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Neurospora culture and basic imaging

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protocol .

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Details about culturing Neurospora, imaging, making freezer stocks, and media recipe

gamclaug 2022. Neurospora culture and basic imaging. **protocols.io** https://protocols.io/view/neurospora-culture-and-basic-imaging-b47cqziw

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Neurospora spores can travel through the air, so work with the spores/cells in a fume hood to avoid contamination. Only open slants and plates in the fume hood.

http://www.fgsc.net/Neurospora/neurospora.htmlis a good neurospora resource

Vogel's Minimal Media recipe:
http://www.fgsc.net/neurosporaprotocols/How%20to%20choose%20and%20prepare%20media.pdf

1. *Medium N* "Vogel's Medium" (Vogel 1956, 1964)

Recipe for 1 liter 50× salts:

water	755 ml
Na_3 citrate.$2H_2O$	125 g
KH_2 PO_4	250 g
NH_4NO_3	100 g
$MgSO_4$.7 H_2O	10 g
$CaCl_2$. $2H_2O$ (dissolved)	5 g
trace element solution	5 ml
biotin stock solution	2.5 ml

Conveniently prepared in a large Erlenmeyer flask with magnetic stirrer.
Dissolve constituents successively. Make certain that everything is dissolved before adding the next component.
Moderate heating is useful in speeding solution of the citrate and phosphate.
Dissolve the calcium chloride separately in 20 ml water and add the solution slowly. (Alternatively, powdered calcium chloride can be added slowly, but this takes longer.)
Add about 5 ml Chloroform as preservative and store the 50× stock solution at room temperature.

Single strength Medium N is autoclaved after adding sucrose (1 or 1.5%) and, if desired, agar (1.5%).
pH of the single strength medium is about 5.8. No adjustment is necessary.
Grocery-store sucrose is sufficiently pure for most purposes.

Slant tubes/plates are made by combining Vogel's media with 1.5% agar.

Intro

1 **Neurospora spores can travel through the air, so work with the spores/cells in a fume hood to avoid contamination. Only open slants and plates in the fume hood.**

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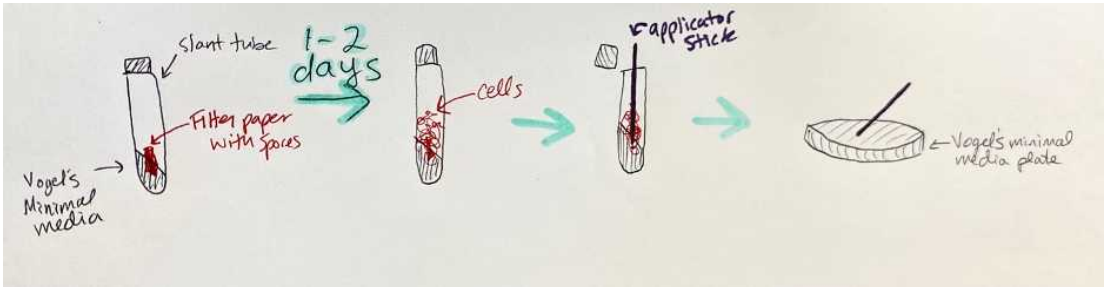
Cell Culture

2 Neurospora spores are generally mailed between labs on small pieces of filter paper. To start a new slant tube from them, push the filter paper into the agar at the bottom of the slant tube. Put on the lid, and leave the tube at room temp. There will be plenty of mycelia in 1-2 days.

To start a new plate, simply insert a sterile applicator stick into the slant tube and then touch that to the center of a plate. The plate can then be left to grow at room temp. This slant tube can be used many times for this for about 2 weeks.

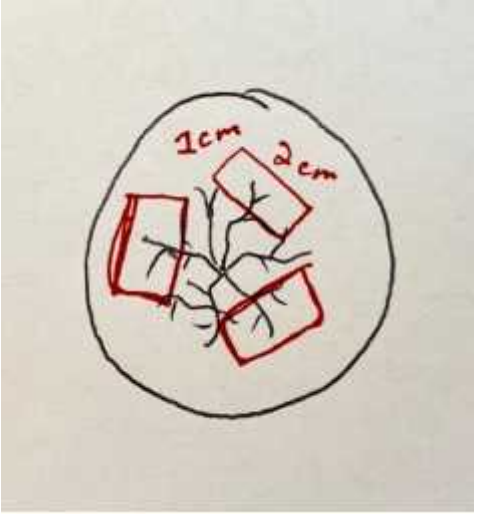


After 2 weeks, transfer the cells from the original slant tube to a new slant tube (with the same method as above- except stick the applicator stick into the new slant instead of the plate).

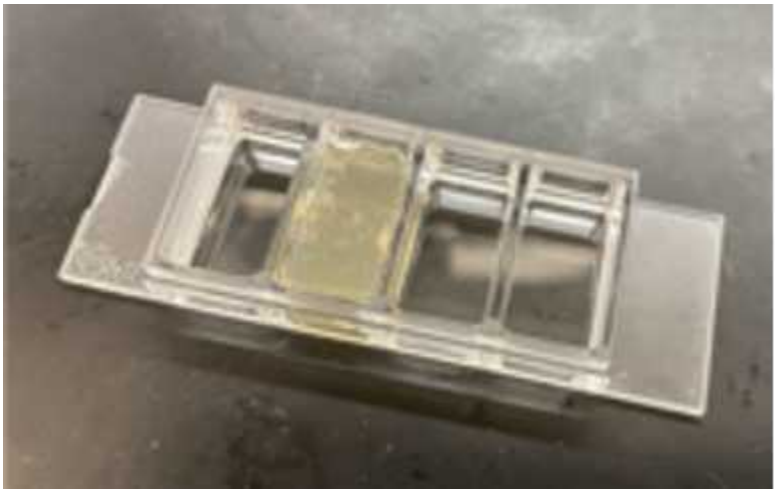
Imaging

3 Grow the cells on the plate at room temp until they cover about 2/3 of it. This usually takes about 12h.

Cut out 2cm x 1cm pieces of agar around the edge of the colony (if your intention is to image the apical regions):



Put 20uL of Vogel's Minimal Media in a 15u-Slide 4 well Ibidi chamber (80426), and then put the agar pieces into the wells with cells facing down:



For imaging high SNR things like nuclei using agar plates is fine. Agarose plates might be better for dimmer signals.

It can also work to put the agar pieces onto a coverslip, but this is not as stable as the ibidi chambers. It works okay to do this on Ann, but does not work very well on Bronty when taking z-stacks.

Making freezer stocks

4 Start with a slant tube that has mycelia growing in it. Pour 2mL of 25% glycerol into the tube, put the cap on, and vortex. Pipette this into cryto tubes and store at -80.

To start a new slant from the freezer stock, use a sterile applicator stick to scrape out a small amount of the freezer stock and put in the middle of the plate. Do not let it thaw, just scrape off the top.