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Deciphering Global Dynamics of Microbial Multidrug Resistance Regulation using Multi-Omics Approach

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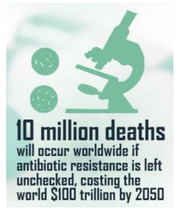
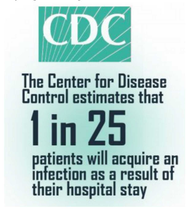
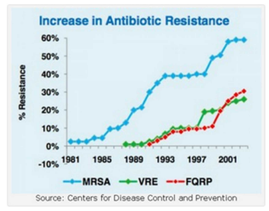
1: Computational Bio & Biophysics, 2: Biotechnology and Bioengineering,3: Systems Biology, 4: Current: BioRad, Hercules, CA

Abstract

The emergence of multi-drug resistant (MDR) bacteria translates to $20 billion per year in healthcare expenses in the US alone. Furthermore, the lack of tools to study these emerging pathogens has made it difficult to devise effective treatments. If left unaddressed, MDR bacteria will pose an enormous threat to US national security by crippling both the economy and the health of the population. For this project, we conducted a study aimed towards understanding MDR bacterial genetic responses to antibiotics, to elucidate the molecular mechanisms underlying the regulation and evolution of antibiotic resistance.





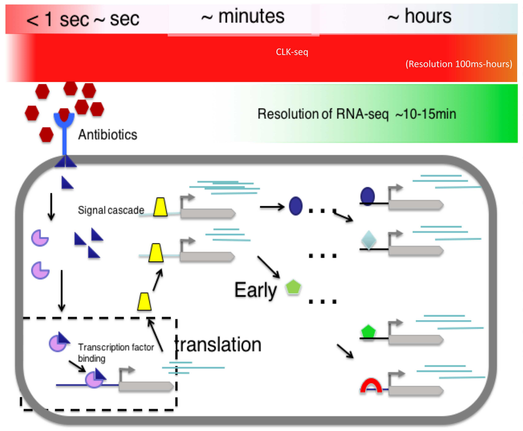


Current omics methods have focused on quantifying transcriptional responses of bacteria to antibiotic challenges and not towards understanding the transcriptional regulation. To address this gap, we use DNase-seq coupled with RNA-seq to better understand resistance mechanisms and identify new biomarkers for drug resistance in MDR bacteria. In addition we introduce CLK-Seq is a novel universal assay to characterize the DNA/protein interactions of any organism without the use of antibodies. We will continue to utilize CLK-seq study with RNA-seq studies to characterize the gene regulation of this pathogenic bacteria to understand the underlying principles of MDR at the molecular level.

We measured gene expression profiles, using RNA-seq, of these MDR bacteria in steady state and in presence five classes of antimicrobial drugs, clinically relevant for Gram-negative bacterial infections. We compare these responses to the untreated samples to see how these pathogens function to survive such harsh treatments. We find that pathogens response to different drugs varies and both in types of genes and also in strength. We further took the samples which showed the greatest gene expression response to an antibiotic drug to apply DNase-seq assay to characterize the gene regulation. We find evidence of changes in major structures in DNA compaction correlated with gene transcriptional changes apart from transcriptional profile changes. Our future efforts include application CLK-seq method to enable collection of kinetic information of protein/DNA interactions to reveal underlying mechanisms of MDR regulation and its dynamics at a time scale of seconds.

This holistic multi-omics approach information is crucial to understand the underlying mechanisms and pathways for development of multi-drug resistance, their functions in infection and pathogen survival. This information arms us with new candidate biomarkers for more effective drugs against these emerging MDR pathogens.

