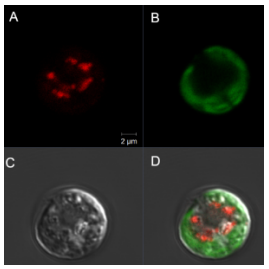
Oct 11, 2022

Prepare agarose pad for microscopy (2022 iGEM)



Forked from Fluorescence microscopy of Chlamydomonas reinhardtii - mCherry | Chlorophyll

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5jze5l1b/v1

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describe the steps to perform fluorescence microscopy for *E. coli*

Guidelines

Cells should be cultured to a late log phase to increase cell number for microscopy.

Before start

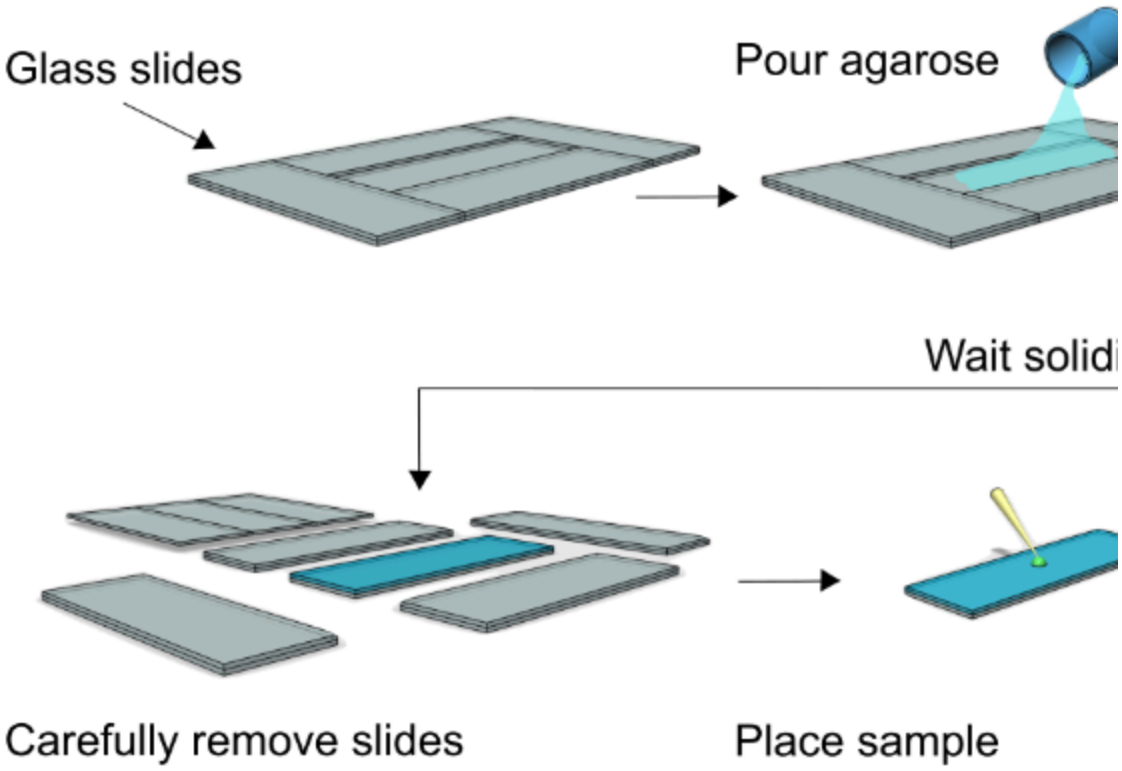
- Culture cells in liquid media

Cell growth

1 Grow cells to late-log phase density (e.g. OD_{600} > 0.6).

Agarose pads

2 Prepare a 1% agarose in distilled water, by adding sufficient low-melting agarose to water and dissolve agarose by heating in a microwave for 30-60 seconds. 16h



3 Prepare a glass slide with a double adjacent slides barrier (see below), forming a wall around the pad slide. Add slides on top of the poured agarose solution (1-1.2 mL) to allow formation of a smooth surface. It takes time for 1% agarose solution to cool down to ~40 degree, and the solution is viscous but not runny.

4 Wait for 45-60 minutes. Carefully remove adjacent and top slides, leaving the bottom slide.

5 Cut the pad into 15×15 mm square pierces, and carefully transfer the pad pierces to other cleared slide (left one agarose pad on the existing glass slide, if it was clean).

6 Add 2-5 µL bacteria culture onto the agarose pad, and cover it was a 18×18 mm #1.5 square coverslip. Seal with wax if needed.

Microscopy GFP

5m

7 5m

- Place the slide onto the microscope stage, with the coverslip facing the objective.
- Use a laser at 488 nm to excite GFP.
- Record images.

8 Analyze the images. For our microscope with 150x objective, the pixel size is 0.0806 µm.