CORA STYLES NEEDLES 'N BLOCKS

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OPTICAL FIBER DISSECTION NEEDLE KIT CONTENTS AND INSTRUCTIONS

Optical Fiber Dissection Needle Kit Contents

The materials provided are for constructing a support rod joined to a short optical fiber with one or both ends flat for micromanipulation of yeast cells on agar plates.

Kit Includes:

- 15mm fiber-optic strands ("needles") with polished ends, 25-micron diameter or 50-micron diameter
- Aluminum rod
- Stiff wire with a right angle 90 degree bend,short arm = 7mm
- Toothpick with Parafilm Tip
- Superglue
- Fine Sandpaper

Note: A diagram of the product is shown at the end of this document.

Kit Does Not Include:

- Tape
- Elmers Glue
- Duco Cement
- Microscope Slides
- Hacksaw or bolt cutter

The tools in the kit are designed for micromanipulating cells on a level agar surface in 15mm x 100mm plastic petri plates with an agar media volume of 25ml. Level plates will show an agar surface at the same level as the edge of the lid of the closed plate when viewed from the side all the way around. The distance from the edge of the plate to the agar surface will be 7mm.

Instructions

1. **Custom-fit the two-part needle support to your microscope:**
   Measure the distance between (a) the viewing site (i.e., the center of the objective lens) and (b) the micromanipulator's holding clamp where the rod will be inserted.

   Place the wire rod on the flat side of the aluminum rod as shown in Diagram A, and adjust their total length to match your measurement above. Be sure the wire

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rod extends at least 1 inch beyond the aluminum rod. If the needle performance when tapping on the table or stage feels too rigid, you may add more spring by extending the wire even further. You may have to cut the aluminum rod with a hacksaw or bolt cutter.

Attach the two together using tape or glue. The suggested orientation is to have the 7mm wire perpendicular (or parallel) to the flat plane of the aluminum support rod. One wind of tape is enough. Avoid adding thickness to the support rod in an area which may bump the edge of the plate
Put the rod in the holder now to ensure fit, and position it parallel to surface of the stage.

2. **Attach an optical fiber needle to the 7mm wire**:
Remove the rod from the holder.

At a low-power (40 - 50 x) microscope in a quiet place with good light, assemble your materials: the dish of optical fibers, two microscope slides, a Parafilm tip toothpick and the superglue.

First, choose an intact fiber. From this point on you should avoid talking (but continue breathing). A puff of air from your mouth could blow away optical fibers. Having clean, dry, oil-free fingers here could help, too.

OPEN YOUR NEEDLE CONTAINER CAUTIOUSLY.

The glass fibers and the plastic dish pick up static electricity, which means that some fibers may be clinging to the lid. Place a glass slide near the dish. Press the parafilm toothpick down gently on a fiber, lift it and transfer it to the middle of the slide. You may need to use your finger or a second bare toothpick to separate the fiber from the parafilm. Transfer the slide to the microscope and examine the fiber at both ends to be sure they aren’t chipped. Decide which end looks “most perfect,” and note the position of the end which you prefer. (Remember that the microscope image may be inverted.)

Apply a droplet of superglue on the second slide and moisten the 7mm wire in it.

Pick up the fiber again by gently pressing the parafilmed toothpick in its center. Keeping your hands steady, bring the fiber parallel to the wire, with both ends free, and with the good end extending 3-4 mm beyond the tip of the wire as shown in Diagram B. Touch the fiber to the wire. It will usually attach quickly and permanently, so you want to be right the first time. Avoid getting any glue on the ends of the glass fiber. If both ends are flat and free of glue, you will have a light-transmitting bright needle.

The parafilm might stick on, too. Try giving it a twist to free it. If not, lay the needle down carefully on its side and cut off the parafilm with a razor blade, leaving a bit of parafilm behind. It won't make any difference.

Alternative method: Using the parafilmed toothpick and your clean finger, move the fiber so that the "bad" end extends into mid-air off the edge of the slide as far as possible. Bring the bend of the glue-moistened wire to touch the fiber just short of the end, without touching the glass slide. The fiber should attach to the wire. The height can be adjusted by pushing the needle up or down now, while the glue is still wet. If the glue gets on the bad end, it's OK. Your needle will still work fine, but will appear dark.

