Lung PCA Collection Guidelines and Protocols
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- **Prospective Cross-Sectional Adenocarcinoma Cohort – <u>FFPE Only</u> Tissue Requirements and Processing Algorithms**

PROSPECTIVE: *Cross-Sectional LUAD*

Prospective cohorts include participants that are recruited from on-going or new screening cohorts and those seen for suspicion of lung cancer or from other pulmonary clinics will be consented for use of tissue for this study and for future studies including those that involve industry/pharma support. For cases selected it is anticipated that each case will have at minimum, biospecimens from an independent, purely pre-invasive lesion and a pre-invasive (lepidic) component of a part-solid invasive adenocarcinoma along with invasive tumor and access to a genomic control for each case that is enrolled. It is also anticipated that prospective biospecimen tissue samples include frozen tissue from the part-solid lesions with pre-invasive and invasive tumor components. However, in valuable cases if the frozen tissue does not represent both pre-invasive and invasive components, submission of the case with only FFPE derived sections can be considered. For such cases instructions are provided below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the M.D. Anderson Cancer Center (MDACC) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCL, UCLA, VUMC
- **Lung PCA Repository to ship cases:** MDACC - *see details in shipping guidelines for samples going to MDACC*

- **<u>Collection Timepoints:</u>**
  - T0-Resection cases of LUAD where AAH and/or AIS is found in association with the invasive tumor and at a site remote from the tumor

- **<u>Required biospecimen to be collected</u>**
  - T0:
    - Resection- Frozen tissue blocks or sections *(see associated sectioning guides A.1.1)*
      - FFPE Tumor with non-invasive lepidic component (Lesion and Normal area) **AND**
      - FFPE Atypical Adenomatous Hyperplasia/Adenocarcinoma-in-situ remote from tumor
    - Genomic DNA
      - If tumor/pre-malignant lesion is formalin fixed, normal FFPE lymph node sample (or less preferable, normal lung) used for:
        - Preferred: Isolated DNA from FFPE (*see section A.2.2 FFPE)*
        - Alternative: FFPE tissue Scrolls (*see section A.1)*

- **<u>Required metadata to be collected</u>:** *see* the PCA portal/data standards for details: (<u>https://lci-test.whiterivercomputing.com/portal/data-standards</u>)
  - <u>Clinical:</u>
    - **Baseline**
    - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
  - <u>Biospecimen:</u>
    - **Specimen**
    - **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for Multiplex IF

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUAD Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Positively charged glass slides (i.e. Fisher 12-550-109)

SECTIONING FFPE BLOCKS for slides

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut sections based on specimen abundance* - note the section thicknesses and order
5. Sections should be prepared on positively charged (+) slides without baking
6. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
7. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
3. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut 5-20 uM scrolls and store at -80^{o}C until shipment or proceed to extraction
5. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
6. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FFPE LUAD RESECTIONS FROM THE PROSPECTIVE CROSS-SECTIONAL LUAD-PML COHORTS (all tissue)

NOTE: *Adequacy of premalignant and invasive adenocarcinoma associated lesions is determined by the greatest dimension of the lesion where those in which this measurement is >/= 0.5 cm are classified as adequate and those where this measurement is 0.1 – 0.5 cm are classified as borderline. When non-invasive lepidic premalignant tissue contiguous with invasive adenocarcinoma in part-solid nodules is discontinuous, the sum of the greatest dimensions of the areas of lepidic tissue is used to determine the appropriate adequacy classification.*

Adequate lesion or normal lung (>/= 0.5 cm in greatest dimension): 15 fresh sections (~96 um)

- *Section 1 – 4µM Section for H&E (pre-existing acceptable/preferred)*
- *Section 2-4 – 3 x 10µM sections for DNA*
- *Section 5 – 4µM Section for H&E (newly cut)*
- *Section 6-10 – 5 x 4µM sections for in situ multiplex IF*
- *Section 11 – 4µM Section for H&E (newly cut)*
- *Section 12-14 – 3 x 10µM sections for RNA*
- *Section 15 – 4µM Section for H&E (newly cut)*

Borderline lesion (0.1 - 0.5 cm in greatest dimension): 21 fresh sections (~156 um)

- *Section 1– 4µM Section for H&E (pre-existing acceptable/preferred)*
- *Section 2-7 – 6 x 10µM sections for DNA*
- *Section 8 – 4µM Section for H&E (newly cut)*
- *Section 9-13 – 5 x 4µM sections for in situ multiplex IF*
- *Section 14 – 4µM Section for H&E (newly cut)*
- *Section 15-20 – 6 x 10µM sections for RNA*
- *Section 21 - 4µM Section for H&E (newly cut)*

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Prospective Cross-Sectional Adenocarcinoma Cohort – OCT & FFPE Tissue Requirements and Processing Algorithms

PROSPECTIVE COHORTS: *Cross-Sectional LUAD*

Prospective cohorts include participants that are recruited from on-going or new screening cohorts and those seen for suspicion of lung cancer or from other pulmonary clinics will be consented for use of tissue for this study and for future studies including those that involve industry/pharma support. For cases selected it is anticipated that each case will have at minimum, biospecimens from an independent, purely pre-invasive lesion and a pre-invasive (lepidic) component of a part-solid invasive adenocarcinoma along with invasive tumor and access to a genomic control for each case that is enrolled. It is also anticipated that prospective biospecimen tissue samples include frozen tissue from the part-solid lesions with pre-invasive and invasive tumor components (in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an adjacent FFPE lepidic and invasive tumor biospecimen. Frozen normal lung tissue will also be collected. Instructions for selecting and preparing these cases are provided below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the M.D. Anderson Cancer Center (MDACC) Core Repository are provided in the shipping guidelines.

1. Cohort: Prospective Cross-Sectional LUAD

- **Collecting Sites:** CU, RPCI, UCL, UCLA, VUMC
- **Lung PCA Repository to ship cases:** MDACC - *see details in shipping guidelines for samples going to MDACC*

- **Collection Timepoints:**
  - T0-Resection cases of LUAD where AAH and/or AIS is found in association with the invasive tumor and at a site remote from the tumor

- **Required biospecimen to be collected**
  - T0:
    - Resection- Frozen tissue blocks or sections *(see associated sectioning guides A.1.1)*
      - Frozen Tumor with non-invasive lepidic component (Lesion and Normal area)
      - FFPE Tumor with non-invasive lepidic component (Lesion and Normal area) **AND**
      - FFPE Atypical Adenomatous Hyperplasia/Adenocarcinoma-in-situ remote from tumor **or**
      - Frozen remote (in invasive tumor block or additional separate block) if collected
    - Genomic DNA
      - If tumor/pre-malignant lesion is frozen: (*see section A.2.1)*
        - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat
        - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) or tissue scrolls (*see sections A.1 for sectioning guidelines*)
      - If tumor/pre-malignant lesion is formalin fixed, normal FFPE lymph node sample (or less preferable, normal lung) used for:
        - Preferred: Isolated DNA from FFPE (*see section A.2.2 FFPE)*
        - Alternative: FFPE tissue Scrolls (*see section A.1)*

- **Required metadata to be collected:** *see* the PCA portal/data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - Baseline
    - Off schedule data is *optional but may be populated any follow up or additional unscheduled visits*
  - Biospecimen:

- Specimen
- Site Pathology

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:
All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections for Bulk RNA isolation from Frozen tissue and biopsy samples.
- Sections on slides for Multiplex IF

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUAD Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Positively charged glass slides (i.e. Fisher 12-550-109)

SECTIONING OCT BLOCKS for slides

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
5. Sections should be prepared on positively charged (+) slides
6. All cut slides should be stored at -20 ^{o}C and shipped on dry ice.
7. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(single nuclei RNA and genomic DNA isolation)*

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut 5-20 uM scrolls and store at -80^{o}C until shipment or proceed to extraction. If tissue is prepared for single nuclei sequencing sections in to 1mL RNA protect and store in 1.8mL cryovial at -80^{o}C
5. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
6. All tissue scrolls should be sectioned into a 1.5 mL tube with 1 mL of RLT buffer with BME and stored at -80^{o}C until shipment
7. All samples should be shipped on dry ice as detailed in shipping guidelines.
8. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides

8. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
9. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
10. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
11. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order

12. Sections should be prepared on positively charged (+) slides without baking
13. All cut slides should be stored at -20 °C and shipped at 4°C (with cold packs)
14. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

7. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
8. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
9. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
10. Proceed to cut 5-20 uM scrolls and store at -80°C until shipment or proceed to extraction
11. All cut samples should be stored at -20 °C and shipped at 4°C (with cold packs)
12. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN LUAD RESECTIONS FOR THE PROSPECTIVE CROSS-SECTIONAL LUAD-PML COHORTS (all tissue – usually just part-solid nodule)

NOTE: When Frozen tissue is available, DNA and RNA sections will be derived from the OCT embedded frozen tissue and sections for multiplex IF will be derived from the FFPE tissue of the same lesion.

