

Feb 16, 2023

Version 1

TMA-TNP Section Map and Slide Processing - Phase 4 V.1

Forked from a private protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.ewov1o7w0lr2/v1>

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Protocol Citation: Heidi S Feiler 2023. TMA-TNP Section Map and Slide Processing - Phase 4. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.ewov1o7w0lr2/v1>

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Protocol status: Working

We use this protocol and it's working

Created: February 16, 2023

Last Modified: February 16, 2023

Protocol Integer ID: 77069

Keywords: biospecimen, FFPE, tissue processing, spatial mapping, TMA, Nanostring GeoMx, DSP, human tumor atlas tissue microarray tnp, breast tumor tma, breast tumor sample, tma section, anonymized breast tumor tma, tma sections for phase, tma design, custom tma design, tissue microarray, pantomics for custom tma ffpe block generation, custom tma design for this project, tma, custom tma ffpe block generation, basic clinical data, clinical sample description, tnp characterization

Funders Acknowledgements:

NCI Human Tumor Atlas Network

Grant ID: U2C CA233280

Abstract

Human Tumor Atlas Tissue MicroArray TNP (TMA-TNP)

The Tissue MicroArray (TMA) TNP will extend the SARDANA-TNP characterization and analytics methodologies for evaluation and validation on a large array of breast tumor samples that provide a broad spectrum of disease state and subtype. A commercially available anonymized breast tumor TMA was purchased and sections distributed to participating HTAN Centers. Some deidentified basic clinical data will also be provided. The participating HTAN Centers will characterize the FFPE specimens (e.g. by imaging) and generate a spatially resolved cell type/state census using each Center's method of choice. Data will then be recorded in a common repository to enable joint analysis.

This TNP has two specific Aims focused on (i) Data Collection and (ii) Data Coordination and Analysis. Individual Centers can participate in one or both aims.

The custom TMA design for this project was generated by Dr. Koei Chin (OHSU) and sent to Pantomics for custom TMA FFPE block generation and sectioning. The TMA design and clinical sample descriptions can be found here: dx.doi.org/10.17504/protocols.io.bn2fmgbn.

This protocol describes the procedure by which the OHSU OMS Atlas HTAN Center sectioned and distributed TMA sections for Phase 4 analysis.

Materials

MATERIALS

Thermo Fisher Superfrost Plus microscope slides: **Catalog No.12-550-15**

<https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-microscope-slides-2/1255015>

Preparation

- 1 Verify the identity of the FFPE block to be cut against written request for sectioning. The FFPE block (TMA1) will be utilized for TMA-TNP Phase 4.
- 2 Each slide was labeled with a unique OHSU Slide ID corresponding to the FFPE section map (below).

	A	B	C	D	E
	Slide Label Name	Slide #	Description	Institution	Thickness (µm)
	OHSU_TMA1_037	37	GeoMx DSP Assay (Nanostring) protein ½ TMA (top 4 rows)	OHSU	5
	OHSU_TMA1_038	38	GeoMx DSP Assay (Nanostring) WTA ½ TMA (top 4 rows)	OHSU	5
	OHSU_TMA1_039	39	GeoMx DSP Assay (Nanostring) protein ½ TMA (bottom 4 rows)	OHSU	5
	OHSU_TMA1_040	40	missing - bad section - Pantomics did not send to OHSU		5
	OHSU_TMA1_041	41	GeoMx DSP Assay (Nanostring) WTA ½ TMA (bottom 4 rows)	OHSU	5

Sectioning

- 3 Align block on microtome to minimize tissue loss.
- 4 Face into block at 5µm until full section of tissue is achieved.
- 5 Cut adequate ribbon at 5µm to cover all serial sections.



- 6 Mount tissue sections onto ThermoFisher Superfrost Plus slides to maintain serial order and orientation of sections. Sections for Nanostring must be placed in a shifted orientation to capture the cores of interest (2 sections/slides to be combined for full representation of TMA1).

Slide Processing and Shipping

12h 30m

- 7 All Slides are immediately baked in a 45 °C oven (~45-48C) for 2 hours prior to distribution.

- 8 In the lab, the slides are baked in an oven at 55 °C Overnight (12-16 hours) (except for #30-33 for Nanostring) , and then at 65 °C for 00:30:00 (30-45 min)

12h 30m

Note: Slides should be baked at 65 °C for at least 30 minutes.

The slides are stored in a 4 °C cold room until deparaffinization or shipping.

- 9 Slides were shipped on 4 °C ice packs by express shipping per the FFPE spatial map noted above.