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Molding microchambers in agar with PDMS stamps for live imaging

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We use this protocol and it's working

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Abstract

This is a step-by-step guide for preparing agar microchambers using a patterned stamp. The protocol includes detailed instructions on selecting the appropriate stamp, preparing the setup, centrifuging the agar into the stamp, and mounting cells of interest for live imaging.

Image Attribution

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Materials

Silicone stamp (opaque PDMS) from https://researchmicrostamps.com/
Noble agar - Thermo scientific CAS 9002-18-0
TAP medium - UTEX Media
Six-well plate - VWR Cat# 10062-892
Glass slides (75 × 25 mm) - Corning 2947
#1.5 square coverslips (22.5 × 22.5 mm) - Electron Microscopy Sciences (EMS) Cat# 72204-01
VALAP made from equal parts:
Vasoline - Sigma-Aldrich 16415
Lanolin - Sigma-Aldrich L7387
Paraffin wax - Sigma-Aldrich 76242
Microcentrifuge tube (1.5 mL) - VWR #20170-038
Small paint brush - Crayola 53516
Forceps - EMS 78317-2
Lab tape - VWR #89087-920
Nitrile gloves - Halyard Ref 55082
Heat block - Benchmark BSH 1001
Metal piece of a heat block removed used as a weight
Pipette and tips to transfer sample - Rainin
Razor blade - Bates Choice Pro
Plastic transfer pipette - HEA206373A
Kimwipe - Kimtech 34155
Centrifuge - Sorvall X Pro Series X1R Pro-MD
Insert for plate - Thermo Scientific 75003624

Troubleshooting

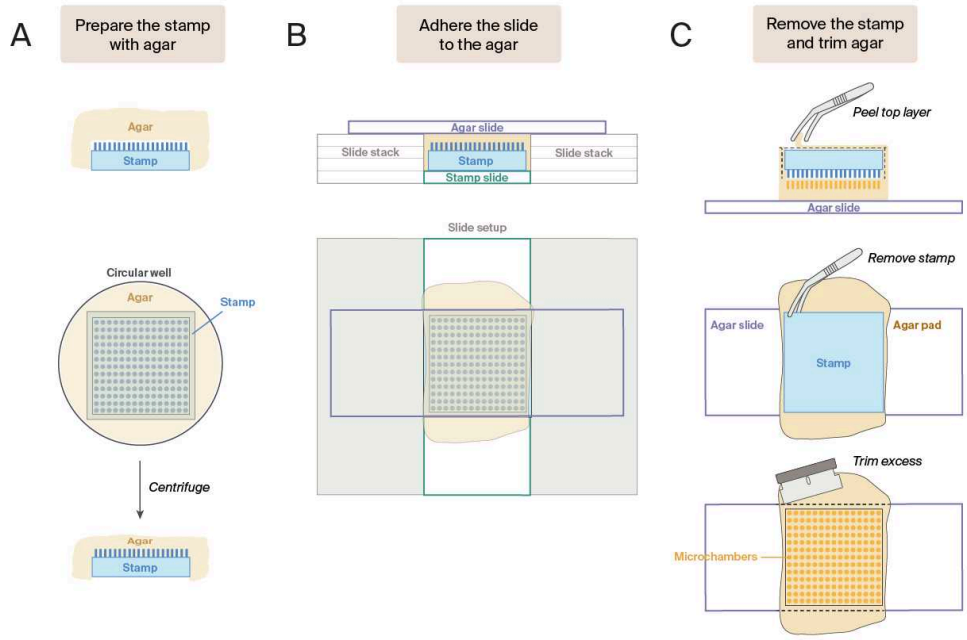
1 Familiarize yourself with the protocol and make sure to click the appropriate tab based on whether this is your first time doing this, a subsequent time, or your first time using a new stamp.

STEP CASE

First time preparing microchambers 24 steps

Use these instructions the first time you run this protocol or when you need to prepare the slide setup and aliquots of agar.

2 Review the workflow below. We refer back to this diagram a few times throughout the protocol.



This figure comes from the **publication** linked to this protocol. For your first time using a new stamp, see the "New Stamp" protocol that includes the centrifugation step.

