Using Spatial Transcriptomics to Discover Genetic Causes of Extranodal Extensions

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Background

Methodology Background – Visium FFPE

- Combines single cell transcriptomic data and tissue staining to consider space as a variable in gene expression[2]
- **Gene expression results and dimensionality reduction clustering trace back to exact spots on the tissue[2]**

Image   Visium Data   Whole Transcriptome Data
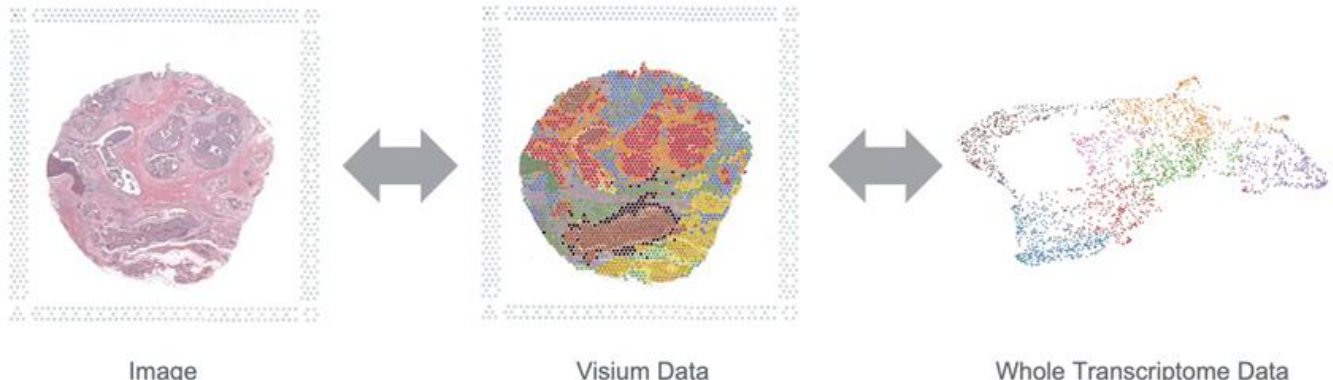
Figure 1. Visium FFPE allows for data analysis to start from histology images and/or from transcriptome clustering[2]
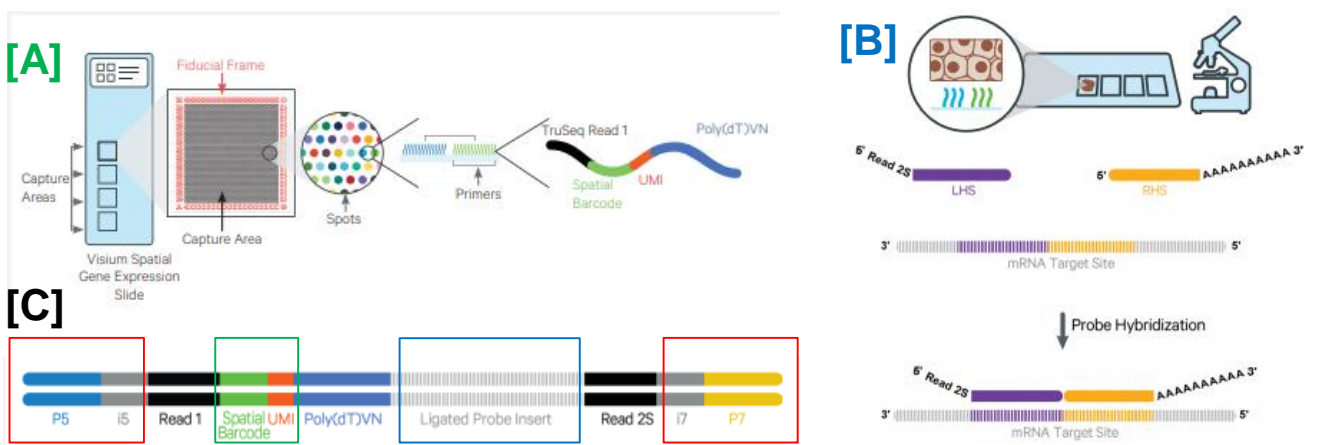
