

Jan 28, 2022

Staging colonial ascidians

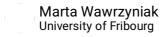
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dx.doi.org/10.17504/protocols.io.bsgznbx6

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This protocol has been successfully used to stage *Botrylloides diegensis* in our laboratory and was based on the following publication:

Ontology for the Asexual Development and Anatomy of the Colonial Chordate

Botryllus schlosseri

DOI

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Simon Blanchoud, Marta Wawrzyniak 2022. Staging colonial ascidians. **protocols.io**

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colonial ascidians, Staging, Botrylloides diegensis, tunicates

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1 glass Petri dish with a slide holder high magnification stereoscope Artificial Seawater (ASW)

Staging 30s

1 Select the slides to be staged.

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Citation: Simon Blanchoud, Marta Wawrzyniak Staging colonial ascidians https://dx.doi.org/10.17504/protocols.io.bsgznbx6

- 1.1 Clean the colony. See Cleaning colonial ascidians V.2 2 Fill the Petri dish with ASW. See Artificial sea water V.2 3 Observe the organization of the zooids and the systems. Check for signs of takeover: 3.1 No signs of active filter-feeding. 3.2 Zooids within systems are not properly fused yet. 3.3 Remnants of resorbed zooids are still visible. Turn the colony upside-down. Look for buds to stage. 4 4.1 Start at low magnification, look for well-visible buds (the one that are not too much underneath the zooid-parent, typically posterior to the adult). 4.2 Once the buds are identified, zoom in.
 - 4.3 Monitor the activity of the heart. Check for signs of regular beating:

Flow will be flowing through the bud at all stages, even without heart beats.

At early stages, heart will be erratic and weak.

Every minute or so, flow will be reversed and heart beat will stop
transiently.

- 5 Look for secondary budlets to stage.
 - 5.1 Budlets are often hidden or difficult to observe, focus on the most evident ones.
 - 5.2 Variability can be observed within a colony. Monitor multiple buds and use the average stage as your reference.
- 6 Use the provided identification scheme to infer the stage from your observations. staging_scheme.png
- 7 The best method to ensure the current stage is to confirm it by staging on consecutive days. Stages should proceed approximately by one per day (at 20°C).
- 8 Take a couple of representative images for archiving.