Paraffin embedding, microtomy and fluorescence in situ hybridization (FISH) of whole adult Drosophila

Carolyn Elya,Ciera Martinez

Abstract

Paraffin embedding and sectioning is a classic technique used in a variety of biological disciplines. Though the fruit fly *Drosophila melanogaster* are one of the most commonly-employed model organisms, the immense *Drosophila* literature is lacking in current protocols for sectioning whole, adult flies. Here, we adopted a plant histology protocol to embed entire adult flies for thin sectioning and present our protocol for performing FISH on the subsequent sections. In the interest of reproducibility, we have tried to include details that are normally omitted from published protocols.

Citation: Carolyn Elya,Ciera Martinez Paraffin embedding, microtomy and fluorescence in situ hybridization (FISH) of whole adult Drosophila. **protocols.io**
dx.doi.org/10.17504/protocols.io.k5ecy3e
Published: 11 Dec 2017

Guidelines

You will need the following reagents, consumables and equipment for each of the three steps as follows:

I. Paraffin Infiltration of Adult Flies

Reagents

- Ethanol (VWR)
- Chloroform (Fisher)
- Acetic acid (Sigma)
- ddH2O
- Histoclear (National Diagnostics #HS2001GLL)
- Paraplast paraffin (McCormick #39501006)

Consumables

- Glass scintillation vials (#FS74500-20)
- Glass serological pipettes
- Glass pasteur pipettes with rubber bulbs
- Graduated plastic transfer pipettes (Fisher Scientific #13-711-9BM)
- Embedding rings (Electron Microscopy Sciences #62350W)
- Embedding base molds (Fisher Scientific #22-363-553)
-

Equipment

- Cold room or refrigerator
- Orbital shaker
- 42C incubator
- 60C incubator or paraffin oven, shaking optional
- Pyrex liquid measuring cups
- Fine forceps (Dumont #5 or #55)
- Teasing needle (or similar tool)
- Pencil, for labeling
- Microslide warming table (Eberbach corporation)

II. Sectioning embedded flies via microtome

Reagents	Consumables	Equipment
• ddH2O	• Graduated plastic transfer pipettes (Fisher Scientific #13-711-9BM) • 818 high profile microtome blades (Leica Biosystems) • Razor blades • Polysine slides (Fisher Scientific #12-545-78)	• 42C incubator • Fine forceps (Dumont #5 or #55) • Teasing needle (or similar tool) • Pencil, for labeling • Microtome • 42C heat block • Paraffin candle and lighter

III. FISH on paraffin sectionsCold room or refrigerator

Reagents	Consumables	Equipment
• Ethanol (VWR) • Histoclear (National Diagnostics #HS2001GLL) • ddH2O • ProLong Gold with DAPI (Fisher Scientific #P36931) • Hydrochloric acid • Hybridization buffer (See Recipes) • PBSTx (See Recipes) • Fluorescently-labeled FISH probe*	• 60 x 24 mm Hybrislips (Sigma-Aldrich #H0784) • 60 x 24 mm #1 coverslips	• 37C incubator • Humid chamber# • Coplin jars or staining dishes • 6” forceps • Fine forceps (Dumont #5 or #55)

*We’ve successfully used an 18mer DNA probe tagged with 5’ Alexfluor-647 (Thermo Fisher) that targets a ~11,000-fold copied sequence in genome.

#Consists of wet paper towels and something to prop up slides above towels in a sealable plastic container. Construction described in protocol.

Materials

✓ Chloroform by Contributed by users

Acetic Acid (Glacial) AC1001.SIZE.1L by Bio Basic Inc.

- Ethanol, 200 proof by Contributed by users
- double distilled water (ddH2O) by Contributed by users
- Histoclear HS2001GLL by Contributed by users
- Paraplast 39501006 by Contributed by users

Glass scintillation vials FS74500-20 by Fisher Scientific

- Glass seralogical pipettes by Contributed by users
- Glass Pasteur pipettes with rubber bulbs by Contributed by users

Graduated plastic transfer pipettes 13-711-9BM by Fisher Scientific

Embedding rings 62350W by Electron Microscopy Sciences

Embedding base molds 22-363-553 by Fisher Scientific

Razor blades 12-640 by Fisher Scientific

Polysine slides 12-545-78 by Fisher Scientific

- ProLong Gold with DAPI P36931 by Contributed by users

Hydrochloric acid by Sigma Aldrich

- Tris-HCl by Contributed by users
- Sodium chloride by Contributed by users
- Sodium dodecyl sulphate by Contributed by users
- Formamide by Contributed by users
- Potassium chloride by Contributed by users
- Disodium phosphate by Contributed by users
- Monopotassium phosphate by Contributed by users
- Triton X-100 by Contributed by users
- 818 high profile microtome blades by Contributed by users

Protocol

I. Paraffin Infiltration of Adult Flies: Day 1 - Fixation

Step 1.

Prepare fresh Carnoy’s solution (6:3:1 ethanol:chloroform:acetic acid) and chill to 4C before use.

I. Paraffin Infiltration of Adult Flies: Day 1 - Fixation

Step 2.

For each sample, anesthetize adult flies and transfer to appropriate glass scintillation vial containing ice cold Carnoy’s.

I. Paraffin Infiltration of Adult Flies: Day 1 - Fixation

Step 3.

Incubate samples in Carnoy’s solution at 4C at least overnight and no longer than 36 hours.

I. Paraffin Infiltration of Adult Flies: Day 2 - Dehydration

Step 4.

Wash each sample three times in 70% ethanol as follows:

- Aspirate solution from each scintillation vial with glass pipette and discard.
- Add 5-10 mL 70% EtOH and incubate at RT 5 min.

I. Paraffin Infiltration of Adult Flies: Day 2 - Dehydration

Step 5.

Incubate samples in the following ethanol concentrations for 1 hour each at RT:

- 70%
- 85%
- 95%
- 100%*
- 100%

NOTES

Carolyn Elya 04 Dec 2017

*Samples can be stored after this step overnight at 4C

I. Paraffin Infiltration of Adult Flies: Day 2 - Dehydration

Step 6.

Replace 100% ethanol and incubate at 4C overnight.

I. Paraffin Infiltration of Adult Flies: Day 3 - Tissue infiltration with Histoclear (Xylene alternative)

Step 7.

Incubate samples in the following solutions for 2 hours each at RT using glass pipettes and beakers (Histoclear melts plastics):

- 3:1 Ethanol:Histoclear
- 1:1 Ethanol:Histoclear
- 1:3 Ethanol:Histoclear
- Histoclear*
- Histoclear
- Histoclear

NOTES

Carolyn Elya 04 Dec 2017

*Samples can be stored after this step overnight at 4C

I. Paraffin Infiltration of Adult Flies: Day 4 - Paraffin infiltration

Step 8.