Figure 2. [A] Each capture area has 5000 spots, each spot with a unique spatial barcode and each probe with a unique UMI [B] Human genome probes enter the tissue to bind to target mRNA site, and they later bind to the spatial barcodes [C] finished library consists of unique sequencing indexes, spatial information from the slides, and genomic information from the human probes
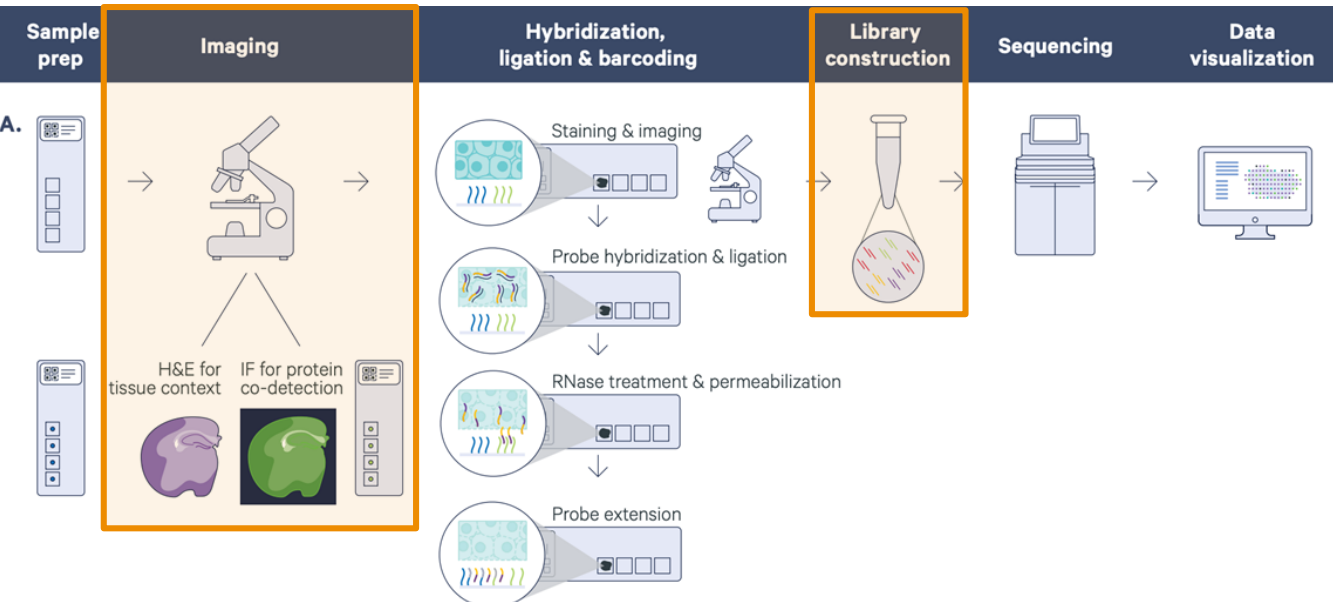
Figure 3. 10X Genomics Visium FFPE protocol overview[2]. Two sets of results will be generated from this protocol (highlighted boxes) -- H&E stains and library of transcriptomes. These two sets will be compiled using SpaceRanger and visualized using the Loupe Browser.

