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TMA-TNP Section Map and Slide Processing - Phase 3

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Human Tumor Atlas Tissue MicroArrary 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 3 analysis.

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biospecimen, FFPE, tissue processing, spatial mapping

protocol ,

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MATERIALS
Tanner slides

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 3.

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_024	24	H&E	OHSU	5
OHSU_TMA1_025	25	t-CycIF (Tumor Panel)	HMS	5
OHSU_TMA1_026	26	t-CycIF (Immune Panel)	HMS	5
OHSU_TMA1_027	27	mIHC (Discovery Panel)	OHSU	5
OHSU_TMA1_028	28	cmIF (G3A Panel) Cyclic IF	OHSU	5
OHSU_TMA1_029	29	cmIF (G3B Panel) Cyclic IF	OHSU	5
OHSU_TMA1_030	30	GeoMx DSP Assay (Nanostring) protein ½ TMA (top 4 rows)	OHSU	5
OHSU_TMA1_031	31	GeoMx DSP Assay (Nanostring) WTA ½ TMA (top 4 rows)	OHSU	5
OHSU_TMA1_032	32	GeoMx DSP Assay (Nanostring) WTA ½ TMA (bottom 4 rows)	OHSU	5
OHSU_TMA1_033	33	GeoMx DSP Assay (Nanostring) protein ½ TMA (bottom 4 rows)	OHSU	5
OHSU_TMA1_034	34	H&E	OHSU	5
OHSU_TMA1_035	35	extra	OHSU	5
OHSU_TMA1_036	36	extra	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 Tanner 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.

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

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.

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