Subcloning colonial ascidians V.2

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1 Works for me
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ABSTRACT

We have been subcloning colonial ascidians since some time already, till now - with a lot of success. This protocol has been ruinously used by many of our co-workers.

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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)

Subcloning colonial ascidians 2d 0h 35m

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

Cleaning colonial ascidians
by Marta Wawrzyniak,
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PREVIEW RUN

1.1 1. Select the slides to be cleaned.

1.1.1 Place them in the first container in a slide rack.

1.2 Take one slide.

1.3 1. Using the single-edge razor blade, wipe clean as much surface as possible.

1.3.1 Make sure to avoid the colonies and the vascular system.

1.3.2 Do not forget to clean the sides of the slides too.

1.4 1. Using the wet paintbrush, wipe gently the systems and the tunic of the colonies.

1.4.1 Try brushing from the center of the colony towards its rim, to avoid detaching it.

1.4.2 Brush out all the weird-looking tissue.

1.5 1. Using the curved-edge scalpel blade, ablate the detached or decaying tissue.

1.5.1 Use a rolling movement to avoid detaching more tissue: press the tip of the blade on the glass, do not slide it, roll the blade to press the edge through the tissue.

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1.6 Place the clean slide into the second container inside another slide rack.

1.7 If the colony as grown too much or outside of the center of the slide, subclone it (see protocol).

1.8 1. **Optional:** If slide needs to be super extra clean:

1.8.1 Place the cleaned slide under the stereoscope.

1.8.2 Inspect the slide.

1.8.3 Use the wet paintbrush to clean the remaining dirt.

1.8.4 Use the plastic tweezers to pluck recalcitrant pieces of foreign tissue.

1.8.5 Let the slide rest in FASW (filtered artificial sea water) for 10 min

1.9 Once all slides are clean, put them back in the system.

2 Take one slide.

3 Decide how many additional slides you want to propagate your colony onto.

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.

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4 Wet the new slides with system water, 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.

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

6.1 Put the subclone upside down on your fingers.

6.2 Hold it gently with your thumb.

6.3 Slice the excess tunic perpendicularly

7 Dry the excess water on the colony using the microfiber tissue.

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

9 Transfer the subclone onto the new slide.

9.1 Gently push the subclone from the surface of the razor blade using a wet paintbrush.

9.2 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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10 Dry the excess water around the subclone.

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

12 Let the subclone dry vertically in the plastic tray for **00:05:00** . 5m

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

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

15 Repeat steps 12 and 13

16 Take the slide, enter the water diagonally and place it into the second container vertically.

17 Let it rest for **00:30:00** . 30m

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

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

19 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.

20 Let it rest for **48:00:00** . 2d

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

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22 Move the subcloned colonies to the main system.

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