PARAFFIN EMBEDDING TISSUE

1. Starting point is fixed tissue that has been stored in 70% Ethanol.
   Pass tissue through following dehydration series:

Day 1

85% Ethanol	1 Hr	on ice
95% Ethanol + 0.1% Eosin	OVERNIGHT	4°C

Day 2

100% Ethanol	30 min	room temp
100% Ethanol	30 min	room temp
100% Ethanol	1 Hr	room temp
100% Ethanol	1 Hr	room temp
25% Histoclear/75% Ethanol	1 Hr	room temp
50% Histoclear/50% Ethanol	1 Hr	room temp
75% Histoclear/25% Ethanol	1 Hr	room temp
100% Histoclear	1 Hr	room temp
100% Histoclear	1 Hr	room temp

Transfer sections to a basket made from a plastic scintillation minivial. (Cut bottom of vial off and remove all but the sides of the lid. Use the remainder of the lid to secure a piece of nylon mesh over the top of the vial). Place basket in 30 ml centrifuge tube containing:

100% Histoclear/1/4 vol wax	OVERNIGHT	room temp

Day 3

Place at 42°C until wax melts completely and then add 1/4 volume fresh chips. Keep at 42°C until wax melts and then move to 60°C for several hours. Transfer basket into tube containing 5 ml fresh molten Paraplast. Incubate 60°C overnight in dri-block or oven.

Day 4-6

Change wax twice daily. Avoid bubbles.

2. Prewarm disposable plastic mold (Polysciences Inc. Cat #18985 or 18646a) on 60°C Dri-Block. Transfer fresh Paraplast and section to

mold using a cut-off prewarmed Pasteur pipette. (Heat pipette in burner and cool with spare molten Paraplast immediately before use – see Note 1.) Make sure section is entirely covered in Paraplast.

3. Align section in mould using warmed forceps. Once surface begins to solidify, float block on ice cooled water (about 18°C). After about 1 minute, push the block below the surface of the water. This ensures that the paraffin solidifies evenly but it takes some practice – the block will crack if chilled too soon.

Notes

1. Sectioning is impaired if Paraplast is heated over 62°C at any stage.