Incubate samples in new Histoclear for 2 hours at RT.

I. Paraffin Infiltration of Adult Flies: Day 4 - Paraffin infiltration

Step 9.

Add 10-15 paraffin chips to each sample and incubate at least 3 hours at RT with gentle orbital shaking.

I. Paraffin Infiltration of Adult Flies: Day 4 - Paraffin infiltration

Step 10.

Add 15 paraffin chips to each sample and incubate overnight at RT with gentle orbital shaking.

I. Paraffin Infiltration of Adult Flies: Day 4 - Paraffin infiltration

Step 11.

Melt paraffin chips overnight at 60C in Pyrex measuring cup.

I. Paraffin Infiltration of Adult Flies: Day 5 - Paraffin infiltration cont'd

Step 12.

Unscrew scintillation vial caps by ¼ turn. Incubate samples at 42C until paraffin is dissolved (about 1.5 hours).

I. Paraffin Infiltration of Adult Flies: Day 5 - Paraffin infiltration cont'd

Step 13.

Aspirate Histoclear/paraffin solution from samples and discard.

I. Paraffin Infiltration of Adult Flies: Day 5 - Paraffin infiltration cont'd

Step 14.

Cover samples with 10 mL molten paraffin and replace cap leaving ¼ turn open. Incubate at least 3 hours at 60C (with optional 50 rpm shaking).

I. Paraffin Infiltration of Adult Flies: Day 5 - Paraffin infiltration cont'd

Step 15.

Perform paraffin change as follows:

- Remove samples one at a time from incubator.
- Make sure your flies have sunk to the bottom of the scintillation vial.
- Press bottom of scintillation against gloved palm until the paraffin at the bottom of the vial hardens (it will become opaque), holding your flies in place.
- Carefully decant and discard the molten paraffin.
- Add a new 10 mL of molten paraffin and replace cap leaving ¼ turn open.

I. Paraffin Infiltration of Adult Flies: Day 5 - Paraffin infiltration cont'd

Step 16.

Continue incubating samples at 60C (with optional 50 rpm shaking) overnight. Melt additional paraffin as needed.

I. Paraffin Infiltration of Adult Flies: Day 6 - Paraffin infiltration cont'd

Step 17.

Perform paraffin change in the morning. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 6 - Paraffin infiltration cont'd

Step 18.

Perform paraffin change in the afternoon. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 6 - Paraffin infiltration cont'd

Step 19.

Perform paraffin change in the late afternoon/evening. Continue incubating samples at 60C overnight. Melt additional paraffin as needed.

I. Paraffin Infiltration of Adult Flies: Day 7 - Paraffin infiltration cont'd

Step 20.

Perform paraffin change in the morning. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 7 - Paraffin infiltration cont'd

Step 21.

Perform paraffin change in the afternoon. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 7 - Paraffin infiltration cont'd

Step 22.

Perform paraffin change in the late afternoon/evening. Continue incubating samples at 60C overnight. Melt additional paraffin as needed.

I. Paraffin Infiltration of Adult Flies: Day 8 - Paraffin infiltration cont'd

Step 23.

Perform paraffin change in the morning. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 8 - Paraffin infiltration cont'd

Step 24.

Perform paraffin change in the afternoon. Continue incubating samples at 60C for at least 3 hours.

I. Paraffin Infiltration of Adult Flies: Day 8 - Paraffin infiltration cont'd

Step 25.

Perform paraffin change in the late afternoon/evening. Continue incubating samples at 60C overnight. Melt additional paraffin as needed.

NOTES

Carolyn Elya 04 Dec 2017

Embedding should be performed as soon after fly infiltration has been completed. Flies can be safely left in molten agar for up to 3 days following infiltration. Flies can remain in molten agar beyond this, but sample integrity may decline (tissues may soften). How long a sample can remain in molten agar should be determined on case-by-case basis.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin

Step 26.

Pre-warm microslide warming table. Pre-warm two transfer pipettes with last “segment” snipped off by placing in 60C incubator.

NOTES

Carolyn Elya 04 Dec 2017

Experience suggests embedding only one fly per ring/base, though this is left to the user’s discretion. The orientation in which a sample is embedded is also up to the user.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin

Step 27.

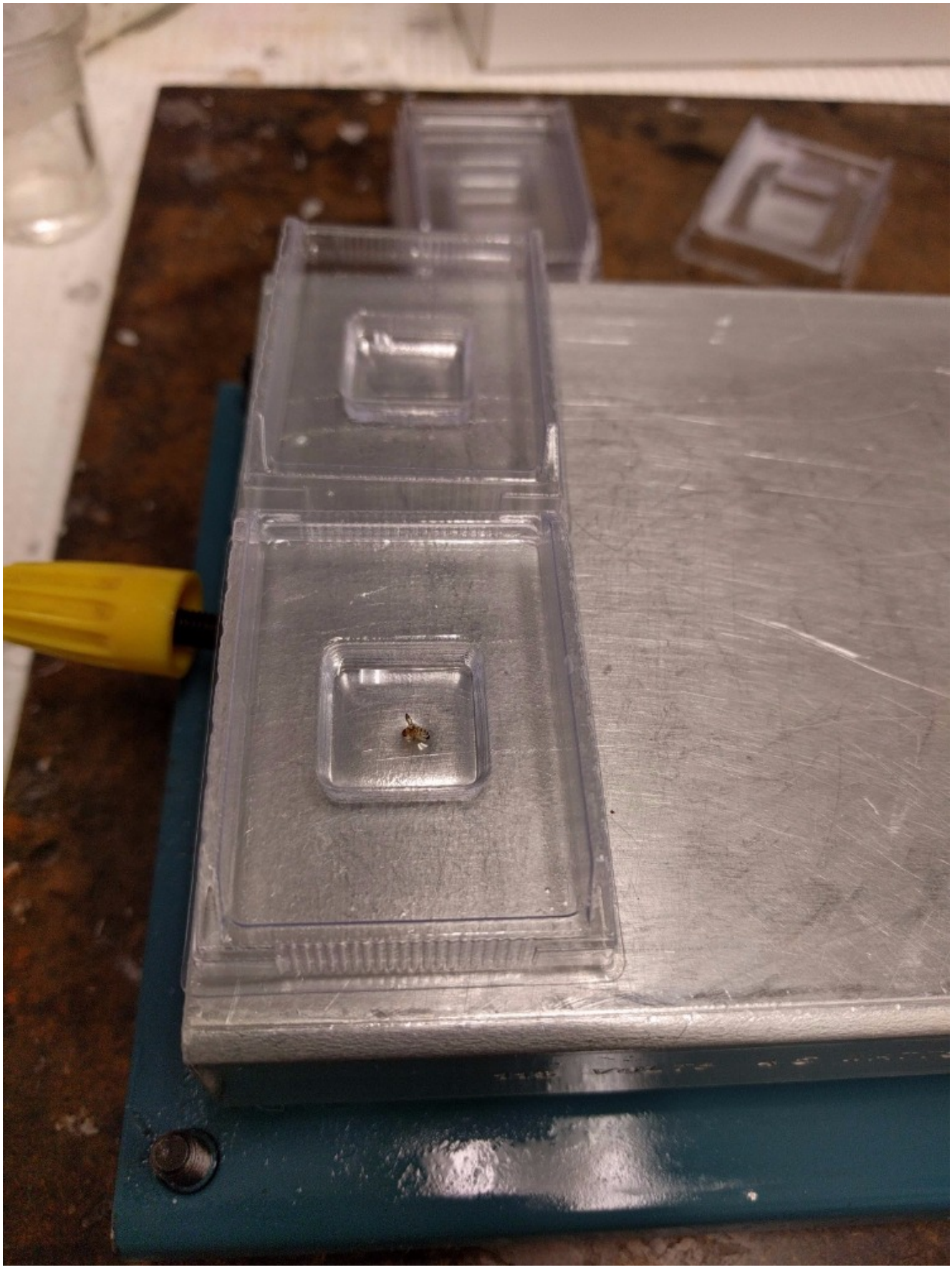
Place embedding mold base onto hottest side of microplate warmer to heat.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin

