



Mitomycin C stem cell ablation V.1

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Works for me

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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. A number of samples have died following this treatment, hence multiple pieces of tissue were treated per slide.

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MATERIALS TEXT

- A cleaned *Botrylloides* colony on a slide
- Mitomycin C (MMC) powder: CAS 50-07-7
- Filtered sea water (FSW)
- Pap pen or other water repellent liquid for IHC
- Dark humidity chamber: closed box with moist tissue paper
- Q-tips

MMC solution

- 1 Dissolve 1 mg of MMC in 500 µl of dH₂O.

- 2 This is the 1000x stock solution (**6 Milimolar (mM)**).
- 3 Aliquot this volume (we did it in **100 µl**) and store at **-20 °C** .
- 4 Mix **10 µl** of MMC stock solution with **990 µl** of FSW.
- 5 This is the working solution (**60 Milimolar (mM)**).

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.
- 8 Dry the whole slide as much as possible.
- 9 Circle the regenerating pieces of tissue using the Pap pen.
- 10 Wait for the liquid to fully dry (~ **00:02:00**).
- 11 Cover one tissue with as much FSW as possible (> **200 µl**) as control.
- 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.

2m

15 Leave the slide to incubate for 🕒 **24:00:00** .

16 Rinse the slide in FSW.

17 Clean the Pap pen circles using a wet Q-tips.

18 Place the slide back in the aquarium.

19 Monitor daily.