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Title: Optimization of Breast Biopsy and Mastectomy Sample Collection Procedures for Biobanking, Personalized Medicine, and Research Applications

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Author Questionnaire

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☑ Correct

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Current Protocol Length

Number of Steps: 17
Number of Shots: 33

Introduction

AUTHORS: Please note that only 5 statements may be presented

1.1. **Rodney B. Dofitas:** We aim to improve breast cancer sample collection for biobanking, enabling better personalized treatment through high-quality RNA sequencing and organoid culture from Filipino patient tissues.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.3*

What are the most recent developments in your field of research?

1.2. **Ma. Easter Joy V. Sajo:** Recent advances include using patient-derived organoids and RNA sequencing to predict treatment response in breast cancer, particularly for cases with lymphovascular invasion linked to drug resistance.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

What technologies are currently used to advance research in your field?

1.3. **Ma. Easter Joy V. Sajo:** We use ultrasound-guided biopsies, RNA sequencing, tumor percentage estimation, and 3D organoid cultures to analyze breast cancer tissues precisely.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.7*

What are the current experimental challenges?

1.4. **Ma. Easter Joy V. Sajo:** Main challenges include obtaining high-quality RNA from small biopsy samples, quality tissues for organoid cultivation, and ensuring consistent cold storage and transport.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Rodney B. Dofitas:** We'll explore gene expression further in larger Filipino cohorts and use organoids to test therapies for thyroid, ovarian, pancreatic, and rare cancers like phyllodes tumors.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been registered and approved by the Research Ethics Board at the University of the Philippines Manila (UPMREB 2020-822-01)

Protocol

2. Breast Core Needle Biopsy and Tissue Processing for RNA and Histopathology

Demonstrator: Rodney B. Dofitas

2.1. To begin, set up a 14-gauge core needle biopsy gun under ultrasound guidance in an outpatient clinic, operating room, or other aseptic environment **[1]**. For each core, collect a breast sample approximately 2 centimeters in length from the center of the tumor and 1 centimeter away for adjacent normal tissue **[2]**. Collect 2 types of specimens, normal tissue and tumor tissue, from each patient **[3].**

2.1.1. WIDE: Talent holding a 14-gauge core needle in a clinical room.

2.1.2. Talent obtaining core samples, showing targeted locations of tumor center and adjacent tissue.

2.1.3. Shot of 2 samples per participant.

2.2. Hand each sample to the research assistant for placement into a petri dish containing PBS solution **[1-TXT]**.

2.2.1. Talent handing samples to research assistant who places them into petri dishes with PBS. **TXT: Obtain up to 9 breast tissue**

2.3. Sort the nine tumor tissue samples into 6 cores for RNA analysis and 3 cores for biobanking **[1]**. Cut each core lengthwise or crosswise using a sterile blade **[2-TXT]**.

2.3.1. Talent sorting tumor cores into RNA and biobanking groups in labeled petri dishes.

2.3.2. Talent cutting the tissue with a sterile blade. **TXT: Send half the tissue core for RNA analysis and the other half for histopathological analysis**

2.4. Place the three normal tissue cores in petri dishes **[1].** Cut the cores in half and allocate them for analysis **[2]**.

2.4.1. Talent placing normal tissue in petri dishes.

2.4.2. Talent cutting normal tissue cores and placing halves into study and pathology containers.

2.5. Distribute samples among the tubes according to priority **[1]**. Place half of the cores into designated 2 milliliter cryovials with 1.25 milliliters of RNA stabilization solution **[2]**.

2.5.1. Talent sorting samples for tube distribution.

2.5.2. Talent placing cores into cryovials with RNA stabilization solution.

2.6. Fix the other halves in specimen vials containing 10 percent neutral buffered formalin **[1]**. Complete and submit the surgical pathology forms with the fixed samples to the pathology department **[2]**.

2.6.1. Talent placing matching core halves into formalin vials.

2.6.2. Talent filling out pathology forms and submitting vials.

3. Breast Tumor and Normal Tissue Handling for Biobanking, RNA Sequencing, and Transport

3.1. After mastectomy, placed the sample into a clean specimen bag after documentation and transport immediately to the Department of Laboratories, Pathology without formalin to reduce cold ischemic time **[1]**.

