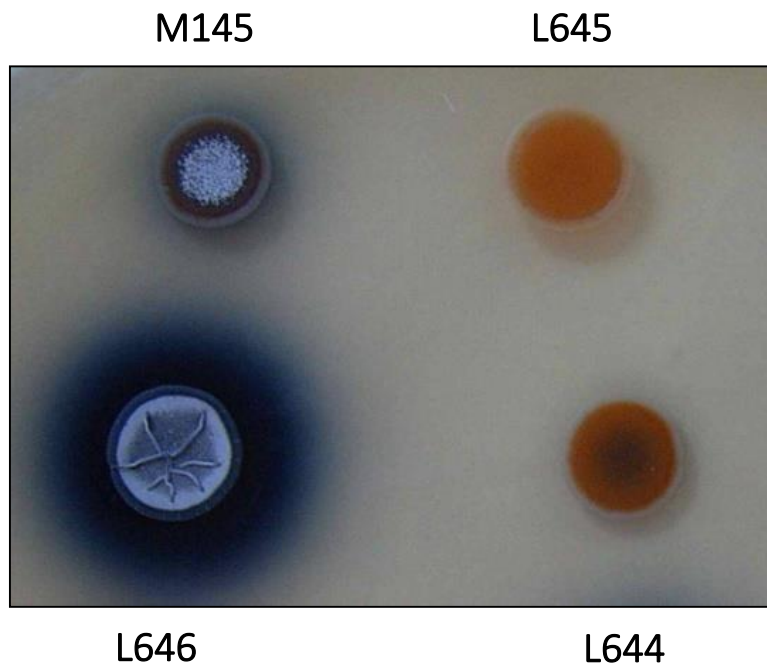
## A regulator of regulators



- AtrA involved in post-transcriptional initiation control?
- Pausing of RNA pol. may affect ribosome coverage, alternative secondary structure formation and anti-termination

