

## Small-scale culture of the ascidian *Ciona intestinalis*

Yasunori Sasakura

9th June 2021

We can culture a few (1-4) *Ciona* until reproductive maturation in 5L aquarium. The growth rate is dependent on the number of animals in an aquarium and smaller number will yield better growth.

The culturing condition is as follows.

Day 1: Fertilization. Start culturing in a 9 cm plate. Do not add too many eggs in a plate.

Day 3: Metamorphosis. Remove *Ciona* on the side. Exchange seawater.

\*In the following steps, please flush the bottom of the plates with seawater in a cleaning bottle to remove feces and remaining diatom cells.

Day 5: Selection. Remain 3 *Ciona* per plate that grow well and emit fluorescence (if available). Remove other animals.

Day 7 (1 week after fertilization): Dilute 20ul of Sun-culture (company-made, living diatom *Chaetocerus calcitrans*; 50,000,000cell/ml) in 3L seawater. Exchange seawater with the diluted diatom.

Exchange seawater every other day with the diluted diatom in this week. We perform this exchange on Monday, Wednesday and Friday.

Day 14 (2 weeks after fertilization): Dilute 100ul of Sun-culture in 3L seawater. Exchange seawater with this diluted diatom.

Exchange seawater every other day with the diluted diatom in this week. We perform this exchange on Monday, Wednesday and Friday.

Day 21 (3 weeks after fertilization): Enter *Ciona* plate in a 5L aquarium. We place up to two plates in an aquarium. Give water flow with a motor (30 rpm). Exchange seawater at least twice a week. We do this on Tuesday and Friday.

The quantity of food is as follows:

Day 21-27: 0.5ml Sun-culture per plate once a day.

Day 28-41: 0.5ml Sun-culture per plate twice a day.

Day 42-48: 1.0ml (one plate) or 1.5ml (two plates) Sun-culture twice a day.

Day 49-55: 1.5ml (one plate) or 2.0ml (two plates) Sun-culture twice a day.

Day 56- : 2.0ml Sun-culture twice a day.