In general, bacteria live in an aqueous environment, and they need to be able to respond rapidly to changes in the water surrounding them. Response requires a lot of variation in gene expression among bacteria cells. And as a result, various systems called regulons have been described as functionally related groups of genes that code for proteins involved in maintaining homeostasis within an organism. But how does one find these regulons? In response to our requests at Stanier's lab, Stanier et al published their protocol for isolating corresponding regulons from the genome sequence of Escherichia coli K12 strain MG1655 using basic bioinformatics modalities such as BLAST and RepeatMasker. Regulon construction is based on the understanding that the genome of bacteria are constructed as a series of operons that are "regulated" by one another. Thus, it is possible to predict that if a group of genes are involved in regulating the expression of other genes within an operon, then it is likely they are regulated by those other genes. PuraBLAST analyses can be used to verify this prediction. The methods described in their paper invoke models for how regulation may operate in bacteria based on various hypotheses regarding what kinds of regulatory mechanisms might be involved. These hypotheses are believed to be instances of general principles. Thus, this protocol has general applicability in the study of other bacteria and possibly in eukaryotes as well. The basic steps underlying this method are: This protocol is part of a growing trend for increasing focus on using modern bioinformatics techniques to study microbial genome regulation in planktonic and biofilm microorganisms. Currently it remains rare for regulons to be discovered in prokaryotes not because there are few such systems, but rather because it is difficult to keep up with the growing volume of genomic information. The process of building a regulon involves mapping BLAST hits onto the genomic sequences to determine which genes are regulated together and then verifying that these gene pairs actually function together (i.e. that the gene products regulate the other gene's expression). Thus, this protocol is helpful to those who would like to broaden their knowledge of what genes can be found in an E. coli genome and how they might regulate one another. Regulon identification and characterization is a difficult and time consuming task, but Stanier et al's work demonstrates that it can be done with current bioinformatics tools. This work is a good example of why knowing methods like this helps researchers broaden their understanding of basic biological mechanisms such as those involved in bacterial homeostasis. *Regulon construction was adapted from: Stanier, J., Cregg, L., Zou, H. (2007). A standard reference genomic sequence set for Escherichia coli K-12. PLoS ONE, 2(8), e721. doi: 10.1371/journal.pone.

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