Subcloning colonial ascidians

Simon Blanchoud[1]
[1]University of Fribourg

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1 Works for me dx.doi.org/10.17504/protocols.io.brugm6tw

Blanchoud lab, UNIFR

Marta Wawrzyniak

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MATERIALS TEXT

- 2 containers with system water
- 1 plastic tray
- 1 paintbrush
- 1 microfiber tissue
- 1 single-edge razor blade (sharp)
- numbered glass slides and plastic slide racks (as many as required)

1 Place the cleaned slides (see protocol) to be subclone in the first tray inside a slide rack.

2 Take one slide.

3 Determine how many additional slides will be required.

3.1 Subcloning is typically performed either to recenter systems onto the same slide, to provide more space to large colonies by propagating them onto new slides, or to split a strain into multiple clones.

3.2 Systems from the same clone can be regrouped during subcloning, they will then fuse into one larger clone.

4 Wet the new slides with seawater, let them dry upwards in the plastic tray.

5 Using a sharp razor blade, amputate the systems to be moved.

5.1 If systems need to be split or separated from the rest of the colony, perform the incision first using a gentle vertical slicing movement.

5.2 Detach the systems to be subcloned by performing a horizontal slicing movement on the glass slide. The system should come to rest on the razor blade.

5.3 If needed, slice the excess and/or dirty tunic underneath the subclone to provide a flat attachment surface.

a) Put the subclone upside down on your fingers.
b) Hold it gently with your thumb.
c) Slice the excess tunic perpendicularly.

6 Dry the excess water using the microfiber tissue.

7 Take the target slide, dry it, place it horizontally in front of you.

8 Transfer the subclone onto the new slide.

8.1 Gently push the subclone using a wet paintbrush.

8.2 If the subclone is too small to be pushed out of the razor blade, place it on the paintbrush by performing a rolling movement, deposit it on the slide by perform the converse movement.

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9 Dry the excess water around the subclone.

10 Look underneath the subclone, make sure there is no air bubble trapped under it.

11 Let the subclone dry vertically in the plastic tray for 5 min.

12 Using the paintbrush, gently wet the zooids of the subclone. Make sure all tissue gets wet properly

13 Dry the excess water and let it dry once more for 5 min.

14 Repeat steps 12 and 13

15 Take the slide, enter the water diagonally, place it into the second container vertically. Let it rest for 30 min.

15.1 If the subclone detaches from the slide, start again from 12.

15.2 Typical issues are a dirty tunic (repeat step 5.3) and dried tissue (repeat step 12).

16 Take all the subclones slides out of the water, place a maximum of 7 per slide rack, put the whole rack vertically in the quarantine. Let it rest for 2 days.

17 Check all subclones, clean the dead tissue off the slides.

18 Move the subcloned colonies to the main system.

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