Jun 03, 2025 Version 2

Protocol for combining immunofluorescence and hematoxylin and eosin staining, and spatial transcriptomics using the 10X Genomics Visium HD pipeline. V.2

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbr9e3lpk/v2

Alberto Pappalardo[1]

[1]Columbia University

Alberto Pappalardo
Columbia University

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbr9e3lpk/v2

Protocol Citation: Alberto Pappalardo 2025. Protocol for combining immunofluorescence and hematoxylin and eosin staining, and spatial transcriptomics using the 10X Genomics Visium HD pipeline.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbr9e3lpk/v2Version created by **Alberto Pappalardo**

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

Created: June 02, 2025

Last Modified: June 03, 2025

Protocol Integer ID: 219370

Keywords: 10x genomics visium hd pipeline, engineered skin construct, immunofluorescence, combining immunofluorescence, combination of immunofluorescence, skin construct, spatial transcriptomic, cell signaling technology 2947, antibody, hematoxylin, eosin staining

Funders Acknowledgements:
NIH
Grant ID: CA275686

Abstract

This protocol allows for the combination of immunofluorescence staining, hematoxylin and eosin (H&E) staining, and spatial transcriptomics using the 10X Visium HD pipeline. **It can only be applied for tissue sections that do not require decrosslinking prior to immunofluorescence staining**.
We employed this protocol to analyze sections of formalin-fixed paraffin-embedded (FFPE) human engineered skin constructs (HSCs), which we stained with anti-p16 (SantaCruz #sc-1661) anti-p21 (Cell Signaling Technology 2947S) and anti lamin-b1 (LS-Bio LS-B11184) antibodies.

Summary

1 This protocol allows for the combination of immunofluorescence staining, hematoxylin and eosin (H&E) staining, and spatial transcriptomics using the 10X Visium HD pipeline. **It can only be applied for the analysis of tissue sections that do not require decrosslinking prior to immunofluorescence staining**.
We employed this protocol to analyze sections of formalin-fixed paraffin-embedded (FFPE) human engineered skin constructs (HSCs), which we stained with anti-p16 (SantaCruz #sc-1661) anti-p21 (Cell Signaling Technology #2947S) and anti lamin-b1 (LS-Bio #LS-B11184) antibodies.

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 ºC.

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 ºC - 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 ºC:
- 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 CG000684 for FFPE blocks (section) and perform RNA quality analysis.

To achieve a permanent tissue immobilization, we used the glass slides Nexterion Slide H 3-D (Schott North America Inc #NC0782819). **Warm up the Nexterion slides at room temperture for 30 minutes before using**.

Immunofluorescence staining

3 Deparaffinize and stain the sections with primary and secondary antibodies as described in the 10X Genomics handbook CG000684 (section 4. Deparaffinization, Decrosslinking, & IF Staining). **Skip the decrosslinking step between deparaffinization and immunostaining**.

See the table below for optimized antibody dilutions for HSCs sections.

	A	B	C	D	E	F
	Primary antibody	Manufacturer and SKU	Host	Dilution	Secondary antibody	Dilution
	P16	SantaCruz sc-1661	Mouse	1:400	Invitrogen # A32754	1:250
	P21	Cell Signaling Technology 2947S	Rabbit	1:400	Invitrogen # A32754	1:250
	Lamin B1	LS-Bio LS-B11184	Goat	1:100	Invitrogen # A32849	1:250

Immediatelly proceed to whole section scanning using a confocal microscope (e.g.; Leica Stellaris 5). We reccomend using a 200X magnification, with 2048×2048 pixel image tiles, speed 200 Hz, bit depth 16.

Immediatelly proceed to demounting, as described in the 10X Genomics handbook (CG000684), and to H&E staining.

Hematoxylin and eosin staining

4 After demounting, start the H&E staining as described in the 10X Genomics handbook (CG000684) from the post-deparaffinization step (i.e.; section immersed in RNAse-free water before applying the hematoxylin).

Scan the stained tissue section at 400X magnification. We reccomend 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, immediatelly demount the slide bearing the section and proceed to decrosslink as described, or dry and store in the dark at 4 ºC for up to 2 weeks. If the slide will be stored, we reccomend using a vacuum sealed packaging to encase the slide holder, and include silica gel packs inside.

Spatial transcriptomics with 10X Genomics Visium HD pipeline

5 From here, follow the standard 10X Genomics Visium HD pipeline according to the 10X Genomics handbooks CG000684 and CG000685.