Dissociate tumor tissue

1. Mince tumor into very tiny pieces (approx. 1mm) and place into dissociation buffer.
2. Dissociate the samples in 5X Dissociation buffer for 4 hours or 1X for 17-20 hrs at 37 degrees Celsius on a rotator.
3. Spin down the cells at 1000rpm for 5 min and discard the supernatant.
4. Wash the cells 3 times by adding 30mls of PBS, re-suspending the cells, and then spin down the cells at 1000RPM for 5 min, discarding the supernatant.
5. Resuspend pellet in 4 ml 10% FBS, 1X anti-anti media.
6. Lyse RBC by adding 10 ml of RBC lysis buffer.
7. Incubate for 15 min at 37 degree Celsius water bath.
8. Add 20 ml of PBS, centrifuge at 1000rpm for 5 min and aspirate supernatant.
9. Add warm trypsin (same volume as pellet) to tube.
10. Pipette up and down 15-20 times.
11. Incubate for 10 min in 37 degree Celsius water bath.
12. Pipette up and down again.
13. Inactivate trypsin with FBS medium (4x the volume of Trypsin used).
14. Spin down cells then wash with 20 ml PBS.
15. Add 1 ml of 10% FBS, 1X anti-anti and 1 ml of DNAse Solution (1mg/ml in PBS) . Incubate for 5 min in 37^0C water bath.
16. Add PBS (20 ml) and centrifuge at 1000 RPM for 5 min.
17. Resuspend the cells in appropriate volume of FBS free media and pipette the solution up and down before transferring it through a 40um filter on a new 50ml conical tube.
18. Count cells.
19. Spin down cells then aspirate supernatant. Freeze cells @ $5x10^6$ to 10^7 per vial. Resuspend cells in freezing buffer as indicated below.

Freeze down cells by resuspending in freezing buffer (containing 20% FBS and 10% DMSO and 70% DMEM media with 10% FBS). Place in -80 degree freezer then transfer to liquid nitrogen tank after 24 hours.