Adequacy of premalignant and invasive adenocarcinoma associated lesions is determined by the greatest dimension of the lesion where those in which this measurement is >/= 0.5 cm are classified as adequate and those where this measurement is 0.1 – 0.5 cm are classified as borderline. When non-invasive lepidic premalignant tissue contiguous with invasive adenocarcinoma in part-solid nodules is discontinuous, the sum of the greatest dimensions of the areas of lepidic tissue is used to determine the appropriate adequacy classification.

Adequate lesion or normal lung (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-4** – 3 x 10µM sections for DNA
- **Section 5** - 4µM Section for H&E (newly cut)
- **Section 6-8** - 3x10µM sections for RNA
- **Section 9** - 4µM Section for H&E (newly cut)

Adequate lesion FFPE tissue (>/= 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

Borderline lesion (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-7** – 6 x 10µM sections for DNA
- **Section 8** - 4µM Section for H&E (newly cut)
- **Section 9-14** – 6 x 10µM sections for RNA
- **Section 15** - 4µM Section for H&E (newly cut)

Borderline lesion FFPE tissue (0.1 - 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Retrospective Cross-Sectional Adenocarcinoma Cohort – FFPE Only Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Cross-Sectional LUAD*

Retrospective cohorts include participants that are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. For cases selected it is anticipated that each case will have at minimum, biospecimens from an independent, purely pre-invasive lesion and a pre-invasive (lepidic) component of a part-solid invasive adenocarcinoma along with invasive tumor and access to a genomic control for each case that is enrolled. It is also anticipated that retrospective biospecimen tissue samples may include frozen tissue from the part-solid lesions with pre-invasive and invasive tumor components. However, in valuable cases if the frozen tissue does not represent both pre-invasive and invasive components, submission of the case with only FFPE derived sections can be considered. For such cases instructions are provided below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the M.D. Anderson Cancer Center (MDACC) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCL, UCLA

- **Lung PCA Repository to ship cases:** MDACC - *see details in shipping guidelines for all samples going to MDACC*

- **Collection Timepoints:**
    - Baseline-Resection cases of LUAD where AAH and/or AIS is found in association with the invasive tumor and at a site remote from the tumor

- **Required biospecimen to be collected**
    - **Baseline**
        - Resection- Frozen tissue blocks or sections *(see associated sectioning guides A.1.1)*
            - FFPE Tumor with non-invasive lepidic component (Lesion and Normal area) **AND**
            - FFPE Atypical Adenomatous Hyperplasia/Adenocarcinoma-in-situ remote from tumor
        - Genomic DNA
            - If tumor/pre-malignant lesion is formalin fixed, normal FFPE lymph node sample (or less preferable, normal lung) used for:
                - Preferred: Isolated DNA from FFPE (*see section A.2.2 FFPE)*
                - Alternative: FFPE tissue Scrolls (*see section A.1)*

- **Required metadata to be collected:** *see* the PCA portal/data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
    - Clinical:
        - **Baseline**
        - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
    - Biospecimen:
        - **Specimen**
        - **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for Multiplex IF

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUAD Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Positively charged glass slides (i.e. Fisher 12-550-109)

SECTIONING FFPE BLOCKS for slides

15. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
16. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
17. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
18. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
19. Sections should be prepared on positively charged (+) slides without baking
20. All cut slides should be stored at -20 and shipped at 4°C (with cold packs)
21. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

13. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
14. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
15. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
16. Proceed to cut 5-20 uM scrolls and store at -80°C until shipment or proceed to extraction
17. All cut samples should be stored at -20 and shipped at 4°C (with cold packs)
18. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FFPE LUAD RESECTIONS FROM THE RETROSPECTIVE CROSS-SECTIONAL LUAD-PML COHORTS (all tissue)

NOTE: *Adequacy of premalignant and invasive adenocarcinoma associated lesions is determined by the greatest dimension of the lesion where those in which this measurement is >/= 0.5 cm are classified as adequate and those where this measurement is 0.1 – 0.5 cm are classified as borderline. When non-invasive lepidic premalignant tissue contiguous with invasive adenocarcinoma in part-solid nodules is discontinuous, the sum of the greatest dimensions of the areas of lepidic tissue is used to determine the appropriate adequacy classification.*

Adequate lesion or normal lung (>/= 0.5 cm in greatest dimension): 15 fresh sections (~96 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable/preferred)*
- ***Section 2-4*** *– 3 x 10µM sections for DNA*
- ***Section 5*** *- 4µM Section for H&E (newly cut)*
- ***Section 6-10*** *– 5 x 4µM sections for in situ multiplex IF*
- ***Section 11*** *- 4µM Section for H&E (newly cut)*
- ***Section 12-14*** *– 3 x 10µM sections for RNA*
- ***Section 15*** *- 4µM Section for H&E (newly cut)*

Borderline lesion (0.1 - 0.5 cm in greatest dimension): 21 fresh sections (~156 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable/preferred)*
- ***Section 2-7*** *– 6 x 10µM sections for DNA*
- ***Section 8*** *- 4µM Section for H&E (newly cut)*
- ***Section 9-13*** *– 5 x 4µM sections for in situ multiplex IF*
- ***Section 14*** *- 4µM Section for H&E (newly cut)*
- ***Section 15-20*** *– 6 x 10µM sections for RNA*
- ***Section 21*** *- 4µM Section for H&E (newly cut)*

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Retrospective Cross-Sectional Adenocarcinoma Cohort – <u>OCT & FFPE</u> Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Cross-Sectional LUAD*

Retrospective cohorts include participants that are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. For cases selected it is anticipated that each case will have at minimum, biospecimens from an independent, purely pre-invasive lesion and a pre-invasive (lepidic) component of a part-solid invasive adenocarcinoma along with invasive tumor and access to a genomic control for each case that is enrolled. It is also anticipated that some retrospective biospecimen tissue samples may include frozen tissue from the part-solid lesions with pre-invasive and invasive tumor components (in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an adjacent FFPE lepidic and invasive tumor biospecimen. Frozen normal lung tissue may also be available. Instructions for selecting and preparing these cases are provided below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the M.D. Anderson Cancer Center (MDACC) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCL, UCLA, VUMC

- **Lung PCA Repository to ship cases:** MDACC *see shipping guidelines for all samples going to MDACC*

- **<u>Collection Timepoints:</u>**
  - Baseline-Resection cases of LUAD where AAH and/or AIS is found in association with the invasive tumor and at a site remote from the tumor

- **<u>Required biospecimen to be collected</u>**
  - **Baseline:**
    - Resection- Frozen tissue blocks or sections *(see associated sectioning guides A.1.1)*
      - Frozen Tumor with non-invasive lepidic component (Lesion and Normal area)
      - FFPE Tumor with non-invasive lepidic component (Lesion and Normal area) **AND**
      - FFPE Atypical Adenomatous Hyperplasia/Adenocarcinoma-in-situ remote from tumor **or**
      - Frozen remote (in invasive tumor block or additional separate block) if collected
    - Genomic DNA
      - If tumor/pre-malignant lesion is frozen: (*see section A.2.1)*
        - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat
        - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) or tissue scrolls (*see sections A.1 for sectioning guidelines*)
      - If tumor/pre-malignant lesion is formalin fixed, normal FFPE lymph node sample (or less preferable, normal lung) used for:
        - Preferred: Isolated DNA from FFPE (*see section A.2.2 FFPE)*
        - Alternative: FFPE tissue Scrolls (*see section A.1)*

- **<u>Required metadata to be collected:</u>** *see* the PCA portal/data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - <u>Clinical:</u>
    - **Baseline**
    - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
  - <u>Biospecimen:</u>

- **Specimen**
- **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections for Bulk RNA isolation from Frozen tissue and biopsy samples.
- Sections on slides for Multiplex IF

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUAD Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Positively charged glass slides (i.e. Fisher 12-550-109)

SECTIONING OCT BLOCKS for slides

8. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
9. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
10. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
11. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
12. Sections should be prepared on positively charged (+) slides
13. All cut slides should be stored at -20 and shipped on dry ice.
14. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for genomic DNA isolation)*

9. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
10. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
11. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
12. Proceed to cut 5-20 uM scrolls and store at -80oC until shipment or proceed to extraction, If tissue is prepared for single nuclei sequencing sections in to 1mL RNA protect and store in 1.8mL cryovial at -80^{o}C
13. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
14. All tissue scrolls should be sectioned into a 1.5 mL tube with 1 mL of RLT buffer with BME and stored at -80^{o}C until shipment
15. All samples should shipped on dry ice as detailed in shipping guidelines.
16. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides

22. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
23. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
24. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
25. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
26. Sections should be prepared on positively charged (+) slides or coverslips without baking

27. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
28. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

19. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
20. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
21. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
22. Proceed to cut 5-20 uM scrolls and store at -80^{o}C until shipment or proceed to extraction
23. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
24. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN LUAD RESECTIONS FOR THE RETROSPECTIVE CROSS-SECTIONAL LUAD-PML COHORTS (all tissue – usually just part-solid nodule)

