



Cryopreservation of intestinal biopsies (whole)

Last updated: 4/20/2023 (TK)

Reagents and equipment:

- Intestinal biopsy punches (2-3) placed in Basal Medium (AdvancedDMEM/F12+HEPES+GlutaMax+antibiotic/antimycotic+gentamicin) in an Eppendorf tube, kept on ice)
- Sterile PBS, cold
- Cryovials
- CryoStor cryopreservation medium, cold (StemCell #100-1061)
 - (alternatively, can use 10%DMSO-90%FBS)
- Cryopreservation jar (e.g. Corning CoolCell, Thomas Scientific # 1200P52; or Mr. Frosty, Fisher # 15-350-50)
- -80 freezer

Protocol:

1. Label the cryovials (Patient ID, tissue type and location, date)
2. Dispense 1mL CryoStor medium per cryovial (we typically freeze one biopsy fragment per vial)
3. Remove the medium the tissue came in (do not use a vacuum aspirator)
4. Wash the tissue fragments 3x by adding 1mL of sterile PBS, pipetting up and down 5-10 times and aspirating with a P1000 pipet
5. Using a P1000 tip, gently transfer the tissue into CryoStor (try to carry over as little PBS as possible), let the tissue settle to the bottom
6. Place the cryovials in the cryopreservation jar, filled with isopropanol as instructed
7. Place the cryopreservation jar in a -80 freezer
8. After an overnight incubation at -80, samples can be moved to liquid nitrogen storage

Important when cryopreserving tissue for future for maximum viability (e.g. for scRNA-seq):

- Biopsies must be delivered to lab ASAP and cryopreserved immediately
- To minimize handling time, all vial labeling, etc., must be done in advance
- Record the time tissue arrives at the lab and time cyo jar is placed in -80 (the difference should be under 15 min)