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Spatial Gene Expression for Formalin-Fixed Paraffin-Embedding (FFPE)-Lung Tissue using 10x Genomics Visium Spatial Transcriptomics



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Cellular Senescence Net...



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We use this protocol and it's working

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Abstract

We present a method to map gene expression in formalin-fixed paraffin-embedded (FFPE) lung tissue sections using the 10x Genomics Visium Spatial FFPE v1 protocol. This approach utilizes whole-transcriptome probes to achieve precise localization of mRNA within tissue structures. By preserving tissue morphology and ensuring reliable detection, this protocol enables comprehensive studies of lung biology and pathology. The spatial transcriptomics data obtained offer valuable insights into gene regulation and cellular diversity, enhancing our understanding of lung tissue organization and disease mechanisms.

Preservation of the samples:

- 1 Store formalin-fixed paraffin-embedded (FFPE) lung tissue blocks at 4 degrees Celsius to preserve RNA integrity.

Tissue Quality Assessment:

- 2 RNA quality: High quality FFPE sections will have well-preserved RNA for accurate measurement.
 - 2.1 For Visium Spatial, the tissue preparation guide by 10x Genomics (**CG000408, Rev D**) recommends using the percentage of RNA fragments greater than 200 nucleotides (DV200) from extracted tissue sections for assessment.
 - 2.2 Place three sections of 10µm thickness each in a 1.5ml microcentrifuge tube and proceed to RNA extraction using Qiagen RNeasy FFPE Kit (Cat no. 73504) and following the the steps outlined in the RNeasy Handbook (**RNeasy Handbook**).
 - 2.3 Determine the DV200 value of your samples utilizing the Agilent 2100 bioanalyzer system.
- 3 Morphological integrity: Tissue sections should be free from excessive tearing, folding, or artifacts that could compromise spatial information.
- 4 Hematoxylin and eosin (H&E) staining: H&E staining allows visualization of cellular morphology and how it changes in response to disease, which can be used to interpret the spatial gene expression data.

Sample preparation:

- 5 To trim the tissue section of ≤6.5 x 6.5 mm and a thickness 5 µm for sectioning onto the Visium Spatial Gene Expression Slide, follow the tissue preparation guide by 10x Genomics (**CG000408, Rev D**).
- 6 Perform all deparaffinization, staining, imaging, and decrosslinking steps using the test slide according to the protocol described by the 10x Genomics (**CG000409, Rev C**).

Visium spatial v1 FFPE library construction:

- 7 To perform probe hybridization, ligation, release and extension, and library construction, follow the user guide by 10x Genomics (**CG000407, Rev D**).



- 8 After obtaining DNA libraries, follow the sequencing requirements of 10x Genomics (**CG000407, Rev D**).