NOTE: *When Frozen tissue is available, DNA and RNA sections will be derived from the OCT embedded frozen tissue and sections for multiplex IF will be derived from the FFPE tissue of the same lesion. Adequacy of premalignant and invasive adenocarcinoma associated lesions is determined by the greatest dimension of the lesion where those in which this measurement is >/= 0.5 cm are classified as adequate and those where this measurement is 0.1 – 0.5 cm are classified as borderline. When non-invasive lepidic premalignant tissue contiguous with invasive adenocarcinoma in part-solid nodules is discontinuous, the sum of the greatest dimensions of the areas of lepidic tissue is used to determine the appropriate adequacy classification.*

Adequate lesion or normal lung (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-4** – 3 x 10µM sections for DNA
- **Section 5** - 4µM Section for H&E (newly cut)
- **Section 6-8** - 3x10µM sections for RNA
- **Section 9** - 4µM Section for H&E (newly cut)

Adequate lesion FFPE tissue (>/= 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

Borderline lesion (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-7** – 6 x 10µM sections for DNA
- **Section 8** - 4µM Section for H&E (newly cut)
- **Section 9-14** – 6 x 10µM sections for RNA
- **Section 15** - 4µM Section for H&E (newly cut)

Borderline lesion FFPE tissue (0.1 - 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Prospective Longitudinal Squamous Cohort – <u>FFPE only</u> Tissue Sectioning Requirements and Processing Algorithms

PROSPECTIVE: *Longitudinal LUSC bronchoscopy cohort*

Prospective cohorts include participants that are recruited from on-going or new screening cohorts and those seen for suspicion of lung cancer or from other pulmonary clinics that will be consented for use of tissue for this study and for future studies including those that involve industry/pharma support. For cases selected it is anticipated that each case will have at minimum, biospecimens from a pre-invasive lesion and/or tumor along with access to a genomic control for each case that is enrolled. It is also anticipated that prospective biospecimen tissue samples include fresh sorted/processed lesions for single cell assays, frozen tissue from the pre-invasive/tumor lesion (alone, in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an FFPE biospecimen. However, in valuable cases if the frozen tissue does not represent both pre-invasive and invasive lesion for the site (as seen in the FFPE tissue), submission of the case with only FFPE derived sections can be considered. FFPE blocks are either shipped to the core repository for processing/sectioning or sectioned locally following the sectioning guidelines detailed below. Below we have provided detail for the prospective longitudinal cohort with FPPE specimens that outlines the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the University of Colorado Core Repository are provided in the shipping guidelines.

- **Enrolling/Collecting Sites:** CU, RPCI, UCL, VUMC

- **Lung PCA Repository to ship cases:** CU- *see details in shipping guidelines for all samples going to CU.*

<u>Longitudinal Sample Collection Overview:</u> For the ***Prospective Longitudinal LUSC*** cohort, a minimum of 1 lung location will be longitudinally biopsied (up to 6-8 being followed at baseline) and the sampled at T1 (6-12 mos) and T2 (6-12 mos), where each location being followed will have at the minimum a formalin fixed and OCT frozen biopsy collected. For 1-3 selected sites followed overtime a minimum of 3 biopsies will be collected where 1) is collected in formalin, 2) is frozen in OCT, 3) is collected fresh for single cell sorting and where possible a fourth biopsy flash frozen in RNA protect (not in OCT) should be collected. The formalin and frozen samples will be shipped overnight on the day of collection to the LUSC core repository at the University of Colorado for processing where possible if formalin samples can't be sent they should be processed with in 24 hours at the site of collection and tissue blocks shared. The fresh tissue will be processed and single cell plates stored on site until the location to be further studied has been decided and plates will then be shipped to the BU team.

- **<u>Collection Timepoints:</u>** (*scheduled visits as part of the Lung PCA study)*
  - T(-1) (optional) Pre-screening sputum collection and potential pre-screening bronchoscopy
  - T0 - Baseline bronchial biopsies
  - T1 - Bronchial biopsies including follow-up fresh tissue from designated 2-3 sites of special interest
  - T2/incident LUSC - Bronchial biopsies or tumor block including follow-up fresh tissue from designated 2-3 sites of special interest
  - *Optional → T+ additional time points pre baseline, interim between T0-T1 or T1-T2 or post T2 can be considered when available – authorization by repository required.*

- **<u>Required biospecimen to be collected: :</u>** *(Additional details can be found in The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal along with the single cell processing protocol)*
  - **T0: Baseline**
    - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections
      - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations deemed to have highest grades of BD or that have shown persistent BD (see sections A.3 & C.3) **AND**
      - Frozen: OCT from all sites, and flash frozen in RNA protect from selected sites of highest grade dysplasia (to parallel those for fresh tissue when possible) **AND**

- Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)

- Genomic DNA
  - If tumor/pre-malignant lesion is frozen: (*see section B.1)*
    - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat *(see section A2.1)*
    - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) (*see sections A.1 for sectioning guidelines*)

- **T1** (same lung location (s) at T0 1-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically nearest location)
  - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guidelines)*
    - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
    - Frozen: OCT/RNA protect from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection, when possible, **AND**
    - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)

- **T2** (same lung location T0/T1 1-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically location)
  - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guideline)*
    - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
    - Frozen: OCT from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection, when possible, **AND**
    - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)
  - NOTE: For any sites at any time point, if invasive LUSC is present, collection of FFPE, OCT frozen, fresh tissue and flash frozen tissue in that order of priority should be undertaken with goal of collecting all

- **Optional Samples:**
  - *Please see The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal for additional samples and processing information for this cohort.*

- **Required metadata to be collected:** *see the Lung* /data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - **Baseline**: T0
    - **On Study Follow-Up**: T1, T2
    - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 2 follow up or additional unscheduled visits*
  - Biospecimen:
    - **Specimen**: T0, T1, T2 *(any T+ samples)*
    - **Site pathology**: T0, T1, T2 *(any T+ samples)*

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Cores for inclusion in TMAs for sections on coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E, RNA isolation and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING FFPE BLOCKS for slides/coverslips

29. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
30. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
31. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
32. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
33. Sections should be prepared on positively charged (+) regular or frame slides or coverslips without baking
34. All cut slides should be stored at -20 and shipped at 4°C (with cold packs)
35. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

25. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
26. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
27. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
28. Proceed to cut 5-20 uM sections in and stored at -80°C until shipment or proceed to extraction
29. All cut samples should be stored at -20 and shipped at 4°C (with cold packs)
30. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 – A.1.5. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FFPE BRONCHIAL BIOPSY FOR NUCLEIC ACIDS AND IN SITU ASSAYS FROM THE PROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *Biopsy lesion adequacy classification is based on the number of 40X fields the dysplastic epithelium traverses. Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. If the invasive SCC or normal tissue are collected from a resection specimen, two categories are used with adequate showing a greatest dimension of .>/= 0.5 cm and borderline 0.1 – 0.5 cm and the collection algorithms listed at the bottom of this section should be employed.*

Adequate dysplastic lesion IN FFPE – BD or SCC (Lesion spans >/= 7 40X fields): 25 fresh sections (~232 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-13** – 12 x 10µM sections for DNA (*DNA derived from micro-dissected epithelium*)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 15-24** – 10 x 10µM sections collected into a 1.5mL tube for RNA (*RNA from whole sections*)
- **Section 25** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 33 fresh sections (~312 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-17** – 16 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 18** - 4µM Section for H&E (newly cut)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 19-32** - 14 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 33** - 4µM Section for H&E (newly cut; NOT necessary if pre-cut scrolls used for RNA)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields): 47 fresh sections (~452 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-25** – 24 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 26** - 4µM Section for H&E (newly cut)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 27-46** – 20 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 47** - 4µM Section for H&E (newly cut; NOT necessary if pre-cut scrolls used for RNA)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 *SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation* for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Prospective Longitudinal Squamous Cohort – <u>Fresh, OCT & FFPE</u> Tissue Requirements and Processing Algorithms

PROSPECTIVE: *Longitudinal LUSC bronchoscopy cohort*

Prospective cohorts include participants that are recruited from on-going or new screening cohorts and those seen for suspicion of lung cancer or from other pulmonary clinics that will be consented for use of tissue for this study and for future studies including those that involve industry/pharma support. For cases selected it is anticipated that each case will have at minimum, biospecimens from a pre-invasive lesion and/or tumor along with access to a genomic control for each case that is enrolled. It is also anticipated that prospective biospecimen tissue samples include fresh sorted/processed lesions for single cell assays, frozen tissue from the pre-invasive/tumor lesion (alone, in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an FFPE biospecimen. Frozen OCT and samples in formalin or FFPE blocks are either shipped to the core repository for processing/sectioning or sectioned locally following the sectioning guidelines detailed below. Below we have provided detail for the prospective longitudinal cohort with both OCT frozen and FPPE specimens that outlines the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the University of Colorado Core Repository are provided in the shipping guidelines.

