Setting up primary culture from mouse tumor tissues

Prior to conducting necropsy:

- Autoclave tools required for handling and cutting tumor tissues into smaller pieces (e.g., tweezers, pincers, scissors)

Day of necropsy (in tissue culture hood):

- Plan how many tumors to harvest, and aliquot PBS into 50 mL conical tube and place on ice.

Day of necropsy (in the vivarium):

- Conduct necropsy and harvest desired tissues into 50mL conical tube submerged in PBS. One should harvest enough tissues for (i) histology, (ii) material to freeze for downstream analysis and (iii) set up primary culture.

In the tissue culture hood:

1. In a six well plate, add 3 mL 70% EtOH to two wells, and 3 mL PBS to one well
2. Pick the tumor tissue submerged in ice-cold PBS from the 50 mL Falcon using sterile pincers and place into the well with 70% ethanol.
3. Gently swirly the 6 well plate to ensure it covers the entire tumor tissue.
4. Pick up the tumor from the first well and place into second well of 70% ethanol, repeat step 3.
5. Pick up tumor tissue and place in well with PBS, gently swirl the 6-well plate to ensure the entire tumor tissue is rinsed.
6. Pick up tumor tissue and place into an empty well.
7. Proceed to mince tissue with a blade or scissors, for approximately two minutes. The finer the tissues are, the better the chances of getting cells to adhere and grow as primary cells.
8. Once mincing is complete, add 200U of collagenase to each well and place in the incubator for approximately 4 hours. It may be possible to lower the collagenase amount and left the tissue overnight, although it is unclear how well the tissues survive without media.
9. Once collagenase treatment is over, resuspend the tissues using a 25mL stripette polystyrene serological pipets gently. Chunks of small tissues may clog or forcefully enter the opening of the pipette, so lower the suction and dispense speeds. Resuspend 10-15 times.
10. Once complete, put the resuspended solution of minced tissue through a cell strainer into a fresh 50 mL conical tube.
11. Centrifuge at 1,500 rpm for 5 mins.
12. Resuspend the cell suspension in 3mL complete media with 2X antibiotic, and plate into one well of a 6-well plate. Depending on the pellet size, you may choose to resuspend into two wells, instead of one.
13. Following day, gently aspirate media and replace with fresh media with 2X antibiotic.
14. Repeat step 13 for three to four days.