3 Purchase a stamp from **researchmicrostamps.com** (or equivalent) with protrusions in the desired dimensions. See Figure 9 in our main **publication** to estimate the appropriate size. $200–300

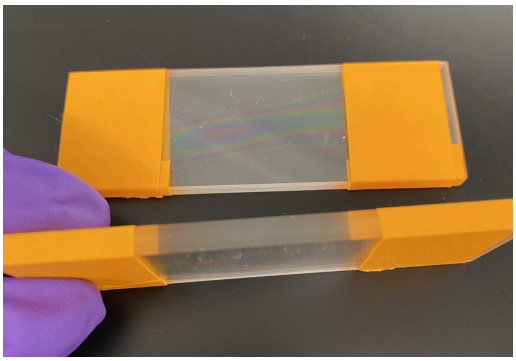
Once you have the stamp, make sure you can tell which side has the protrusions.

4 Wash the stamp with 70% ethanol in deionized (DI) water and allow to air-dry completely. You can dry in a one-well plate to minimize dust. If small fibers or dust remain on the stamp, try using an air blower/duster.

Note

When not in use, store the stamp in a plastic case to keep it from collecting dust.

5 Stack four glass slides and bind each end of the stack with a single layer of lab tape. You will need two bound stacks of four to make one agar pad. If you are making two agar pads at once, you will need four slide stacks total.



Two stacks of four slides each, bound by a single layer of lab tape.

Note

If the agar pad ends up being too thick, try using slide stacks with only three slides.

Prepare the agar

6 Make up 5% agar in the appropriate medium (for more detail see 6.1).

5% Noble agar solution. The clear portion is liquified. The translucent portion is material that we hardened in the six-well plate and then added back to the bottle for reuse.

Note

We used Noble agar and TAP medium (2.5 g noble agar in 50 mL tris-acetate-phosphate). Noble agar is clear and TAP medium is optimal for observing *Chlamydomonas* cells in their vegetative state. You can substitute other types of agar and different media or water more appropriate for other organisms. Adjust the percentage of agar if the stamp does not form uniform protrusions into the agar bed.

6.1 To make up 5% agar in a solution, first tare a balance with the weigh boat or paper you will use. Then measure 2.5 g of Noble agar and transfer to a glass container (large enough to hold the volume needed with room for the agar to boil and short enough to fit into the microwave). Add 50 mL of water or medium to the jar. Microwave in 30 s intervals until you see bubbling. Gently swirl the bottle to make sure the agar is fully dissolved. Be careful not to overheat and spill the solution.

In the photo in step 6, we show 50 mL of agar in a 250 mL glass jar.

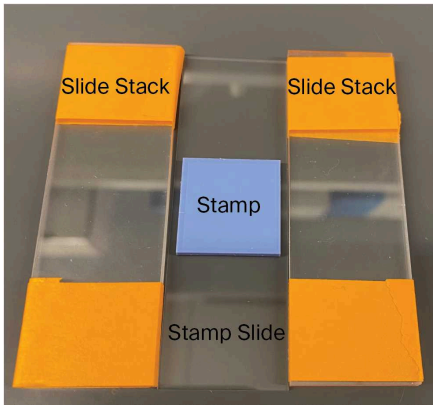
Safety information

Make sure the cap is only loosely screwed onto the bottle while microwaving the solution. Handle the hot bottle with protective gloves and remove from the microwave slowly.

7 Place the melted agar into a bead bath or water bath set to 80 °C for 00:03:00 or until the bubbles dissipate. 3m

Prepare slide sandwich

8 Place a single glass slide on a flat surface lined by each stack of slides (from step 5). This will become the "stamp slide" coded in teal in panel B in our overview figure from step 2.



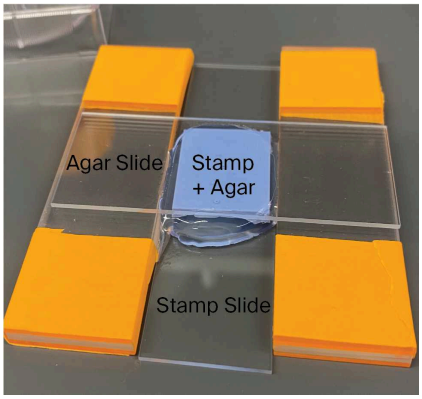
Slide setup with the stamp facing up.

Note

If you are making more than one set of agar microchambers at a time, label the corners of the slides to keep track of each stamp type. If you cannot tell which side of the stamp the protrusions are on, use a stereo microscope to check.