- **Enrolling/Collecting Sites:** CU, RPCI, UCL, VUMC

- **Lung PCA Repository to ship cases:** CU- *see details in shipping guidelines for all samples going to CU.*

<u>Longitudinal Sample Collection Overview:</u> For the ***Prospective Longitudinal LUSC*** cohort, a minimum of 1 lung location will be longitudinally biopsied (up to 6-8 being followed at baseline) and the sampled at T1 (6-12 mos) and T2 (6-12 mos), where each location being followed will have at the minimum a formalin fixed and OCT frozen biopsy collected. For 1-3 selected sites followed overtime a minimum of 3 biopsies will be collected where 1) is collected in formalin, 2) is frozen in OCT, 3) is collected fresh for single cell sorting and where possible a fourth biopsy flash frozen in RNA protect (not in OCT) should be collected. The formalin and frozen samples will be shipped overnight on the day of collection to the LUSC core repository at the University of Colorado for processing where possible if formalin samples can't be sent they should be processed with in 24 hours at the site of collection and tissue blocks shared. The fresh tissue will be processed and single cell plates stored on site until the location to be further studied has been decided and plates will then be shipped to the BU team.

- **<u>Collection Timepoints:</u>** (*scheduled visits as part of the Lung PCA study)*
  - T(-1) (optional) Pre-screening sputum collection and potential pre-screening bronchoscopy
  - T0 - Baseline bronchial biopsies
  - T1 - Bronchial biopsies including follow-up fresh tissue from designated 2-3 sites of special interest
  - T2/incident LUSC - Bronchial biopsies or tumor block including follow-up fresh tissue from designated 2-3 sites of special interest
  - *Optional → T+ additional time points pre baseline, interim between T0-T1 or T1-T2 or post T2 can be considered when available – authorization by repository required.*

- **<u>Required biospecimen to be collected:</u>** *(Additional details can be found in The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal along with the single cell processing protocol)*
  - **T0: Baseline**
    - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections
      - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations deemed to have highest grades of BD or that have shown persistent BD (see sections A.3 & C.3) **AND**
      - Frozen: OCT from all sites, and flash frozen in RNA protect from selected sites of highest grade dysplasia (to parallel those for fresh tissue when possible) **AND**

- Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)

- Genomic DNA
  - If tumor/pre-malignant lesion is frozen: (*see section B.1)*
    - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat *(see section A2.1)*
    - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) (*see sections A.1 for sectioning guidelines*)

- **T1** (same lung location (s) at T0 2-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically nearest location)
  - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guidelines)*
    - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
    - Frozen: OCT/RNA protect from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection when possible, **AND**
    - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)
- **T2** (same lung location T0/T1 2-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically location)
  - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guideline)*
    - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
    - Frozen: OCT from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection when possible, **AND**
    - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)
- *NOTE: For any sites at any time point, if invasive LUSC is present, collection of FFPE, OCT frozen, fresh tissue and flash frozen tissue in that order of priority should be undertaken with goal of collecting all*

- **Optional Samples:**
  - *Please see The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal for additional samples and processing information for this cohort.*

- **Required metadata to be collected:** *see the Lung* /data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - **Baseline**: T0
    - **On Study Follow-Up**: T1, T2
    - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 2 follow up or additional unscheduled visits*
  - Biospecimen:
    - **Specimen**: T0, T1, T2 *(any T+ samples)*
    - **Site pathology**: T0, T1, T2 *(any T+ samples)*

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections for Bulk DNA or RNA isolation from Frozen tissue and biopsy samples.
- Cores for inclusion in TMAs for sections on coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E, RNA isolation and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING OCT BLOCKS for slides

15. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
16. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
17. Pre label all slides with PCA ID and section number *NO MRNs or DATES*
18. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
19. Sections should be prepared on positively charged (+) regular or frame slides
20. All cut slides should be stored at -20 and shipped on dry ice.
21. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for RNA and genomic DNA isolation)*

17. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
18. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
19. Pre label all slides and 1.5 mL tubes with PCA ID and section number *NO MRNs or DATES*
20. Proceed to cut 5-20 uM sections for DNA and for RNA refer to details below* - note the section thicknesses and order and store at -80^{o}C until shipment or proceed to extraction
21. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
22. All tissue scrolls should be sectioned in to a 1.5 mL tube with 1 mL of RLT buffer with BME and stored at -80^{o}C until shipment
23. All samples should be shipped on dry ice as detailed below.
24. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides/coverslips

36. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
37. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
38. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
39. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
40. Sections should be prepared on positively charged (+) regular or frame slides or coverslips without baking
41. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)

42. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for RNA or genomic DNA)*

31. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
32. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
33. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
34. Proceed to cut 5-20 uM for DNA and for RNA refer to details below and store at -80^{o}C until shipment or proceed to extraction
35. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
36. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 – A.1.5. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN BRONCHIAL BIOPSY FOR NUCLEIC ACIDS AND FFPE BRONCHIAL BIOPSY FOR IN SITU ASSAYS FROM THE PROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *Biopsy lesion adequacy classification is based on the number of 40X fields the dysplastic epithelium traverses. Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. If the invasive SCC or normal tissue are collected from a resection specimen, two categories are used with adequate showing a greatest dimension of .>/= 0.5 cm and borderline 0.1 – 0.5 cm and the collection algorithms listed at the bottom of this section should be employed.*

Adequate dysplastic lesion IN OCT – BD or SCC (Lesion spans >/= 7 40X fields): 25 fresh sections (~232 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-13** – 12 x 10µM sections for DNA (*DNA derived from micro-dissected epithelium*)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Section 15-24** – 10 x 20µM (can cut down to 10 uM if limited) sections collected into a 1.5mL tube for RNA (*RNA from whole sections*)
- **Section 25** - 4µM Section for H&E (newly cut)

Adequate dysplastic lesion IN FFPE – BD or SCC (Lesion spans >/= 7 40X fields):

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

FOR MORE LIMITED SPECIMENS, COLLECTION OF DNA FROM OCT FOLLOWED BY COLLECTION OF IN SITU/MULTIPLEX *AND* RNA FROM FFPE CAN BE CONSIDERED AS OUTLINED BELOW

Intermediate dysplastic lesion IN OCT – BD or SCC (Lesion spans 4-6 40X fields): 18 fresh sections (~168 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-17** – 16 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 18** - 4µM Section for H&E (newly cut)

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 16 fresh sections (~148 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 2-15** - 14 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 16** - 4µM Section for H&E (newly cut; NOT necessary if pre-cut scrolls used for RNA)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion IN OCT – BD or SCC (Lesion spans 2-3 40X fields): 26 fresh sections (~248 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-25** – 24 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 26** - 4µM Section for H&E (newly cut)

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields): 22 fresh sections (~208 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 9-21** – 20 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 22** - 4µM Section for H&E (newly cut; NOT necessary if pre-cut scrolls used for RNA)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Adequate resected SCC or normal lung IN FFPE or OCT (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-4*** *– 3 x 10µM sections for DNA*
- ***Section 5*** *- 4µM Section for H&E (newly cut)*
- ***Section 6-8*** *– 3 x 10µM sections for RNA*
- ***Section 9*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline resected SCC or normal lung IN FFPE or OCT (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-7*** *– 6 x 10µM sections for DNA*
- ***Section 8*** *- 4µM Section for H&E (newly cut)*
- ***Section 9-14*** *– 6 x 10µM sections for RNA*
- ***Section 15*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 *SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation* for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA
  - Minimum DNA amount (in 30uL): 250 ng, 500-1000 ng is optimal

SECTION A.3 PROTOCOL FOR PROCESSING FRESH SAMPLES FOR SINGLE CELL PROFILING:

- **Fresh biopsy samples processed at the site of collection day of the bronchoscopy**
  - Basic prep: (*for detailed protocol see: Section C).*
    - Bronchial biopsy samples are collected into in 1-2 mL of RPMI in 1.5mL cryovial. * Post-collection, if waiting is required before sample prep (1-4 hours), keep sample at 4 °C. Samples can be stored overnight IF the cryovial is filled up to the top with complete medium (RPMI/10% FBS added to the existing medium) immediately upon receipt. This may not work for very small biopsies.
    - Refer to Bronchial Biopsy Dissociation and Sorting Protocol/Guidelines document to complete single cell sorting of fresh specimens

Retrospective Cross-Sectional Squamous Cohort – FFPE only Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Cross-Sectional LUSC*

All cases identified for the retrospective cohorts are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. The cases selected for the Lung PCA are anticipated to have at the minimum biospecimens from an independent pre-invasive bronchial dysplasia and a pre-invasive lesion contiguous with invasive squamous cell carcinoma (SCC) along with access to a genomic control for each case that is enrolled. Tissue from the invasive SCC is also required. When frozen tissue with associated matched Formalin Fixed Paraffin Embedded (FFPE) tissue is available, either as OCT embedded or flash frozen (alone or with RNA preservative) tissues that are then prepared in Optimal Temperature Cutting (OCT) media blocks, these tissues will be sectioned and punched for tissue microarrays (TMAs) locally following the guidelines detailed below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the University of Colorado (CU) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCLA
- **Lung PCA Repository to ship cases:** CU- *see shipping guidelines for all samples going to CU.*

  - **Collection Timepoints:**
    - T0: Resection cases of LUSC where bronchial dysplasia is found in the tissue margin or remote airway from tumor AND in a tissue adjacent (contiguous with) the invasive SCC.

