1.0 Purpose
The purpose of SOP 2.10 is to provide details on how to dissociate primary tumor tissue to get CAFs.

2.0 Scope
SOP 2.10 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Description</th>
<th>Storage Location</th>
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<tbody>
<tr>
<td>1.0</td>
<td>Collagenase</td>
<td>Enzymatic Dissociation</td>
<td>Freezer #2</td>
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<td>2.0</td>
<td>Medium 199</td>
<td>Media</td>
<td>Walk in 4C</td>
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4.0 Procedures
4.1 Obtain primary tumor tissue or freshly harvested xenograft.
4.2 Using two scalpels mince tissue into very fine pieces.
4.3 Transfer minced tissue into a 50 mL conical tube, add prepared tumor Collagenase in 1:1 ratio to the tissue (equal amounts of tissue to Collagenase). Ensure that the tissue is well suspended in the enzyme mixture.
4.4 Gently dissociate the minced tissue on a rotary shaker (200-22 RPM) until all larger tissue fragments are digested, for 30 – 60 min. Longer digestion times may be required for large tough fibrous tissue or shorter digestion times for softer tissue.
4.5 After dissociation: Obtain tube from shaker, and spray outside with ethanol. Shake the tube and centrifuge at 40 x g for 2 minutes and 20 degrees C. Then pipette the supernatant carefully (careful not to disturb the pellet at the bottom which contains the organoids) into a new 50 mL conical tubes and label it as FIBROBLASTS.
4.6 Spin down all fibroblast tubes at 250 x g for 5 minutes and 4 degrees C.
4.7 You should see a reddish-white pellet at the bottom, which are the fibroblasts. Remove and discard the supernatant carefully (the pellet is loose), and rinse the pellet with HBSS (red or white) and centrifuge the sample between 1000-1200 rpm for 5 minutes.
4.8 After centrifugation, remove the supernatant and re-suspend the pellet in Fibroblast media. Plate the fibroblasts in fibroblast media in a suitable cell culture flask and after 1.5 hours, change the media.
4.9 Expand CAFs by in vitro passage for two or three population doublings in Fibroblast media in an incubator at 37 °C and in 5% CO2.
4.10 Check the purity of isolated cells using appropriate markers through flow-cytometry and immunocytochemistry.

5.0 Applicable References

6.0 Change Description