Step 28.

Remove one sample from 60C. Using pre-warmed pipette, aspirate up a fly and quickly deposit into base mold, filling base mold to cover fly in paraffin.

EXPECTED RESULTS



NOTES

Carolyn Elya 04 Dec 2017

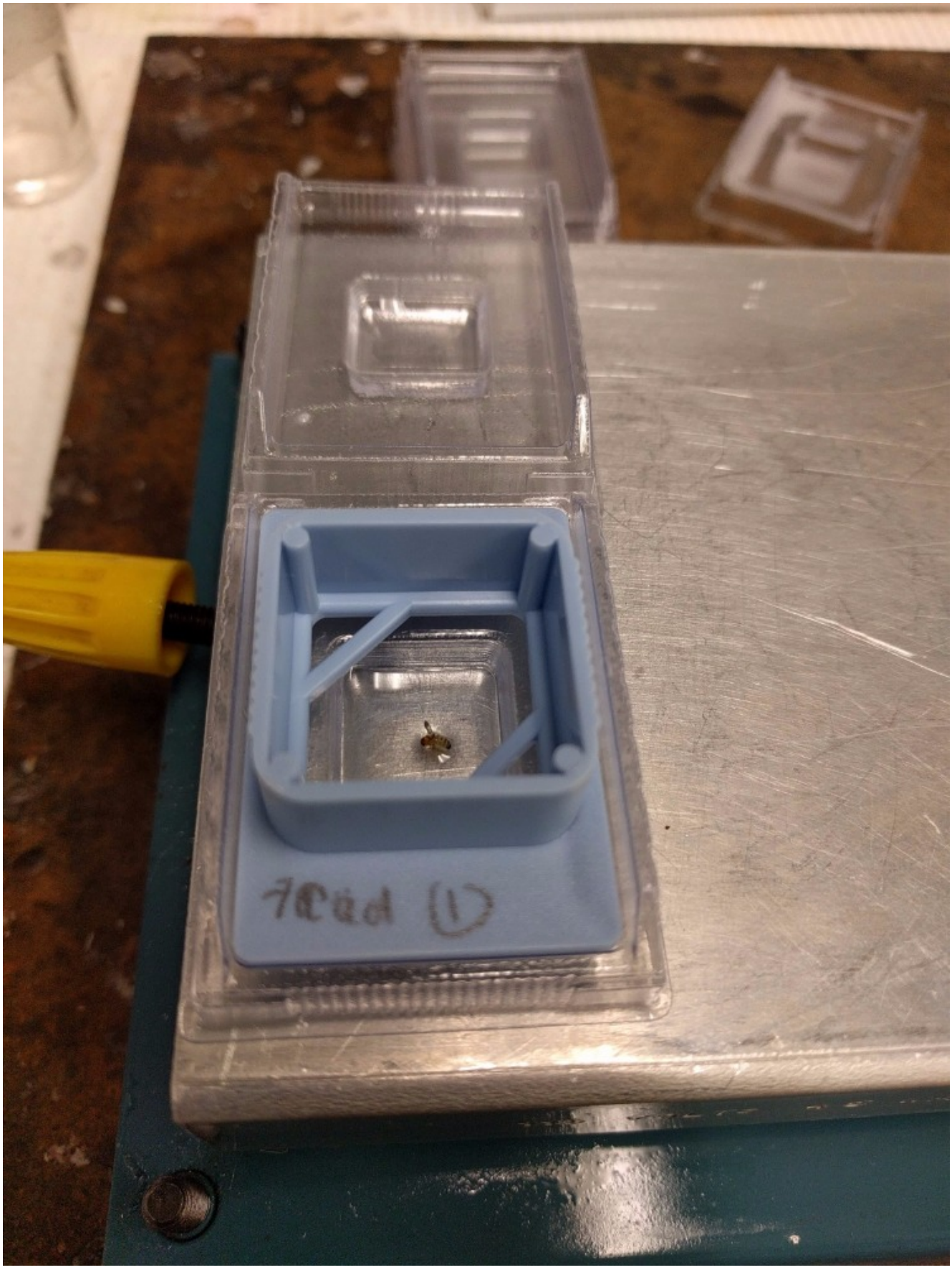
Make sure that your fly is touching the bottom of the embedding base and as close to the middle as possible. Samples that are embedded next to the side tend to break or otherwise section poorly.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin

Step 29.

Top base mold with labeled embedding ring.

EXPECTED RESULTS



I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin

Step 30.

Slide base mold to the middle of the heat gradient. Quickly use tools (forceps or poker) to orient fly in desired position.

NOTES
Carolyn Elya 04 Dec 2017

Clean wax off of tools periodically using flame to prevent unwanted sticking.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin
Step 31.

Using pre-warmed pipette, add a mL or so of molten paraffin to begin filling the embedding ring.