Important note. The wire can be used repeatedly, but it must be cleaned each time (with fine sandpaper or emery cloth) to remove the superglue. Superglue does not stick to itself. Seal your tube of superglue with Parafilm to keep it from polymerizing in the tube.

3. Install the support rod with fiber needle in micromanipulator holder.
Be sure to swing the objective out of the way, and keep one eye on the needle during installation. Follow the instructions of your manipulator sales rep for centering the needle. You can check for tilt (toward the operator or away from) by looking from the side, or by looking through the lens and focusing on the needle tip and then downward, watching the needle shaft. If it seems to displace quickly away from center, it has a tilt which should be corrected.

The probability of contamination from the needle is negligible. You can sterilize it by plunging it once or twice into the agar plate if you wish. That's probably a good idea just to clean debris off the tip anyway. Contamination is more likely to come from air-borne mold spores and bits of lint and lab air detritis which the static electricity attracts when you place your plate on your microscope. If you have compressed air in a can, you might want to blow out your dissecting chamber. Check with your microscope sales person whether you could apply a light spray of alcohol. (I think it's hard on the microscope, as a standard practice, and I would resort to this only in very dirty environments.) Contaminant colonies can be "surgically removed" from your plate conveniently with the thin flat blade of an artist's palette knife dipped in alcohol and flamed... a handy tool. (Cut all the way down to the bottom of the plate and lift out a small chunk of agar with the offending contaminant.) If contamination is really bad, try to set your microscope up somewhere else.

Comments and Tips:

Will your microscope be multi-purpose? If you are looking at microcolonies on agar plates, you can still leave the needle in place. It is safest to leave the needle alone, just lowered out of the way. Some people rotate the needle 90 degrees to avoid bumping it when doing other work. If you need to remove the needle and support, it is

worth considering the Singer model for its durability. This is not an ideal position to be in. It would be better to join forces with another lab and share two microscopes with one dedicated to micromanipulation.

The needle support shaft ideally is parallel to the surface of the stage. This allows the greatest up-down range. If the 7mm wire bend is a right angle, then your needle will be perpendicular to the agar in this plane.

Centering the needle: First position it roughly by looking sideways at the objective. Then refine position by using the low-power objective. If not, bring the objective close to the needle to be sure of seeing it. Set the “east-west” position and fan the needle north-south (or vice versa). Reset position and continue fanning till you find it.

Tilt adjustment: Focus on the needle surface and then focus downward. Does the bore of the needle shaft change position more than just a little? North-south tilts can be corrected by rotating the support shaft. (Take into account that the visual image is inverted.)

YPD is the standard medium for dissecting ascospores. Some poorly germinating mutants germinate better on GNA; some do worse. If you want to keep a plasmid in, I still recommend dissecting on YPD, and printing the colonies to selective medium afterward. Dissecting on selective medium (SC-X) will prevent full tetrads (4 spores) from growing, and further, this medium is harder to pick up cells from (because of electrostatic properties?).

Be selective about the plate you choose to work on. Is the agar surface parallel (level) to the edge of the lid all the way around? Try the squeeze test for moisture content: remove the lid and hold the bottom between thumb and middle finger 180 degrees apart. Tilt the surface to reflect light and squeeze firmly. A fresh plate will form tiny beads of water on the agar just at the two pressure points. When the pressure is released, the droplets disappear immediately. (This is also a good test for replica plates.)

Before you pour your plates (for *Saccharomyces*) you can get rid of bubbles by spritzing with alcohol.

Positioning your agar plate on the stage is like flying a helicopter, not a jet. For landing, come over high, then descend. Taking off, go straight up, then depart. The needle stands higher than the platform!

Tetrad digestion: I put 50 ? l of 1 mg/ml Zymolyase 100T in water, filter sterilized, into an eppendorf. I add a barely visible tad of sporulated cells and twirl the toothpick. I leave the tube on my bench till I’m ready to dissect later in the day. I give digestion a minimum of 15 minutes. I personally have had no problem with germination viability

putting digested tetrads into the refrigerator overnight — even over 2 or 3 nights! (But I don't recommend it.)
You can check for digestion by placing a small amount (2-3 ? l) of digestion under a coverslip. If you see sets of 4 ascospore cells barely clinging to each other, looking "strung out," perhaps one dangling, that's a good digestion. Anything that looks tighter will not be dissectable. Spores are sticky and will cling to each other in their original set.

