

## How to Cross Chlamydomonas

### Materials:

TAP-minus-nitrogen (made with Beijerinck's without  $\text{NH}_4\text{Cl}$ )

Large and small tubes, specify small, ask Janette

TAP or TAP + arginine plates with thick 4% washed agar (made at least one day in advance).

Use TC agar and wash ~2-3x with MilliQ  $\text{H}_2\text{O}$

Drawn out Pasteur pipets:

- Draw out a half-diameter short region in the hot (blue) part of the tiny candle-like flame.
- Draw out this region into a very thin thread of glass in the white part of the flame.
- Break
- Briefly, in the white cool part of the flame, melt the tip.

Glass Petri plate with chloroform.

### Preparation of the Strains

1. Have strains to be crossed growing well. It is best to have both mating types of each. Streak them every three to seven days. Streak them on Day 3. Include wild type tester stains for each crossing experiment.

2. To induce gametogenesis, on Day 1 inoculate 5 ml of TAP-minus-N in a large tube. Look at each strain under the microscope for bacterial contamination and to ensure that they are in good condition. Ensure that the clumps of cells are dispersed and that the culture is fairly dense, about  $2 \times 10^6$  per ml. (Clumped cells make it difficult to verify the mating the following day).

3. Incubate cultures on the rotating wheel overnight.

(Make at least one 4% washed agar TAP plate for each cross.)

### Mating Day 2

4. On day two test mate the strains to be crossed with wild type tester strains of both mating type to ensure they will mate and to verify their mating types.

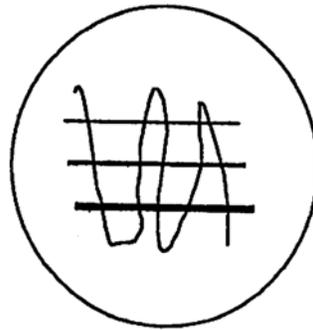
- Disperse the cultures to be tested by hitting the tube against the palm of one hand.
- Spot one drop of this to each side of a slide labeled “-” and “+”.
- Mix one drop of each of the tester strains with the drop of cells to be tested.
- Cover with a coverslip and look for mating under the scope.

5. In a small test tube, mix 1.0 ml of each of the strains to be crossed.

6. Let these cells mate in the laminar flow hood for 2 hours with occasional agitation.

7. To verify that mating has occurred, treat a drop of each mating on a slide with 4  $\mu$ l of freshly diluted 1% gluteraldehyde. Cover with a cover slip. Score the cells with four flagella. They should be greater than 10% of the total. (*Too much gluteraldehyde—the flagella drop off!!*)

8. On a TAP plate with 4% agar, pipet ~0.1-0.2 ml of the mated cells in three equidistant parallel lines (Janette). Then, with a sterile loop, spread the mating culture across the plate with quick, light strokes perpendicular to the three lines. Michel spreads about 0.2 ml of mated cells in three equidistant lines; one thin, one medium, and one thick, and then passes the pipet across. The three lines should be spread within the width of the razor blade. *I like to do 1-2 thick lines and leave agar clear between. – Beth. The point is to leave areas of clean agar as working space to either dissect zygotes or to gather together zygotes for transfer to dissecting plates.*



9. Leave these plates covered in the hood overnight, lids up, and with the hood light on.

10. On Day 3, wrap each of the plates individually in aluminum foil and incubate at 24°C for five to six days. *Incubate upside down. No parafilm.*

### **Sporulation of the zygotes**

11. On Day 8, unwrap the plates and check for growth and contamination. *Once you expose to light you can't stop the process, don't scrape more plates than you want to dissect!*

12. Remove the vegetative cells from the zygotes.

- Plant a razor blade (sterilized by ETOH flaming) in the agar at the edge of the plate. *Hold razor blade with hemostat clamps located in hood.* Then gently with some pressure, in one slow continuous motion, scrape cells from the surface of the plate to the opposite edge. This preferentially removes the vegetative cells from the zygotes which stick to the agar.
- Then invert this plate over a glass Petri plate containing chloroform for 30 seconds. This preferentially kills vegetative cells.

13. Leave plates (lids up) in the light over night to induce germination of the zygotes. *Either in hood or in incubator.*

(At this stage, Michael picks up potential zygotes on to little pieces of agar to a fresh 2.4% TAP plate. This allows a cleaner area for dissection and reduces the risk of contamination.) *I use 2% plates. Colonies appear sooner on lower % plates than 4% plates. Zygotes can be scraped into a*

*pile using microspatula and then lifted by adhesion to flat side. Be sure to mark where you place zygotes on new plates, to be able to find them the next day. I make a line with my glass tool down the center of plate and then move zygotes to either side along the line. –BD*

### **Dissections of the tetrads**

14. On Day 9 (or 10), dissect the tetrads. Under the highest magnification of the dissection scope, gently touch potential tetrads at the border of patches of cells with a finely drawn out Pasteur pipet. Tetrads separate into four tiny spores when they are ready for dissection. If they do not separate, wait an hour or two. Check periodically. Separate these spores by gentle poking. They should be sufficiently far apart (*greater than a field of vision is best!*) that the colonies which grow from them can be isolated. Carve a moat around them. *I pull the spores out to a line and then mark as such. I mark each spore as soon as I have placed it. – BD*

15. Germination occurs over the next few days. Keep photosynthetic mutants in dim light.

### **Picking the progeny** (after about five days)

16. Under the dissecting scope and using the point of a finely drawn out Pasteur pipet, gently carve into the agar around each of the four progeny colonies of a tetrad. *Can also use a round pointed tip toothpick, and just pick colony directly to fresh plate.*

17. Then pick up the colony on its piece of agar with the point and deliver it either to the grid of a TAP plate or to a small glass tube with 1.0 ml of TAP. Label each of the tubes.

18. Incubate 24°C.

0.5 – 1 mg/ml Kazugamycin plates or 0.15 mg/ml Amp plates can be used to eliminate bacterial contamination.