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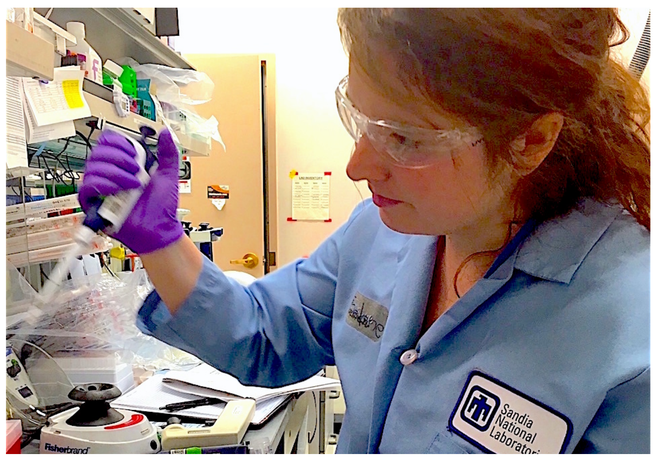

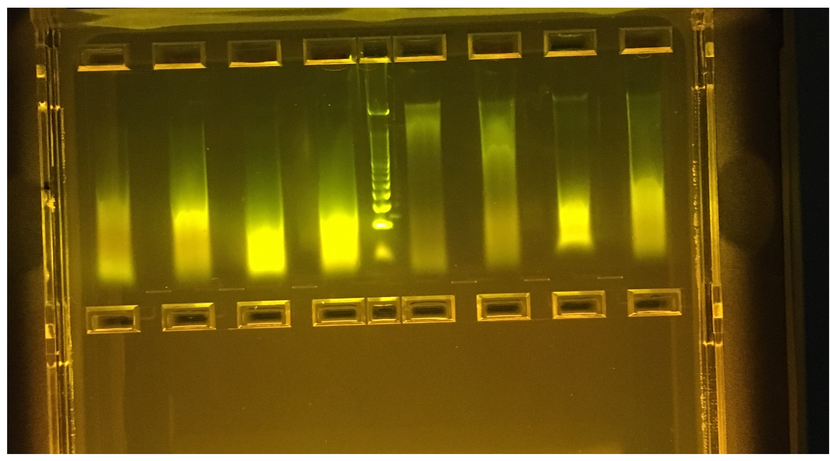

Deciphering Antibiotic Resistance

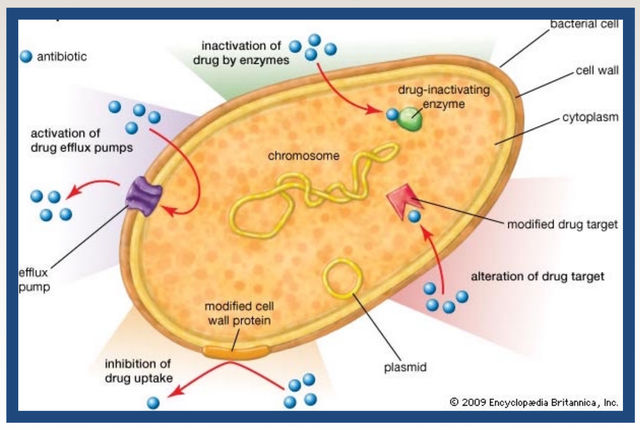

Figure 1: Mechanisms of antibiotic resistance in a prokaryotic, bacterial cell include activation of drug efflux pump, inactivation of drug by enzyme, alteration of drug target, and inhibition of drug uptake.

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Abstract

Wide use of antibiotics has resulted in evolution of multidrug resistant (MDR) bacteria. To counter MDR bacterial infections, we must find new biomarkers to target and develop new drugs. For this we need to investigate the functional genomics of these pathogens to understand the mechanisms involved in antibiotic resistance. We have developed μCLK-seq with microfluidics to capture the dynamics of the genome and transcription regulation in any organism of interest and identify critical gene regulators as targets for countermeasures. We demonstrated method's success by applying it to *Klebsiella pneumoniae* and correlating protein-binding events to transcription.

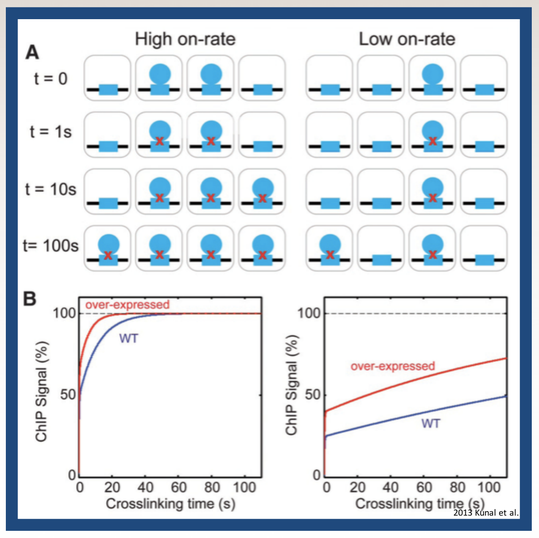

Figure 2: Hypothetical situation in which a gene has a high on-rate (k_a) versus a low on-rate. Off-rates are the same. **(A)** Transcription factors (blue circles) and binding sites (blue boxes) interacting while being crosslinked with formaldehyde (red x) at time t. **(B)** ChIP vs. crosslinking time simulation using CLK model (blue lines, wild type) with red lines showing a 3x increase in TF.

