Ref: 08/LCP

Project Title: The Tet-OFF system and the potential importance of transformation markers

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Project Summary:
The student will rear different transgenic mosquito lines and characterise the expression pattern of the selected strains to help identify key features of the Tet-OFF system. The findings will allow the optimisation of the design of future constructs, working towards the development of a Killer-Rescue drive in *Aedes aegypti*.

Details:
Mosquito-borne diseases are a severe public health burden worldwide. The mosquito *Aedes aegypti* is a widely distributed vector of arboviruses like chikungunya, dengue and Zika. As there are no safe and effective vaccines for these diseases, the development of genetics-based control methods such as gene drives to modify populations to make the mosquitoes less able to transmit pathogens is very attractive. One of such methods, which is localised, with low invasiveness and self-limitation, is a Killer-Rescue drive (1). This system requires effective lethal genes and efficient rescues, and the Arthropod Genetics group uses the Tet-OFF system to develop and test each component.

The Tet-OFF system is widely used, for example, to analyse the expression pattern of promoters of interest, and direct the expression of selected effectors in specific tissues or at a specific moment. It consists of two components, a tetracycline transactivator (tTA) and a TetO response element upstream of a minimal promoter. A selected promoter will express the tTA, which will bind to the TetO to activate the expression of the gene or genes downstream the minimal promoter (i.e. effector gene/s). The Arthropod Genetics Group uses the Tet-OFF system to coordinate the temporal and spatial expression of the different elements used in the variety of genetic control systems under development within the group.

Each of the transgenic lines carrying the Tet-OFF components also has its own transformation marker, i.e. a selected promoter that expresses a fluorophore in a constitutive or tissue specific manner. One of the promoters used to express the fluorescent marker is hr5-IE1, which includes the IE1 promoter derived from the Autographa california multicapsid nuclear polyhedrosis virus and the hr5 enhancer element that stimulates transcription from the IE1 promoter (2).

Although the Tet-OFF component and the transformation marker are included in the same transformation plasmid, they constitute separate units and are supposed to have no interaction. It is relevant, however, to test if the presence of the hr5 enhancer sequence influences the Tet-OFF system – either by expressing more tTA than what the selected promoter would generate, or by facilitating the expression of the effector by the stimulation of the minimal promoter.

This proposal has two objectives:

i) to determine if the presence of the hr5 enhancer has an impact on the Tet-OFF system.
ii) to analyse if the Tet-OFF system has an amplification effect on its own.

In order attain these, the Arthropod Genetics group is developing a variety of transgenic lines that include a transgenic marker that expresses the fluorophore DsR under the hr5-IE1 or the eye specific 3xP3 promoters (3), and both components of the Tet-OFF system using Carboxypeptidase A (midgut specific) as selected promoter and the fluorophore AmC as effector. The developed constructs also include lox sites to remove the tTA-TetO-minimal promoter segment via Cre-lox recombination, allowing the comparison of the expression of AmC when indirectly driven by Carboxypeptidase A via the Tet-OFF system, and when directly controlled by the promoter.

The student will compare the different lines by taking pictures of dissected midguts to compare the expression of AmC between them, and perform quantitative PCR experiments to detect correlations between the expression of tTA and AmC. The information obtained will allow the optimization of future constructs, and shed light on the interaction of these widely used genetic components.

References for Suggested Reading:

1. Alphey (2014) Ann Rev Entomol 59:205-224.
2. Ren et al. (2011) Afr J Biotechnol 10(44):8930-8941
3. Berghammer & Klingler (1999) Nature 402:370371

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