  - **Required biospecimen to be collected**
    - T0:
      - Resection- FFPE tissue blocks or sections *(see sectioning guide: A.1.2)*
        - FFPE Tumor with contiguous Bronchial Dysplasia (Lesion and Normal area) **AND**
        - FFPE Bronchial Dysplasia remote from tumor (Lesion area)
      - Genomic DNA
        - If tumor/pre-malignant lesion is formalin fixed normal FFPE lymph node sample (or less preferable, normal lung) used for:
          - Preferred: Isolated DNA from FFPE *(see section A.2.2)*
          - Alternative: FFPE tissue Scrolls, Blood derived gDNA

  - **Required metadata to be collected:** *see* the PCA portal/data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)

    - Clinical:
      - **Baseline**
      - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
    - Biospecimen:
      - **Specimen**
      - **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:
All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Tissue cores in TMAs to be cut onto coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING FFPE BLOCKS for slides/coverslips

43. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
44. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
45. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
46. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
47. Sections should be prepared on positively charged (+) regular or frame (DNA/RNA sections) slides or coverslips without baking
48. All cut slides should be stored at -20 and shipped at 4°C (with cold packs)
49. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

37. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
38. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
39. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
40. Proceed to cut 5-20 uM sections in and stored at -80°C until shipment or proceed to extraction
41. All cut samples should be stored at -20 and shipped at 4°C (with cold packs)
42. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 below. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FFPE LUSC RESECTIONS FOR NUCLEIC ACIDS AND IN SITU ASSAYS FROM THE RETROSPECTIVE CROSS-SECTIONAL LUSC - PML COHORT (All samples)

NOTE: *Bronchial dysplasia adequacy classification is based on the number of 40X fields the dysplastic traverses. Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. For tissue collection from invasive SCC or normal tissue two categories are used with adequate showing a greatest dimension of .>/= 0.5 cm and borderline 0.1 – 0.5 cm and the collection algorithms listed at the bottom of this section are employed.*

Adequate dysplastic lesion – BD or SCC (Lesion spans >/= 7 40X fields): 17 fresh sections (~152 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-9** – 8 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 10** - 4µM Section for H&E (newly cut)
- **Section 11-16** - 6 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 17** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Intermediate dysplastic lesion – BD or SCC (Lesion spans 4-6 40X fields): 23 fresh sections (~212 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-13** – 12 x 10µM sections for DNA (DNA derived from microdissected epithelium)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Section 15-22** – 8 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 23** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion – BD or SCC (Lesion spans 2-3 40X fields): 31 fresh sections (~292 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-17** – 16 x 10µM sections for DNA (DNA derived from microdissected epithelium)
- **Section 18** - 4µM Section for H&E (newly cut)
- **Section 19-30** – 12 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 31** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Adequate resected SCC or normal lung (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-4*** *– 3 x 10µM sections for DNA*
- ***Section 5*** *- 4µM Section for H&E (newly cut)*
- ***Section 6-8*** *– 3 x 10µM sections for RNA*
- ***Section 9*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline resected SCC or normal lung (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-7*** *- 6x10µM sections for DNA*
- ***Section 8*** *- 4µM Section for H&E (newly cut)*
- ***Section 9-14*** *- 6x10µM sections for RNA*
- ***Section 15*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see Appendix D (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
    - If using an airway brushing
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Retrospective Cross-Sectional Squamous Cohort – <u>OCT & FFPE</u> Tissue Requirements and Processing Algorithms

RETROSPECTIVE *Cross-Sectional LUSC*

All cases identified for the retrospective cohorts are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. The cases selected for the Lung PCA are anticipated to have at the minimum biospecimens from an independent pre-invasive bronchial dysplasia and a pre-invasive lesion contiguous with invasive squamous cell carcinoma (SCC) along with access to a genomic control for each case that is enrolled. Tissue from the invasive SCC is also required. When frozen tissue with associated matched Formalin Fixed Paraffin Embedded (FFPE) is available, either as OCT embedded or flash frozen (alone or with RNA preservative) tissues that are then prepared in Optimal Temperature Cutting (OCT) media blocks, these tissues will be sectioned and punched for tissue microarrays (TMAs) locally following the guidelines detailed below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the University of Colorado (CU) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCLA
- **Lung PCA Repository to ship cases:** CU- *see shipping guidelines for all samples going to CU.*

  - **<u>Collection Timepoints:</u>**
    - T0: Resection cases of LUSC where bronchial dysplasia is found in the tissue margin or remote airway from tumor AND in a tissue adjacent (contiguous with) the invasive SCC.

  - **<u>Required biospecimen to be collected</u>**
    - **T0:**
      - Resection- OCT and FFPE tissue blocks or sections *(see sectioning guide: A.1.2)*
        - OCT and FFPE Tumor with contiguous Bronchial Dysplasia (Lesion and Normal area) **AND**
        - FFPE Bronchial Dysplasia remote from tumor (Lesion area). NOTE: if OCT available for remote lesion this would be priority tissue though we expect this would be rare.
      - Genomic DNA
        - If tumor/pre-malignant lesion is frozen: (*see section B.1)*
          - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat *(see section A2.1)*
          - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) (*see sections A.1 for sectioning guidelines*)
        - If tumor/pre-malignant lesion is formalin fixed or no frozen normal tissue is available, normal FFPE lymph node sample (or less preferable, normal lung) used for:
          - Preferred: Isolated DNA from FFPE *(see section A.2.2)*
          - Alternative: FFPE tissue Scrolls, Blood derived gDNA

  - **<u>Required metadata to be collected:</u>** *see* the PCA portal/data standards for details: (<u>https://lci-test.whiterivercomputing.com/portal/data-standards</u>)
    - <u>Clinical:</u>
      - **Baseline**
      - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
    - <u>Biospecimen:</u>
      - **Specimen**
      - **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:
All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections for Bulk RNA isolation from Frozen tissue and biopsy samples.
- Tissue cores in TMAs to be cut onto coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING OCT BLOCKS for slides

22. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
23. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
24. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
25. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
26. Sections should be prepared on positively charged (+) regular or frame (DNA/RNA sections) slides
27. All cut slides should be stored at -20 and shipped on dry ice.
28. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for RNA and genomic DNA isolation)*

25. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
26. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
27. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
28. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
29. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
30. All samples should be shipped on dry ice as detailed below.
31. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides/coverslips

50. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
51. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
52. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
53. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
54. Sections should be prepared on positively charged (+) regular or frame (DNA/RNA sections) slides or coverslips without baking
55. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
56. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

43. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
44. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
45. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
46. Proceed to cut 5-20 uM sections in and stored at -80^{o}C until shipment or proceed to extraction
47. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
48. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 below. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN LUSC RESECTIONS WITH FFPE LUSC RESECTIONS FOR NUCLEIC ACIDS AND IN SITU ASSAYS FROM THE RETROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *Bronchial dysplasia adequacy classification is based on the number of 40X fields the dysplastic traverses. Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. For tissue collection from invasive SCC or normal tissue two categories are used with adequate showing a greatest dimension of .>/= 0.5 cm and borderline 0.1 – 0.5 cm and the collection algorithms listed at the bottom of this section are employed.*

Adequate dysplastic lesion IN OCT – BD or SCC (Lesion spans >/= 7 40X fields): 25 fresh sections (~252 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-13** – 12 x 10µM sections for DNA (*DNA derived from micro-dissected epithelium*)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Section 15-24** – 10 x 10µM sections collected into a 1.5mL tube for RNA (*RNA from whole sections*)
- **Section 25** - 4µM Section for H&E (newly cut)

Adequate dysplastic lesion IN FFPE – BD or SCC (Lesion spans >/= 7 40X fields): 1 fresh sections (~4 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

FOR MORE LIMITED SPECIMENS, COLLECTION OF DNA FROM OCT FOLLOWED BY COLLECTION OF IN SITU/MULTIPLEX *AND* RNA FROM FFPE CAN BE CONSIDERED AS OUTLINED BELOW

Intermediate dysplastic lesion IN OCT – BD or SCC (Lesion spans 4-6 40X fields): 18 fresh sections (~168 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-17** – 16 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 18** - 4µM Section for H&E (newly cut)

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 16 fresh sections (~148 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-15** – 14 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 16** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion IN OCT – BD or SCC (Lesion spans 2-3 40X fields): 26 fresh sections (~248 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-25** – 24 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 26** - 4µM Section for H&E (newly cut)

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields): 22 fresh sections (~208 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-21** – 20 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 22** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Adequate resected SCC or normal lung IN FFPE or OCT (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-4*** *– 3 x 10µM sections for DNA*
- ***Section 5*** *- 4µM Section for H&E (newly cut)*
- ***Section 6-8*** *– 3 x 10µM sections for RNA*

- ***Section 9*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline resected SCC or normal lung IN FFPE or OCT (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-7*** *- 6x10µM sections for DNA*
- ***Section 8*** *- 4µM Section for H&E (newly cut)*
- ***Section 9-14*** *- 6x10µM sections for RNA*
- ***Section 15*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see Appendix D (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
    - If using an airway brushing
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

Retrospective Longitudinal Squamous Cohort – <u>FFPE Tissue</u> Requirements and Processing Algorithms

RETROSPECTIVE *Longitudinal LUSC bronchoscopy cohort*

All cases identified for the retrospective cohorts are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. The cases selected for the Lung PCA are anticipated to have at the minimum biospecimens from a pre-invasive lesion and/or tumor along with access to a genomic control for each case that is enrolled. It is also anticipated that retrospective biospecimen tissue samples will primarily be prepared as Formalin Fixed Paraffin Embedded (FFPE) blocks that can be sectioned locally following the sectioning guidelines detailed below or as FFPE blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Alternatively, where possible, tissue may also be frozen, whereas tissue (pre-invasive/tumor lesion) samples are flash frozen (alone or with RNA preservative) that are then prepared in Optimal Temperature Cutting (OCT) media blocks that are sectioned locally following the sectioning guidelines detailed below or as blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Below we have provided detail for the retrospective longitudinal cohort with FPPE specimens only that aims to outline the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols and the details for shipping cases to our biorepositories.