Clinical Background on Extranodal extensions (ENE)

- ENE are essential diagnostic tools to determine tumor aggressiveness[1]
- ENE is the metastasis of neoplastic cells through the lymph node capsule into the perinodal adipose tissue; the genetic causes need to be further studied[1]
- **Signals worse prognosis and elevated occurrence rates, thus ENE significantly impacts treatment plan for patient[1]**
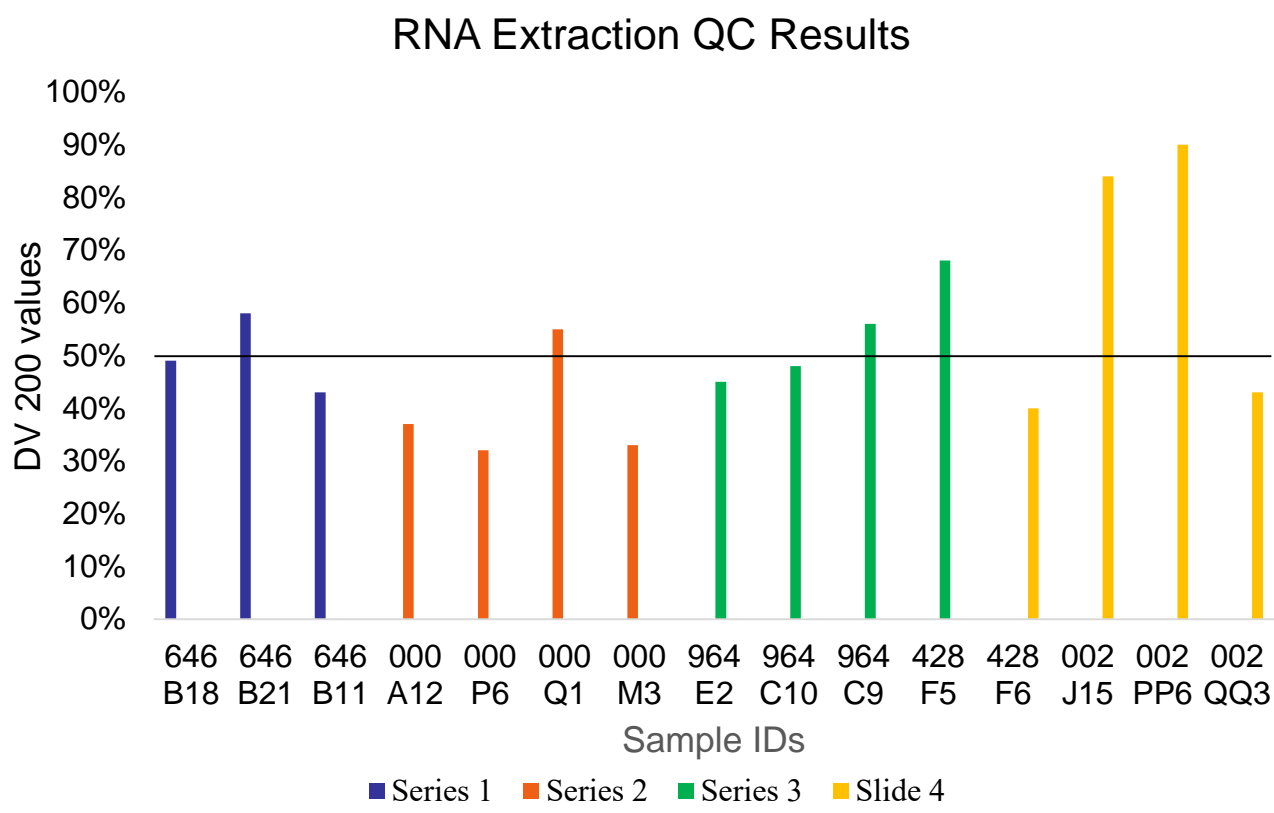
Hypothesis

