Sex biased collecting strategy for gene expression in mealybugs

Laura Ross

Abstract

This protocol was used to determine when to collect sex biased samples for RNA extraction and establish expression profile throughout mealybug development.

Sex ratio throughout oviposition was established first in Planococcus citri (Ross et al., 2010). We used the procotol that the authors established and tried it on Planococcus kraunhiae. The sex ratio variation follow a similar pattern (Vea et al., 2016).

Citation: Laura Ross Sex biased collecting strategy for gene expression in mealybugs. **protocols.io** dx.doi.org/10.17504/protocols.io.nkndcve
Published: 02 Mar 2018

Materials

- Chloroform by Contributed by users
- DAPI (2.5mg/mL) by Contributed by users
- 1.5 mL Eppendorf tubes by Contributed by users
- 10 x PBS Solution (Phosphate Buffered Saline) PD8117.SIZE.4L by Bio Basic Inc.
- 100% Ethanol by Contributed by users
- 100% Acetic acid by Contributed by users
- Microscope slides by Contributed by users
- Coverslip by Contributed by users
- Fluorescence compound microscope (x200) by Contributed by users

Protocol

Egg collection

Step 1.

Separate mated females that are about to lay eggs on sprouted potatoes or sprouted beans and wait until the first eggs.

Egg collection

Step 2.

Collect embryos every 24 hours as they are laid, and fix them in 200-500 uL Carnoy fixative in a 1.5mL Eppendorf tube and store at 4ºC for maximum 4 days. If the embryos can only be prepared after 4 days, replace the fixative with 90-100% ethanol and store at 4ºC.

Carnoy fixative:

- 4 parts chloroform

- 3 parts ethanol

- 1 part acetic acid

DAPI staining

Step 3.

Transfer the embryos on a microscope slide, let the fixation or ethanol evaporate enough to have the slide dry but the embryo still humid.

DAPI staining

Step 4.

Add 20 uL of DAPI diluted in 1xPBS at 1:1000

DAPI staining

Step 5.

Add coverslip, seal with nailpolish and keep in the dark for 10-15 min

DAPI staining

Step 6.

The slides can be visualized with a fluorescence compound microscope. The heterochromatin is best seen at 200x

Counting embryos

Step 7.

Each slide will have all the embryos from one day of oviposition.

For each slide, identify male and female embryos (see result section) and count the numbers of males and female embryos

Counting embryos

Step 8.

Report the sex ratio for each day of oviposition.

If possible repeat the process for at least 5 females.

Counting embryos

Step 9.

Use Excel or R to make a graph of sex ratio