- **Enrolling/Collecting Sites:** CU, UCL

- **Lung PCA Repository to ship cases:** No shipping of tissue required (all cases processed on site at either CU or UCL)

  - **<u>Collection Timepoints:</u>** *(estimated time between the collection of samples fresh or banked)*
    - T0 - Baseline bronchial dysplasia/CIS,
    - T1 - Bronchial dysplasia/CIS from the same lung location or invasive LUSC at 12months post baseline (at least 6M post BD, but later times also acceptable)
    - *Optional → T+ additional time points pre-baseline and post baseline (i.e. at separate site from T1) or post T1 can be considered when available – authorization by repository required.*

  - **<u>Required biospecimen to be collected:</u>**
    - **T0: Baseline**
      - Bronchial Biopsy- FFPE and/or Frozen (if available) tissue blocks or sections
        - Frozen: (OCT/RNA protect) dysplastic lesions (*see OCT & FFPE sectioning guide)*
        - FFPE: dysplastic lesions see sectioning guide A.1.2 below
          - **If providing frozen, FFPE is required for in situ assays** *(see OCT & FFPE sectioning guide)*
      - Genomic DNA
        - For formalin fixed tumor/pre-malignant lesion, normal FFPE lymph node sample (or less preferable, normal lung) used for:
          - Preferred: Isolated DNA from FFPE (*see section A.2.2)*
          - Alternative: FFPE tissue Scrolls, Blood derived gDNA

    - **T1:follow-up longitudinal** (tumor from approximately the same lung location as T0/baseline biopsy)
      - Bronchial Biopsy or resected tumor block - FFPE and/or Frozen (if available) tissue blocks or sections*:*
        - Frozen: (OCT/RNA protect) Incident squamous cell carcinoma tumor (*see sectioning OCT & FFPE guide)*
        - FFPE: Incident squamous cell carcinoma tumor see sectioning guide A.1.2 below

- **If providing frozen, FFPE is required for in situ assays** *(see sectioning OCT & FFPE guide)*

- **Required metadata to be collected:** *see the Lung* /data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - **Baseline**: T0
    - **On Study Follow-Up**: T1
    - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 1 follow up or additional unscheduled visits*

  - Biospecimen:
    - **Specimen**: T0, T1 *(any T+ samples)*
    - **Site pathology**: T0, T1 *(any T+ samples)*

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be on labels)***

Sections needed for:

- Sections on slides for DNA isolation via LCM isolation for bulk sequencing
- Sections on slides for RNA isolation via macro-dissection (whole sections) isolation for bulk sequencing
- Sections on coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***All Sections for DNA isolation:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING FFPE BLOCKS for slides/coverslips

57. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
58. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
59. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
60. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
61. Sections should be prepared on positively charged (+) slides or coverslips without baking
62. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
63. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

49. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
50. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
51. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
52. Proceed to cut 5-20 uM for DNA and for RNA refer to details below and store at -80^{o}C until shipment or proceed to extraction
53. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
54. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.2. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.2 FFPE BRONCHIAL BIOPSIES FROM THE PROSPECTIVE (IF NO FROZEN OR FROZEN IS NEGATIVE FOR DYSPLASIA) AND RETROSPECTIVE LUSC - PML COHORTS (All samples)

NOTE: ***Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible.***

Adequate dysplastic lesion – BD or SCC (Lesion spans >/= 7 40X fields): 17 fresh sections (~152 um)

- **Section 1 - 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-9 - 8 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)**
- **Section 10 - 4µM Section for H&E (newly cut)**
- **Section 11-16 - 6 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 17 - 4µM Section for H&E (newly cut)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

Intermediate dysplastic lesion – BD or SCC (Lesion spans 4-6 40X fields): 23 fresh sections (~212 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-13 – 12 x 10µM sections for DNA (DNA derived from microdissected epithelium)**
- **Section 14 - 4µM Section for H&E (newly cut)**
- **Section 15-22 – 8 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 23 - 4µM Section for H&E (newly cut)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion – BD or SCC (Lesion spans 2-3 40X fields): 31 fresh sections (~292 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-17 – 16 x 10µM sections for DNA (DNA derived from microdissected epithelium)**
- **Section 18 - 4µM Section for H&E (newly cut)**
- **Section 19-30 – 12 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 31 - 4µM Section for H&E (newly cut)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 *SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation* for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA
  - Minimum DNA amount (in 30 L): 250 ng, 500-1000 ng is optimal

Retrospective Longitudinal Squamous Cohort – <u>OCT & FFPE</u> Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Longitudinal LUSC bronchoscopy cohort*

All cases identified for the retrospective cohorts are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. The cases selected for the Lung PCA are anticipated to have at the minimum biospecimens from a pre-invasive lesion and/or tumor along with access to a genomic control for each case that is enrolled. It is also anticipated that retrospective biospecimen tissue samples will primarily be prepared as Formalin Fixed Paraffin Embedded (FFPE) blocks that can be sectioned locally following the sectioning guidelines detailed below or as FFPE blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Alternatively, where possible, tissue may also be frozen, whereas tissue (pre-invasive/tumor lesion) samples are flash frozen (alone or with RNA preservative) that are then prepared in Optimal Temperature Cutting (OCT) media blocks that are sectioned locally following the sectioning guidelines detailed below or as blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Below we have provided detail for the retrospective longitudinal cohort with both OCT frozen and FPPE specimens that aims to outline the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols and the details for shipping cases to our biorepositories.

- **Enrolling/Collecting Sites:** CU, UCL

- **Lung PCA Repository to ship cases:** No shipping of tissue required (all cases processed on site at either CU or UCL)

  - **<u>Collection Timepoints:</u>** *(estimated time between the collection of samples fresh or banked)*
    - T0 - Baseline bronchial dysplasia/CIS,
    - T1 - Bronchial dysplasia/CIS from the same lung location or invasive LUSC at 12months post baseline (at least 6M post BD, but later times also acceptable)
    - *Optional → T+ additional time points pre-baseline and post baseline (i.e. at separate site from T1) or post T1 can be considered when available. – authorization by repository required.*

  - **<u>Required biospecimen to be collected:</u>**
    - T0: Baseline
      - Bronchial Biopsy- FFPE and/or Frozen (if available) tissue blocks or sections
        - Frozen: (OCT/RNA protect) dysplastic lesions (*see sectioning guide A.1.1)*
        - FFPE: dysplastic lesions **(if providing frozen, FFPE is required for in situ assays)** *(see sectioning guide: A.1.2 (if providing frozen block see A.1.5))*
      - Genomic DNA
        - If tumor/pre-malignant lesion is frozen: (*see section A.2)*
          - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat
          - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node (or less preferable, normal lung/ airway) or tissue scrolls (*see sections A.1 for sectioning guidelines*)
          - Alternatives: non-isolated blood samples, isolated DNA from FFPE normal lymph node (or less preferable, normal lung/ airway) or tissue scrolls (*see sections A.2 for sectioning guidelines*)

    - T1: Follow-up longitudinal (tumor from approximately the same lung location as T0/baseline biopsy)
      - Bronchial Biopsy or resected tumor block - FFPE and/or Frozen (if available) tissue blocks or sections*:*

- Frozen: (OCT/RNA protect) Incident squamous cell carcinoma tumor (*see sectioning guide: A.1.1)*
- FFPE: Incident squamous cell carcinoma tumor **(if providing frozen, FFPE is required for in situ assays)** *(see sectioning guide: A.1.2)*

- **Required metadata to be collected:** *see the Lung* /data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - **Baseline**: T0
    - **On Study Follow-Up**: T1
    - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 1 follow up or additional unscheduled visits*
  - Biospecimen:
    - **Specimen**: T0, T1 *(any T+ samples)*
    - **Site pathology**: T0, T1 *(any T+ samples)*

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for pre-invasive lesions associated with tumors for bulk sequencing
- Sections on coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All FFPE Sections for H&E. RNA & Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING OCT BLOCKS for slides

29. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
30. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
31. Pre label all slides with PCA ID and section number *NO MRNs or DATES*
32. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
33. Sections should be prepared on positively charged (+) slides
34. All cut slides should be stored at -20 and shipped on dry ice.
35. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for RNA and genomic DNA isolation)*

32. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
33. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
34. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipmen
35. Pre label all slides and 1.5 mL tubes with PCA ID and section number *NO MRNs or DATES*
36. H&E Sections for biopsy samples should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
37. Proceed to cut 5-20 uM sections for DNA and for RNA of biopsy samples refer to details below* - note the section thicknesses and order and store at -80^{o}C until shipment or proceed to extraction
38. All tissue scrolls for RNA isolation should be sectioned in to a 1.5 mL tube with 1 mL of RLT buffer with BME and store at -80^{o}C until shipment.
39. All samples should be shipped on dry ice as detailed below.
40. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides/coverslips

64. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
65. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
66. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
67. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
68. Sections should be prepared on positively charged (+) slides or coverslips without baking
69. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
70. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for RNA or genomic DNA)*

55. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
56. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
57. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
58. Proceed to cut 5-20 uM sections for DNA and for RNA refer to details below and store at -80^{o}C until shipment or proceed to extraction
59. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
60. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 – A.1.5. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN BRONCHIAL BIOPSY FOR NUCLEIC ACIDS AND FFPE BRONCHIAL BIOPSY FOR IN SITU ASSAYS FROM THE RETROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. Can be applied for the Prospective LUSC cohort if processing locally*.