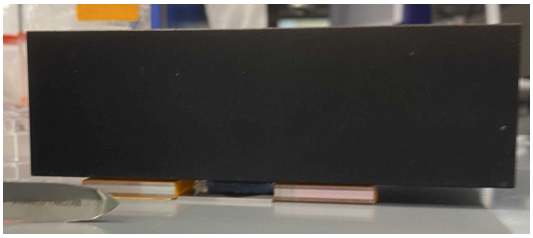
9 Pour the liquified agar on top of the stamp. ~2 mL should be sufficient to cover it.

10 Place a clean slide perpendicularly on top of the agar.



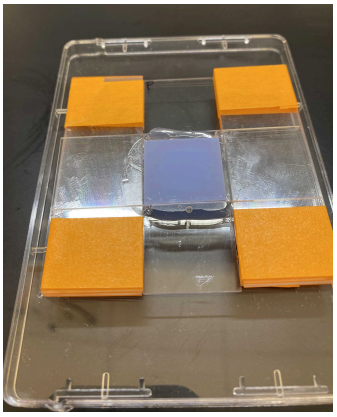
Slide setup with liquid agar and slide placed on top.

11 Place a metal object on top of the agar+slide sandwich to apply even pressure for at least 00:01:00 or up to 00:05:00 . 6m



Metal insert from a heat block that we use to apply pressure to the sandwich.

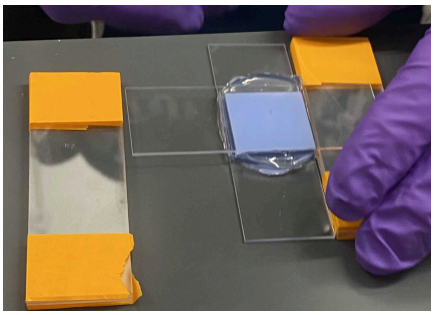
12 Transfer the slide sandwich to cool at 4 °C for at least 00:30:00 or up to 01:00:00 . You can move the entire setup to the lid of a well plate. 1h 30m



The entire setup in a lid for transfer.

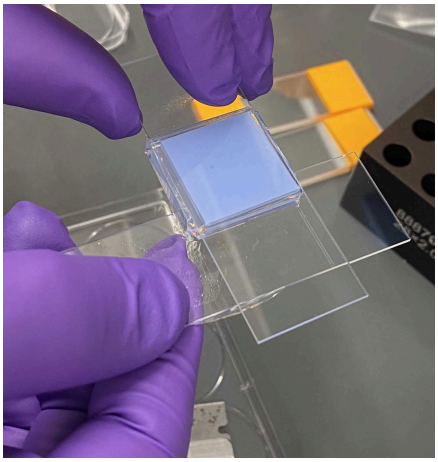
Remove stamp and trim agar

13 Remove the slide stacks from both sides of the perpendicular slide sandwich.



A user removing the slide stacks.

14 Use a razor blade to gently pry the bottom slide off of the sandwich.

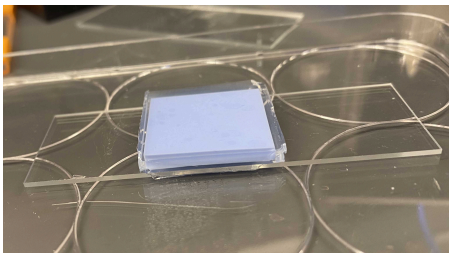


Prying the stamp slide from the agar sandwich.

Note

It may be easier to flip the sandwich and remove this slide from the top. The goal is to separate the slide that had the stamp placed on it. This is the teal "stamp slide" from the diagram in step 2.

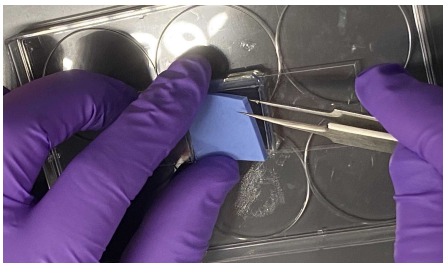
15 Use the razor blade to score (cut through the top, about halfway down) all four sides of agar along the edges of the stamp.



Scored agar prior to stamp removal.

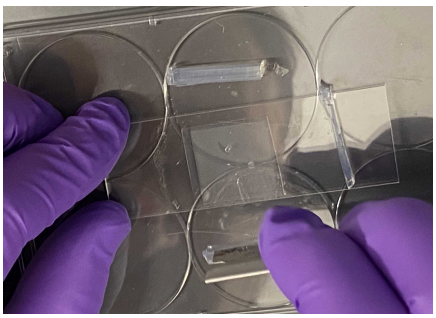
16 Once the agar is scored around all edges, you should be able to use forceps to gently peel off and discard or recycle the thin layer of agar on top of the stamp.

