# Isolation of live bacteria from adult insects

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**Method Article**

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# Abstract

# Introduction

Many experiments such as transfection of cells and organisms, live imaging and certain biochemical assays, require isolation of live, viable bacteria from insects. This protocol was developed to isolate Wolbachia from adult Drosophila, but it can be adapted for other insects. In some insects leg removal prior to isolation facilitates hemolymph extrusion [1]. This is not necessary for Drosophila. Conventionally, for transfection experiments, Wolbachia are isolated from embryonic cytoplasm [2-5]. In comparison, the main advantages of this method are: Better elimination of debris from ultra-filtration. Faster; requires less manipulation. Less yolk is co-purified with the bacteria. Due to the associated auto fluorescence, yolk becomes an issue for imaging \(see also “Live imaging of Wolbachia”).

# Reagents

Reagents: 1. Drosophila \(or other insect) infected with Wolbachia. Many stocks from the Bloomington stock center are infected with Wolbachia [6]. Have also a stock free of Wolbachia to be used as control. A Wolbachia free control stock can be easily obtained by using fly food containing tetracycline \(0.25 mg/ml), for three generations. 2. Ultrafree – MC Centrifugal Filter Unity. This low binding microporous filter assembled on a microcentrifuge tube allows filtration of biological solutions \(Millipore Corporation, Bedford, MA). Wolbachia diameter sizes vary from 0.5 to 1.5 micrometer. Two filters size were tested. In our hands, even the smaller one did not seem to reduce yield and produced a cleaner product: 0.65 micrometers, Catalogue No. UFC3 ODV 5.0 micrometers, Catalogue No. UFC3 OSV 3. 70% EtOH \(v/v) 4. Sterile H20

# Equipment

1. Microcentrifuge.

# Procedure

\(Use autoclaved microcentrifuge tubes and gloves) 1- Collect 50-100 adult insects \(for Drosophila, females will give higher yield). Anesthetize on CO2. Keep the flies and 70% EtOh on ice. 2- Transfer anesthetized flies to the Centrifugal Filter \(5 or 0.65 micrometer). 3- Wash 5 times with 70% ethanol. Invert the tube a couple of times between washes. This step is to collect as much debris and sterilize the fly surface as much as possible. 4- Spin for 5-10 seconds in tabletop microcentrifuge at low speed setting \(around 1,000 g) to dispose most of the EtOH 5- Wash 3x with sterile H2O. 6- Spin 5-10 seconds to eliminate most of the water 7- Transfer the filter unit to a sterile microcentrifuge tube. 8- Spin for 3 minutes at around 10,000 g. In case yolk is not desired \(e.g. for visualization) low speed centrifugation is recommended to avoid disruption of eggs inside females. Speeds can go as low as 1,000 g for 3 minutes. 9- From 50 to 100 flies the yield is around 10- 20 µl. To maximize the bacteria viability, keep on ice all the

time. The exudates can be used for transfection into other hosts \(see “Transfection of Wolbachia into adult hosts”), cell culture or imaging. The presence of bacteria can be quickly verified with a DNA stain \(see “Live imaging of Wolbachia”). As a control, always compare with hemolymph isolated from an uninfected stock.

## Timing

30 minutes.

## References

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