THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Crypt Culture Protocol – Adapted from Stepppenbach and Warner labs

Day 1 Before Starting:

Make sure there is **thawed Conditioned Media** (place in fridge night before)

Make 200 mL PBS, 800ul 0.5M EDTA (no Ca or Mg)
Make 100 mL HBSS with 400 uL 0.5M EDTA (no Ca or Mg)
Thaw Matrigel on ice (for 6 wells in duplicate: ~200 uL)
Start Vortexe in cold room because delayed with being cold
Number of tubes:
2mL of PBS without EDTA in 5 mL tubes: 1 x number of mice
2mL of PBS + EDTA in 5 mL tubes: 1 x number of mice
5 mL HBSS + EDTA in 15 ml conical: 1 total times 3 washes = 3 x number of mice
30mL PBS + EDTA in 50 mL conical to flush intestine: 1 x number of mice
50 mL conical empty for crypts straining: 1 x number of mice
1.7 mL eppendorf tubes : 2 x number of mice (not counting FACS staining or RNA tubes)

1. Weigh mice before sac.
2. Take out intestine (jejenum only) and wash with PBS +EDTA
3. Measure length. Take 4 cm (5 cm from stomach is starting point)
4. Put in 2 mL PBS, keep on ice until ready to move on.
5. Do second mouse steps 2-6.
6. Shake gently to remove feces
7. After all mice are harvested, move intestines to PBS+EDTA (keeping each in own tube)
8. Incubate on ice for 10 minutes.
9. Move each sample to 15 mL conical with 5 mL HBSS+EDTA
10. Vortex 1 – Jej 1600rpm,– 5 minutes, do not save sup
11. Transfer intestines to new tubes
12. Vortex 2 - Jej 1600rpm,– 3 min, save sup
13. Vortex 3 - Jej 1600rpm,– 8 minutes, save sup
14. Strain sup through 70 μm strainer (will likely clog and need a few)
    14a. Wash all tubes with 5mL HBSS + EDTA and strain
15. Spin at low speed – 700-1000 rpm for 5 minutes, discard sup, if not pelleted re-spin
16. Gently add washing medium, 5 mL, spin 2-3 minutes at 1000-1500 rpm
17. R/S in 500uL washing medium
18. Transfer samples to eppendorf tubes for plating/freezing
19. Adjust to make sure all volumes of pellets are equal
20. Aliquot to tubes for different plates/freezing

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