Mammary gland tumor digestion protocol

Materials:
Dissecting tools Autoclaved
Cotton swabs Autoclaved
Sterile scalpels disposable
F12 media (Invitrogen) 4°C
DMEM/F12 (Invitrogen) 4°C
Fetal Bovine Serum (FBS) JHR biosciences -20°C
Gentamycin 10 mg/ml (Invitrogen) 4°C
100X Antibiotic-antimycotic (Invitrogen) -20°C
Collagenase type 3 (Worthington, 4182) 4°C
HEPES buffer solution 1M (Invitrogen) 4°C
HBSS (Invitrogen) 4°C
Miscellaneous disposables (50 ml tubes, pipettes, pipette tips etc.)

Equipment:
Reserve hood.
Reserve 37°C incubator/125 RPM.

Procedures:
1. Prepare wash buffer:
   F12 media + Gentamycin (50 µg/ml) + 5% Fetal Bovine Serum; (500 ml F12+2.5 ml Gentamycin+25 ml FBS)
   Weigh 50ml tubes + 25ml wash buffer and place on ice.
2. Take out tumors, place dissected tumor tissues in 50ml tubes containing wash buffer at 4°C
3. Weigh tubes containing wash buffer + harvested tissue. Subtract original weight to determine the weight of the tumor tissues.
4. Using sterile technique, mince tissue into very small pieces (as small as possible) with a scalpel.
5. Make digestion medium (Need to use 10 ml/gm tissue)
   DMEM:F12
   100 X Anti-Fungi Antibody (1 X)
   Gentamicin (100 µg/ml)
   Collagenase type 3 (225 U/ml)
6. Using filter bottle. Place minced tissue in digestion medium and shake at 37°C for approximately 2.5 hr at 125rpm.
   Small volumes can be dissociated in a 50ml tube placed at a 45° angle.
7. Pipet up and down with 25 ml pipette every 30 min.
8. At the end of incubation, cells were filtered through 40-ul nylon mesh by gravity.
   All subsequent spins are done in 35 ml total volume
   Sup: Spin sup at 800 rpm 3 min in 35ml → Pellet #1
   Sup: Spin at 1500 rpm 10 min (Discard Sup)→ Pellet #2
Combine pellet #1 and #2 and wash at 800 rpm for 3 min in 35 ml wash buffer until buffer is clear (~4 times). Then washed once with HBSS+ 800 rpm for 3 min. Cells are ready for labeling. (HBSS+: HBSS with 2% FBS and 10 mM HEPES Buffer)
Freezing down pieces of tumor for later transplantation

TO FREEZE:
1. Excise tumor to be frozen
2. Mince into approx. 1-mm-sized pieces (the size that you would transplant).
3. Place tissue in medium containing 10% DMSO and 90% freezing medium (5% FBS)
4. Slowly freeze aliquots, lowering temperature at the rate of 1°C/min. (Put the cryotubes into 15 ml foam rack in -20°C, O/N)
5. For short-term storage, freeze to -80°C and for longer-term storage, store in liquid nitrogen

TO THAW:
1. Quickly melt frozen aliquots by submersing in a 37°C water bath.
2. Add extra medium to dilute concentration of DMSO. Centrifuge at 500 rpm for 3 mins to get rid of the medium with DMSO.
3. Transplant pieces into the cleared fat pads of 3-week-old recipient mice.

Freezing medium for p53 null mammary gland tumors

<table>
<thead>
<tr>
<th>(stock)</th>
<th>Final [con]; dilution</th>
<th>100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin (10 mg/ml)</td>
<td>5 µg/ml; 2,000X</td>
<td>50 µl</td>
</tr>
<tr>
<td>Insulin (1 mg/ml)</td>
<td>10 µg/ml; 100X</td>
<td>1 ml</td>
</tr>
<tr>
<td>EGF (10 µg/ml)</td>
<td>5 ng/ml; 2,000X</td>
<td>50 µl</td>
</tr>
<tr>
<td>AA (100X)</td>
<td>1X; 100X</td>
<td>1 ml</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>5%</td>
<td>5 ml</td>
</tr>
<tr>
<td>DMEM/F12</td>
<td></td>
<td>92.9 ml</td>
</tr>
</tbody>
</table>