2/28/2006
Elisha Wood-Charlson
Rodriguez-Lanetty, M., Krupp, D.A. and Weis, V.M. (2004) *Mar Ecol Prog Ser* 275: 97-102.

**Hawaii – Infecting *Fungia scutaria* with Algae (quantitative measure of infection success)**

**Materials/Reagents**

0.25µm filtered seawater (fsw)

Hemocytometer, coverslips, cell counter

6-well culture dish

Frozen *Artemia* sp.

40µm cell strainers (BD Falcon: 352340, blue)

1.5ml tubes

1ml plastic pipettes

**10X PBS**

| | |
|---|---|
| Make up in 800 ml | 800 ml |
| 0.02M $NaH_2PO_4$ (monobasic) 120 g/m | 1.92 |
| 0.077M $Na_2HPO_4$ (dibasic) 142 g/m | 8.8 |
| 1.4 M NaCl 58 g/m | 69.6 |

add $DH_2O$ to 800 ml
Autoclave

**1X PBS**
100 ml 10X PBS
900 ml $DH_2O$ water

**Paraformaldehyde fix**
1 g paraformaldehyde
12.5 ml $DH_2O$
2.5 ml 10X PBS
10 ml $DH_2O$

a) Heat 12.5 $DH_2O$in a small beaker with vigorous stirring.
b) Weigh out paraformaldehyde making sure to wear a mask and use gloves.
c) Add paraformaldehyde to fast stirring, heated water – stir for about a minute, do not allow to boil. Powder will not dissolve.
d) Remove from heat and continue to stir.
e) Add 1 drop of 1 N NaOH to solution. The solution should clear.
f) Once clear, filter the solution through a sterile filter and check that volume is 12.5 ml – if not add up to 12.5 with water.
g) Add 2.5 ml of 10X PBS and 10 ml of additional $DH_2O$ water.
h) Check pH – should be around 7.0 – 7.5. If not start over.

**Make fresh every time (use w/in 24h, store at 4°C and bring to room temp before using)**
**Keep away from LIVE material, only work with under the hood!!**

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**Procedure**

A. Titer algae (see algal cell count protocol for more detail)

1. Use clean, recently extracted algae
2. If really dark brown, dilute to a pale brown color (keep track of dilutions)
3. Place coverslip on hemocytometer
4. Mix sample well
5. Load both sides
    a. 10µl with a 10µl pipetteman into the hemocytometer
    b. Load at a 45° angle w/ constant speed – should fill uniformly
6. Count squares depending on density
    a. Dense – count middle only
    b. Medium – count corners and middle
    c. Light – count all nine
7. Record # algae counted in # of squares
8. Repeat with other side
9. Repeat load and counts one more time
10. Calculate cells/ml
    a. (# cells/# squares) x (# squares) x (10,000) = # cells/ml
    b. Average the 4 replicates to get a final conc. of cells/ml
11. If diluted original solution, calculate cells/ml in original solution
12. Titer to $10^6$ cells/ml for infections (inoculate w/ 1ml – $10^6$ total algae)

| | | |
|---|---|---|
| ml | l | ml |
| l | dml | l |
| ml | l | ml |

B. Infecting *Fungia scutaria* larvae

1. Day 3: examine larvae under microscope to determine if mouths are beginning to form.

**Do not infect before Day 4**

2. Morning: conc. larvae during cleaning (see protocol "Cleaning *Fungia scutaria* larvae")
    a. Choose a populated, healthy larval bowl
    b. Clean, but do not add fsw to rinsed larvae
    c. Choose another bowl, clean and add larvae to previous bowl
    d. Repeat if necessary – should be thick!
    e. Use within 4-6hrs
3. Afternoon: infect w/ titered algae
4. Thaw *Artemia* sp., homogenize and filter to create an 'essence of *Artemia*'
5. Add to each well in 6-well culture dish
    a. 4ml fsw
    b. 5ml conc. larvae
    c. 1ml algae (= $10^6$ cells minimum) + 'essence of *Artemia*'
6. Cover and place in watertable on bricks so immersed but not submerged
7. Feed for 3h

C. Rinsing infected *Fungia scutaria* larvae

1. After 3h, remove 6-well plates from watertable
2. Set up a clean 6-well plate, fill 4 wells w/ fsw
3. Using a clean plastic pipette, withdraw larvae from well and squirt onto 50µm cell filter over empty well

4. Rinse larvae on the filter by dipping and squirting w/ fsw
5. Rinse original 6-well plate with di-water
6. Replace larvae to original well by inverting filter and squirting w/ fsw
7. Cover, return to watertable and repeat
8. When finished – this is TIME 0

D. Collecting and fixing *Fungia scutaria* larvae

1. At set time point, remove 1ml of larvae with plastic pipette and place in a LABELED 1.5ml tube
2. Spin 1min, 1000x*g*
3. Remove sup to conc. larvae
4. Fill with paraformaldehyde
5. Parafilm and store at 4°C

**Comments:**