Tissue (tumor) cryopreservation –

Use sterile instruments (autoclaved forceps, hemostats, and scalpel handle, wrapped blade)

Place sterile 6.5cm^2 petri dish under hood. Separate the petri dish halves. Lean the instruments (handle side down) on the walls of the taller of the halves.

Open the scalpel blade packaging and remove the blade, touching it only with the hemostats. Use the hemostats to attach the blade to the handle and return the scalpel to rest, keeping the blade from touching anything.

In the shorter of the petri dish halves, pipet a small amount of the media from the tissue container and place the tissue in this media (do not let it dry out)

Use the forceps to move a piece of the tissue to a dry spot on the petri dish and cut it into small blocks (~1mm^3) and return the pieces to the media until all of the tissue is sliced. DO NOT LET THE TISSUE DRY OUT.

Fill the cryo-tubes with tissue freezing fluid and split the tissue pieces into roughly equal amounts. Place tissue in cryo-tubes and store them overnight at -80^{o}C. The following day, move them to liquid nitrogen storage.

CLEAN-UP

If the tissue is a xenograft – spray the instruments with 70% ethanol and leave them to dry on a paper towel on the lab bench. Throw the dish parts into the biohazard waste.

If the tissue is human – leave the dish parts and the instruments in the culture hood overnight, exposed to the UV light. Then treat them like the xenograft above.

Cryopreservation freezing fluid – 10ml

9ml FBS

1ml DMSO