Ref: 04/JK

Project: Discovery and characterisation of constitutive promoters in mosquitoes
Supervisor: Dr Jaroslaw Krzywinski
Research Group: Vector Molecular Biology

Project Summary:

Recent technological advances in next generation sequencing have dramatically accelerated acquisition of sequence information from genomes of numerous organisms. To give biological meaning to this deluge of data, location of individual genes and other DNA features needs to be identified. Gene sequences can be detected with considerable precision, especially with support of sequence data from the transcriptomes (expressed portions of the genome). However, identification of the DNA elements that regulate gene expression, such as gene promoters, remains notoriously difficult and unreliable, and requires experimental validation. Promoters with ubiquitous expression are critically important for the analysis of gene function *in vivo*, to drive knock-down or overexpression of the desired transcripts irrespective of tissue localization. To date, only a couple of ubiquitous promoter sequences have been described in mosquitoes. The proposed work will contribute to filling in this major knowledge gap, by experimental validation of selected candidate promoter sequences from the African malaria mosquito *Anopheles gambiae*.

Details:

We have recently developed a robust system of *in silico* identification and subsequent testing of promoters in *A. gambiae* cell lines, which allowed us to molecularly characterize several new promoters. The placement student will work on characterisation of additional promoters using the above system. Each candidate promoter will be isolated from the genome, and cloned in front of a fluorescent marker gene or luciferase gene in a custom-built reporter plasmid. The plasmids will be individually transfected into a cell line, and the expression of the marker, indicative of the promoter activity, will be measured. Then, sequences homologous to the validated promoters from *Anopheles gambiae* will be identified in a mosquito *Aedes aegypti* (major vector of arboviruses, such as dengue and Zika), and their promoters tested in the *Aedes* cell line, as described above. The validated promoters will be subsequently used for experiments in transgenic mosquitoes, which forms a coherent part of the group's activity, however, due to technical complexity, this aspect of research is beyond the scope of the student's project.

The student will receive a comprehensive, hands-on training in all parts of the study, and will be exposed to the relevant aspects of bioinformatics, primer design, DNA isolation, PCR, restriction digestion, cloning, plasmid isolation, cell line transfection, fluorescence microscopy, and use of a microplate reader. In addition, the student will be introduced to mosquito colony maintenance, to get the feel of a variety of activities involved in the lab focusing on insect functional genomics. All the technical steps are of moderate complexity, to allow the student to perform all the experiments with sufficient guidance. All the necessary training will be provided by the head of the group and a postdoctoral researcher.

References for Suggested Reading:

- Behura et al. BMC Genomics (2016) 17:341.
- Lycett et al. Insect Molecular Biology (2012) **21**(1), 79–88.
- Anderson et al. Insect Molecular Biology (2010) **19**(4), 441–449.