Sample preparation
Learning objectives

• Understand the differences in sample preparation for different sample formats

• Appreciate the critical steps for embedding and cryosectioning of tissue samples for LCM

• Embed, section, and stain representative tissues and adherent cells
Three main sample preps

- Suspension cells: isolate and lyse

- Cultured adherent cells: fix, stain, and microdissect

- Tissues: embed, cryosection, fix, stain, and microdissect
Suspension preparations

• Add 0.1% (w/v) saponin to the first step of poly(A) amplification
• Permeabilize cells and release mRNAs
• Does not interfere with downstream enzymatic steps
Cultured adherent cells

- EtOH fixation on glass
- Histologic staining (nuclear fast red, hematoxylin, toluidine blue) < 2 min aqueous!
- EtOH dehydration and xylene clearing

HT-29 cells

NFR = brownish red
Tissue embedding

• Snap freeze tissue pieces immediately in liquid nitrogen or dry ice-isopentane (store at –80ºC)
• Embed in a small cryomold with NEG50 (like OCT but less flaky at low temps)—make sure that the tissue piece is completely covered
• Work quickly and accept some thawing on the exterior of the tissue piece (do not sample on the edge)
• Blocks can be stored long term, but sectioned ONCE
Cryostat nomenclature

Object head

Anti-roll plate

Chuck

Pressure platform
Before starting

• Equilibrate at a cold temperature (< −20°C)

Fresh blade

Moderate platform angle and move platform to object head
Setting up the cryoblock

Diagonal (to reduce friction)
Trim, trim, trim... section

Refreeze sections quickly inside the cryostat box!
Cryosection storage and staining

• Transport slides to –80ºC on dry ice
• EtOH fix slides at the –80ºC (no thawing)
• Histologic staining (nuclear fast red, hematoxylin, toluidine blue) < 2 min aqueous!
• EtOH dehydration and xylene clearing

Human melanoma
Questions?