NOTES
Carolyn Elya 04 Dec 2017

Act quickly! You want all of the paraffin to harden together in a continuous mass. If you allow a layer of paraffin to cool before adding the next, your sample will not cut evenly.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin
Step 32.

Slide sample all the way to the cool end of the gradient.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin
Step 33.

Continue adding molten paraffin as the previous paraffin has just set but not hardened to continue filling the mold.

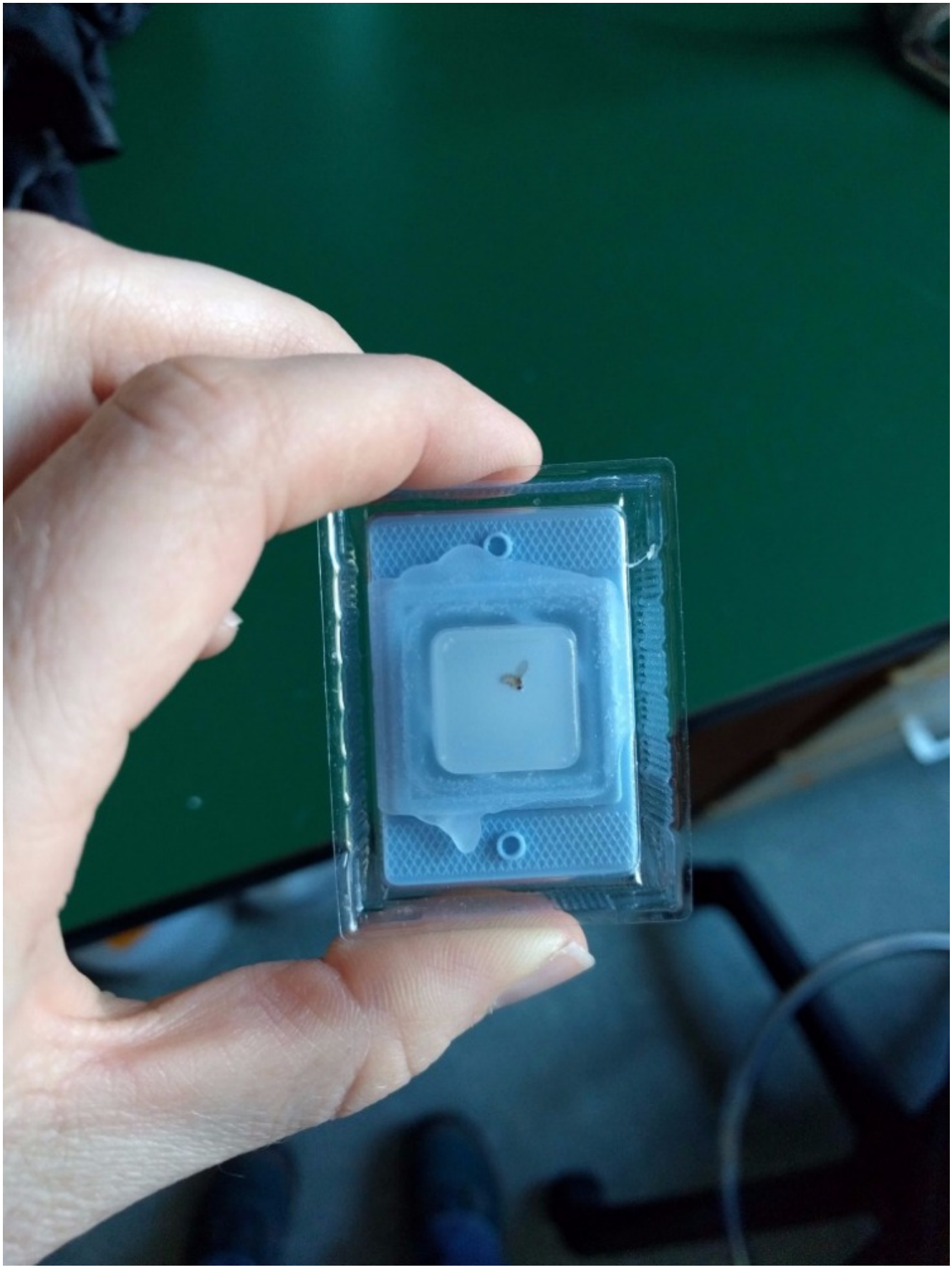
I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin
Step 34.

Once mold is almost full, allow it to set completely before transferring to the coolest end of the plate for further cooling.

I. Paraffin Infiltration of Adult Flies: Day 9 - Embedding flies in paraffin
Step 35.

Allow samples to completely set overnight before sectioning.

EXPECTED RESULTS



II. Sectioning embedded flies via microtome

Step 36.

Tape 5 or 10 mL serological pipette to the heat block.

II. Sectioning embedded flies via microtome

Step 37.

Heat slide block to 42C.

II. Sectioning embedded flies via microtome

Step 38.

Clean the area adjacent to the microtome with 70% ethanol.

II. Sectioning embedded flies via microtome

Step 39.

Place up to 6 slides on the heat block, making sure that polysine side is facing up.

II. Sectioning embedded flies via microtome

Step 40.

Cover polysine-coated area with ddH2O.

II. Sectioning embedded flies via microtome

Step 41.

Prepare a sample for sectioning by carefully removing ring from base (do this gently so as not to break your sample off into the plastic base).

II. Sectioning embedded flies via microtome

Step 42.

Starting from the edge of the paraffin block, use a razor blade to slowly cut away excess paraffin on each side of your sample.

NOTES

Carolyn Elya 04 Dec 2017

If you try to make a direct cut next to your sample, the paraffin will break and your sample will be lost.

II. Sectioning embedded flies via microtome

Step 43.

Clamp your sample onto the microtome. Do not over tighten the clamp.

II. Sectioning embedded flies via microtome

Step 44.

Move the blade cassette away from your sample before installing a fresh blade.

II. Sectioning embedded flies via microtome

Step 45.

Move the blade cassette toward your sample until you are a few hundred microns away.

II. Sectioning embedded flies via microtome

Step 46.

In “coarse” mode set to 30 um intervals, progress the blade until you make contact with the paraffin.

II. Sectioning embedded flies via microtome

Step 47.

Change microtome to “fine” mode and set to 8 um. Make one slice then use your pointy tool to gently hold the slice down.

II. Sectioning embedded flies via microtome

Step 48.