I give the eppendorf a finger snap or two and a shake down to re-suspend the cells. Do not vortex! Most workers add water (5 volumes) to dilute and get good separation. I apply cells to my agar plate using a P-20 pipetman set at 2.3 ? l, holding it at a low angle and drawing the liquid bead across the agar once. I try not to gouge too much. That is the lazy way.

Troubleshooting:

"I know the needle is centered, but I can't see it when I put my plate on."

In this case, the needle is too short to reach the agar. The tell-tale sign is that the support rod strikes the edge of the plate when the operator tries to bring the needle up to see it. If the support rod sections are taped together, perhaps the tape is too thick and it hits the edge of the plate.

"I can't focus on my cells."

The agar is too thick and exceeds the focal distance of the objective. The objective pushes against the bottom of the plate as the operator tries to focus.

"The cells I picked up have disappeared. Did they fall off?"

Cells do not fall off into outer space. They are somewhere, either on the needle or on the agar. If they are on or near the surface of the needle, continued patience will coax them off. They might be down on the side of the shaft. These just won't come off. If you decide to give up on this tetrad and go on to another, be sure you start with a clean needle. In an area away from the ascospore grid, plunge the needle into the agar a few times. You may even drag the needle across the agar, too. Agar doesn't break needles. Plastic does.

"My needle seems to swallow cells. I'm always losing cells."
Check to make sure the needle is perpendicular.

The needle surface may be dirty or defective. With repeated use, needles build up a film of agar on their surface. Remove this by soaking/plunging the tip in agar and leaving it there a few minutes. Then plunge some more times and drag it across the agar surface. Can you see a trail of something? Try to get rid of it.

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If the laboratory cleaning agent Chromerge (a mixture of chromic and sulfuric acids) is available, it's handy to keep a small tightly-closed bottle at the microscope table. Place a drop inside an empty plastic petri plate and position the plate on the stage. Immerse the needle tip in the droplet for 10-20 seconds. This must be done cautiously and carefully and with conscious attention to whether the needle is moving up or down. The needle is dangerously close to the plastic. No false moves. Then switch plates and plunge the needle into the agar a few times to remove the Chromerge. It will be squeaky clean again.

Defects of the needle are usually visually observable, especially if the needle transmits light. There may be a spur or a chip on one edge. Such a needle needs to be replaced.

Cells vary in the amount of handling they can survive. Ascospores are very durable. Stationary phase cells and zygotes are quite tough, too. Log phase cells are sensitive. My personal rule for log phase cells is that if I can't pick up the cell on the first attempt, I leave it and go on to another. I don't want to waste my time carrying around dead bodies.

Manipulation technique: When the flat surface of the needle touches the surface of the agar, a thin layer of water forms between them, and surface tension binds the surfaces together. You can see a dark ring suddenly appear surrounding your needle. Be sure to focus sharply on the cells or on your needle touching the agar. When the needle is pulled away, the water connection stretches and finally breaks. A cell in the water will either end up on the needle or on the agar.

Most often it is on the agar, and you have to apply some additional technique. Cells can be coaxed on to the needle surface better if the needle is moved east-west or north-south at the same time it is being pulled away. Try different maneuvers to see what works best for you. Sometimes pulling, sometimes pushing, sometimes a rather abrupt snatch. You may find a "sweet" side of your needle. Stick with it.

Put cells down gently. Approach with caution because the surface at the new position may be out of focus. You simply cannot work out of focus. If the cells don't come off the needle, try positioning the needle just short of the agar and tapping on the table or stage to make the needle bounce gently on the agar, then snatch the needle away quickly to avoid picking the cells up again. This maneuver also separates ascospores from each other.

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DIAGRAM A

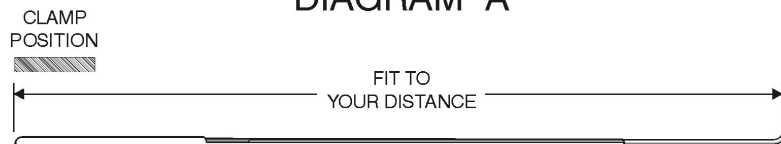

DIAGRAM B