Human Breast Tissues Dissociation

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Human Cell Atlas Method Development Community

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Prepare 4mg/mL collagenase solution before start and place on 37C shaker to dissolve.

**Materials**

- **Collagenase Type I powder**: Thermo Fisher Scientific Catalog #17100017
- **DMEM and Hams F-12 50/50**: Thermo Fisher Scientific Catalog #MT10090CV
- **Deoxyribonuclease I**: Sigma Aldrich Catalog #D4263-5VL
- **Dulbecco Phosphate-Buffered Salt Solution 1X**: Fisher Scientific Catalog #MT21031CV
- **0.05% Trypsin**: Fisher Scientific Catalog #MT25051CI
- **Antibiotic-Antimycotic**: Thermo Fisher Scientific Catalog #15240062
- **Fetal Bovine Serum**: Omega Scientific Catalog #FB-12

**Prepare materials**

1. **Collagenase Solution**
   - 100mg/mL Collagenase Type I in DMEM/Ham's F12

2. **Media**
   - DMEM/Ham's F12
   - 10% FBS
   - 1% Antibiotic
   - DMEM/Ham's F12
   - 5% FBS
   - 1% Antibiotic

3. **DNase**
   - 1mg/mL DNase in PBS

**Initial Tissue Preparation**

2. Transfer tissues to 150cm plate.
   - Wash with large quantities of PBS to remove blood and old storage media.
   - Amount of PBS varies depending on volume of tissues washed.
   - Aspirate off PBS.
   - Repeat PBS wash twice, for a total of three washes.
   - Collect small pieces of tissues for FFPE or OCT Blocks.

**Mechanical Dissociation of Tissue**

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Use two number 10 scalp to chop tissue. Chop tissue until epithelial tissues are about 2-3mm in size.
-Depending on stating tissues sample, removal of excessive adipose tissues will help in the chopping and digesting process.

**Enzymatic Dissociation of Tissue**

Transfer about 20mL in volume of chopped tissue to each 50mL conical tube. Add 20mL of DMEM with 5% FBS with 4mg/mL Collagenase Type I. Place on 37°C shaker at about 180-200rpm. Leave on heated shake for 12-16 hours.

**Organoid Collection**

Centrifuge tubes at 400gx5min. Aspirate to remove supernatant. Wash with 50mL of PBS. Centrifuge at 400gx5min to remove PBS.

**Single Cell Preparation**

Add 2mL of 0.05% Trypsin to pellet of organoids. Place at 37°C for 6 minutes. Take tube out every 2 minutes to pipette up and down with P1000, for a total of three times.

Add 10mL of DMEM with 10% FBS to tube. Centrifuge at 400gx5min. Aspirate off supernatant. Resuspend pellet in 1mL of DMEM with 10% FBS.
Add 100uL of DNase, incubate for 5min.
Add 10mL of DMEM with 10% FBS. Centrifuge at 400gx5min. Remove supernatant. Resuspend in 10mL of DMEM with 10% FBS. Count cells. Centrifuge at 400gx5min. Bring back up to appropriate concentration for FACS staining.

**Single Cell FACS Preparation**

Prepare appropriate tubes for FACS staining. To all tube add the following antibodies:
-PE-CD49f
-APC-EpCAM
-DAPI-CD45/CD31
-Use antibody concentration suggested by manufacture.

Stain for 20min at 4C. Take out and wash cells with DMEM. Pass through FACS tube filter. Before sort begins add SytoxGreen to tube.
Sort for all live cells, anything that is SytoxGreen negative.