



Apr 22, 2025

Protocol for performing spatial transcriptomics in engineered human skin constructs using the 10X Genomics Visium Cytassist FFPE pipeline.

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygx75n4l8j/v1>

Alberto Pappalardo¹

¹Columbia University



Alberto Pappalardo

Columbia University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kxygx75n4l8j/v1>

Protocol Citation: Alberto Pappalardo 2025. Protocol for performing spatial transcriptomics in engineered human skin constructs using the 10X Genomics Visium Cytassist FFPE pipeline.. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.kxygx75n4l8j/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: April 20, 2025

Last Modified: April 22, 2025

Protocol Integer ID: 131008

Keywords: engineered human skin construct, engineered skin construct, 10x genomics visium cytassist ffpe pipeline, human skin construct, skin construct, performing spatial transcriptomic, spatial transcriptomic, ffpe

Funders Acknowledgements:

NIH

Grant ID: CA275686

Abstract

This protocol describes sample processing with the 10X Genomics Visium Cytassist FFPE pipeline, for the analysis of formalin-fixed paraffin-embedded (FFPE) human engineered skin constructs (HSCs) sections stained with H&E.

Guidelines

Depending on the source of cells employed in HSCs, this protocol may require prior approval by the users' Institutional Review Board (IRB) or equivalent ethics committee(s).

Summary

- 1 This protocol describes sample processing with the 10X Genomics Visium Cytassist FFPE pipeline, for the analysis of formalin-fixed paraffin-embedded (FFPE) human engineered skin constructs (HSCs) sections stained with H&E.

Tissue preparation

- 2 Fix the tissue samples - HSCs - in 4% paraformaldehyde (PFA) diluted in phosphate buffered saline (PBS) for 12-16 hours at 2-4 °C.

Wash and dehydrate the samples through progressive immersion in the following solutions at room temperature under agitation:

- PBS for 30 minutes (1)
- PBS for 30 minutes (2)
- RNase free water for 30 minutes
- 50% ethanol (molecular grade) diluted with RNase free water for 30 minutes
- 70% ethanol for at least 2 hours (or longer at 4 °C - up to 1 month)
- 85% ethanol for 30 minutes
- 95% ethanol for 45 minutes (1)
- 95% ethanol for 60 minutes (2)
- 100% ethanol for 30 minutes (1)
- 100% ethanol for 45 minutes (2)
- 100% ethanol for 60 minutes (3)
- 100% xylene for 30 minutes (1)
- 100% xylene for 30 minutes (2)

Then proceed with sequential immersion in liquid paraffin at 60 °C:

- paraffin for 30 minutes (1)
- paraffin for 30 minutes (2)
- paraffin for 60 minutes (3)

If using different tissues than HSCs, particularly intact human/animal tissue, consider increasing the incubation time indicated above according to the density of the tissue (double the time for intact human skin biopsies).

Embed the samples in paraffin block and proceed to sectioning.

Section the samples at 5 µm according to the 10X Genomics handbook CG000660 for FFPE blocks (section) and perform RNA quality analysis.



Nexterion H 3-D glass slides (Schott North America Inc #NC0782819) can be used to achieve a permanent tissue immobilization, which is very helpful with collagen-rich tissues such as the skin. **Warm up the Nexterion slides at room temperature for 30 minutes before using.**

Hematoxylin and eosin staining

- 3 Deparaffinize the tissue and perform the H&E staining as described in the 10X Genomics handbook (CG000658).

Scan the stained tissue section at 400X magnification. We recommend the Leica Aperio AT2 system for its high throughput performance, with the ability to scan entire tissue sections fitting the Visium HD dimension (6.5 × 6.5 mm) in less than one minute at 400X magnification.

After scanning, immediately demount the slide bearing the section and proceed to decrosslink as described, or dry and store in the dark at 4 °C for up to 2 weeks. If the slide will be stored, we recommend using a vacuum sealed packaging to isolate the slide holder, and include silica gel packs inside to absorb humidity.

Spatial transcriptomics with 10X Genomics Visium Cytassist FFPE pipeline

- 4 Follow the standard 10X Genomics Visium Cytassist FFPE pipeline according to the 10X Genomics handbooks CG000658 and CG000494.