# $\Delta$ atrA “bld” phenotype

Phenotype on TSB



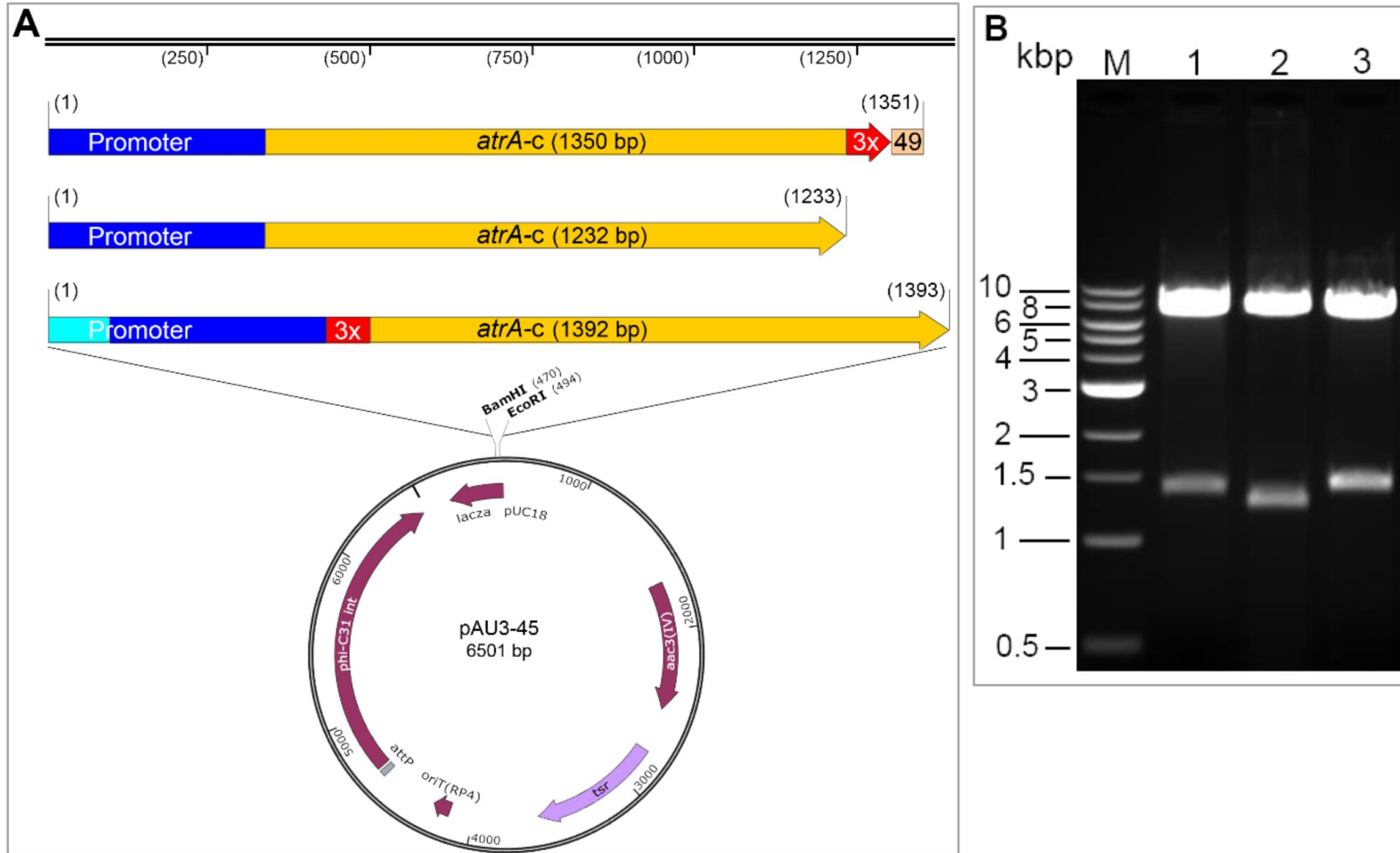
Phenotype on R2YE



# Unpolished data and work in Progress

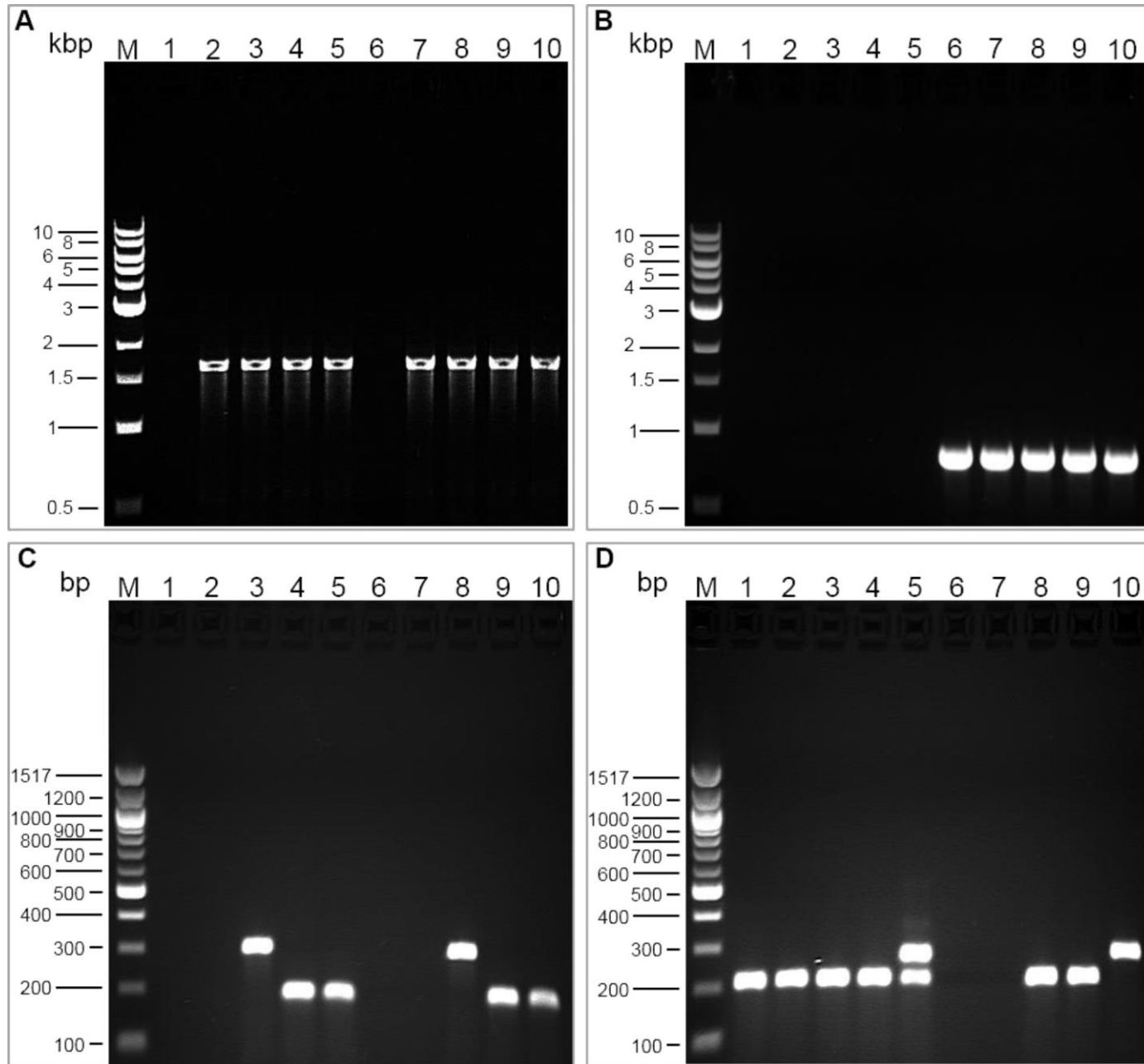
- Chip on Chip

# Construction and description of plasmid



Schematic representation and enzymatic analysis of constructs based on pAU3-45.

