Fly Sindbis Virus Treatment Protocol

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Protocol reference: V. Avadhanula et al. 2009

Feeding Larvae:

Approximately 50 (mixed sex) young mated adults will be transferred to fresh food vial and maintained for 12 hours. Vials are cleared and allowed to age 3.5 to 4 days. Vials are then rinsed into aa series of sieves using tepid water and feeding third instar larvae are collected form the #40 sieve, transferred to a hard agar plate with a pot of yeast to induce crawling. Prior to reaching the yeast larvae are captured and 50 larvae are transferred to new food vials containing the virus stock and allowed to feed for 12 hours. Treated larvae are captured in the same way as described above, transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. The number of survivors is recorded and the mean lethality calculated for each treatment.

Sindbis Virus treatment:

A transgenic stock was obtained from Dr. Richard Hardy, Indiana Universtiy, Bloomington. This strain contained two transgenic elements. The first pUAST- SINrep: GFP is a construct in the pUAST vector containing pSINrep/GFP that encodes the non-structural proteins of the Sindbis virus tagged with GFP preceded by 5 UAS sequences. The GFP tag marks the production of Sindbis viral RNA but the RNA is incapable of producing capsid protein and therefore no mature virus van be made by the flies expressing this transgene. The second is a standard Act5C::Gal4 driver (P{Act5C-GAL4}17bFO1) obtained from the Bloomington Drosophila Stock Center. Both inserts are on the third chromosome and in the original stock the chromosome is homozygous lethal and balanced over TM3, Sb^1. Males from this stock were crossed to Oregon R virgin females and progeny Sb^+ adult males expressing GFP collected. These were crossed to Oregon R virgin females and the GFP positive male progeny collected. This procedure was continued for 10 further generations in order to place the Act5::Gal4=>UAS::SIN system into the same genetic background as previously used for RNA extraction. After the 12^{th} generation adult male and female progeny expressing GFP were collected, aged for four days and were flash frozen in liquid nitrogen. GFP positive larvae and pupae (each containing mixed ages and sexes) were also collected and were flash frozen in liquid nitrogen for RNA preparation.

The genotypes of the starting stock and the characterization of the transgenic lines is described in: V. Avadhanula, B. P. Weasner, G. G. Hardy, J. P. Kumar, R. W. Hardy. 2009. A novel system for the launch of alphavirus RNA synthesis reveals a role for the Imd pathway in arthropod antiviral response. PLoS Pathogens. 5 (9). e1000582