

# Induced spawning of gametes in *Ciona intestinalis*

Light-induced spawning is a procedure utilized when adults are needed to be kept alive for repeated embryo collection. It is based on the observation that a number of ascidians spawn in response to light following darkness (see Lambert and Brandt, 1967). This method is similar to that successfully utilized for genetic screens.

1. Select healthy and mature specimens (**mature animals are usually detected by the fullness of the gonoducts, as observed through the transparent test** (ASSEMBLE – JRA 1 – Protocol – 04.00).
2. Place the animals under continuous illumination, lighting the tank with a lamp from above, until the dark adaptation period, that is the next step. **This light condition prevent spontaneous spawning and is also applied for obtaining animals with more ripeness gonoducts.**
3. Shift the animals in the dark condition for one hour. Take care to cover the vessel correctly in order to avoid the light entering.
4. Re-expose animals to light. It is sufficient to uncover the vessel; to strengthen the effect, a light can be switched on above the vessel. Spawning generally occurs within about 30 min after the onset of illumination.
5. Remove the animals and collect embryos, moving them in clean Petri dishes with filtered seawater.  
The induced spawning leads to the release of both male and female gametes from the animals. **Use a single animal per vessel for self-fertilization. Use more animals (2 or more) per vessel for cross-fertilization.**
6. Water should be changed 1 hour after the transfer to the dishes, in order to remove surplus sperm (see also Protocol for fertilization tests in *Ciona intestinalis* ASSEMBLE – JRA 1 – Protocol – 02.00).

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**Paola Cirino**

*Stazione Zoologica Anton Dohrn, It.*

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*Animals: Adult ascidians collected from natural population.*

*Apparatus: a plastic box or a vessel with dark (black) sides and cover, a lamp, filtered seawater 0,22μ, Pasteur pipets and pipet bulbs, Petri dishes, eppendorf tubes.*

*Parameters: T 18-20 °C*

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*Additional information:*

**Cirino P., Toscano A., Caramiello D., Macina A., Miraglia V. and Monte A.** Nov.27, 2002. *Laboratory culture of the ascidian *Ciona intestinalis* (L.): a model system for molecular developmental biology research.*

*Mar. Mod. Elec. Rec.[serialonline].*  
<http://www.mbl.edu/html/BB/MMER/CI R/CirTit.html>

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*Contact e-mail: [paola.cirino@szn.it](mailto:paola.cirino@szn.it)*

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