Visium FFPE can be used to characterize gene expression variability contributing to the formation of Extranodal Extension (ENE) in head and neck cancers.
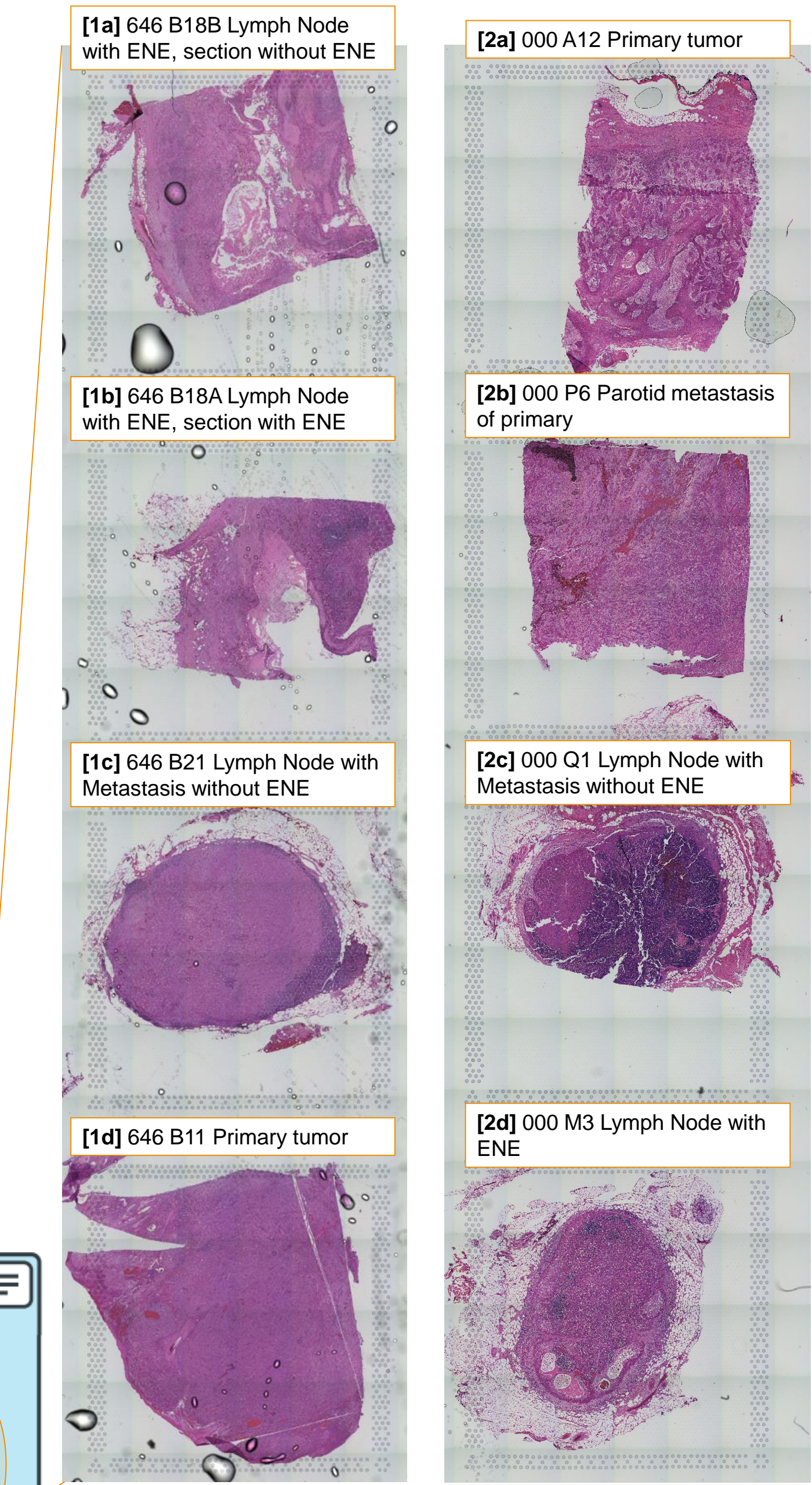
Methods
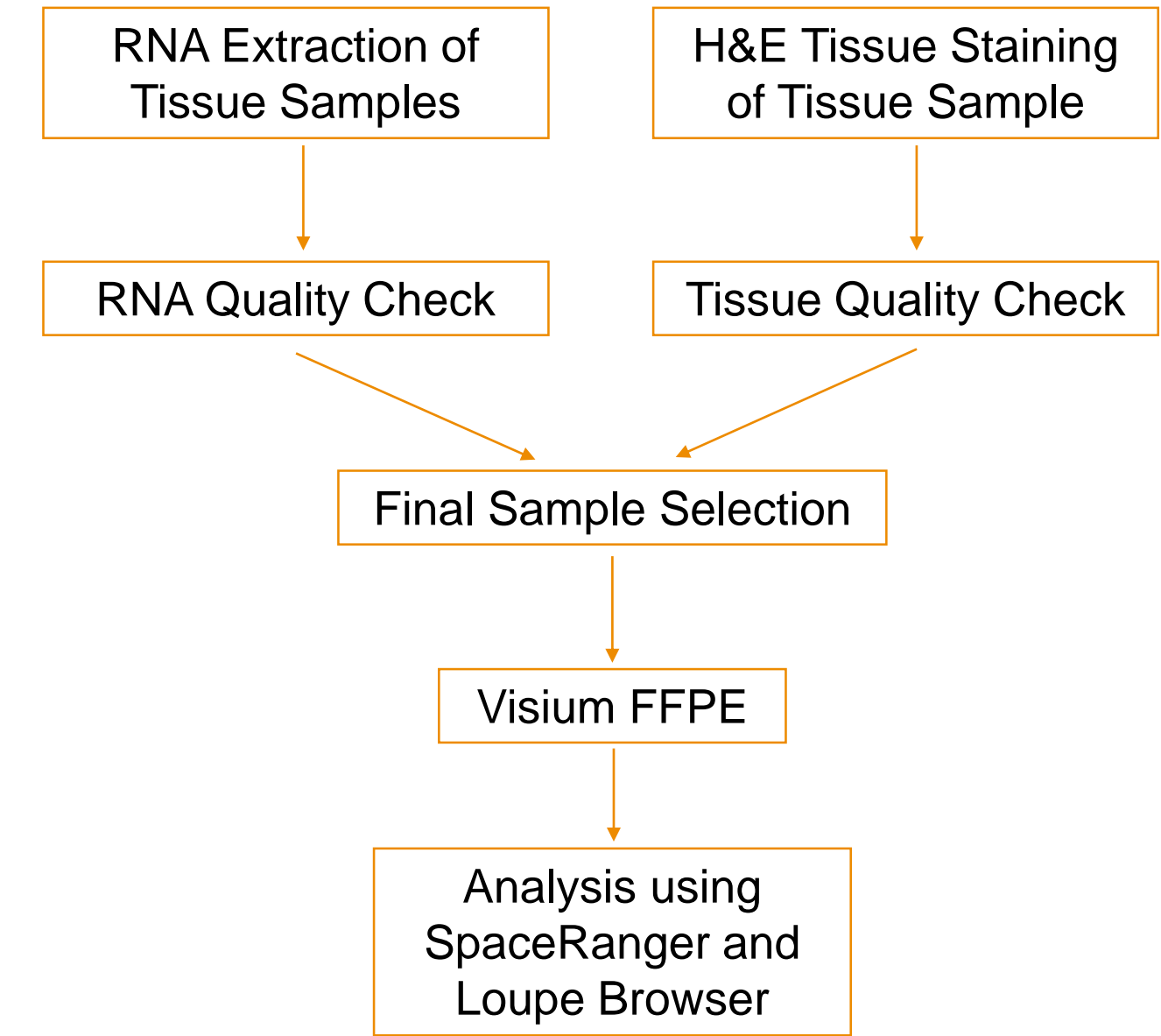
Figure 4. Flow chart of work flow for Visium FFPE used to analyze ENE.