Background

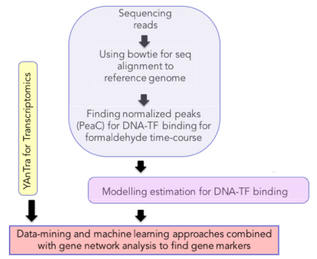


A schematic of a hypothetical scenario of a multidrug-resistant pathogenic bacterial response to a drug. When drug interacts with the pathogen, the quorum sensing receptors sense the toxins/drugs and activates a signaling cascade to activate the first cascade of gene expression change. These mRNAs will translate to proteins which will either nullify the effect of the drugs and/or regulate the next wave of genes further and this goes on as long as the pathogen is stressed. The top bar of the figure shows the times line of the events from seconds to hours.

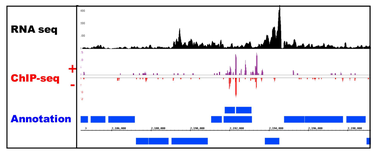
Early Regulators

It is important to understand the DNA promoter activity to understand the transcriptional response at the mechanism level. With quantitative information of the transcriptional dynamics it becomes relatively easier to understand the dynamic response of pathogen defenses to drugs. The time resolution of RNA-Seq assay is about 10-15 minutes at best, but based on previous studies CLK-seq can provide more time-resolved useful information than RNA-seq to decipher and understand the ABR mechanism including crucial early regulators.

Strategy and Tools



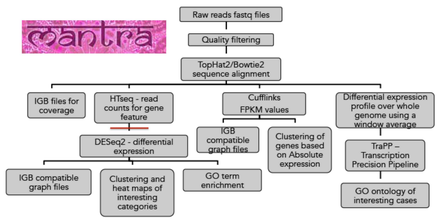
Protein – DNA interaction captured in *Klebsiella pneumoniae* treated with sublethal dose of antibiotics. We apply biophysical models to explain transcription factor binding and its correlation with transcription



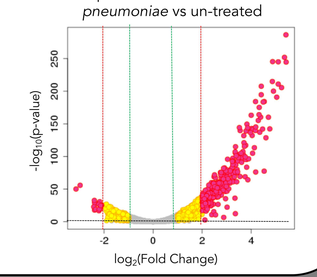
Research strategy used in the analysis of DNase-seq datasets.

Different sections correspond to the different stages in the development of assay. At the top, grey section shows the initial processing of the sequencing data of the different crosslinking time points of DNase-seq assay. The yellow box for RNA-seq analysis. The blue section is the development of the kinetic model. Pink section denoted the development of software for model fitting and parameter estimation and finally the salmon color box for integration and data mining of the collected datasets for interesting biology and interpretation

RNA-seq Analysis by MAnTra (Multi Analysis Transcriptomics pipeline) ©

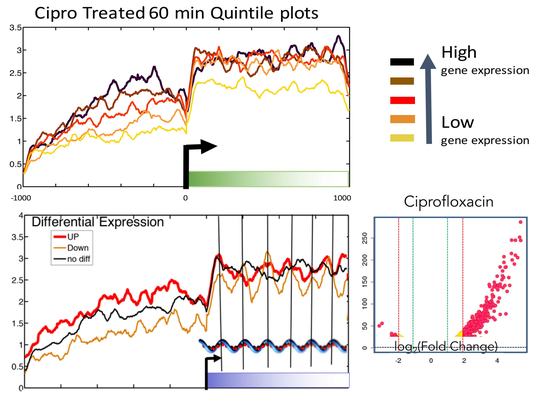


Ciprofloxacin Treated *K. pneumoniae* vs un-treated



Results : DNase-seq reveals that DNA activity and transcriptional response are correlated

