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Dirty Spore Prep

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Preparation of an A. gossypii spore mixture that can be used to germinate mycelia for Ashbya transformations or other procedures requiring large numbers of germinated spores.

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Triton appears to have a negative effect on spore germination. For live cell microscopy, make sure most of the Triton is removed from the spores either by washing gently with media, or diluting to at least 1:1000 in culture.

AFM plate of Ashbya mycelia, grown for 5-7 days (has gone most of the way to the edge of the plate)
15mg/ml Zymolyase stock (see recipe in protocol)
15ml conical tube
Tube rotator or nutator in a 37°C incubator
Sterile 0.03% Triton X-100
Sterile 50% glycerol
2ml screw cap tubes with o-ring for storing spores (any brand is fine, the o-ring screw cap is the important part)

Preparation of 15mg/ml Zymolyase stock for dirty spores

1 Add 300mg Zymolyase 20T powder (Sunrise Scientific, N0766391) to 20ml 1M Sorbitol.

2 Mix to dissolve.

3 Bring final volume to 25ml with 1M Sorbitol.

4 Filter sterilize with a 0.22µM filter (a Steriflip 50ml conical filter works well for this, do not use nitrocellulose filters).

5 Aliquot 1ml each into 1.5ml microcentrifuge tubes. Store at -20°C. For this purpose a freeze-thaw or two is not a problem. **Note: Thaw stocks of Zymolyase gently, do not shake or vortex!**

Dirty Spore Procedure

6 Grow Ashbya mycelium on an AFM plate until it grows to the edge of the plate (about 7-10 days depending on conditions).

7 Scrape mycelium from the plate with a flat toothpick and suspend in 10ml sterile milliQH_2O in a 15ml conical tube. Shake/vortex vigorously to disrupt mycelium.

8 Add 200µl 15mg/ml Zymolyase and incubate on a rotator or nutator at 37°C for 1-2 hours. The mixture should look homogeneous with no chunks of mycelia left.

9 Spin mixture in benchtop centrifuge at 3000rpm for 5 min, carefully pour off supernatant. A little bit of the pellet may pour off, but the majority of the pellet will remain in the bottom of the tube.

10 Wash pellet 3 times with 5ml filter sterile 0.03% v/v Triton X-100.

11 Resuspend spores in 1ml filter sterile 0.03% v/v Triton X-100 + 1ml sterile 50% glycerol and transfer to screw cap tubes (100-200µl per tube depending on your usage). Label tubes with the strain, date, and “dirty” to differentiate from clean spore preps.

12 Store at -80°C (no need to flash freeze).

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