Introduction

Problem

- Because of the wide use of antibiotics, multi-drug resistance in bacteria has evolved **(Figure 1)**
- MDR mutations, regulator, and plasmids are being discovered all the time
- Current popular methods for measuring protein-DNA interactions (such as ChIP methods, FRAP, ChEC-seq, etc.), only quantitatively measure binding location OR kinetics
- Without a better method, it will be difficult to determine targets for new drugs

Solution

- We suggest a method called Cross Linking Kinetics with sequencing or CLK-seq
- Is a modified ChIP-seq method that can determine binding location AND kinetics of the protein-DNA interactions of interest **(Figure 2)**
- It will allow for identification of significant sites of gene regulation by transcription factors (TFs) for antibiotic resistance
- New drugs can target these sites for countermeasures

Methods

Microorganism (i.e. *Klebsiella pneumoniae)* grown in antibiotics (i.e. Ciprofloxacin) → Active protein-DNA interactions crosslinked with formaldehyde at different time points (CLK) → Bacteria permeablized and DNA fractured with DNase I and sonication *in situ* → DNA extracted and DNA of interest separated using size-selection electrophoresis gel to 50 bp → DNA sequenced and DNA library constructed (ChIP-seq)

Results

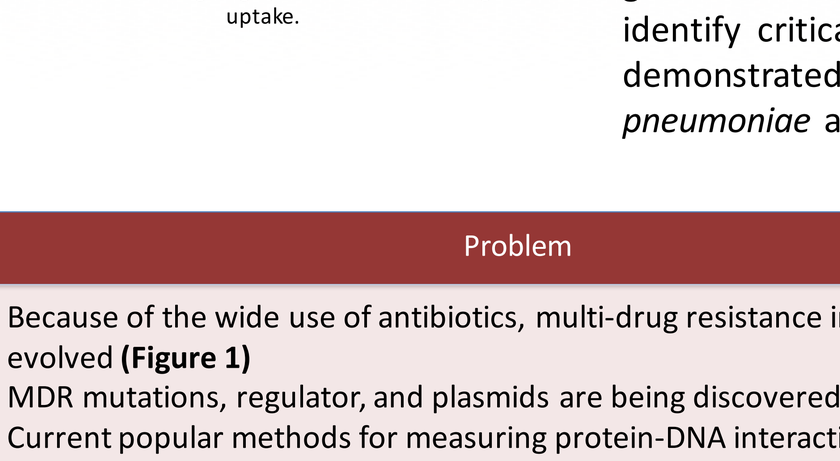

Figure 3: High Sensitivity DNA Assay of sample A2 containing the non-crosslinked DNA of *Klebsiella pneumonia* precipitated using beads. Peak at 125 corresponds to indexing adaptors while peak at 163 indicates that adaptor ligated to the DNA isolated from size selection.

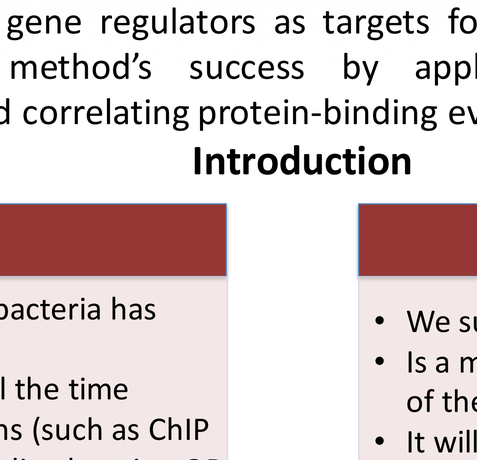

Figure 4: High Sensitivity DNA Assay of 11 samples

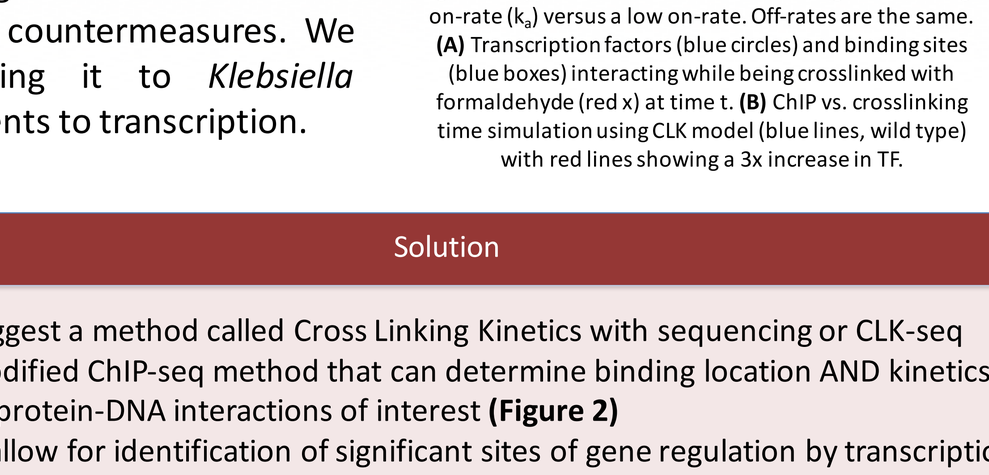

Figure 5: Protein binding events on *K. pneumoniae* genome shown in a genome browser. Magenta track at the top shows binding at the positive strand and red track the the bottom shows binding at the negative strand. Note that the bindings are clustered along the promoters of the genes.

Discussion

CLK-seq is used to measure specific protein-DNA interactions and has a significant advantage over previous methods such as the ability to determine precise binding locations of target transcription factors (TFs). This method can also be used to calculate the equilibrium binding constant (k_a) and determine the half-life ($t_{1/2}$) of the DNA-protein complex. Also, because CLK-seq is based off the popular ChIP method it is readily adaptable. Future directions of this project include employing CLK-seq to further study the effects of ciprofloxacin on *Klebsiella pneumonia* transcriptional dynamics.

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