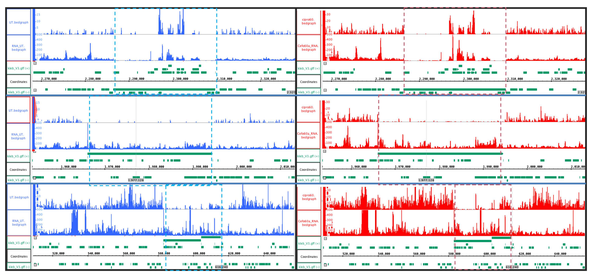
The protein-DNA interactions increase with the transcriptional response. For this analysis, genes in the genome were split in quintiles based on gene expression measured from RNA-seq analysis using MAnTra pipeline (from low to high). The average profile of DNase-seq read counts for these genes at the promoter and within the body of genes is shown in the figure. These five quintiles are shown from the genes least expressed (yellow) to highest expressed (black). It can be noted that the signal for protein-DNA increases as the transcriptional response increases in the promoter region, which suggests that the regulatory activity of the genes with high transcriptional response is higher. In the gene body, only the low expressed genes (yellow) show a depletion in signal than the others groups. Also, the profile for DNA-protein interaction shows a dip in the signal at the gene start site. This profile resembles the Nucleosome-free region in Eukaryotes.



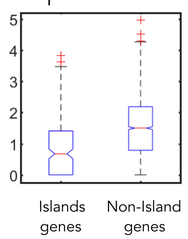
Average profile for DNase-seq for differentially expressed genes in presence of drugs is shown in the bottom figure. These average plots highlighted an interesting pattern in the differentially downregulated genes. These genes have a periodic accumulation of signal in the gene body at an amplitude of 100-150 nt. This pattern also resembles profile for nucleosome protection assay where the DNA locations associated with nucleosome are protected in Eukaryotic organisms. We speculate that these patterns are related to DNA compaction proteins which are associated with gene repression. Further experiments and studies are needed to explore this possibility

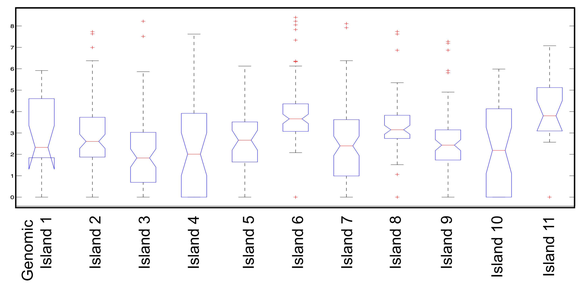
Results: Genomic Islands have fewer DNA activity recorded

DNase-seq and RNA-seq read maps at genomic islands



log_2(counts) at promoters





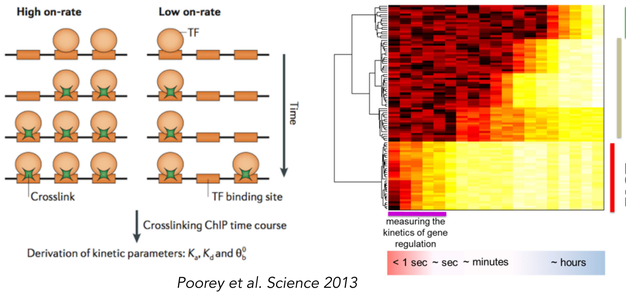
Using Genome Browser we noticed that for DNase-seq read counts for genomic islands were depleted. In the six figures showed in top figure, the top track is DNase-seq data, the middle tier is transcriptomics data and bottom track shows genes.

The boxed region shows the genomic islands. We observe that the overall DNA binding activity in the Genomic Islands is greatly reduced despite a great abundance of promoters and transcriptional activity. This indicates that either there is lack of DNA compaction in these regions or/and altered mode of transcription regulation than non-mobile elements.

Discussion and Future Direction

Preliminary data for our multi-omics study has revealed new observations in transcriptional regulation mechanisms for prokaryotic genomes and discovered new evidence of bacterial chromatin and its novel effect on gene regulation at the global scale. We observed that the there is large pathogen transcriptional response in the presence of drug and it also correlates with differential change in the DNA-binding activity. Further research is needed to study the in-dept dynamics of this response with CLK-seq, a novel assay which has proved to be a valuable tool in studying dynamics of locus specific gene regulation.

Measuring DNA interaction timescale with CLK-seq CrossLinking Kinetic ChIP sequencing
(pronounced "clock")



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We aim to continue the study of time sensitive MDR response with application of CLK-seq to strengthen our findings

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Acknowledgements

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