

3/23/00

In Situ Hybridization of DNA to Polytene Chromosomes

I. Cleaning of slides.

1. Use frosted No. 3050 slides.
2. Place in a rack and dip in 1 N HCl approximately 1 hour. Rinse HCl off with tap water. Soak in very hot 50% soap (heat over bunsen burner until beginning to boil) for approximately 1 hour. Then rinse in hot tap water and dry with a piece of fine cheese cloth. Store in a clean slide box.

II. Cover slips.

Use 18 X 18 #2 cover slips and use directly from the box without cleaning. #2 cover slips are heavy and useful for squashing chromosomes.

III. Care of embryos.

Try to avoid overcrowding bottles with embryos. For larger chromosomes feed embryos daily with yeast paste:

- 145 g Dry Yeast
- 400 ml Sterile H₂O
- 1.36 ml Propionic Acid

Yeast paste can be stored in the cold room for weeks large beaker covered with seran wrap. Add to fly bottles with a pasture pipette with the tip broken off.

IV. Preparation of chromosomes.

1. Use moving 3rd Instar larvae. They have fewer puffs than later, sedentary animals.
2. Remove the larva with a dissection needle. Place into 45% acetic acid in a small depression slide (three place) or small glass petri dish. Examine under low power under a dissecting microscope.
3. Hold the head with one forceps and grab the tail with the other and pull the tail. The glands should be left with the head parts and the rest of the embryo will be pulled away. Clean off the fat body from the glands (To make this easier one can move the gland to a second well in the depression slide, containing 45% acetic acid, using a pasture pipette. Although with practice the fat can be removed in the same well that the dissection is done, this is faster and easier.).

3/23/00

4. Take a short Pasteur pipet and suck up some of the debris resulting from the dissection. This will coat the pipet with protein, etc. and keep the salivary glands from sticking. Pick up the glands with the pipet and transfer them to a cover slip. Remove most of the liquid with the pipet. Carefully place the slide over the cover slip.
5. Firmly hold the corners of the cover slip while using a dissecting needle handle end to tap the cover slip 3-5 times firmly then tap gently in expanding spiral patterns for 15-30 seconds. One can check the chromosomes by examining under phase optics. If further chromosome spreading is desired resume gentle tapping.
6. Place the slide over blotting paper with the cover slip down and press with your thumb. Apply good pressure (weight of hand plus a small amount of arm pressure) twice for 10 seconds each.
7. Freeze the slide by placing on relatively flat surface of a slab of dry ice for at least 30-60 minutes. Then immerse in a very cold dry ice/ethanol bath for at least 1 hour. Use a razor blade and quickly flip off the cover slip. Immediately immerse the slide in a tray of 95% ethanol that has been cooled to -20°C . Store at -20°C overnight (it is not certain whether this step is absolutely necessary). Slides may be stored at -20°C for several days.

V. Heat Treatment.

1. Remove the slides from the ethanol and allow to air dry.
2. Place them in a slide tank at 65°C of 2 X SSC and incubate for 30 minutes. While slides are in water bath, heat two tanks of 70% EtOH and one of 95% EtOH to 65°C .
3. Incubate slides in a 65°C 70% EtOH tank 10 minutes then move to the second 70% EtOH tank for 10 minutes.
4. Place slides in 95% EtOH tank and remove tank from water bath. Allow to cool to near room temperature (may still be warm to the touch).
5. Air dry slides. (They may now be stored at 4°C for months if necessary.)

VI. Denature Slides.

3/23/00

1. Immerse slides for 2-3 minutes in 1.4 ml of 10N NaOH in 200 ml of H₂O. Rinse three times in 2x SSC for 5 minutes each.
2. Run the slides through two 70% ethanol washes for 5 minutes each and one 95% ethanol wash for 5 minutes. Air dry.
3. It may be helpful to mark slides to show chromosome location.

VII. Hybridization.

1. Label the probe with biotinylated nucleotides. After ethanol precipitation, washing and drying, dissolve the probe in 15 µl of hybridization solution. Boil the probe for 5 - 10 minutes, then quick cool in an ice-water bath. When cool, collect the liquid into the bottom of the microfuge tube by spinning 15 seconds in the microfuge.
2. Add the solution to the slide and cover with a 22 X 22 #1 cover slip. Add liquid glue around the cover slip using a 10 ml syringe.
3. Take a plastic tray and place wet (H₂O) paper towels in the bottom of it. Rest plastic 1 ml pipets on top of the paper towels.
4. Place the slides on top of the 1 ml pipets. Place the cover on top of the tray and seal with Parafilm. Do not use Vaseline.
5. Put in the 42° C oven and incubate overnight.

VIII. Washing.

1. Remove the glue with a forcep and place the slide into a baking dish of 2 X SSC. Use a forceps and slide the cover slip a little bit. Then grab one end of the cover slip and slowly remove it.
2. Wash in 500 ml trays.
3. Wash 2 times in PBS for 10 minutes at room temperature.
4. Wash for 2 minutes in PBS/0.1% triton X-100.
5. Rinse for 2 minutes in PBS.

IX. HRP/DAB Reaction.

3/23/00

1. Prepare a tray as for hybridization.
2. Use the Enzo Biochem Kit reagents. Prepare a 1/250 dilution of the Streptavidin/biotin/HRP complex in 1X Enzo dilution buffer. The amount required is 100µl/slide.
3. Pipet 100 µl of the solution onto each slide. Cover each slide with a 22 X 40 #1 cover slip. Place into the tray, add its lid and wrap with Parafilm. Incubate at 37° C for 2 hours.
4. Wash 2 X with PBS for 5 minutes each at room temperature.
5. Wash for 2 minutes with PBS/0.1% triton X-100.
6. Rinse for 2 minutes in PBS.
7. Prepare a solution 0.5 mg/ml of DAB in PBS. Add 17 µl of 3% H₂O₂ /ml.
8. Add 0.5 ml of the solution/slide and incubate at 37° C for 30 minutes.
9. Wash in water.
10. Add drop of sterile water and #1 22x22 cover slip to area where chromosomes lie. Observe with phase optics.

X. Solutions:

Hybridization buffer:

5 X SSPE	0.5 ml 20 X SSPE
50 % Formamide	1 ml Formamide
1 X Denhardt's	40 µl 50 X Denhardt's
5 µg/ml poly (dU)	10 µl 1 mg/ml poly (dU)
200 µg/ml sheared Salmon sperm DNA	40 µl 10 mg/ml DNA
10 % dextran sulfate	0.2 g
	0.4 ml H ₂ O

2 ml

3/23/00