3.1.1. Talent placing transporting specimen from the Operating Room Complex to Pathology for grossing.

3.2. Obtain a minimum 1 cubic centimeter tumor tissue block during grossing and cut into two halves **[1-TXT]**.

3.2.1. Talent obtaining and slicing a 1 cubic centimeter tumor block. **TXT: Use half for biobanking and the other half for pathology; Dimensions: 1 cm x 0.5 cm x 0.5 cm**

3.3. Evenly trisect the fresh half block for RNA sequencing, biobanking, and organoid culture **[1]**.

3.3.1. Talent trisecting the half block and placing parts into corresponding containers.

3.4. Immediately place tissue for RNA sequencing in 5-milliliter pre-chilled and pre-labeled cryovials pre-filled with 2.5-milliliters of RNA stabilization solution **[1]**. For biobanking, use 5-milliliter cryovials with the same solution **[2]**.

3.4.1. Talent placing RNA sequencing sample in 5-milliliter cryovials.

3.4.2. Talent placing biobanking sample in 5-milliliter cryovials.

3.5. For organoid culture, use 15 milliliter conical vials pre-filled with 5 milliliters tissue storage solution and 0.01 milliliters Primocin **[1]**. Pool one-third of all tumor blocks into one conical tube **[2]**.

3.5.1. Talent placing organoid culture sample in 15 milliliter conical vials.

3.5.2. Talent pooling tissue blocks into one tube.

3.6. Obtain a minimum 1 cubic centimeter normal tissue block **[1]** and slice it in half for RNA biobanking and routine histopathology **[2]**.

3.6.1. Shot of a normal tissue block.

3.6.2. Talent slicing normal tissue block and distributing samples. **TXT: Dimensions: 1 cm x 1 cm x 0.5 cm**

3.7. Transfer the tissue block into 5-milliliter pre-chilled and pre-labeled cryovials pre-filled with 2.5-milliliters of RNA stabilization solution for RNA sequencing and biobanking **[1]**. Then seal all cryovials with parafilm **[2]**.

3.7.1. Talent placing samples in cryovials.

3.8. Store at 4 degrees Celsius for 24 hours then transfer to minus 20 degrees Celsius for 2 to 4 hours and finally store at minus 80 degrees Celsius **[1]**.

3.8.1. Talent storing vials at successive temperatures. **AUTHORS: Perform any 1 storage for demonstration**

3.9. Seal the cryovial caps with parafilm **[1].** Place sealed cryovials in test tube rack and cover with absorbent and bubble wrap **[2]**.

3.9.1. Talent wrapping the vial caps with parafilm.

3.9.2. Talent placing tubes in rack and wrapping them.

3.10. Place the wrapped rack in a resealable bag with a temperature logger **[1-TXT].** Position gel packs on the bottom of a polystyrene box **[2]** then place the bag over the gel packs **[3]**. Put another layer of gel packs on top of the bag. Make sure the tubes are sandwiched between gel packs to ensure the specimens will be kept cold during transport **[4].**

3.10.1. Talent packing tubes with logger in a resealable bag.**TXT: Ensure temperature logger is logging temperature and tubes are intact and upright**

3.10.2. Talent placing gel packs at the bottom of a polystyrene box.

3.10.3. Shot of the bag being placed over gel packs in a polystyrene box.

3.10.4. Talent placing gel packs over the bag.

3.11. Put the closed polystyrene box into a cardboard shipping box and include the manifest in a ziplock bag **[1]**. Seal and label the box for shipping **[2-TXT]**. Before courier handoff, note the number of vials and the departure time from the biobank **[3]**.

3.11.1. Talent placing box inside shipping box with manifest.

3.11.2. Talent sealing and labeling the box. **TXT: Inform recipient about the collection schedule and immediately courier the samples**

3.11.3. Talent documenting number of vials and time courier leaves.

Results

4. Results

4.1. 2 samples with varying RIN *(Rin)* values and concentration failed in the library preparation step during sequencing and were excluded in the final analysis **[1].** All samples had great sequencing quality scores, where more than 90% of base calls had an accuracy of at least 99.9% **[2].**

4.1.1. LAB MEDIA: Table 1 **TXT: RIN: RNA Integrity Number**
Video Editor: Please highlight the 6th and 11th row , "RIN Value" and "Reasons for Failing QC" columns

4.1.2. LAB MEDIA: Table 2 *Video Editor: Please highlight the columns for "%>Q30"*

4.2. Bright-field imaging showed successful organoid formation from Stage III breast cancer mastectomy tissues after neoadjuvant therapy, with abundant spheroid-like structures visible at low **[1]** and high magnification **[2].**

