Thesis project and research project offers in Microbiology at Uppsala University (30-45 hp)

Mycobacteria can cause various infections, including tuberculosis and leprosy, which may be difficult to treat and cure. We are interested in understanding the biology of mycobacteria, and in particular how they respond to various stresses they encounter during infection of humans and animals and how they evade being detected by the immune system. If you are interested in doing a project (Thesis project/Research project 30-45hp) in this field of research, please contact us.

Dynamics of gene regulatory networks in response to different stresses in Mycobacteria

Gene regulatory networks play important roles in *e.g.* the cell cycle, signal transduction, cellular differentiation, and adaptation to varying environments. Understanding the dynamics of these networks will lead to a better understanding of mycobacterial pathogenesis. Mycobacteria encounter numerous stresses during infection inside the host body. To adapt to the stressful environment, they alter their gene expression in a complex manner involving DNA-binding transcription factors and other regulatory factors. Studying the gene regulatory network during specific stresses will increase our understanding of adaption and survival of mycobacteria. Analysis of total transcriptome (RNA-Seq) data derived from 10 different stress conditions and growth phases, as well as of the sigma factor over expression transcriptomes from the fish and opportunistic human pathogen *Mycobacterium marinum* (a *Mycobacterium tuberculosis* model system) resulted in identification of stress specific transcription regulators. However, what the target genes of these transcription regulators are and how they are regulated during different stress responses has not yet been explored. In this project, we want to identify active DNA binding sites of these transcription regulators using CLIP-Seq under different stress conditions. Based on these data, we will be able to construct active gene regulatory modules during different stress conditions. This information may further lead to the identification of new drug targets for different mycobacterial diseases.

More specifically, we want to address the following questions:
1. How many transcription factors are present in *M. marinum* and what are the genes these factors regulate?
2. How are the gene regulatory networks perturbed in response to different stress conditions?
3. Are there genes regulated by multiple transcription factors?
4. How does the gene regulatory network of *Mm* differ compared with the *M. tuberculosis* gene regulatory network?
5. Finally, are there any stress specific modules present in the network?

Student projects (preferentially 45hp, but 30hp may also be considered)
The projects will involve growing *M. marinum* carrying plasmids with 6xHis tagged transcription sigma factors. The overexpression of these sigma factors should then be optimized during non-stressed conditions. Once accomplished, these proteins have to be cross-linked with their DNA targets and that too will have to be optimized. These cross-linked DNA-protein complexes then has to be purified with the help of the 6xHis tag. Degradation of the DNA by DNases will then destroy all the DNA except the region that is protected by the cross-linked protein. The cross-linking will then be reversed and the DNA fragments purified.

These will then be sequenced and mapped to the chromosome of *M. marinum*. When the technique has been established, the procedure will be repeated after exposing the bacteria to various stresses. This can then be correlated with existing transcriptomic gene expression data obtained after exposure to the same stresses and will lead to an understanding of how the gene expression pattern is accomplished and which strategy the cells use to adapt to the stresses.

Desired student background/skills
The students should have a background in microbiology/molecular biology/microbial genetics/biotechnology or similar. In addition to these skills, knowledge and experience in advanced bioinformatics (transcriptome/genome mapping and assembly) is an advantage. Practical experiences in 6xHis tag purification, cross-linking, and standard bacterial work are also desirable.

Contact
Interested students are welcome to contact Fredrik Pettersson (Fredrik.Pettersson@icm.uu.se). The work will be carried out at the Department of Cell- and Molecular Biology (ICM) in BMC, UU. Starting time is flexible, but the sooner the better… (from Fall 2018 onwards).