17 Use the forceps to gently dislodge the stamp without piercing the agar microchamber pad.



A user lifting the stamp by holding one corner with forceps.

18 Trim the walls of the agar pad using the razor blade to leave a uniform square.



A user trimming the agar pad.

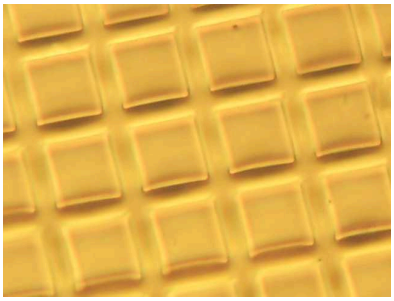
Note

If desired, cut the agar pad into four squares to place onto four different slides to image different groups of organisms or different conditions.

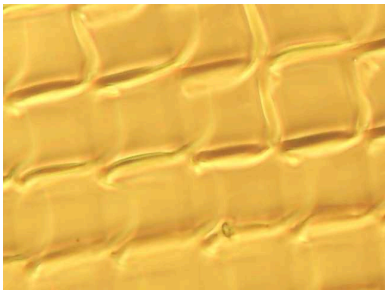
Mount samples for imaging

5m

19 Use a microscope to check that you have clean, intact agar microchambers before mounting.



Intact chambers. Chamber dimensions: 100 µm width, 100 µm spacing, and 40 µm depth. Phase contrast image taken with a 20× objective.



Botched chambers due to stamp movement or underpolymerization.

20 Melt an aliquot of VALAP at 80 °C using a heat block. It should be liquid after 00:05:00 . 5m

A 1 mL aliquot of VALAP (marked V) in a heat block.

Note

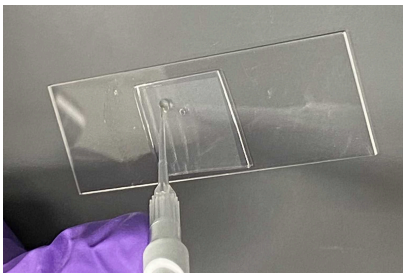
VALAP is made from a 1:1:1 mixture of vasoline:lanolin:paraffin. You can purchase vaseline from a drugstore, just be sure that it is unscented. You can melt equal parts of each ingredient, mix them together, aliquot, and store at room temperature for later use.

21 Prepare your cells or organisms of interest at the desired density.

Note

Adjust the volume and concentration of cells or organisms as needed for your desired experiment (for example, choose whether you want a single cell per confined microchamber or several). Finding the right concentration may take a bit of trial and error — you can clean the microchambers by flushing with water or media and try again.

22 Pipette 2–5 µL of cells or organisms onto the agar pad, spreading the liquid across to cover multiple microchambers.



Transferring 4 µL of algal cells to the surface of an agar microchamber.

Note

If you want to maximize the number of cells or organisms in wells, you can check them using a phase microscope and then try adding more culture, gently brushing the pipette to spread cells/organisms across the pad, and then using a Kimwipe to decrease the liquid volume by absorbing water at the edge of the pad.

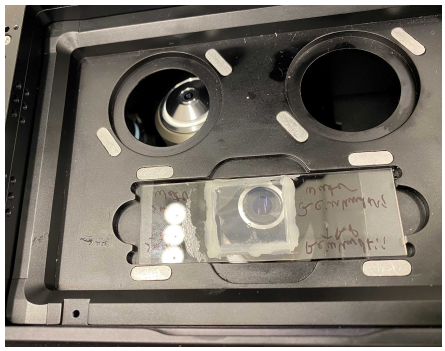
23 Once the ratio of cells/organisms in the wells is satisfactory, place a clean, square coverslip (#1.5 thickness) and seal with VALAP.

Note

VALAP will harden quickly once in contact with the slide at room temperature. Hold your slide close to the opened tube of VALAP on the heat block to keep it liquified and avoid dripping.

If you want to pipette media into the chamber just prior to sealing, leave an opening on either side of the agar pad. You can seal this opening with additional VALAP after flooding the chamber.

24 Mount the slide onto the microscope stage and begin imaging.



A slide with coverslip facing down on an inverted microscope.