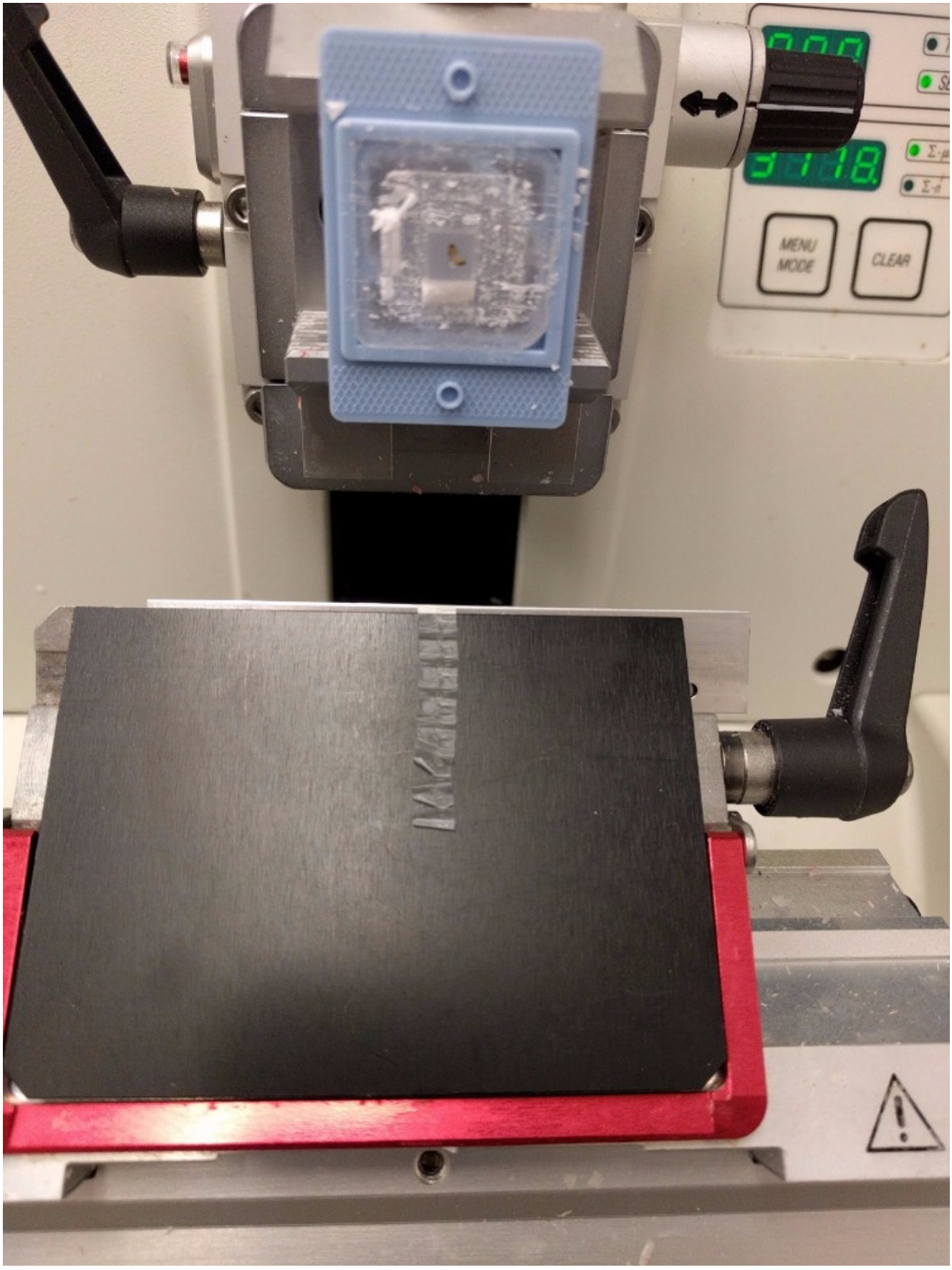
Make an additional slice, checking that your first slice adheres to the second to start forming a ribbon. If it does not, discard unattached slices and try again.

II. Sectioning embedded flies via microtome

Step 49.

Continue slicing slowly, taking care to keep the ribbon coming straight away from the blade. As the ribbon grows, you will need to use your teasing needle (or similar tool) to gently pick up and support the growing ribbon.

EXPECTED RESULTS



II. Sectioning embedded flies via microtome

Step 50.

Continue slicing, supporting the ribbon until you have sliced through the entire sample.

II. Sectioning embedded flies via microtome

Step 51.

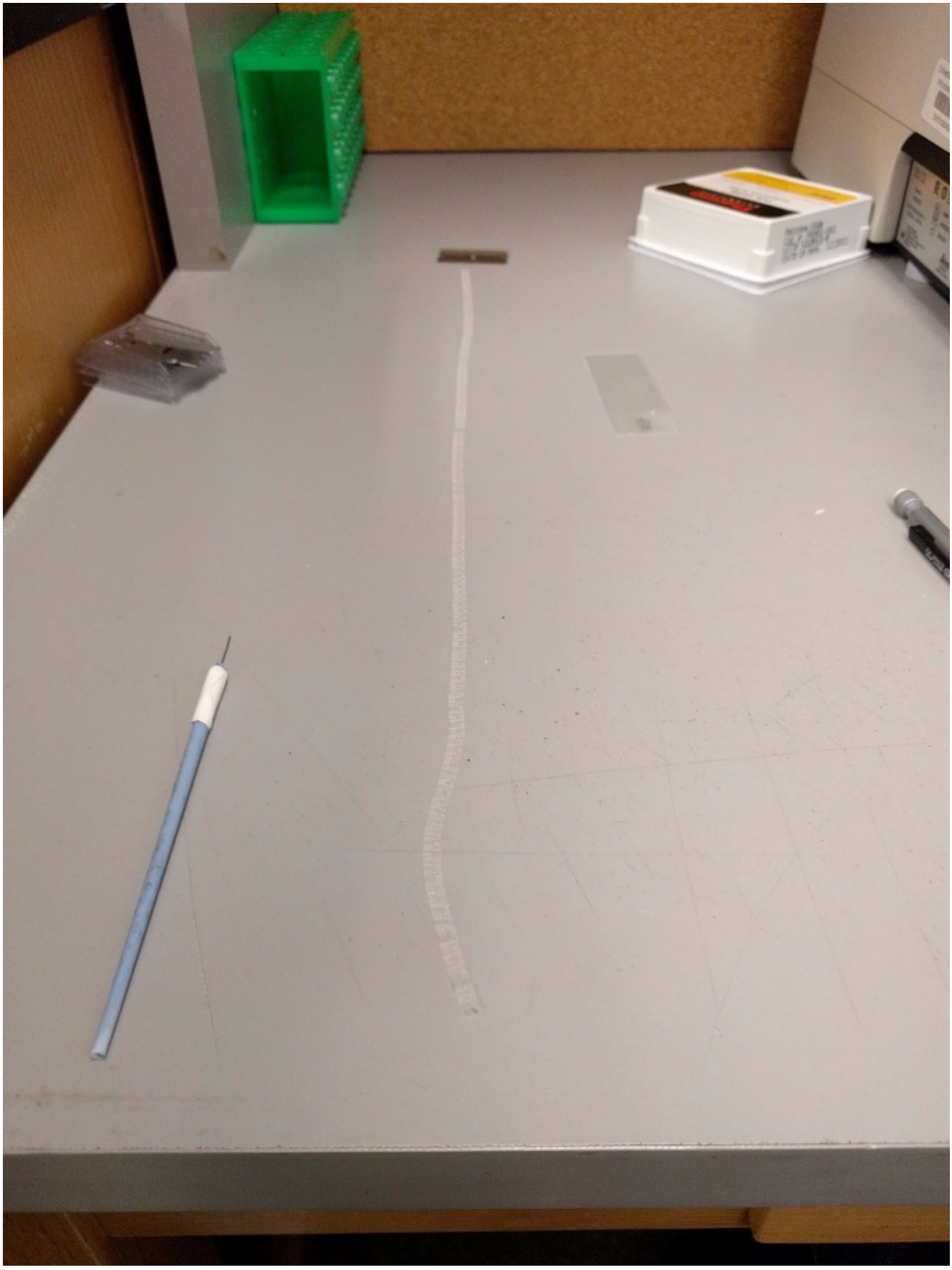
Use a new razor blade to cut the ribbon away from the paraffin block.