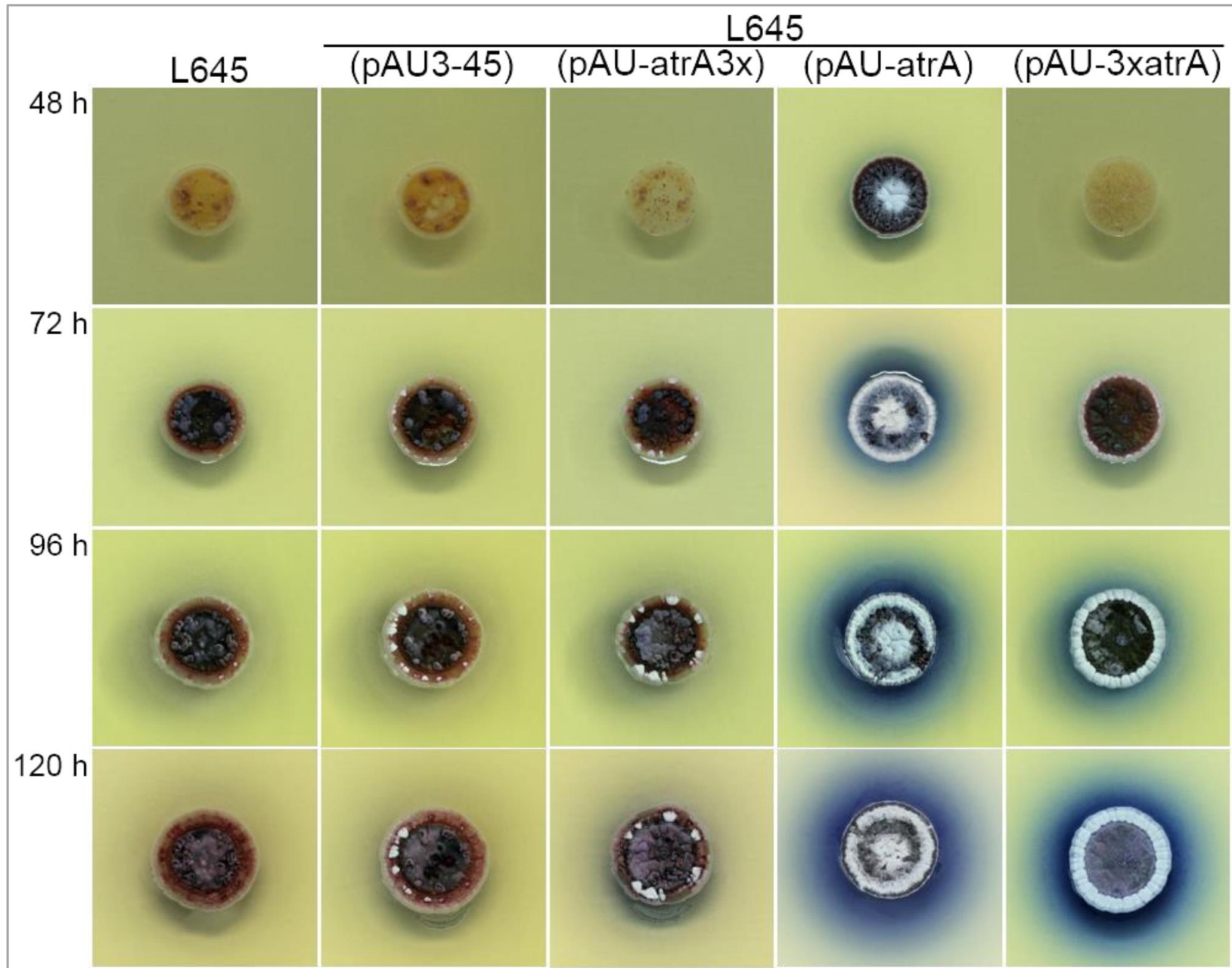
## Analysis of transconjugants and chromosomal integration



**Lane 1** corresponds to M145, while **lane 6** corresponds to L645. **Lanes 7 to 10** correspond to transconjugants (L645) containing pAU3-45, pAU-atrA3x, pAU-atrA and pAU-3xatrA, respectively. **Lanes 2 to 5** correspond to transconjugants (M145) containing pAU3-45, pAU-atrA3x, pAUatrA and pAU-3xatrA, respectively. Lane M corresponds to the 1kb DNA ladder

(A), (B), (C) and (D) were attB-F and attP-R, atrA56Dn-R and Apra-F, atrAF3 and pSET-R2, and 3xatrA-R and 3xatrA159-F, respectively.

# Analysis of the phenotypes of the transconjugants



# Summary

- AtrA coordinate and regulate multiple metabolic processes during the transition between vegetative growth and sporulation.
- A substantial proportion of the targets of AtrA appear to influence acyl CoA metabolism.
- AtrA regulates *actII-ORF4*, *nagE2*, *zur*, gamma-butyrolactones, and others.
- Random SELEX can initiate or fast track the identification of sites of binding of transcription factors based on strategies that utilise *in silico* predictions.
- Comparison with output from ChIP-based approaches should be informative.

# Acknowledgements



Keneth McDowall



Gilles van Wezel



Bin Hong

**Thanks to!**

Mia

Aqeel

Bayan

Shaaban

The *SELEX* team

Thank you for your attention