## Tissue processing

1. Add 5 ml of serum free RPMI media into a C tube (purple top)
2. Use sterile forceps and sterile scalpel to cut the tumor (<1g) into 1 mm pieces on ice.
3. Transfer the samples into the C tube, add the three enzymes (H, R, A) to the media.
4. Place the C tube upside down on the octo dissociator and be sure the cap is clicked into place. Place the heating jacket over the C tube.
5. Use the 37C\_h\_TDK\_3 program and let sit for half hour.
6. After the dissociation is over, place a 100-micron filter on a 50-ml falcon tube and add ~5 mL of RPMI + FCS to wet the filter. Pour the sample over the filter. **ON ICE**
7. Use the back of a syringe to push the cells through the filter.
8. Periodically add RPMI + FCS to the sample to pass the rest of the cells though the filter and to inactivate the enzymes.
9. Spin cells down at 1500 rpm for 4 minutes at 4OC, discard supernatant and resuspend in 2mL of RBC lysis buffer (ACK lysing buffer).
10. Incubate at room temperature for 3 minutes, and quench with 8mL RPMI + FCS.
11. Run sample through 70-micron filter.
    1. FOR BARCODING: Spin cells down at 1500 rpm for 4 minutes at 4OC

Resuspend in 1-2mL RPMI + FCS (or PBS), bring to Rachel for sorting

* 1. FOR FREEZING: Count cells and spin cells down at 1500 rpm for 4-5 minutes at 4OC.

Add 10% DMSO solution (9 ml FCS 1 ml DMSO).

Freeze into number of vials depending on cell concentration desired (1 ml and ~5 million cells per cryo tube).

*Formula for counting on hemocytometer:*

(Cells counted / 4 ) X 10,000 X Dilution factor = cells / mL

For total # of cells, multiply by total volume.

## Ascites processing

1. Spin cells down at 1500 rpm for 4 minutes at 4OC, discard supernatant and resuspend in 2mL of RBC lysis buffer (ACK lysing buffer).
2. Incubate at room temperature for 3 minutes, and quench with 8mL RPMI + FCS.
3. Run sample through 70-micron filter. Rinse filter with 5ml RPMI + FCS.
4. Count cells