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Staging colonial ascidians

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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](#)

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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.

- 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.
- 4 Turn the colony upside-down. Look for buds to stage.
 - 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.



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.