APPENDIX F: Tissue Microarray Generation

SECTION F.1 TMA for Biopsy & Resection Cohorts

SECTION F.1.1 Block and Sample ID Generation

SECTION F.1.2 H&E Annotation

SECTION F.1.3 Coring, Assembly and Sectioning.

SECTION F.1.1 Block and Sample ID Generation

TMA Block IDs:

Each TMA block will require a unique **tma_block_ID** to be created and **tma_block template or GUI section to be complete**. TMAs may contain cores from 1 or more participants. Below are examples for resected and biopsy tissue cases where the TMA is from a single or multiple (use participant (site) 9999 for participant when multiple) participants.

For each ***tma_block_ID*** you will enter the following in either a ***LPCA_biospecimen_TMA_block template (under Data Standers> biospecimen> templates)*** or enter into the ***Lung PCA Registry***.

1. Program : **PCA**
2. Cohort: **Retrospective Cross-sectional LUSC**
3. tma_block_ID: ie - **HTA3_11013_2901901 * (see example below)**
4. Number of cores: 1-20
5. Processing location
6. TMA_core_coordinates_control_row_1 (ie location of Tonsil): **A**
7. TMA_core_coordinates_control_column_1 (ie location of Tonsil):**5**
8. TMA_core_coordinates_control_row_2 (ie location of Tonsil):**E**
9. TMA_core_coordinates_control_column_2 (ie location of Tonsil):**5**
10. TMA_creation_date: **11-01-2023**

TMA_Block_IDs * ***for each TMA should be entered as a biospecimen and requiries:***

Sample type	Example tma_block_ID	*Study ID* (PCA=HTA3)	*Participant ID* (site & participant ID)	*Block ID* TMA FFPE	tma_block_ID
TMA has ONLY 1 participant use participant ID					
FFPE Res. Tissue	HTA3_1XXXX_ 29XXXXX	*HTA3*	*11013*	*2901901*	HTA3_11013_2901901
TMA has more than 1 participant use participant 9999					
FFPE Res. Tissue	HTA3_19999_ 29XXXXX	*HTA3*	*19999*	*2900001*	HTA3_19999_2900001

Core IDs:

For each **TMA core** to be punched create a unique *"**derivative" biospecimen_ID** **using the LPCA_biospecimen_TMA_core_Template(under Data Standers> biospecimen> templates)*** or enter as a biospecimen into the ***Lung PCA portal/Registry***.

1. For each ***"derivative" biospecimen_ID*** *for a TMA core*, you will provide the TMA specific details:
    1. parent_biospecimen_ID (biospecimen ID of the block cored)
    2. biospecimen_id
    3. **biospecimen_id_local**
    4. Processing location
    5. Protocol
    6. Processing days from index
    7. tissue_type (ie premalignant/ tumor/ normal) *(derivative)*

8. TMA_block_ID
9. TMA_core_coordinates_row
10. TMA_core_coordinates_column
11. morphology *(derivative)*
12. preinvasive_ morphology *(derivative)*

SECTION F.1.2 H&E Annotation

As descried in the Cohort Guidelines, following the sectioning for isolation of RNA & DNA make a new H&E section made (just prior to coring), work with site pathologist to annotate (on slide or image) areas that are to be cored – image file will be required and annotations to be shared via scanned image or annotated within the portal. This confirms the tissue is still adequate, inadequate or intermediate (*using the same schema described when sectioning samples*) to confirmed there is still Dysplasia / cancer / normal left after cutting slides for DNA and RNA.

SECTION F.1.3 TMA Coring, Assembly and Sectioning.

