Mariya Nov 2020

**GUT prep**

1. Take out Intestines and remove fat with fingers. Remove PPs if doing LP. Fillet open in dissection board.
2. Wash in 2 sequential dishes of cold HBSS
3. Cut into 50mL falcon with ~5mL cold HBSS. Keep on ice until done with all.
4. Shake or briefly (1sec) vortex tube. Remove soup with strainer, put back into same tube and add **20mL DTT mix** to intestine. **15min 37C 225-ish rpm**.
5. 2min shake.
	1. For **IEL**: Transfer **supernatant** to 50mL falcon with **10mL cRPMI-10%FCS**. **Spin** down
	2. Remove supernatant. Proceed to **Percoll**. (**Resuspend pellet** in 7mL 40% Percoll, **filter** through soft mesh to 15mL Falcon and underlay with 2mL 80% Percoll)
	3. For **LPL**: Keep **tissue**. Proceed to **#6**
6. Put Tissue back to 50mL Falcon. Add **10mL EDTA-only mix**. **10min 37C 200rpm**.
7. 2min shake.
8. Discard supernatant. **Wash** tissue with **cold HBSS**. (pour over metal strainer)
9. Transfer tissue to plate lid and **cut** into sauce using curved scissors.
10. Transfer minced tissue into new 50mL Falcon tube with **6mL Digest mix**. **30min 37C 200-ish rpm**.
11. 1min shake.
12. Filter contents via mesh into LPL 15mL Falcon with 5mL cRPMI-10%FCS. Spin down. 5min 4C 1600rpm
13. Discard supernatant. Proceed to Percoll. (**Resuspend pellet** in 7mL 40% Percoll, **filter** through soft mesh to 15mL Falcon and underlay with 2mL 80% Percoll)

**Percoll**:

Resuspend and filter (if not already) pellet in **7mL 40% Percoll**. Underlay with **2mL 80% Percoll** using glass pipette.

Spin: **22C. 22min. 2,2000rpm. BRAKE OFF** (Deceleration=0, but max acceleration). Takes about **40min** to spin and stop.

Very top layer is fat. The layer between the 40% and 80% Percoll is where your cells are! Aspirate the top layers/as much of the 40% Percoll as you can without disrupting the cell layer. If red blood cell pellet on bottom, can stick p200 pipette straight down and suck it out. (otherwise can just collect the layer of cells with a transfer pipette). Fill tube to almost top with fresh cRPMI, mix to disrupt the layer, spin down, and collect cell pellet at bottom and proceed to staining.