Texas A&M
Molecular Cytogenetics Lab
Raudsepp

Collection biopsies and tissues to establish primary cell cultures for karyotyping

I COLLECTION MEDIUM

In a sterile cell culture hood prepare 15 ml sterile conical tubes containing
- 10 ml Hank's Balanced Salt Solution (sterile)
- 200 µl of 100XAntibiotic-Antimycotic solution (sterile)

Store the tubes *ready-to-go* at -20ºC.

Hanks' Balanced Salt Solution (HBSS) (1X), liquid
Invitrogen SKU# 24020-117
500 ml
Contains calcium and magnesium.

Antibiotic-Antimycotic (100X), liquid

ThermoFisher (Gibco SKU# 15240-062 100 mL; #15240-096 20 mL)

Contains 10,000 units of penicillin (base), 10,000 µg of streptomycin (base), and 25 µg of amphotericin B/ml utilizing penicillin G (sodium salt), streptomycin sulfate, and amphotericin B as Fungizone® Antimycotic in 0.85% saline.

II SETTING UP PRIMARY CULTURES

1. Collect skin biopsy/necropsy **as sterile as possible**; transport and store in 10 mL collection media (see above).
2. Change collection medium a few times during 1-2 days. Do not keep tissues in in collection tubes longer than 3-4 days.
3. Pass pieces of skin/biopsy/other through multiple (min 6X) petri dishes containing HBSS & anti-anti; cut pieces small (0.2x0.2 cm). Take time in cleaning each piece individually.
4. Set up cultures in T25 flasks by planting 8-9 small pieces of tissue per T25 flask. Many pieces on a small territory is important for cell-cell communication via growth factors and will facilitate cell emergence and proliferation. Add about 1 mL of sterile alpha MEM containing 20% FBS and anti-anti into each.
5. Keep on checking cultures for cell growth every day. Change medium in every 2-3 days.
6. Once the cells emerge and start to proliferate, remove pieces of tissue.
7. Grow T25 flasks to confluency. Passage into 2 T75 for chromosome perps, otherwise freeze in LN2.