Agarose gel pads for live Ashbya imaging

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ABSTRACT

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

PROTOCOL CITATION

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MATERIALS TEXT

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

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

SAFETY WARNINGS

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

BEFORE STARTING

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

Resuspend in 4 mL 1X LFM. Spin down again. **00:05:00** 300 RPM

1.3  30m

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

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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 **200 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.

2.6

When ready, add **50 µl** of the cells to the center of the pad. Gently cover it with a coverslip, and press with a kimwipe to squish the cells. Cells are now ready to image.

Imaging

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