# Transgenic technologies in support of the IAEA mosquito SIT project

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The ability to genetically engineer mosquitoes is likely to have major implications for the development and improvement of genetic vector control systems such as the sterile insect technique (SIT). In particular, genetically transformed mosquito strains can be created for genetic marking and sexing, two key factors influencing the success of SIT programmes. In the context of malaria transmission, female elimination is of critical importance. Field releases of biting females, which transmit the parasite, during a SIT campaign is totally proscribed. Classical approaches to developing a sexing strain for *An. arabiensis* have proven alternatively simple or difficult during previous efforts (Lines and Curtis 1985, Andreasen, 2003) and their success cannot be guaranteed. Furthermore, the development of a sexing strain can be a long process. For example, the development of the Vienna-8 *Ceratitis capitata* genetic sexing strain currently used in several SIT programmes for the control of the Mediterranean fruitfly necessitated 10-15 years of research. Parallel to the creation of a conventional genetic sexing strain based on radiation-induced Y-translocation of a resistance marker, our group is undertaking a transgenic approach to genetic sexing. The use of germline transformation as a supporting technology for genetic sexing may help reduce the traditionally long R&D phase.

The sexing strategy currently tested in our laboratory exploits the differential splicing properties of the *An. gambiae dsx* gene to express sex-specific transcripts in both males and females. It is hoped that this approach will achieve the high sexing efficiency (above 99%) required for safe, and efficient releases.

Our initial efforts have concentrated on developing injection methods appropriate for *An. arabiensis*. Embryo microinjections of *piggybac* vectors are now performed routinely in the laboratory. Although originally developed for *An. arabiensis*, this simplified protocol should be useful for other mosquito species (protocol details can be accessed through the MR4 website, see:http://www2.ncid.cdc.gov/vector/Tech%20Tips/Microinjection%20Method%20for%20Anopheles%20Embryos%20V2.pdf).

Using this method, transient transgene (GFP) expression is readily observed in injected G0 larvae. Once established, transgenic sexing strains will require a thorough molecular and bio-ecological characterization. The latest include testing the effects of genetic transformation on fitness (i.e. longevity, dispersal, and lifetime mating success). Such processes are difficult to study in laboratory settings or may deliver results that are non-representative. Moreover, the release of genetically-transformed organisms would present unique challenges in terms of public health and ecological safety. Therefore, a comprehensive knowledge of the long-term stability, viability and reproductive competitiveness of genetically modified mosquito strains must first be obtained. To this end, we intend to use semi-field environments (i.e. greenhouses), which have proven to be a good intermediate between the laboratory and open field (Knols, 2003). Full control over the number released, their physiological status and genetic make-up enables rapid insight in the nature of critical determinants of mating success of mass-produced and sterilized (transgenic) males. This will allow simulation of inundative SIT releases and modeling of population-induced sterility.

## References

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