Mar 09, 2022

Agarose gel pads for live Ashbya imaging

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1

protocol .

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A protocol to prepare agarose gel pads for live Ashbya imaging.

ameya.jalihal , Grace 2022. Agarose gel pads for live Ashbya imaging. **protocols.io** https://protocols.io/view/agarose-gel-pads-for-live-ashbya-imaging-bythpwj6

protocol ,

Oct 05, 2021

Mar 09, 2022

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2X LFM
autoclaved dd H2O
Overnight Ashbya culture
Depression slide
Agarose

Large beaker for water bath
2 50 mL conicals
1 15 mL conical

Agarose is hot. Use gloves when handling the beaker and the tube.

Rinse a depression slide with ethanol and have coverslips on hands.

Resuspension of Ashbya 10m

1 ⚠

- Make 20 mL 1X LFM stock before you start
- Always check LFM tube for contamination
- Reconstitute with ddH2O

1.1 5m

Spin down overnight Ashbya culture to pellet cells in 15 mL conical tubes **00:05:00** 300 RPM

1.2 5m

Use a pipette to remove as much of the AFM as you can while leaving the cells at the bottom. Resuspend in 4 mL 1X LFM. Spin down again. **00:05:00** 300 RPM. Either pipette pelleted cells from here onto the finished gel pad (steps 2.1-2.6), or do step 1.3 if the cells are too dense.

1.3 30m

Optional: Resuspend in 1 mL 1X LFM. Incubate on the rotator at **30 °C** until ready to make gel pad, or for **00:30:00** .

Making agarose pads 30s

2

depression slide

Depression slides live on Grace's desk. These are expensive, and we reuse them. Don't throw these out! Wash thoroughly with water and ethanol, dry them, and replace them in the

box.

2.1

Aliquot **10 mL** of 1X LFM from step 1 into a 50 mL conical.

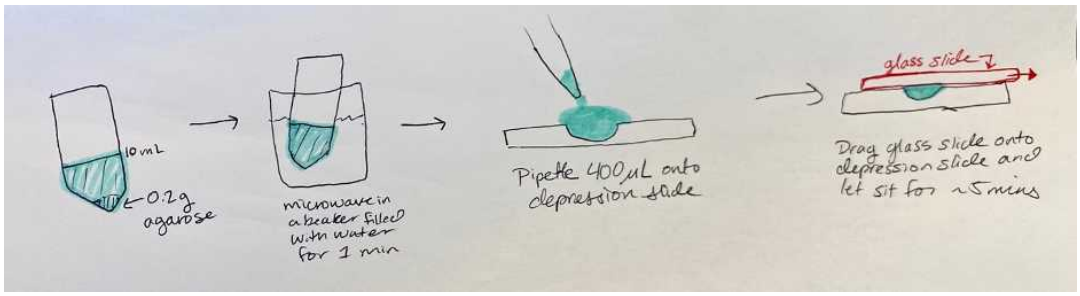
2.2 Measure **200 mg** agarose.

2.3 Fill a 500 mL beaker with water to make a water bath and microwave the tube in **00:00:30** increments until the agarose is dissolved. Shake the tube to dissolve agarose. 30s

2.4

Pipet **400 µL** of the agarose solution into the depression on a depression slide.

2.5 Drag a coverslip across the depression to create the pad. After about 2-5 min, gently lift the coverslip off. The gel pad is now ready to use.



steps 2.1-2.5

2.6

When ready, add **50 µL** of the cells to the center of the pad. Gently cover it with a coverslip, and press gently with a kimwipe to squish the cells.

2.7 Stick a glass Pasteur pipette into a beaker of valap (you want to get the wax into the pipette). Then hold this over a Bunsen burner until the wax melts, and then apply around the edges of the coverslip to seal it:

The slide is now ready to image.