Adequate dysplastic lesion IN OCT – BD or SCC (Lesion spans >/= 7 40X fields): 25 fresh sections (~232 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-13 – 12 x 10µM sections for DNA (*DNA derived from micro-dissected epithelium*)**
- **Section 14 - 4µM Section for H&E (newly cut)**
- **Section 15-24 – 10 x 20µM sections collected into a 1.5mL tube for RNA (*RNA from whole sections*)**
- **Section 25 - 4µM Section for H&E (newly cut)**

Adequate dysplastic lesion IN FFPE – BD or SCC (Lesion spans >/= 7 40X fields):

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

FOR MORE LIMITED SPECIMENS, COLLECTION OF DNA FROM OCT FOLLOWED BY COLLECTION OF IN SITU/MULTIPLEX *AND* RNA FROM FFPE CAN BE CONSIDERED AS OUTLINED BELOW

Intermediate dysplastic lesion IN OCT – BD or SCC (Lesion spans 4-6 40X fields): 18 fresh sections (~168 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-17 – 16 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)**
- **Section 18 - 4µM Section for H&E (newly cut)**

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 16 fresh sections (~148 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-15 – 14 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 16 - 4µM Section for H&E (newly cut)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion IN OCT – BD or SCC (Lesion spans 2-3 40X fields): 26 fresh sections (~248 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-25 – 24 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)**
- **Section 26 - 4µM Section for H&E (newly cut)**

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields): 22 fresh sections (~208 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 9-21 – 20 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 22 - 4µM Section for H&E (newly cut)**
- **Core remainder of tissue for placement in TMA on 22 x 22 coverslip**

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 *SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation* for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

APPENDIX B: SHIPPING BIOSPECIMENS TO CORE LABORATORIES

SECTION B.1 FROZEN TISSUE/BLOOD/DNA

SECTION B.1.1 BLOOD & OCT BLOCKS, FLASH FROZEN, AND TISSUE IN RNA PROTECT

SECTION B.1.2 TISSUE SECTIONS

SECTION B.2 FIXED TISSUE

SECTION B.2.1 TISSUE IN FORMALIN OR FFPE BLOCKS

SECTION B.2.2 TISSUE SECTIONS

SECTION B.3 SINGLE CELL PLATES

Core repositories: *Biospecimens collected at Lung PCA Collection sites will be sent to the University of Colorado (CU) for all LUSC cases or MD Anderson Cancer Center (MDACC) for all LUAD cases collected specimens for participants in the Lung Pre-Cancer Atlas study.* ***Exceptions:*** *Single cell plates should be sent directly to BU, DNA isolated for genomic controls sent to UCLA and tissue sectioned on coverslips for CODEX at site sent to Stanford.*

Frozen samples: *All freshly sorted plates, OCT blocks, OCT sections, flash frozen (+/- protect agents), genomic DNA/ blood samples are to be shipped on dry-ice. All biospecimens should not undergo freeze-thaw cycles prior to shipping, so please aliquot volume appropriately at the time of storage. All biospecimen samples must be shipped* ***overnight for next day delivery, shipped Monday through Wednesday only for batched specimens and Monday through Thursday for prospective bronchoscopy specimens.*** *Shipping methods should take seasonal temperatures into account, and include the use of extra insulated packaging and be shipped using dry ice.*

Formalin/FFPE samples: Formalin tissue, FFPE tissue blocks or sectioned FFPE blocks. *All biospecimen samples must be shipped* ***overnight for next day delivery, shipped Monday through Wednesday only for batched specimens and Monday through Thursday for prospective bronchoscopy specimens.*** *Shipping methods should take seasonal temperatures into account, and include the use of extra insulated packaging and be shipped using cold packs.*

Ship to Addresses:

CU: (core) Attn: Andrea Osypuk

University of Colorado– AMC, 12801 E. 17th Ave. L18-5400A, Aurora, CO 80045

MDACC: (core & MIF) Attn: Junya Fujimoto/ Humam Kadara
MD Anderson Cancer Center, 2130 W Holcombe Boulevard, Life Science Plaza, SUITE 910, LSP9.4227, Houston TX 77030

BU: (single cell plates- RNA & DNA) Attn: Austin Potter / Sarah Mazzilli,

Boston University School of Medicine, E-624, 72 East Concord St Boston, MA 02118

Stanford: (CODEX Coverslips) Attn: Wilson Kuswanto

Stanford University, 269 Campus Drive CCSR 3220, Stanford, CA 94035

UCLA: (genomic DNA) Attn: Kostyantyn Krysan

University of California -Los Angeles 650 Charles Young Dr. South, 10-240B Factor Bldg., Los Angeles, CA 90095

Broad: (snuc samples) Attn Xian Adiconis Broad Institute of MIT and Harvard 75 Ames Street, 9085A Cambridge, MA 02142

NOTE: *for the prospective LUSC Cohort Samples only the CU core will provide:*

- Biopsy Collection Kit
- Dual compartment Shipper with Ice packs
- Specimen transport bags with Zorbs
- FedEx Shipping Label (Priority Overnight)
- Dry Ice Label
- Recipient Label

SECTION B.1.1 FROZEN TISSUE/BLOOD/DNA *

*If preparing shipping for prospective longitudinal LUSC-PML cohort refer to the *Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual*

1. Upload all required biospecimen and clinical data for the case to be shipped to synapse or Lung PCA portal
2. Complete all fields in the provided PCA shipping manifest
    a. PCA ID sample # (PID)
    b. Sample type
    c. Confirm required biospecimen and clinical data has been uploaded
3. When ready to ship, fill a **cooler box (bottom if using CU shipper)**, about 1/3 full of dry ice.
4. Place frozen specimens in the specimen transport bag with Zorbs (3"x3" Zorb Sheets from Medline Manufacture # SFE44001) and seal each specimen bag, removing as much air as possible, and then place on top of the dry ice.
5. Add dry ice on top of the frozen specimen bags until cooler is full. Make certain the lid fits properly. Do not tape the container top down to allow vapors to escape.
6. Record the FedEx tracking number and the date shipped
7. Close and seal the cardboard box with packaging tape.
8. Affix the Biological Substance Category B, Dry Ice, FedEx shipping label and Recipient label to the box.
9. Ship immediately following packaging. Make certain package is available at FedEx pick up site to ensure overnight shipment and next day delivery.
10. Email notification of shipment including the FedEx tracking number in the subject line of the email, include: **PCA Study, Site ID#**, **PID #, Fresh Shipment**
    a. CU → to BCF.Schedule@cuanschutz.edu, cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu
    b. MDACC → to BSanchez2@mdanderson.org, , HKadara@mdanderson.org, jfujimoto@mdanderson.org, cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu
    c. BU → to jackcunn@bu.edu cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu

SECTION B.1.2 FROZEN TISSUE SECTIONS

1. Upload all required biospecimen and clinical data for the case to be shipped to synapse or Lung PCA portal
2. Complete all fields in the provided PCA shipping manifest
    a. PCA ID sample # (PID)

   b. Sample type
   c. Confirm required biospecimen and clinical data has been uploaded
3. When ready to ship, fill a **cooler box (bottom if using CU shipper)**, about 1/3 full of dry ice.
4. Package slides into the slide box with padding material on top to make sure nothing breaks in transit.
5. Tape the slide box closed and place the slide box into the Ziploc bag.
6. Add dry ice on top of the frozen specimen bags until cooler is full. Make certain the lid fits properly. Do not tape the container top down to allow vapors to escape.
7. Record the FedEx tracking number and the date shipped
8. Close and seal the cardboard box with packaging tape.
9. Affix the Biological Substance Category B, Dry Ice, FedEx shipping label and Recipient label to the box.
10. Ship immediately following packaging. Make certain package is available at FedEx pick up site to ensure overnight shipment and next day delivery.
11. Email notification of shipment including the FedEx tracking number In the subject line of the email, include: **PCA Study, Site ID#**, **PID #, Fresh Shipment**
    a. CU → to BCF.Schedule@cuanschutz.edu, cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu
    b. MDACC → to BSanchez2@mdanderson.org, cc: dan.merrick@cuanschutz.edu, HKadara@mdanderson.org, jfujimoto@mdanderson.org, erinkane@bu.edu, mazzilli@bu.edu