Results

Graph 1. RNA Quality check. DV 200 score gives % RNA with at least 200 nt. Ideally for Visium FFPE, DV 200 for sample is at least 50% (threshold line). Candidates for Slides 3 & 4 have been identified.
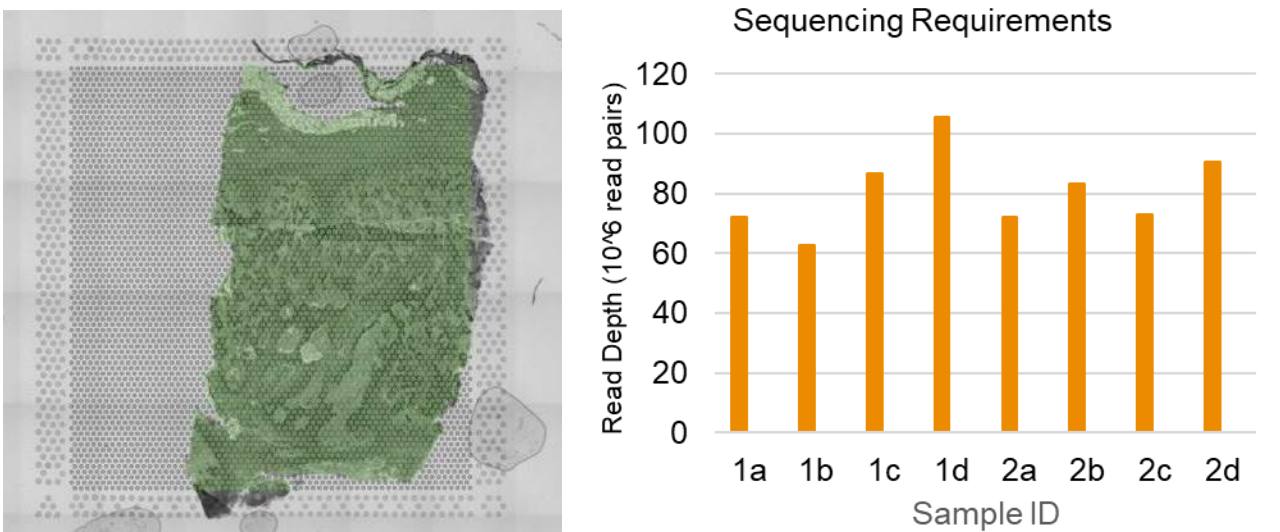
Results (continued)

[1a] 646 B18B Lymph Node with ENE, section without ENE

[1b] 646 B18A Lymph Node with ENE, section with ENE

[1c] 646 B21 Lymph Node with Metastasis without ENE

[1d] 646 B11 Primary tumor

[2a] 000 A12 Primary tumor

[2b] 000 P6 Parotid metastasis of primary

[2c] 000 Q1 Lymph Node with Metastasis without ENE

[2d] 000 M3 Lymph Node with ENE

Figure 5. Slide 1 and Slide 2 H&E images from during Visium FFPE

Results (continued)

Sequencing Requirements

Slide	Sample	%coverage	Read Depth (10⁶ read pairs)
1a	646 B18B	57.66	72.075
1b	646 B18A	50.26	62.825
1c	646 B21	69.34	86.675
1d	646 B11	84.54	105.675
2a	000 A12	57.58	71.975
2b	000 P6	66.64	83.3
2c	000 Q1	58.3	72.875
2d	000 M3	72.4	90.5

Figure 6. Using Loupe Browser, tissue coverage % was calculate for each frame. Example alignment on Loupe shown for sample 2a.
Table 1. Using the coverage% found with Loupe the necessary Read Depth for each sample was calculated for further sequencing.
Graph 2. Graphical representation of Read Depth for each sample

Conclusions

The innovative Visium FFPE technique has been a necessary development to fully understand the magnitude of gene expression variance contributing to the development of ENE. Previous technologies only allow for sequencing of transcriptomes at the single-cell level without considering the cells' locations in the tissue. In the future, using Visium FFPE will help isolate a transcriptome or a gene expression motif correlated and possibly contributing to the development of ENE. Clinically, this discovery will help with accurate characterization of tumor aggressiveness, significantly impacting treatment decisions.

References

1. Aziz, S., Wik, E., Knutsvik, G., Klingen, T. A., Chen, Y., Davidsen, B., Aas, H., Aas, T., & Akslen, L. A. (2017).. PloS one, 12(2), e0171853.https://doi.org/10.1371/journal.pone.0171853
2. Spatial Gene Expression. 10x Genomics. (n.d.).https://www.10xgenomics.com/products/spatial-geneexpression.