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Inoculating and harvesting fungal isolates on cellophane overlay

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1 Works for me dx.doi.org/10.17504/protocols.io.qtedwje

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SUBMIT TO PLOS ONE

ABSTRACT

Some fungal isolates will embed into the solid media during growth, which makes it difficult to harvest agar-free mycelium for downstream applications such as DNA and RNA extraction. Here, we describe a protocol to (1) inoculate fungal isolates onto solid media with sterile cellophane overlays and (2) harvest the tissue for later DNA or RNA extraction.

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GUIDELINES

- If at any point you feel the forceps has become compromised via either touching the outside of the glass beaker, touching the outside of the Petri dish, or you held it too closely to the tip of the forceps-- please re-sterilize it and use another sterilized forceps.
- Make sure to record sample information in chart in notebook.

MATERIALS TEXT

MATERIALS

- 4 cm x 4 cm cellophane **Contributed by users**
- 250 ml beaker **Contributed by users**
- Aluminum foil **Contributed by users**
- Nanopure water **Contributed by users**
- Bactoincinerator **Contributed by users**
- 95% ethanol **Contributed by users**
- Forceps **Contributed by users**
- Sterilized beaker **Contributed by users**
- Sterilized cellophane **Contributed by users**
- Petri dishes with media of choice PDA/MEA/OA **Contributed by users**
- Scalpel **Contributed by users**
- Cellophane plates **Contributed by users**
- Permanent marker **Contributed by users**
- Fungal cultures **Contributed by users**
- Parafilm **Contributed by users**
- 1.5 ml sterile RNase free tubes with caps such as Qiagen or NAVY **Contributed by users**
- ICE block -20C **Contributed by users**
- Dewar with liquid nitrogen **Contributed by users**

Preparing Cellophane

1 Wipe down bench top and scissors with RNase away solution.

2 Cut cellophane into approximately 1 inch squares, taking care to measure squares to ensure they will fit in a 60mm Petri dish.

3 Place ~75 pieces into a 250mL glass beaker.

4 Fill beaker with 100 mL nanopure water to fully cover the cut cellophane pieces.

5 Cover beaker with aluminum foil and autoclave indicator tape. Autoclave for 30 minutes on liquid cycle at 121°C and allow to cool to room temperature.

Preparing Plates with Cellophane

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6 Sterilize the biosafety cabinet and preheat the bactoincinerator.

7 Pre-sterilize fine-point forceps by submerging in 95% EtOH and placing for 5 seconds in the hot bactoincinerator.

8 While forceps cool, retrieve media plates from refrigerator and remove from sleeves, taking care to keep sleeves and media sterile since they will be re-bagged for future use.

9 In the biosafety cabinet, remove aluminum cover from cellophane beaker and place in the back of the biosafety cabinet to ensure that it does not become contaminated.

10 Utilize sterilized, cooled forceps to remove cellophane from beaker.
- Note: Scoop a large portion of cellophane over to the glass wall of the beaker. Carefully remove one piece of cellophane per media plate, taking care that the cellophane is not hanging over the media plate.

11 Place the square of cellophane in the center of the plate. Be careful to lay the cellophane flat.

12 Once all plates are inoculated with cellophane, carefully re-bag media and place pink tape with initials and date to indicate that cellophane has already been added to plates.

13 If cellophane remains in the beaker and the beaker has been kept sterile in the process of inoculating plates, place aluminum cover back on beaker and place on shelf for future use.
- Note: If there is no more cellophane in beaker, please wash dish as normal. If there is cellophane remaining in beaker and you think you might have contaminated the jar, it can be autoclaved again as described above.

Inoculating Cellophane Plates

14 Sterilize the biosafety cabinet and preheat the bactoincinerator.

15 In your lab notebook, record the names of isolates to be inoculated onto cellophane plates.

16 Pre-sterilize fine-point forceps by submerging in 95% EtOH and placing for 5 seconds in the hot bactoincinerator.

17 While forceps are cooling, retrieve media plates from refrigerator and begin labeling plates with isolates to be inoculated.
- Note: Labeling isolates in reverse order (from the last isolate to the first) is useful because then you will end up with your first isolate on the top of the stack.

18 Remove the Petri dish containing the fungal isolate to be inoculated from the stack. Remove the parafilm from the plate

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and discard. Place on the side of the hood, out of the way of where culturing is to occur.

19 Remove top lid from the new agar plate containing the cellophane overlay. Place the lid such that when you work your hands/arms are not crossing over any of the plates.

20 Using the sterile scalpel, cut small piece of mycelium/agar from culture plate and carefully transfer it to the center of the piece of cellophane. Replace lid onto new cellophane plate and move to the side of the hood.

21 Replace lid on the source culture plate and stack away from samples that still need to be subcultured.

22 Repeat steps 18-21 until all isolates have been inoculated.

23 Once all samples are done, parafilm Petri dishes with two layers of parafilm, ensuring that the mycelium pieces on the new plate do not move. Place the parafilmed Petri dishes on the proper culture tray.

24 Record any relevant notes on samples in lab notebook (i.e. whether the mycelium moved and had to be re-placed)

Harvesting Cellophane Plates

25 Sterilize the biosafety cabinet and preheat the bactoincinerator.

26 Pre-sterilize fine-point forceps by submerging in 95% EtOH and placing for 5 seconds in the hot bactoincinerator.

27 While the equipment is cooling, label the sides of 2 ml screwtop bead tubes with the following information:
- Name of isolate
- Tube number
- If it is for DNA or RNA
- Age of plate
- Type of media it was grown on
- Date
- Your initials

28 Pour liquid nitrogen from large dewar into portable benchtop dewar.

29 Loosen cap(s) of tubes for one isolate (e.g. if one isolate has four voucher, loosen all at the same time.)

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30 Using the sterile scalpel, carefully remove the plug that was used as the source inoculum. Place this piece on the side of the plate. This will ensure that you are only collecting fresh mycelium without any agar.

31 Use the side of the forceps to lightly scrape the new mycelial tissue into a “bundle” (make sure not to tear into the cellophane).

32 Transfer the bundled tissue into labeled tube (this can be achieved by the use of the scalpel or a pair of forceps) and cap the tube.
- Note: the same pair of scalpels and forceps can be used for the same isolate, just make sure to have a separate tray so they don’t contaminate the sterilized equipment.

33 Tap the tube on the benchtop to ensure the tissue is at the bottom of the tube on the beads.

34 Flash freeze tube by placing it in the liquid N in the portable dewar.

35 Repeat steps 30-34 until all samples have been harvested and frozen.

36 When all samples are done, remove the tubes from the dewer using long forceps and place them in their respective boxes in -80°C freezer.

37 Update the spreadsheet with harvesting information.
- Name of isolate
- Tube number
- If it is for DNA or RNA
- Age of plate
- Type of media it was grown on
- Date
- Your name

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