Andrew Tolonen (atolonen@genoscope.cns.fr)

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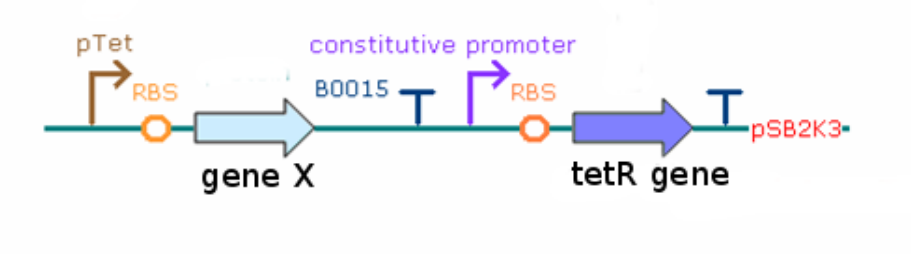
Masters II in Systems and Synthetic Biology (mSSB) Exam

This exam focuses on "The *Mycobacterium tuberculosis* regulatory network and hypoxia" Galagan et al, 2013 Nature (PMID 23823726)

1. The *M. tuberculosis* (MTB) genome (4.41 Mb, 4,000 genes) has 180+ transcription factors. This study uses ChIP-seq to map the binding sites of 50 of these transcription factors. Answer the following questions about how they used the ChIP-seq method (5 pts):



A. In this study, transcription factors were cloned with tet promoters. In the above hypothetical example, explain whether you would expect the tet repressor (tetR) and/or gene X to be expressed in the presence or absence of tetracycline. Note: pTet contains a tet operator site and B00015 is a transcriptional terminator (2 pts).

Minus-tetracycline: expression of gene X and tetR

Minus-tetracycline: tetR gene is expressed and binds the pTet promoter. Gene X is not expressed.

Plus-tetracycline: expression of gene X and tetR

Plus-tetracycline: tetR gene is expressed. Tetracyline binds TetR and prevents it from binding pTet. Gene X is expressed.

B. Why were the transcription factors cloned with FLAG tags? (1pt).

FLAG tag enables purification of the transcription factor and bound DNA by immuniprecipitation.

C. Why is it necessary to cross-link DNA and protein (usually with formaldehyde)? (1 pt).

DNA must be crosslinked to DNA in order to capture the transcription factor complexed

with its DNA binding site.

D. What is chromatin immunoprecipitation? (1 pt).

Chromatin IP is a way to capture and purify chromatin (DNA+ specific protein) in a cell lysate. The cellular lysate is mixed with antibody-coated beads that specifically capture and precipitate the protein of interest (here by FLAG tag). Once it has been purified, the protein-DNA complex is unlinked from the from the bead.

2. What is shown in the figure below (Fig 1F). What is surprising about the red bars and how can they be explained? (2 pts).

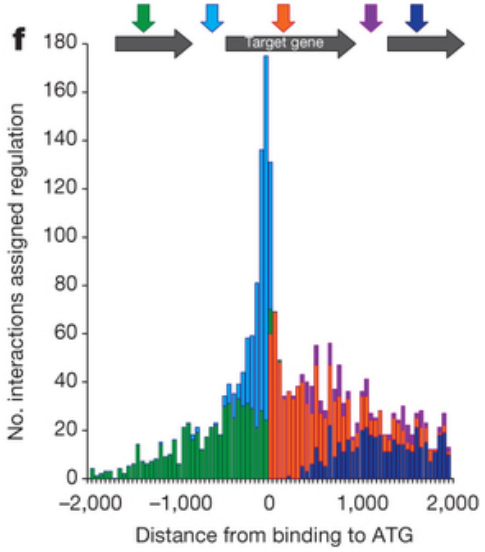
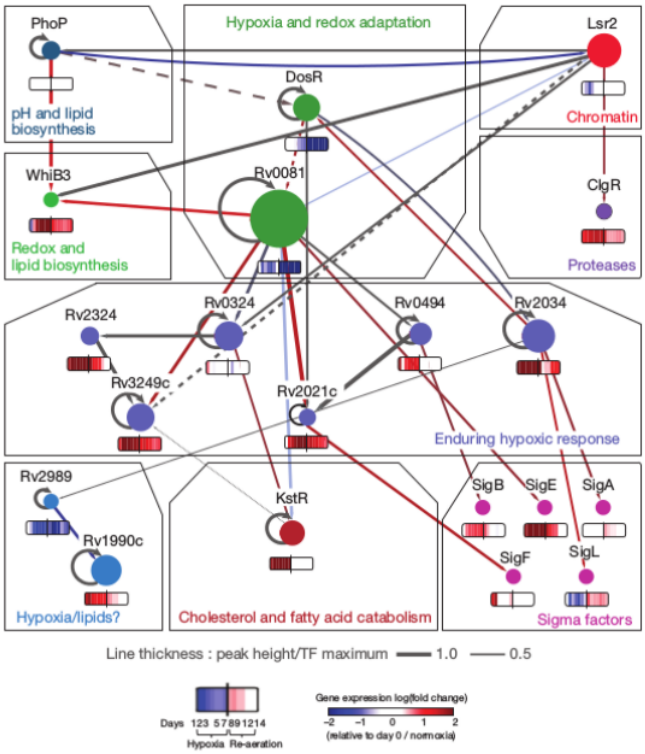


