Inoculation of Holidic Media (HM) with bacteria to generate gnotobiotic Drosophila

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Abstract

This protocol is part of the manuscript: Gonçalves et al. Commensal bacteria and essential amino acids control food choice behavior and reproduction. Plos Biology. 2017 Apr 18.

We thank the Leulier laboratory for their help in establishing this protocol.

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Protocol

Bacterial cultures preparation

Step 1.

Grow the bacterial cultures in liquid media according to the Growing Drosophila gut bacteria protocol.

DURATION
04:00:00

Bacterial cultures preparation

Step 2.

Prepare 1:10 dilutions of each bacterial liquid culture using the corresponding media to do the dilutions. Measure the OD at 600nm using a spectrophotometer and using the corresponding media as a blank.

Bacterial cultures preparation

Step 3.

Allow the bacterial cultures to grow until they reach approximatly the following ODs:

- OD *L. plantarum*^{WJL}=6
- OD *L. brevis*^{EW}=8
- OD *C. intestini*^{A911T}=1.5
- OD *A. pomorum*=0.7
- OD *E. faecalis*=0.8

NOTES

Carlos Ribeiro 10 Apr 2017

If these ODs are much higher than the reference values, a contamination likely occured. If they are much lower, it is likely to mean that the bacteria are no longer healthy. In these cases we advise you to start a new solid culture from the frozen bacterial stock.

Step 4.

Prepare the HM needed for the experiment following the steps in the Holidic Media (HM) preparation protocol.

Calculating the volume of bacterial culture required

Step 5.

For each HM vial you will need the following volume of the liquid culture:

- 0.025 ml of *L. plantarum*^{WJL} culture
- 0.002 ml of *L. brevis*^{EW} culture
- 0.1 ml of *C. intestini*^{A911T} culture
- 0.2 ml of *A. pomorum* culture
- 0.2 ml of *E. faecalis* culture

Calculate the total volume of each culture required for your experiment by multiplying these volumes by the total number of vials being inoculated.

Preparation of the bacterial mix

Step 6.

Once the total volume of each bacterial culture is known, pipette them into a falcon to obtain the required combinations.

Preparation of the bacterial mix

Step 7.

To exclude an effect of residual components of the bacterial media on the experiments, pipette the equivalent volume of the different sterile bacterial media and use them as a control.

Preparation of the bacterial mix

Step 8.

Centrifuge the bacterial mixes and controls at 3000 rpm in an Eppendorf 5810R centrifuge for 10 min at room temperature, remove the supernatant, and re-suspend the pellet in 1x sterile PBS.

DURATION
00:15:00

Preparation of the bacterial mix

Step 9.

Repeat step 8 twice.

DURATION
00:30:00

Preparation of the bacterial mix

Step 10.

After the final centrifugation, discard all the supernatant from the control and the bacterial mixes and re-suspend the pellets in sufficient 1x sterile PBS to obtain a final inoculation volume of 50 µl per vial. Mix by gently pipetting up and down.

Preparation of the bacterial mix

Step 11.

For the experiments with heat-inactivated bacteria: incubate the bacterial mix at 100°C in a dry bath for 10 min before inoculating the HM vials.

DURATION
00:10:00

Inoculation of bacterial mixes in HM

Step 12.

Add the final suspensions of control and bacteria mixes to the surface of the HM (50 µl per tube) and allow it to dry for approximately 1 h before adding the flies.

DURATION
01:15:00