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Protocol status: Working
We use this protocol and it's working

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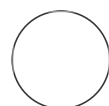**Last Modified:** Jan 26, 2023

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75508

🌍 Spatial Transcriptomics for FFPE utilizing 10x Genomics Visium

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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_0721_WW (1).pdf

     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