II. Sectioning embedded flies via microtome

Step 52.

Using forceps and the teasing needle, carefully lay the ribbon on the bench.

EXPECTED RESULTS



II. Sectioning embedded flies via microtome

Step 53.

Using a slide as reference, cut ribbon 2 sections shorter than the slide length.

II. Sectioning embedded flies via microtome

Step 54.

Carefully transfer the ribbon, shiny side facing down, onto a water-coated slide. Arranged the ribbon as straight as you can.

NOTES

Carolyn Elya 04 Dec 2017

As teasing needle, forceps and razor blade start to retain paraffin, they will grow sticky. Razor blades should be properly disposed of and replaced with a new blade. Paraffin can be removed from forceps and teasing needle by passing the tips through the flame.

II. Sectioning embedded flies via microtome

Step 55.

Continue adding ribbons in rows to the slide until you cannot safely add additional ribbons.

II. Sectioning embedded flies via microtome

Step 56.

Allow a few seconds for all of the ribbons to unwrinkle then use transfer pipette to start removing water from underneath ribbons. Be careful to keep an eye on your ribbons as they will slide when you do this (you can use your teasing needle to hold them in place).

II. Sectioning embedded flies via microtome

Step 57.

Gently tilt the slide and remove as much water as possible from the slide.

II. Sectioning embedded flies via microtome

Step 58.

Place slide to dry leaning against the serological pipette taped to the 42C block.

NOTES

Carolyn Elya 04 Dec 2017

If you place the slide directly on the 42C block, you will damage the tissue.

II. Sectioning embedded flies via microtome

Step 59.

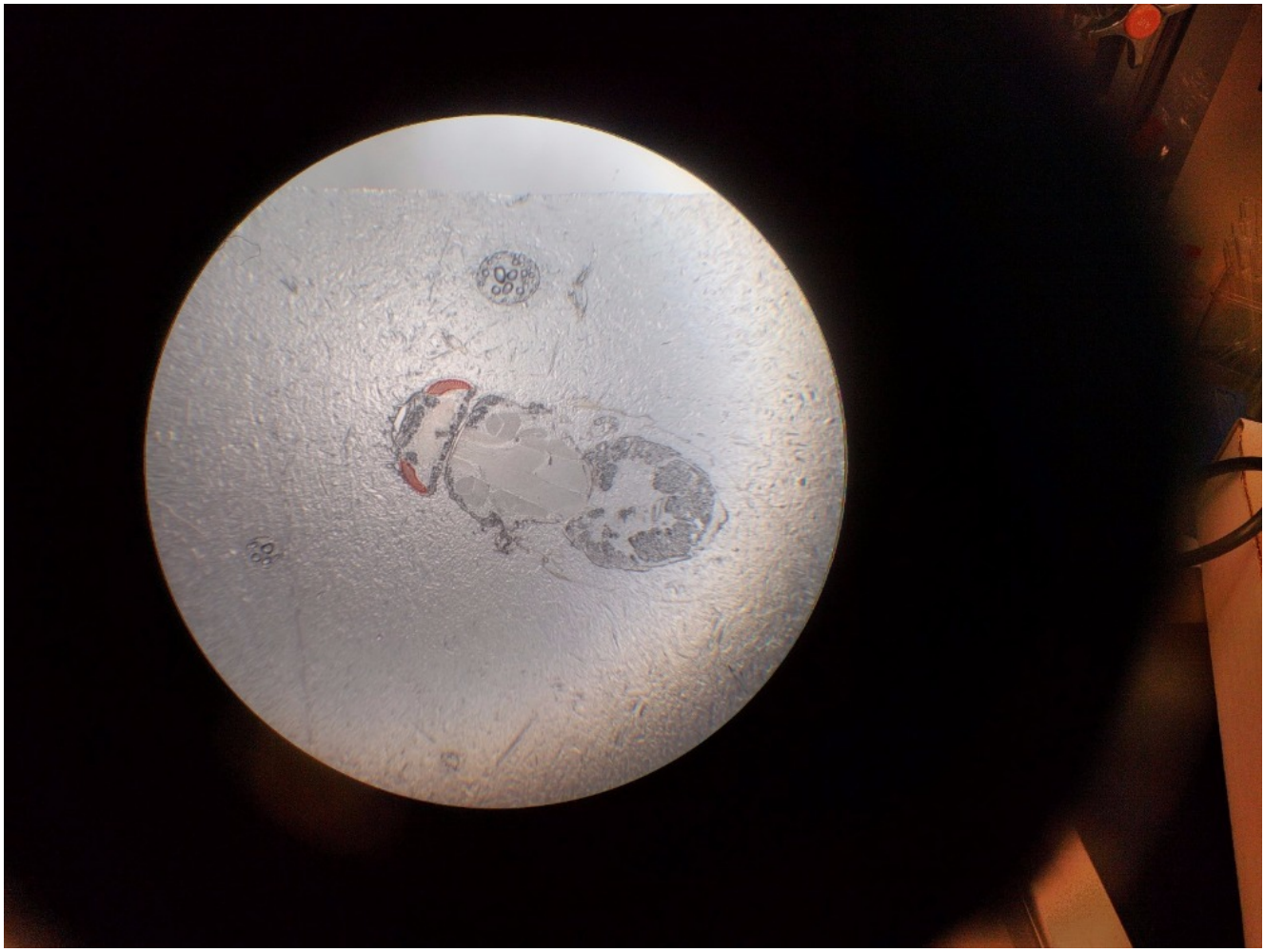
When you are finished slicing your sample, remove paraffin ring. If you wish to slice additional samples, slide the razor so that you are cutting with a unused portion of the blade.

