Computational-based approach to identify quorum sensing regulated genes using *lux* box alignment matrix in *Vibrio fischeri* genome

About Quorum Sensing and *lux* box

The term quorum sensing describes the ability of a microorganism to recognize and respond to others in a population by detecting the concentration of self-produced intercellular molecules commonly known as autoinducers. When the autoinducer reaches a critical threshold concentration, often at high cell densities, it triggers a signal transduction pathway leading to an alteration of gene expression patterns. There are a number of important bacterial processes regulated in this manner, including antibiotic production, release of exoenzymes, production of virulence factors, induction of genetic competency, conjugative plasmid transfer, biofilm formation and bioluminescence.

In the Gram-negative bioluminescent marine bacterium *V. fischeri* a complex signal transduction system controls expression of bioluminescence. However, it is the products of *luxI* and *luxR* that directly activate *lux* operon transcription. LuxI, the autoinducer synthase, produces the diffusible autoinducer 3-oxo-hexanoyl-L-homoserine lactone (3-oxo-C6-HSL). As the levels of 3-oxo-C6-HSL rise, complexes form between it and an autoinducer-dependent activator of transcription, LuxR. The complex can then activate transcription of the *lux* operon by binding to the ***lux* box** in the promoter region, leading to an increase in bioluminescence.

The 252-amino acid, two-domain LuxR polypeptide is one of the most studied members of a family of over 50 transcriptional regulator proteins involved in acyl homoserine lactone mediated quorum sensing. In LuxR, the N-terminal domain is believed to be membrane associated and to function as a receptor for the 3-oxo-C6-HSL ligand. In response to binding the 3-oxo-C6-HSL, the N-terminal domain modulates the activity of the C-terminal domain (CTD). Binding of 3-oxo-C6-HSL permits multimerization of LuxR and subsequent activation of transcription of the *lux* operon, which is carried out by the CTD. The CTD of LuxR has a helix-turn-helix motif and binds to a region of DNA termed the ***lux* box**, which is 20 bp in length with a dyad symmetry centered at a position –42.5 bp upstream of the transcription start site for the *lux* operon.. When LuxR functions as an activator of transcription at the *luxI* promoter, it is proposed to function as a homodimer.

About the Methodology

Much has been learned about quorum sensing and the ability of a microorganism to recognize and respond to others through the quorum sensing regulon. Thus, as part of a continuing effort to better understand about this cell to cell communication, we want to implement a computational approach to identify additional quorum sensing regulon.

As the complete sequences of more and more genomes become available, the extraction of biological information by computational analysis of sequence data is quickly becoming an indispensable tool in biological research. While searches for gene homology are routine, searches for DNA binding sites are less common, in part because the sequences recognized by a DNA binding protein are short and often vary considerably from a simple consensus sequence. Here we will use an alignment matrix constructed from previously identified lux box sequences as a model to search through the entire *Vibrio fischeri* genome for putative lux boxes.

AIM

The aim is to identify orthologs of known quorum sensing regulated genes as well as novel genes not previously known to be regulated by quorum sensing using an alignment matrix.

Methodology

1. Literature and Database search of naturally occurring Lux binding sites among gram-negative bacteria that have been previously confirmed experimentally.

2. Aligning the binding sites collected from step1 using BioEdit

3. Construction of the matrix using BioEdit

4. Searching for Lux binding site and so the regulated genes using genomes-scale-patser (http://rsat.scmbb.ulb.ac.be/rsat/). The search will be done in the regions -300 to +25 bp of the translation start site. Use the options as given in this screen-shot.

5. A cut-off/threshold weight score should be calculated, which is the score that predicts 75% of the binding sites from which the matrix was made.

6. Analyse the predicted Lux binding sites from PATSER results for known as well as the novel Lux regulated genes.

7. Do a BLAST search to identify for orthologs of known Lux regulated genes in the *Vibrio fischeri* genomes. Use Microbial genomes BLAST (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) only against *Vibrio fischeri* genomes.

8. Report the results in a table, with the gene name from *V. fischeri*, whether involved in quorum sensing, is it known to be quorum sensing regulated gene.

Points to remember

1) BioEdit is installed on all machines locally in the mediatek.
2) The excel sheet has to be downloaded locally (may be in some folder) on the machines and used.

3). Check the results to make out how many of the known quorum sensing regulated genes that you also predicted.
4). Are there any novel genes that you found to be quorum sensing regulated and predicted in both the genomes.

Related References

1) **New insights into the regulatory mechanisms of the LuxR family of quorum sensing regulators**, William Nasser and Sylvie Reverchon, Analytical and Bioanalytical Chemistry, September 2006.

2) **Quorum sensing in vibrios: complexity for diversification**, Milton DL, Int J Med Microbiol, April 2006.

3) **LuxR- and Acyl-Homoserine-Lactone-Controlled Non-lux Genes Define a Quorum-Sensing Regulon in Vibrio fischeri,** Sean M. Callahan and Paul V. Dunlap, Journal of Bacteriology, February 2000.

4) **Analysis of LuxR Regulon Gene Expression during Quorum Sensing in Vibrio fischer**i, Qin N, Callahan SM, Dunlap PV, Stevens AM, Journal of Bacteriology, March 2007