Microinjection protocol for Spodoptera Sp. Version 3

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Abstract

Modification on the coventional protocol for insect microinjection was performed. This protocol describe a simple and unique protocol for microinjecting *Spodoptera frugiperda*. Insect eggs with thin chorions such as *S. frugiperda* or with chorions that are removable can be sensitive to desiccation, resulting in death of the embyro. To control or prevent excessive desiccation, eggs can be covered with liquid during the microinjection process. *Spodoptera frugiperda* are relatively large insect eggs (about 400µm in diameter) and because the chorion does not have to be removed it is somewhat less sensitive to desiccation during the microinjection process. However, injecting *S. frugiperda* eggs in the absence of any fluid covering resulted in rapid clogging of injection needles due to rapid drying of residual ooplasm onto the outside of the needle. So, seek for alternative covering liquid became a demand to overcome the lethal effect of the conventional covering liquid in order to developed microinjection and delivering system for the target insect emberyo. New liquids were tested for their compatibility with *S. frugiperda* viability. Halocarbon oils (either low viscosity #27 or high viscosity #700) which are used routinely during the microinjection of some Diptera, allowed for easy injections but were lethal to *S. frugiperda* embryos even after removing as much of the residual oil as possible immediately following injection. Alternatively, an emulsion of halocarbon oil and water; polysorbate 20 (Tween 20®); was used and didn't affect on egg viability (90% hatch rate), while it causes turbidity and made injections more difficult due to the obstructed visibility. Finally, dilution of honey was found to a very effective medium with which to temporarily cover the eggs during injection resulting in a 100% survival rate of properly aged embryos. The solutions viscosity was similar to halocarbon oil #27 and this allowed the honey solution to completely cover the eggs without flowing off of the eggs and onto the glass slide. There were no problems with injection needles clogging due to ooplasm adhering and drying on the outside of the needle, and the honey solution was readily removed immediately after injection. Prolonged (24hrs or longer) exposure of developing embryos to the honey solution was detrimental to embryonic development and led eventually to death of the embryos before hatching and therefore it was removed immediately after eggs were injected.

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Guidelines

1-Spodoptera eggs in the preblastoderm stage of development and were mounted to a glass microscope slide by placing them on a small strip of transparent film-dressing (Tegaderm®, 3M, St. Paul, MN) which had been fixed to a glass slide with its adhesive-containing side facing up using double-sided tape (Scotch®, 3M, St. Paul, MN).

2-Micro-injection needles were produced from quartz-glass capillaries using a P-2000 needle-puller (Sutter Instrument Company, Novato, CA) using the following settings: HEAT = 750, FIL = 1, VEL = 40, DEL = 1215, PUL = 125.

3-Needles were back-filled with the injection solution using a 100 µl capillary and the filled needle was mounted in an MPH3 needle-holder (World Precision Instruments, Sarasota, FL) connected to a Picopump (World Precision Instruments, Sarasota, FL). Pressure used for all injections was between 10-20psi.

4-Microijection process carried out under Olympus SZH dissecting microscrope equipped with trans-illumination.

Before start

1- Collect deposited egg in early stage (1 hour).

2- prepare needles.

3-Prior to injection *Spodoptera Sp.* eggs, it should be covered with a liquid to control desiccation and facilitate the injection process.

Materials

- Natural Honey by Contributed by users
  polysorbate 20 by Sigma-aldrich

Protocol

Step 1.

Collect eggs in the preblastoderm stage of development and mounte to a glass microscope slide by placing them on a small strip of Tegaderm® transparent film, which fixed to a glass slide with its adhesive-containing side facing up using double-sided tape.

Step 2.

Conventional covering liquids used to control desiccation during injection process included holocarbon oils No.27 or No. 700 was applied on the top of eggs surface.

Step 3.

New liquids were examined as alternative covering liquids as follows:

A-diluted honey 1:1 with water.

B-mixture of halocarbon oil No. 700, Tween 20 (1g No. 700), Tween 20 (80 µl) and water (1ml).

The purpose of using these alternative liquids was to prevent desiccation of the embryos and to facilitate microinjection.

Step 4.

Micro-injection process performed using needles mounted in an MPH3 needle-holder (World Precision Instruments, Sarasota, FL) connected to a Picopump (World Precision Instruments, Sarasota, FL). Pressure used for all injections was between 10-20psi under Olympus SZH dissecting microscrope equipped with trans-illumination.

Step 5.

evaluate the tested liuids on egg viability by cheking the development of the emberyo under microscope.

Step 6.