II. Sectioning embedded flies via microtome

Step 60.

When you are finished slicing all samples, incubate slides at 42C overnight to dry. After overnight drying, slides can be used for histology, immunohistochemistry or fluorescence in situ hybridization (FISH).

EXPECTED RESULTS



NOTES

Carolyn Elya 04 Dec 2017

For best results, FISH and immunhistochemistry should be performed as soon after the slides are ready (i.e. the day after sectioning). Note that this protocol has been optimized to work with 8 micron sections.

III. FISH on paraffin sections: Day 1

Step 61.

Prepare solutions:

Hybridization solution

20 mM Tris-HCl [pH 8.0]
0.9M NaCl
0.01% sodium dodecyl sulphate
30% formamide

PBSTx

0.8% NaCl [w/v]
0.02% KCl [w/v]
0.115% Na2HPO4 [w/v]
0.02% KH2PO4 [w/v]
0.3% Triton X-100 [v/v]

III. FISH on paraffin sections: Day 1

Step 62.

Warm 0.2 M HCl in staining dish to 37C.

III. FISH on paraffin sections: Day 1

Step 63.

Remove paraffin by incubating slides in 2x changes of Histoclear for 10 min each.

III. FISH on paraffin sections: Day 1

Step 64.

Rehydrate sections by incubating slides in a graded ethanol series as follows:

- 100% ethanol – 2x 5 min
- 95% ethanol - 2 min
- 85% ethanol - 2 min
- 70% ethanol - 2 min

III. FISH on paraffin sections: Day 1

Step 65.

Unmask antigens by incubating slides in 0.2 M HCl (in water) for 60 min at 37C. Rinse 2-3x in ddH2O to remove acid.

III. FISH on paraffin sections: Day 1

Step 66.

Prepare 80 uL of 100 pmol/µL FISH probe in hybridization buffer for each slide.

NOTES

Carolyn Elya 05 Dec 2017

100 pmol/µL is the optimized concentration for our probe, but it might not be for yours. The best probe concentration should be determined empirically on a case-by-case basis.

III. FISH on paraffin sections: Day 1

Step 67.

Apply 80 uL of 100 pmol/uL FISH probe to each slide and top with Hybrislip.

NOTES

Carolyn Elya 04 Dec 2017

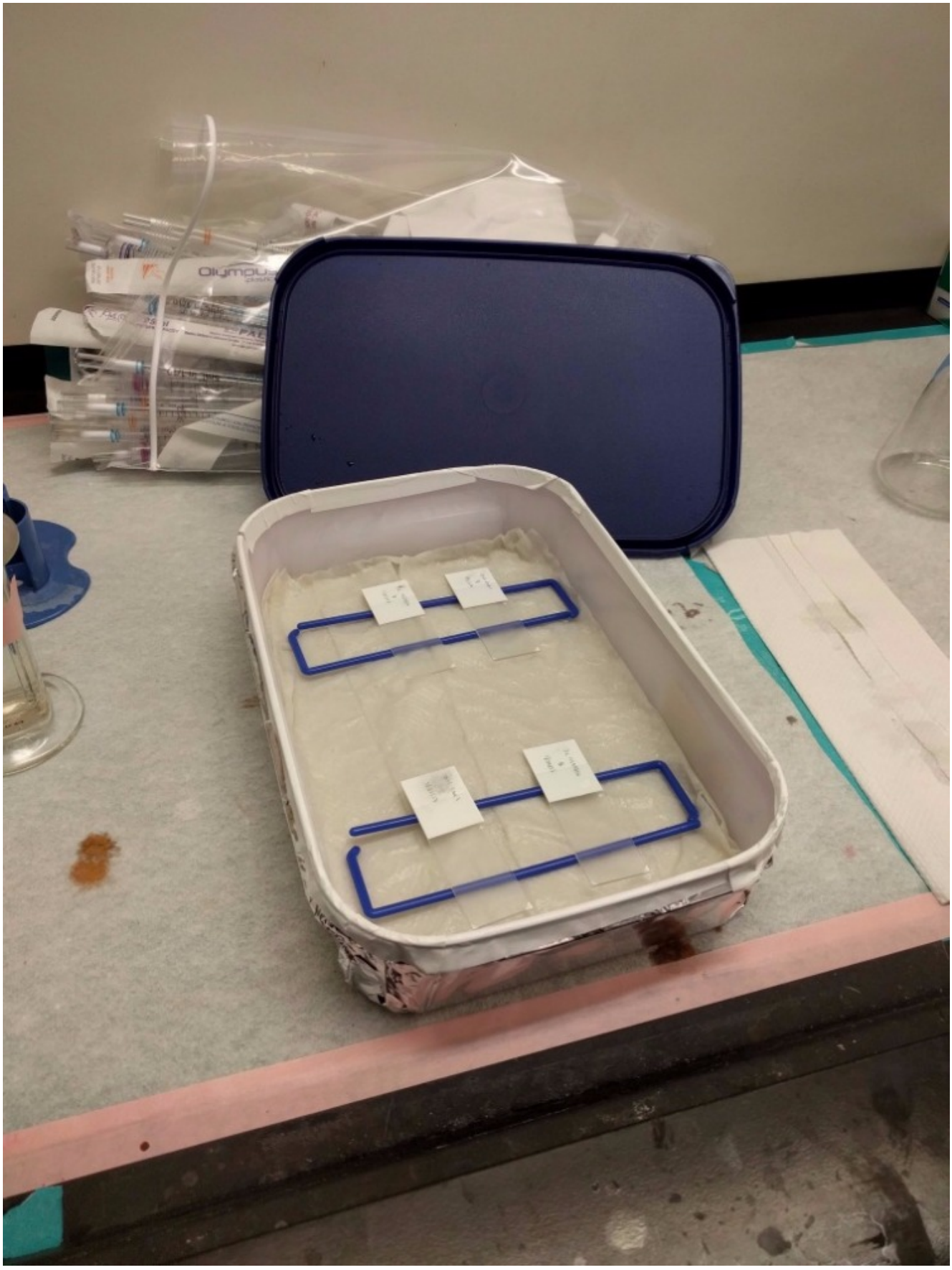
Be careful not to introduce any bubbles! If you wind up with bubbles, you can carefully remove Hybrislip and try to lay down again without bubbles.