4.2.1. LAB MEDIA: Figure 6A.

4.2.2. LAB MEDIA: Figure 6B.

Pronunciation Guide:

1. **Biobanking**
   Pronunciation link: (no single dictionary entry, but "biobank" gives root)
   https://www.sciencedirect.com/topics/medicine-and-dentistry/biobanking
   IPA: /ˌbaɪ·oʊˈbæŋ·kɪŋ/
   Phonetic Spelling: bye-oh-BANK-ing
2. **Biobank**
   Pronunciation link: https://en.wikipedia.org/wiki/Biobank (as reference)
   IPA: /ˌbaɪ·oʊˈbæŋk/
   Phonetic Spelling: bye-oh-BANK
3. **Personalized**
   Pronunciation link: Merriam-Webster "personalized"
   IPA: /ˈpɜr·sə·nəˌlaɪzd/
   Phonetic Spelling: PUR-suh-nuh-lyzd
4. **Organoid**
   Pronunciation link: (less common; some sites)
   https://www.howtopronounce.com/organoid
   IPA: /ˈɔr·gəˌnɔɪd/
   Phonetic Spelling: OR-guh-noyd
5. **Ultrasound**
   Pronunciation link: Merriam-Webster "ultrasound"
   IPA: /ˈʌl·trəˌsaʊnd/
   Phonetic Spelling: UL-truh-sownd
6. **Lymphovascular**
   Pronunciation link: (may not exist in standard dictionary)
   IPA: /ˌlɪmf·oʊˈvæs·kjʊ·lər/
   Phonetic Spelling: lim-foh-VAS-kyuh-lur
7. **Sequencing**
   Pronunciation link: Merriam-Webster "sequencing"
   IPA: /ˈsi·kwən·sɪŋ/
   Phonetic Spelling: SEEK-wuhn-sing
8. **Tumor / Tumour**
   Pronunciation link: Merriam-Webster "tumor"
   IPA: /ˈtu·mər/
   Phonetic Spelling: TOO-mur
9. **Neoadjuvant**
   Pronunciation link: NCI "neoadjuvant therapy"
   https://www.cancer.gov/publications/dictionaries/cancer-terms/def/neoadjuvant-therapy
   IPA: /ˌni·oʊˈæd·ʒu·vənt/
   Phonetic Spelling: nee-oh-AD-joo-vuhnt
10. **Mastectomy**
    Pronunciation link: Merriam-Webster "mastectomy"
    IPA: /mæsˈtɛk·tə·mi/
    Phonetic Spelling: mas-TEK-tuh-mee
11. **Cryovial / Cryovials**
    Pronunciation link: (less common)

IPA: /ˌkraɪ·oʊˈvaɪ·əl/
Phonetic Spelling: cry-oh-VY-uhl

12. **Histopathology**
Pronunciation link: Merriam-Webster "histopathology"
IPA: /ˌhɪs·toʊ·pəˈθɒl·ə·dʒi/
Phonetic Spelling: his-toh-puh-THOL-uh-jee
13. **Ischemic / Ischemia**
Pronunciation link: Merriam-Webster "ischemia"
IPA: /ɪˈski·mi·ə/
Phonetic Spelling: is-KEE-mee-uh
14. **Trisect / Trisecting**
Pronunciation link: Merriam-Webster "trisect"
IPA: /traɪˈsɛkt/
Phonetic Spelling: try-SEKT
15. **Neoadjuvant / Neoadjuvant Therapy**
(Repeated, but useful in context)
Pronunciation link given above
IPA: /ˌni·oʊˈæd·ʒu·vənt/
Phonetic Spelling: nee-oh-AD-joo-vuhnt
16. **RIN (RNA Integrity Number)**

- ***RNA* — /ˌɑr·ɛnˈeɪ/ (ar-en-ay)**
- ***Integrity* — /ɪnˈtɛg·rə·ti/ (in-TEG-ruh-tee)**
- ***Number* — /ˈnʌm·bər/ (NUM-bur)**

17. **Bright-field**
Pronunciation link: (compound word)
IPA: /ˈbraɪt·fiːld/
Phonetic Spelling: BRYTE-feeld
18. **Spheroid**
Pronunciation link: Merriam-Webster "spheroid"
IPA: /ˈsfɪr·ɔɪd/
Phonetic Spelling: SFIR-oid