emulate

Guideline:
Mounting and Sectioning Chip-A1 Culture Chamber Samples

EG-183 Rev A
June 2024

Items Required

- Xylene (ES34411VWR)
- 100% Ethanol (ES34116VWR)
- 95% Ethanol (ES34114VWR)
- 80% Ethanol (ES34112VWR)
- 70% Ethanol (KOPTEC V1401)
- 6mm biopsy (Integra Miltex 12-460-412)
- 10% Formalin (Sigma HT5011)
- Leica RM2235 Microtome
- Warm water bath
- Hydrophobic marker (Sigma Z672548)
- HistoCore ARCADIA H
- Cold plate ARCADIA C
- DI water
- Leica TP1020 Tissue Processor
- Poly-L-Lysine coated slides (Sigma P0425-72EA)

Part I Harvesting Sample

- Perfuse 10% formalin in the bottom channel
- Using a 6mm biopsy (Integra Miltex 12-460-412), punch out the hydrogel including the bottom channel membrane (straight down, then twist to ensure uniform cut)
- Transfer to well plate in 10% formalin bath
- Before proceeding to Part II, ensure the epithelium is still intact

Part II Sample Dehydration

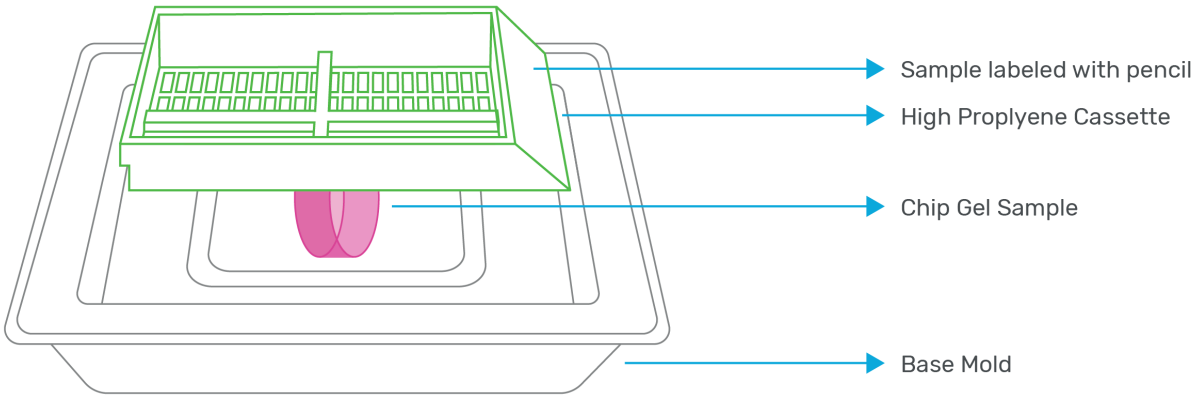
- Ensure Tissue processing carousel has been cleaned from previous runs
    - **NOTE**: Empty wash and chemical waste into properly labeled propylene waste containers for every part of this protocol.
- Using forceps, carefully transfer gels (**without Bottom membrane**) to high propylene cassettes
    - Label cassettes with pencil (does not wash off)
- Place cassettes containing samples in the first carousel jar of the TP 1020
- Run program U01 on Leica TP 1020:
    1. 10% Formalin – 1 hour
    2. 10% Formalin – 1.5 hours
    3. 70% Ethanol – 1.75 hours
    4. 80% Ethanol – 1.75 hours
    5. 95% Ethanol – 2 hours
    6. 100% Ethanol – 2 hours
    7. 100% Ethanol – 2 hours

8. 100% Ethanol – 1.5 hour
9. Xylene – 1.5 hours
10. Xylene – 2 hours
11. Paraffin – 2 hours
12. Paraffin – indefinitely

Part III Paraffin Embedding

- Warm HistoCore ARCADIA H 24 hours in advance to melt the paraffin
- Using forceps, carefully transfer sample to base mold and orient sample to be perpendicular to the mold (Figure 1)
- Pour an initial layer of paraffin, place on cold block, and then ensure sample is oriented correctly
- Fill remainder of mold with paraffin and then cover mold with the cassette
- Place casted blocks on HistoCore ARCADIA C cold surface for at least 30 minutes

Figure 1.



Part IV Sectioning and Slide Preparation

- Turn on warm water bath (45˚ C) and slide warmer
- Remove block from base mold and shave off excess paraffin with a razor blade
- Lock the lever prior to adjusting the microtome
- Insert block into Leica microtome with the block facing the user

- Set dial to 6-8 µm slices, set blade right up against block, and lock in the stage
- Unlock the lever and crank clockwise to begin sectioning
    - Crank lever counterclockwise to reset the stage
- Cut sectioned film with razor, leaving ~ 0.5 inch on the stage to ensure uniformity
- Transfer sectioned film with forceps to warm water bath, laying flat on top of the water
- With a lysine coated slide, gently scoop sectioned film onto it
- Place slide on slide warmer
- Slides can be stored at room temperature until further processing