III. FISH on paraffin sections: Day 1

Step 68.

Put the glass slides in a humid chamber and leave at room temperature, overnight, in the dark.

EXPECTED RESULTS



NOTES

Carolyn Elya 04 Dec 2017

A humid chamber can be easily constructed using a sealable tupperware container or other appropriate vessel. Line the container with paper towels and apply DI water until completely wet (no standing water). Place spacers (e.g. dowels) on the wet paper towels to prop up your slides. Place the slides on top of the spacers, making sure that they are horizontal, before closing the container.

Carolyn Elya 04 Dec 2017

From this point onward, be sure to keep your slides in the dark as much as possible to avoid bleaching of your probe.

III. FISH on paraffin sections: Day 2

Step 69.

The next day, carefully remove the coverslip by gently dunking the slides one-at-a-time in a Coplin jar filled with freshly prepared PBSTx.

III. FISH on paraffin sections: Day 2

Step 70.

Gently dunk the slides 5x in PBSTx to wash off probe.

NOTES

Carolyn Elya 04 Dec 2017

If you find you have high background fluorescence, you can increase the amount of washing by placing slides in a Coplin jar containing 1x PBSTx and incubating on an orbital shaker at low speed for 5 minutes. Be sure to cover the Coplin jar in foil to prevent your probe from bleaching.

III. FISH on paraffin sections: Day 2

Step 71.

Dunk slides in 1x PBS to remove all detergent (3 times)

III. FISH on paraffin sections: Day 2

Step 72.

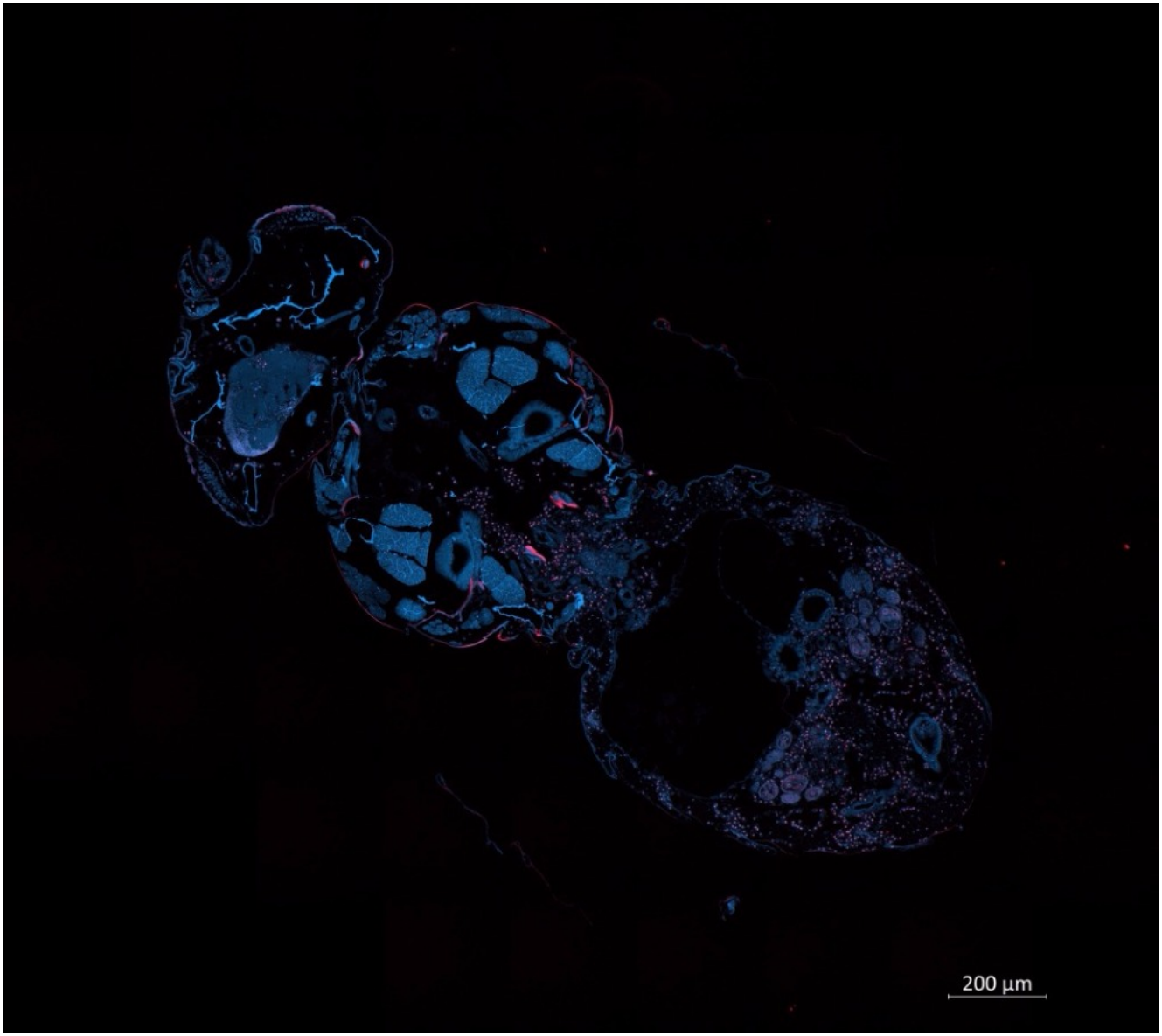
Apply 80 µl of ProLong Gold (antifade reagent) with DAPI directly onto the slide and cover the slide with a 60 x 24 mm coverslip. Avoid air bubbles.

III. FISH on paraffin sections: Day 2

Step 73.

Allow the slides to dry 24 hours in the dark at RT before viewing (to cure the ProLong Gold).

EXPECTED RESULTS



NOTES

Carolyn Elya 04 Dec 2017

Slides should be immediately imaged at 10x-40x with the appropriate excitation and emission filters.

Warnings

- Histoclear is very toxic to marine life. Do not dispose of Histoclear down the drain.
- All chemicals should be used according to SDS recommendations.