Jun 08, 2022

Preparation of agarose pads suitable for viewing filamentous cyanobacterial microbial communities using time-lapse imaging

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dx.doi.org/10.17504/protocols.io.81wgb65p1lpk/v1

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This protocol is for viewing cultures on top of agar pads, e.g filamentous cyanobacterial microbial community (from a freshwater environment) that form biofilms and granular structures, using time-lapse imaging.

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DOI

dx.doi.org/10.17504/protocols.io.81wgb65p1lpk/v1

Jerko Rosko, Mary Coates, Kelsey Cremin, Christian Zerfass, Orkun Soyer 2022. Preparation of agarose pads suitable for viewing filamentous cyanobacterial microbial communities using time-lapse imaging. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgb65p1lpk/v1

time-lapse imaging, agarose pads, filamentous cyanobacterial microbial communities

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Apr 29, 2022

Jun 08, 2022

Apr 29, 2022 maria.s
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Materials for STEP 1:Agarose pad medium

- Gelling agent: MacroSieve Low melt agarose (Flowgen Cat No H15621)
- Agarose percentage: 2%
- Medium volume: **50 mL**

STEP 1: Agarose pad medium (makes approx. 50 pads)

1 

Theoretically any medium solution can be used to make agarose pads. Here, we describe 2% agarose pads made with BG11+ (DSMZ Medium 1593) **Note:** we make the 'BG11-Mix' as individual components.

Into a 100 mL Duran bottle, add **1 g** agarose and make up to **47.35 mL** with DW.

2 Autoclave at **120 °C** for 20 – 30 minutes. Remove at **80 °C** .

For accuracy, you can weigh the bottle before and after autoclaving to calculate any evaporation losses.

3 

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After autoclaving, gentle swirl the bottle to ensure the agarose is fully dissolved.

4 Once removed from the autoclave, keep in a hot water bath (**55 °C**) to prevent agarose from setting.

5 Working in a sterile environment e.g Microbiological safety cabinet (MSC), add the following sterile BG11+ components:

A	B
BG11+ Top Table mix (salts)	2 mL
Trace metals	50 µl
NaNO3 solution (300 gL-1)	250 µl
BG11-Mix components	3 x 100uL

6 At this point, there are two optional routes to take:
Step 6 includes a Step case.
OPTION 1
OPTION 2

step case

OPTION 1

7 Optional route 1

7.1 For agarose pads (Step 8, below), only **10 mL** medium is required per session, thus, it is best to aliquot the media at this stage to avoid repeated solidification/melting cycles of the medium.

7.2 

Using sterile glass bijou bottles with screw cap lids, aliquot **9.995 mL** medium per bijou.

These can be stored, solidified, in the cupboard until needed.

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7.3 

Before using any aliquot, add **5 µL** vitamin B12 (or your preferred vitamin[s]) and swirl to mix.

To melt solidified medium, microwave at 800w for **00:00:30** and swirl to mix. Leave to cool slightly before adding any vitamins.

Pipetting agarose pads (Makes 10 Pads) 1h

8 Sterilise 20 22-mm^2 cover glass slides with 70% Ethanol inside a MSC, allowing time for drying 1h (~ **01:00:00**).

9 Keep the agarose solution at **55 °C** using a water bath before moving into the MSC.

10 Place five glass cover slips in each if 2 x 90 mm petri dish.

11 

Working on one glass cover slip at a time, pipette **800 mL** of cooling agarose solution evenly across the cover slip (as the agarose does not spread well when sandwiched, try to reach as far into the corners without unevenly distributing the gel). Quickly remove any air bubbles using the suction of the pipette.

We found 800 mL to be the ideal volume for this medium but depending on your media components and how viscous your medium is, you may want to adjust this, anywhere up to 1 mL.

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12 Gently lower another cover slip on top of the agarose solution, to sandwich it between the two cover slips. Quickly adjust the cover slip, if required, to ensure the agarose appears level.

Manipulation is not possible once the agarose sets e.g. within **00:00:30** . The warmer the medium, the easier it is to achieve an even distribution, with no adjustments necessary after adding the top slip.

13 Using a new pipette tip each time, repeat steps 11-12 for each pad.

14 Allow the pads to solidify, then seal the lid with parafilm and store in the cupboard until required.

We had condensation issues when storing pads in the fridge.

15 The BG11+ pads are best used the day after making, but we found them also store well for 1-2 weeks (and even longer).

Image 1. Solidified agarose pads in a petri dish.

Agarose 'pads' in 60mm dish (makes 15) (Alternative Step 2) 2h

2h

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16

A	B
Petri dish (Falcon, Cat 353002)	60 mm
Medium volume	7 mL

Pipette **7 mL** medium per dish, leave to dry with lids on in the MSC for **01:00:00** , then a further **01:00:00** with lids off.

17 Parafilm close the dishes and store in the cupboard for use the next day.

Pads can be used on the same day, but you may get 'drift' when imaging due to the continued setting of the agarose.

STEP 3: Microscopy on the agarose pads

18 When required for use with microbial cultures, the top cover slip on an agarose pad should be removed from the selected pad. The pad can then be cut into smaller pieces (if required) or the whole pad placed into a 60mm dish.

You can use the top coverslip to help release the pad from the bottom coverslip and slide it into the petri dish.

19

Add bacteria culture and start imaging.

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