Mitomycin C stem cell ablation V.2

Laura Bugada[1], Simon Blanchoud[1]
[1]University of Fribourg

Version 2
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1 Works for me
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Blanchoud lab, UNIFR

Marta Wawrzyniak
University of Fribourg

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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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)

MATERIALS TEXT

- A cleaned *Botrylloides* colony on a slide

Cleaning colonial ascidians
by Marta Wawrzyniak, University of Fribourg
PREVIEW RUN

- Mitomycin C (MMC) powder: CAS 50-07-7
- Filtered artificial sea water (FASW)

Artificial sea water
by Marta Wawrzyniak, University of Fribourg
PREVIEW RUN

- 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 FASW.

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.

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9 Circle the regenerating pieces of tissue using the Pap pen.

10 Wait for the liquid to fully dry (~ **00:02:00**). 2m

11 Cover one tissue with as much FASW 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.

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.

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