SECTION B.2.1 TISSUE IN FORMALIN OR FFPE BLOCKS*

*If preparing shipping for prospective longitudinal LUSC-PML cohort refer to the *Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual*

1. Upload all required biospecimen and clinical data for the case to be shipped to synapse or Lung PCA portal
2. Complete all fields in the provided shipping manifest
    a. PCA ID sample # (PID)
    b. Sample type
    c. Confirm required biospecimen and clinical data has been uploaded
3. In a **cooler box (top if using CU shipper)**, place in a specimen transport bag with zorbs and seal each specimen bag removing as much air as possible:
    a. Biohazard bag containing: (For all collections)
        i. Formalin Fixed Samples – ensure caps are sealed before shipping.
4. Record the FedEx tracking number and the date shipped
5. Close and seal the cardboard box with packaging tape.
6. Ship immediately following packaging. Make certain package is available at FedEx pick up site to ensure overnight shipment and next day delivery.
7. Email notification of shipment including the FedEx tracking number In the subject line of the email, include: **PCA Study, Site ID#**, **PID #, Fresh Shipment**
    a. CU → to BCF.Schedule@cuanschutz.edu, cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu
    b. MDACC → to BSanchez2@mdanderson.org, cc: dan.merrick@cuanschutz.edu, HKadara@mdanderson.org, jfujimoto@mdanderson.org, erinkane@bu.edu, mazzilli@bu.edu

SECTION B.2.2 FFPE TISSUE SECTIONS

1. Upload all required biospecimen and clinical data for the case to be shipped to synapse or Lung PCA

portal

2. Complete all fields in the provided PCA shipping manifest
    a. PCA ID sample # (PID)
    b. Sample type
    c. Confirm required biospecimen and clinical data has been uploaded
3. When ready to ship, using a **cooler box (bottom if using CU shipper)** add frozen cold packs to the bottom
4. Package slides into the slides/coverslips in a box with padding material on top to make sure nothing breaks in transit.
5. Tape the slide box closed and place the slide box into the Ziploc bag.
6. Record the FedEx tracking number and the date shipped
7. Close and seal the cardboard box with packaging tape.
8. Affix the FedEx shipping label and Recipient label to the box.
9. Ship immediately following packaging. Make certain package is available at FedEx pick up site to ensure overnight shipment and next day delivery.
10. Email notification of shipment including the FedEx tracking number In the subject line of the email, include: **PCA Study, Site ID#**, **PID #, Fresh Shipment**
    a. CU → to BCF.Schedule@cuanschutz.edu, cc: dan.merrick@cuanschutz.edu, erinkane@bu.edu, mazzilli@bu.edu
    b. MDACC → to BSanchez2@mdanderson.org, cc: dan.merrick@cuanschutz.edu, HKadara@mdanderson.org, jfujimoto@mdanderson.org, erinkane@bu.edu, mazzilli@bu.edu
    c. SU → to nmukher1@stanford.edu, cc: dan.merrick@cuanschutz.edu, HKadara@mdanderson.org, erinkane@bu.edu, mazzilli@bu.edu

Appendix C: Bronchial Biopsy Dissociation and Sorting Protocol

Bronchial Biopsy Dissociation and Sorting Protocol

MATERIALS

- DPBS (no calcium, no magnesium) (Gibco 14190-250 or Corning CellGro #21-031-CV)
- FBS (ThermoFisher 10438018)
- HEPES 1M (Gibco 15630-080)
- UltraPure™ 0.5M EDTA (Gibco 15575)
- RPMI- no phenol red (ThermoFisher 11835055)
- Complete RPMI - with 10% FBS (filtered).
- Falcon 70um cell strainers (Falcon 352350)
- Petridishes 60mm (any brand)
- 3-ml syringe plunger with flat back (or equivalent device)
- Liberase TL Research Grade (Roche), (Millipore Sigma 05401020001)– reconstitute 1 vial with 300 uL sterile H_2O. Per biopsy need 50 uL Liberase in 2 ml RPMI. Aliquot 50 uls and keep at -20C.
- 5mL sterile polystyrene round-bottom culture tubes with cap (VWR 60818-565).
- Filter bottles (0.2 um), sterile, any brand or style to fit the volume.
- Sort buffer and Wash buffer:

Sort Buffer (store at 4 °C)	Wash Buffer (store at 4 °C)
PBS (Ca/Mg free)	PBS (Ca/Mg free)
5mM EDTA	5mM EDTA (5 ml/ 500 ml)
25mM HEPES	25mM HEPES (12.5 ml / 500 ml)
1% FBS	0.2um filter sterilize
0.2um filter sterilize	

Cell Staining

Note: the fluorochrome combination is dependent on your instrument configuration so these choices are just for guidance. Do not omit anti-CD235a.

- APC anti-human CD235a (Glycophorin A) antibody (BioLegend 306607) – Lot B242428. Titrated use: Dilute 1:100 to make working stock, from this use 5 ul per sample (<5 million total cells). If the sample appears bloody and a lot of RBCs are visible in the pellet, double or triple the amount.
- APC-Fire750 anti-human CD326 (EpCAM) (BioLegend 324234) – Lot B223438. Titrated use: Dilute to ¼ to make working stock, from this use 5 ul per sample (or use 1.25 ul directly).
- BV510 Anti-Human CD45 (BD Horizon 563204) – as titrated (lots vary).
- DAPI (Sigma Cat. #D9542) 0.5 ug/ml in ddH_2O.
- Filter cap FACS tubes (BD Falcon 352235).

Cell Sorting

- NP-40 collection solution for RNA plates:

  - 1% NP-40 SurfactAmps™ Detergent Solution (ThermoFisher 85124, 10% stock)
  - 5% RNaseOut™ Recombinant Ribonuclease Inhibitor (ThermoFisher 10777019, 5000U/vial, 40U/ul, 125ul/vial).
  - UltraPure™ DNase/RNase-free Distilled Water (ThermoFisher 10977023)
  - Mix: 60 ul NP-40 solution + 30 ul RNaseOut + 510 ul UPwater (600 ul total) to fill two 96-well plates using 2 ul/well. For each additional plate use 200 ul more (a trough needs ~200 ul dead volume with this solution, 150 ul is usually not enough).
- REPLI-g Single Cell Cryo-Protect Reagent for DNA plates (Qiagen 150370).
- Trizole (or Qiazole) or equivalent.
- MicroAmp® Optical 96-Well Reaction Plate with Barcode (ThermoFisher 43-067-37, originally Applied Biosystems).
- Microseal 'F' foil seals (Biorad MSF1001, rated to -70C).

Equipment

- Cell sorter (BD FACSAria II) with Diva 8.0 (capable of index sorting) – or equivalent
- Mini Plate Spinner (Fisher Scientific #14100143)
- Multichannel pipettor (p50 or smaller, accurate in range 2-10 ul).
- Dry ice

PROCEDURE

Sample Collection:

- Collect sample in 1-2 mL of RPMI in 1.5mL cryovial. * Post-collection, if waiting is required before sample prep (1-4 hours), keep sample at 4 °C. Samples can be stored overnight IF the cryovial is filled up to the top with complete medium (RPMI/10% FBS added to the existing medium) immediately upon receipt. This may not work for very small biopsies.

Sample Dissociation:

1. Remove most of the storage medium from the biopsy in the original cryovial (be careful not to remove pieces of biopsy) and transfer to a clear tube (i.e. 5ml polystyrene FACS tube) using a P1000. Inspect the solution and put any visible not-bloody clumps back into the original vial. If any clumps are mainly bloody, they can/should be added to this tube.
2. Transfer the solid biopsy pieces to another clear tube with 1 ml RPMI (no FBS). Use some of the RPMI to wash out the original cryovial to make sure there are no more fragments in the vial. Pipet up and down to wash the biopsy of loosely associated blood cells, but make sure that the biopsy does not get lodged in the pipet tip. Then transfer all medium but not the biopsy pieces to the other clear tube that contains the storage medium. This together is called the "Wash".
3. Add 1 ml **Liberase** in RPMI to the tube with the biopsy pieces.
4. Using the same P1000, gently transfer the biopsy pieces in Liberase to a 15 ml tube labeled with the sample number and "BT" (for Biopsy Tissue).
5. Incubate in waterbath at 37C for 20 mins – swirl up every few minutes (original protocol states to use a shaking waterbath but this works too).
6. Meanwhile, spin the "Wash" tube (all spins 1500 rpm ~400 g x 5 min at RT).
7. Remove most of the sup from the "Wash" tube by aspiration, add 1 ml Liberase in RPMI, resuspend, and transfer to a second 15 ml tube labeled with the sample number and "W" (for Wash). Also place this tube in the waterbath for the remainder of the original 20 minutes (usually 10-15 minutes).
8. After incubation, add 10 ml Complete RPMI (with serum) to both tubes, mix gently by inversion.