

Jan 26, 2023

Spatial Transcriptomics for FFPE utilizing 10x Genomics Visium

 [Nature](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygx95ezg8j/v1>

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DOI: <https://dx.doi.org/10.17504/protocols.io.kxygx95ezg8j/v1>

External link: <https://doi.org/10.1038/s41586-024-08087-4>

Protocol Citation: Andrew Houston, Siqi Chen, Feng Chen 2023. Spatial Transcriptomics for FFPE utilizing 10x Genomics Visium . protocols.io <https://dx.doi.org/10.17504/protocols.io.kxygx95ezg8j/v1>

Manuscript citation:

Mo, CK., Liu, J., Chen, S. *et al.* Tumour evolution and microenvironment interactions in 2D and 3D space. *Nature* **634**, 1178–1186 (2024). <https://doi.org/10.1038/s41586-024-08087-4>



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Protocol status: Working

We use this protocol and it's working

Created: January 19, 2023

Last Modified: January 26, 2023

Protocol Integer ID: 75508

Keywords: spatial transcriptomics for ffpe, 10x genomics visium ffpe v1 protocol, utilizing 10x genomics visium, spatial transcriptomic, 10x genomics visium, 10x genomic, embedded tissue sample, ffpe, gene expression, tissue sample, gene

Abstract

To detect gene expression spatially mapped onto formalin-fixed paraffin-embedded tissue samples adapted from the 10x Genomics Visium FFPE v1 protocol.

Quality Assessment

- 1 Follow the tissue preparation guide as outlined below to determine if your starting FFPE tissue block is acceptable for the 10x Visium platform
Start [here](#)
Utilize Visium Tissue Section Test Slide (PN-2000460) to do a quality assessment of each sample
Be sure to trim a few sections and place in a 1.5ml tube for RNA quality check (no more than 40µm)
Follow the steps starting on pg. 10 of the above user guide
- 2 Perform all deparaffinization/staining/imaging steps utilizing the test slide
Follow the protocol as described by 10x [here](#)
- 3 Perform RNA extraction using the Qiagen RNeasy FFPE kit (Cat no. 73504)
Follow the steps outlined in the RNeasy Handbook which can be found below
 HB-0375-006_HB_RNeasy_FFPE_0...
Start with your pre-cut sections in a 1.5ml tube on pg. 15
- 4 After RNA extraction test the concentration of your samples using a nanodrop
Determine the DV200 value of your samples utilizing the Agilent 4200 TapeStation system or similar quantitative assessment
If utilizing Agilent TapeStation to prepare your RNA samples utilize this [guide](#)
For first time analysis of DV200 you can set up your parameters as described [here](#)

Visium FFPE v1

- 5 If the tissue adheres to the slide and has a DV200 value >50% that sample can be used for the Visium Spatial Transcriptomics platform
- 6 Utilize the CG000408 linked above to section onto the Visium Spatial Gene Expression Slide (PN-2000233)
- 7 After sectioning start the protocol utilizing CG000409 linked above to perform deparaffinization/staining/imaging steps
Be sure to either store your slide or perform decrosslinking to proceed with the rest of the protocol outlined in the next step
- 8 If decrosslinking is complete, utilize the following user guide outlined [here](#)
Start probe hybridization on pg. 39



- 9 Follow the sequencing requirements on pg. 60 to generate your Visium libraries