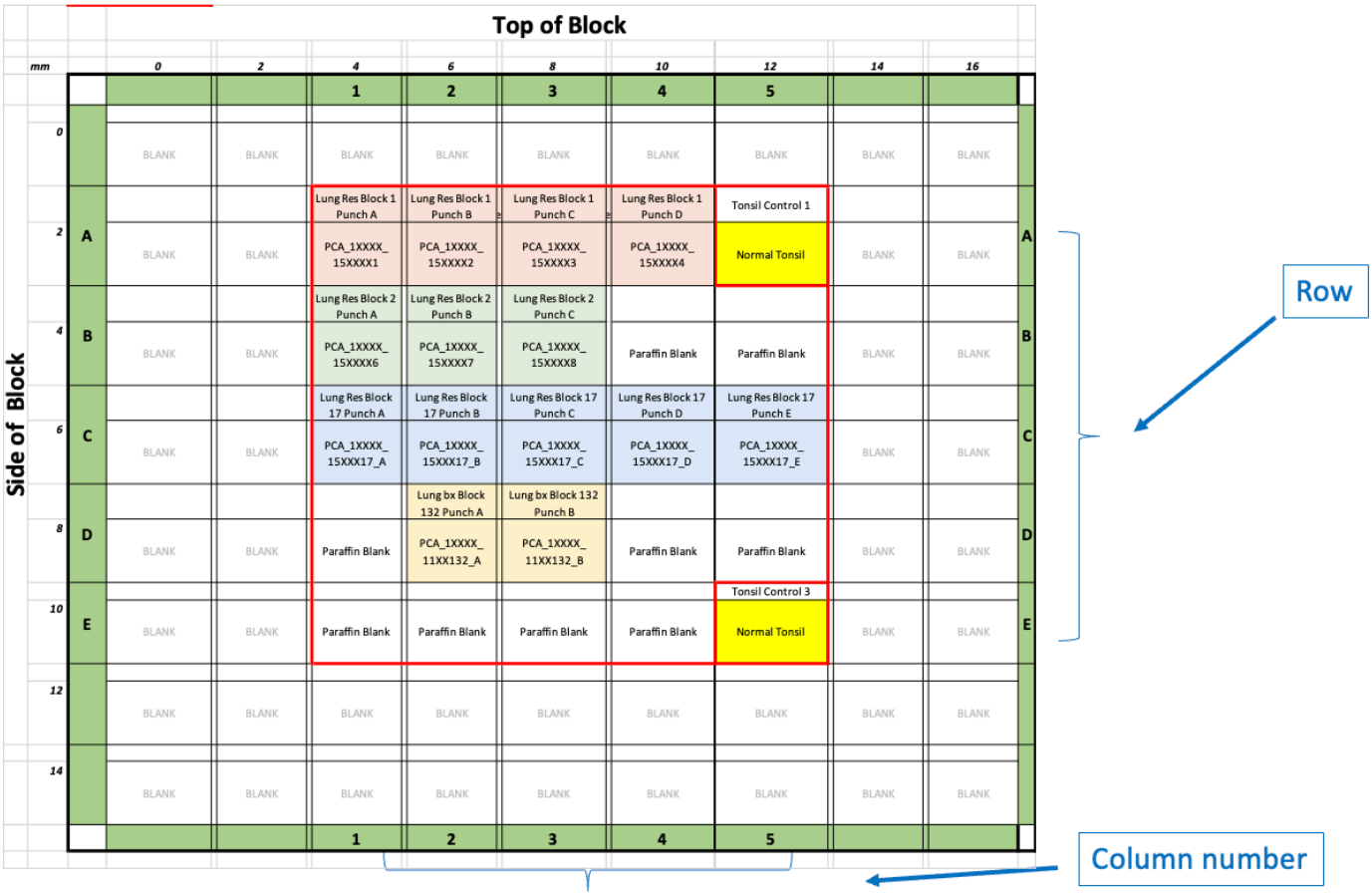
The TMAs will be utilized for CODEX, possible IF, IMC or Spatial Transcriptomics so we would like the keep all TMAs at Using a 1mm punch/corer 5x5 square trying to keep with in ~ 12.5x12.5 mm total. This will allow for sectioning on to any of the needed slides. Generally, more than one punch/core (3-5/lesion) are taken for each lesion area to ensure the same diagnosed histology is represented throughout the core once sanctioned. Blocks can then be sent to the core repository for sectioning for downstream assays. ****For coordinate mapping see map below,*** keep the cores together in the block when possible as to not leave large paraffin areas in the middle to prevent tissue loss or cracking.

General Coring Protocol:

1. Create map and obtain representative H&Es from all donor blocks (donor area circled if needed)
2. Pour blank recipient block
3. Face recipient and donor blocks (skip donor facing if target lesion very small).
4. Affix blank recipient block into arrayer.
5. Remove wax core at first position from recipient block
6. Place donor block on platform and extract core from marked area
7. Place core into hole in recipient block.
8. Repeat for all positions
9. Anneal completed TMA in an oven at a temperature slightly below wax melting point.
10. Face and section TMA (very carefully)

Sectioning of TMA: Cut 2 sections for Codex – Send BU and email to coordinate shipping to the Stanford team, If material is sufficient cut 3 additional slides to send to the BU team.

Core Mapping:



Core Coordinate	TMA Biospecimen ID	organ_type	parent ID	Core ID	tissue_type	*Histology (morphology or preinvasive morphology)*
A - 1	HTA3_11013_2901901	Lung	HTA3_11013_151001	HTA3_11013_1501823	Primary	*Squamous cell carcinoma, Non-Keratinizing*
A - 2	HTA3_11013_2901901	Lung	HTA3_11013_151001	HTA3_11013_1501824	Premalignant	Moderate dysplasia
A - 3	HTA3_11013_2901901	Lung	HTA3_11013_151001	HTA3_11013_1501823	Premalignant	Moderate dysplasia
A - 4	HTA3_11013_2901901	Lung	HTA3_11013_151001	HTA3_11013_1501824	Non-tumor (adjacent normal)	normal