Fig 1F shows the positions of transcription factor binding sites relative to the predicted target gene. The red bars are surprising because they show that many transcription factor binding sites are in the target gene. A few explanations: 1 the current start annotation of gene may be incorrect. 2 the transcription factor may block transcription of the target gene. 3 These binding sites may be disproportionally false positives.

3. Fig 2 transcription factor interaction network.



A. What is the biological significance of red, gray, and blue arrows? (2 pts).

An arrow pointing from transcription factor (TF) X to TF Y says that X is predicted to regulate Y. A red arrow means X up-regulates Y. A blue arrow means X represses Y. A gray arrow means X does not significantly affect expression of Y.

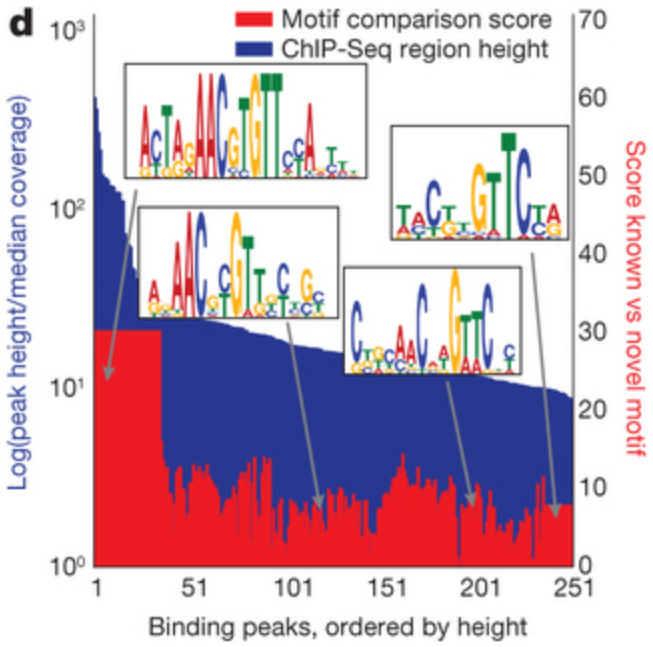
B. What transcription factors are predicted to regulate Rv2021c? (2 pts).

Rv0494, Rv0081, DosR, Rv2021c (autoregulation).

4. One of the exciting aspects of this paper is how it predicts gene expression changes during a shift from hypoxia to aeration (Fig 3). What types of experimental data did they use to make these predictions? (3 pts).

Expression predictions for target genes are based on a modeling approach (Fig S14) that uses in ChIP-seq data and TF induction microarray data.

5. Fig 1D motif structure of KstR binding sites.



The highest scoring DNA binding site (ACTA-**GAAC**-GT-**GTTC**-CA) is more palindromic than lower scoring binding sites. Why are transcription factor binding sites often palindromes? (3 pts)

Transcription factor may be palindromic if the transcription factor binds as a dimer with each subunit binding half of the sequence.

6. Lipid changes during the shift to aerated conditions were monitord by HPLC-MS (Fig 4). How do the authors explain the connection between hypoxia, lipid metabolism, and *M. tuberculosis* virulence? (3 pts).

M. tuberculosis often survives in the human body in a persistent, drug resistant state with low metabolic activity. Survival in this state likely involves survival in hypoxia, during which *M. tuberculosis* alters its lipid metabolism and can use cholesterol as a primary nutrient.