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We use this protocol and it's working

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Preparation Of Bacteria For Optical Microscopy (DAPI)

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ABSTRACT

This protocol explains how to prepare bacterial samples for optical microscopy imaging, with a DAPI staining step.

MATERIALS

Reagents:
M9 buffer: as prepared in dx.doi.org/10.17504/protocols.io.x54v9yo6pg3e/v2
LB powder: LB Broth **Becton Dickinson (BD) Catalog #244620**
1% ultra-pure Agarose in M9
DAPI in PBS 1µg/mL

Supplies:
Microscope slides:
76x26mm glass microscope slide **Contributed by users Catalog #LR90**
Microscope chambers:
20x1.7mm reusable imaging chamber **Grace Bio-Labs Catalog #635021**
Coverslips: Round glass coverslips, 22mm in diameter

Equipment:
Tabletop centrifuge: Thermo Scientific MySpin 6

BEFORE START INSTRUCTIONS

In order to perform this protocol, you will need the following solutions:
- LB medium (25g in 1L of mQ water, autoclaved and then stored at room temperature)
- M9 buffer as prepared in dx.doi.org/10.17504/protocols.io.x54v9yo6pg3e/v2
- DAPI solution (in PBS) (ready-made, stored in freezer)
- Agarose 1% (ultra-pure agarose in M9)

Bacteria preparation

1 Pick a colony with a sterile loop and transfer it to 5mL of LB medium, in a closed 50mL tube, on 300 rpm shaking, for 22-26 hours at room temperature (our room has a controlled temperature around 22.5°C).

2 Take 0.5mL from the culture into a 1.7mL tube. Add 0.5mL of a 1% Triton_100X solution (in M9 buffer) to the bacteria.

3 Vortex briefly, and wait for 3 minutes.

4 Pellet the bacteria by centrifuging it for 5 minutes in a tabletop centrifuge.

5 Remove as much liquid as possible from the pellet, and then resuspend in a DAPI solution. We typically use a 1µg/mL stock, and dilute it from 1:2 up to 1:10.

6 Wait for 5 minutes.

7 Pellet the bacteria by centrifuging it for 5 minutes in a tabletop centrifuge, and resuspend the pellet in 1.3mL (full push of a 1000µL pipette) of M9 buffer.

8 Wait for 5 minutes.

9 Pellet the bacteria by centrifuging it for 5 minutes in a tabletop centrifuge, and resuspend the pellet in M9, up to the desired bacterial density (in the case of our bacterial strains, the amount of M9 to add would typically range from 200µL to 1.3mL).

Slide preparation

10 Melt Agarose 1% stock by putting it in an incubator around 60°C for about an hour.

11 Place your microscope slide on a clean flat surface, and then stick a reusable microscope chamber (see Materials for the model we use) on top of it.

12 Using a 1000µL pipette, put 800µL of liquid Agarose 1% in the chamber, and then place another microscope slide on top of the chamber.
Some agarose should overflow, and there should be no air trapped between the inside of the chamber and the top slide. Press slightly on the top slide, and then wait until the agar solidifies, which should take at least 2 minutes.

13 Once the agarose has dried, remove the upper slide by sliding it horizontally to the side. The surface of the agarose pad left in the chamber should be flat, although it is sometimes slightly irregular.

14 You can now put a 1µL droplet from the sample of bacteria as prepared in the previous section. We use a 2µL pipette, and try to not touch agarose surface.
Using a blade, you can also cut the pad in the middle, in order to separate two samples on the same pad.

15 Wait for the droplet of bacteria to dry, and then drop a round glass coverslip on top of the pad. If there are a lot of air bubbles stuck under the coverslip, it is possible to use an object to gently press it against the pad, and have the bubbles move.