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Transmission assay Cicadella viridis

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Abstract

This protocol is used to determine whether Cicadella viridis can transmit a bacterium of interest.

Troubleshooting

Pre-preparation

1 Make an o/n culture of the bacteria for which you wish to test transmission.

2 Capture the insects required in the experiment.

3 UV sterilise the following:
a.1 PCR tube tray
b.2 x N PCR tubes
c.2 X N pieces of 1 * 16 cm green tape (reference)
d.2 X N pieces of 1 * 2 cm parafilm M
e.M9TG
Where N is the amount of replicates used including negative control. Additionally, alcohol-sterilise a pair of scissors.

Note: it is highly recommended to perform steps 3-7 in a UV hood, to reduce possibilities for contamination.

Preparing setup

4 Cut the last 2 PCR tubes from all rows of 8, and cut off the caps from the remaining 6

5 Fill N/2 lines of 6 tubes with 320 ul M9TG medium per tube.

Note: When using 1250 ul pipette tips, it is recommended to pipette the solution deep into the PCR tube, such that no bubble is formed in its bottom.

6 Stretch parafilm M overthe far ends of the line of PCR tubes allowing the parafilm to come into contact with the solution.

7 Stretch the parafilm M over the sides of the line of PCR tubes, carefully making sure no air bubbles are allowed to form

8 Apply the green tape around the line of tubes to keep the parafilm M in place

9 Take N 250 mL PET containers (flesschenland.com) and use an (injection) needle to make airholes in the screw cap.

10 Wetten one single use paper towel with tapwater. Squeeze the paper to remove excess water, and use it to cover the floor of the container

11 Attach the lines of PCR tubes to one side of the containers with a short stretch (+/- 1 cm) of green tape. Tweezers can be used to access the narrow container.

12 Mark this side of the container as “clean”.

13 Repeat steps 4-7, but instead of M9TG, add 320 uL of a solution of 80% M9TG, 20% o/n culture to every tube.

14 Attach the lines of PCR tubes created in step 13 to the wall of the container opposite to the “clean” line of tubes using short stretches (+/- 1 cm) of green tape. Label this side with an identifier of the bacterium in the tubes.

Introducing insects

15 Take a bottle in which an appropriate amount of insects are caught.

16 Move the insects to the bottom of the bottle by inverting and waiting for the insects to climb upwards, or by gently tapping the bottle.

17 Open the container, and invert the bottle above this container.

18 Unscrew the cap of the bottle and dislodge the insects from the bottle with a few forceful taps

Note: chance of insects escaping can be minimized by tapping the sides of the container with the bottle during this step, as not to provide insects the chance to recuperate and jump out

19 Swiftly close the container with the screw cap

20 Keep the containers at room temperature where sunlight cannot directly hit them

21 Wait for 2-4 days

Plating

22 Sedate or kill the insects by injecting appropriate amounts of a sedative (e.g. ethyl acetate or chloroform) by injecting through the screwcap.

23 Prepare an amount of 1.5 mL eppendorf tubes with 200 uL of LB medium equal to the amount of insects used.

24 Use a scalpel to dissect away the head of the insect by cutting through its thorax. Place the dissected heads in the eppendorf tubes and crush it with the backside of an inoculation loop.

Note: Innoculation loops are usually packaged as to prevent contamination of the “loop” side. It is recommended to take great care not to touch the backside of the inoculation loops, as to prevent contamination, or open the package at the other end.

25 Briefly vortex the eppendorf tubes and plate 100 uL on an agar plate with antibiotics suitable to the resistance(s) of the bacterium used.

26 Plate 100 uL of the “clean” PCR tubes. It is recommended to also plate some spiked PCR tubes as a control.

Note: to reduce the amount of plates required, it is possible to pool samples of PCR tubes from the same container, or insects from the same sex and container.

27 Incubate the plate overnight at 37 C

Collecting results

28 Count the resulting amount of colonies on plates for insects and PCR tubes. It is recommended to verify the identity of colonies by colony PCR.