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Mitomycin C stem cell ablation V.2

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1 Works for me

dx.doi.org/10.17504/protocols.io.brw8m7hw

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ABSTRACT

Treatment is done for 24 h right after WBR (Whole Body Regeneration) induction. Tissue will not regenerate but haemolymph flow should be observable for around 20 days.

In our hands, a number of samples have died following this treatment, hence multiple pieces of tissue were treated per slide.

We have tested concentrations down to [M]6 Micromolar (µM) with good success.

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PROTOCOL CITATION

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KEYWORDS

Whole Body Regeneration, MMC, colonial ascidians, Mitomycin C

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GUIDELINES

Our protocol is based on two publications:

- Kassmer et al. (2020)
- Rinkevich et al. (2010)

mprotocols.io

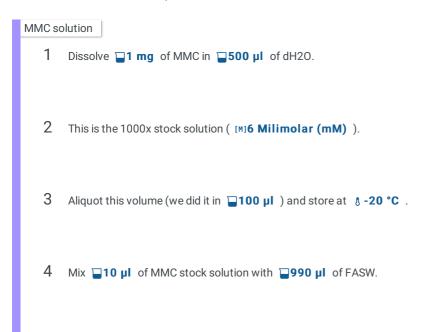
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MATERIALS TEXT

A cleaned Botrylloides colony on a slide



- Pap pen or other water repellent liquid for IHC
- Dark humidity chamber: closed box with moist tissue paper
- Q-tips



MMC treatment 1d 0h 2m

- 6 Induce WBR in at least two portions of the colony.
- 7 Gently dry around the edges of the regenerating pieces of tissue.

This is the working solution ([M]60 Milimolar (mM)).

8 Dry the whole slide as much as possible.

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9	Circle the regenerating pieces of tissue using the Pap pen.	
10	Wait for the liquid to fully dry ($\sim \odot 00:02:00$).	2m
11	Cover one tissue with as much FASW as possible (> $$	
12	Cover the other pieces of tissue with as much MMC solution as possible.	
13	Place the slide horizontally in the humidity chamber.	
14	Place the chamber at a temperature close to their culture temperature.	
15	Leave the slide to incubate for © 24:00:00 .	1d
16	Rinse the slide in FASW.	
17	Clean the Pap pen circles using a wet Q-tips.	
18	Place the slide back in the aquarium.	
19	Monitor daily.	