Procedure for making agar plugs to support growth of axenic mosquitoes

Disposables
Sterile pipette tips
Petri dishes (3)

Equipment
Autoclave
Centrifuge
4 x 500 ml sterile centrifuge bottles

Reagents
1 container of 20 ml sterile LB broth (Difco)
2 1L containers of sterile LB broth (Difco)
Phosphate buffered saline (Dulbecco's Phosphate Buffered Saline; Sigma)
E. coli K-12 grown on an LB agar plate
1% 3:2 liver:yeast solution

Mix 3 g of liver extract (Difco, dessicated, powdered beef liver) and 2 g of yeast extract (Fisher Scientific, granulated yeast extract) in a 50 ml Falcon Tube. Dissolve 1g of mixture per 10 ml of H2O to make a 1% solution

Note: The procedure below is based on agar plugs consisting of stationary phase autoclaved *E. coli*, as this is the simplest procedure. Results in the manuscript were primarily derived from mid-log phase sonicated and autoclaved *E. coli* (see manuscript for details).

Day 1

E. coli starter culture

1. At the end of the day, inoculate 20mL of LB with *E. coli* (wildtype strain K-12). This can be done in a 50 mL sterile conical tube. Leave on shaker overnight at 37°C.

Day 2

1. Inoculate each 1L flask of LB with 5mL of the K-12 starter culture.
2. Leave the flasks on the shaker at 37°Covernight.

Day 3

Collect cell pellets

1. Pour ~500 ml of culture into each of the autoclaved centrifuge jars. Balance to within 0.5 g.
2. Centrifuge the jars for 10 minutes at 10,000 rpm. Pour off the supernatant.
3. Re-suspend the pellets in a total volume of 20 mL sterile PBS. Transfer to an autoclavable container.

Make agar solution

1. Add 0.9g of agar to 40 ml H_2O.
2. Add 2g of Liver:yeast extract

Sterilization and pouring agar plates

1. Sterilize cell pellets and agar solution by autoclaving for 1 hour.
2. After autoclaving: allow to cool, mix the cell pellets and agar solution, pour into 3 Petri dishes.

Agar plates can be stored in the refrigerator for up to 3 weeks.

Agar plugs were supplied to mosquito larvae in an amount of 0.4g per well in a 6 well palte or ½ a plate per 50 larvae in mass rearing experiemnts.