Physical Separation of Forensic Mixtures Using

Laser Microdissection Techniques

Technology Transition Workshop

Slide Preparation

1. Pipette 20 µl of sample cells suspended in **Carnoy's Fixative** onto a membrane slide, 1 mm in thickness, and spread gently into a 1 cm x 1 cm area using a pipette tip.

2. Place the slide above the steam of a water bath for 5 seconds.

3. Place slides onto a slide warmer set to 56°C until completely dry.

4. Bake slides in a biological oven set to 60°C for approximately 3 hours.

5. Store slides in dessicator until ready for pre-treatment.

Slide Pre-Treatment

1. Cover marked sample region with reusable perfusion chamber.

2. Pipette 500 µl **Pepsin Buffer**, pre-warmed to 37°C, into the chamber. Incubate on the ThermoBrite for 2 minutes at 37°C.

3. Remove the perfusion chamber and place the slide into a Coplin jar containing **PBS/$MgCl_2$** solution. Incubate at room temperature for 5 minutes. Shake gently.

4. Fix slides in **Formalin Buffer** for 5 minutes at room temperature. Shake gently.

5. Wash the slides by placing them into a Coplin jar containing room temperature **PBS** for 5 minutes.

6. The slides are then dehydrated through a series of **Ethanol Washes** (70%, 85%, and 100%) for one minute each at room temperature. Following dehydration, the slides should be air dried.

7. Store slides in a desiccator until FISH processing is initiated.

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CEP X and Y Probe Hybridization

1. Turn off lights before handling probe.

2. Combine 7 µl of **CEP Hybridization Buffer®**, 1 µl of **CEP X® probe**, 1 µl of **CEP Y® probe**, and 1 µl of **ddH_2O** in a 0.5 µl microcentrifuge tube.

3. Dehydrate the slides through a series of **Ethanol Washes** (70%, 85%, and 100%) for one minute each. These ethanol washes are performed at room temperature. Following dehydration, the slides should be air dried.

4. Apply 10 µl of the **probe mix** directly to the marked sample region and immediately conceal with a cover slip.

5. Seal the cover slip to the slide with rubber cement.

6. Place slide on the ThermoBrite. Run the pre-programmed denaturation/hybridization program for 45 minutes.

Post-Hybridization

1. Following the 45 minutes incubation period, remove slide from the ThermoBrite

2. Using forceps, remove the rubber cement and cover slip.

3. Cover marked sample region with a perfusion chamber and apply 500µl **SSC/Tween20 Buffer.** Incubate for 2 minutes on the ThermoBrite at 73°C.

4. Remove perfusion chamber. Immerse slide in a Coplin jar containing **SSC Buffer** for one minute at room temperature. Shake gently.

5. Remove slide from Coplin jar. Air dry slide in darkness.

6. Dip slide in a ddH_2O bath 2-3 times.

7. Place 10 µl of diluted **DAPI VectorShield Counterstain** directly onto the marked sample region. Cover with a cover slip. Incubate the specimen in the dark for 15 minutes at room temperature.

8. View the samples using a fluorescence equipped LM microscope with appropriate filters. All three fluorophores may be visualized using a fluorescence capable microscope with DAPI (blue-nucleus), FITC (green-female), and TRITC (red-male) filters.