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GeoMx Whole Transcriptome Atlas (WTA) Analysis of FFPE Human Term Placenta

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We use this protocol and it's working

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Abstract

This protocol describes the use of the GeoMx Digital Spatial Profiler, together with the Whole Transcriptome Atlas (WTA), for spatial transcriptomic analysis of FFPE human term placenta. It outlines slide preparation, manual processing, instrument operation, and region of interest (ROI) selection, with tissue-specific parameter adjustments noted where applicable. The workflow supports compartment-resolved transcriptomic profiling through antibody-guided segmentation and is intended to enable reproducible generation of spatially resolved gene expression data from placental tissue.

Guidelines

- Ensure that all solutions are made **RNase-free**.
- Thoroughly treat all surfaces and Coplin jars with **RNase AWAY** prior to use. High-contact areas - specifically those exposed to concentrated RNA probes **-** must be prioritized to prevent cross-contamination in subsequent runs or downstream library preparation.
- Equilibrate deionized formamide to Room temperature prior to opening to prevent atmospheric condensation and subsequent reagent degradation. Ensure conductivity is **<100 µS** (use a conductivity meter**).** To maintain optimal stability, aliquot upon receipt and store at Room temperature .
- Buffer R contains formamide; equilibrate this reagent to Room temperature prior to opening.
- Use Lite-safe tubes when preparing antibody cocktails.
- Exercise extreme caution when applying the HybriSlip. Precise alignment is critical; an offset coverslip can cause the hybridization buffer to escape its boundaries via capillary action during the overnight hybridization, resulting in a dry slide and failed assay.
- If the HybriSlip does not detach easily after hybridization, submerge the slide in 2X SSC-T. A brief immersion (several seconds) is typically sufficient for the coverslip to slide off. **Do not mechanically force** the removal, as this will result in irreversible tissue damage or detachment.
- Do not allow tissue sections to dry out at any point during slide preparation.
- Ensure instrument Buffers S and H have 20% volume or more prior to starting a run.

Materials

Equipment

Microtome
Tissue floating bath
Water bath
Vortexer
Picofuge
Thermal Cycler (BioRad cat # C1000 Touch with 96–Deep Well Reaction Module); others okay
Baking oven (Amazon # B006H8JNKC)
Digital thermometer (Amazon cat # B078KPHKZD)
5.5 quart digital steamer (Hamilton Beach cat # 37530Z)
RapidFISH Slide Hybridizer (Boekel Scientific cat # 240200)
GeoMx Digital Spatial Profiler (Bruker Spatial Biology)

Materials/reagents

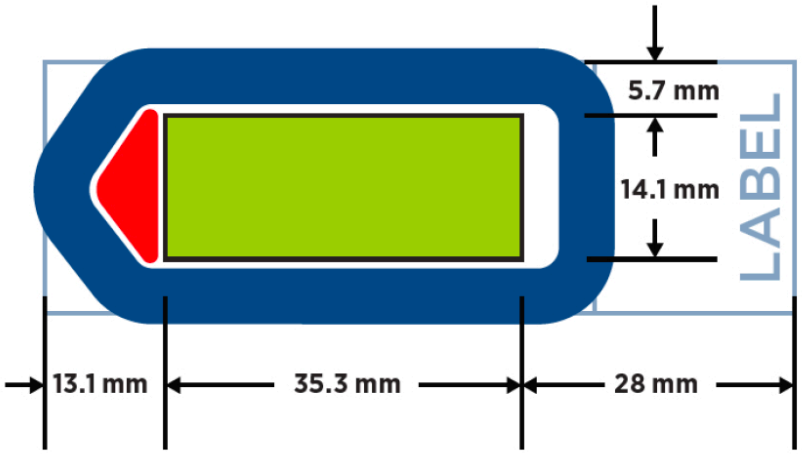
GeoMx DSP Instrument Buffer Kit (Bruker cat # 100474)
GeoMx DSP Collection Plate (Bruker # 100473)
GeoMx RNA Slide Prep Kit (Bruker # 121300313)
GeoMx Human Whole Transcriptome Atlas (Bruker # 121401102)
GeoMx Nuclear Stain Morphology Kit (Bruker # 121300303)
Cytokeratin, pan Antibody (AE-1/AE-3) [Alexa Fluor 532] (Novus Biologicals cat # NBP2-33200AF532)
HLA-G (4H84) Alexa Fluor 594 (Santa Cruz Biotechnology cat # sc-21799 AF594)
Alexa Fluor 647 Anti-CD31 antibody [JC/70A] (Abcam cat # ab215912)
Pipettes
Filter tips (DNase/RNase-free)
Microcentrifuge tubes (DNase/RNase-free)
Forceps (for slide handling)
Aluminum foil
Kimwipes
100% ethanol
FisherBrand Superfrost Plus microscope slides (Fisher cat # 12-550-15)
Plastic Coplin jars (Fisher cat # S89498)
StainTray Slide Staining System (Neta Scientific cat # SIM-M920-2)
HybriSlip hybridization covers (Sigma cat # GBL714022-100EA)
RNase AWAY (Fisher cat # 21-402-178)
DEPC-treated water (Fisher cat # 43-879-37)
10X phosphate buffered saline pH 7.4 (Sigma cat # P5368-10PAK)
10% neutral buffered formalin (NBF) (Fisher cat # 50-980-502)
100% deionized formamide (VWR cat # 97062-010)
20X SSC (DNase, RNase free) (Sigma cat # S6639-1L)

Proteinase K (Fisher cat # AM2546)
Antigen Retrieval Solution (Fisher cat # 00-4956-58)
Citrisolv (Fisher cat # 04-355-121)
10% Tween-20 (Fisher cat # 50-843-255)
Glycine (Sigma cat # G7126-100G)
Tris base (Sigma cat # 10708976001)
Benchtop protectors (Fisher cat # FB12007300)
Lite-safe tubes (Fisher cat # 03-391-161)
AeraSeal (Sigma cat # A9224-50EA)
Adhesive PCR foil sheets (Fisher cat # AB-0626)

Tissue preparation

1d

1 Using a calibrated microtome, trim the FFPE block face and section at 5 µm. Mount sections onto Fisherbrand Superfrost Plus microscope slides. Verify tissue placement is centered within the green scan area (see image below). Avoid the slide gasket (blue) or the tip calibration area (red) to prevent scanning errors.



Reproduced from the Bruker GeoMx DSP Manual Slide Preparation manual.

Tip: Print the slide template at **100% scale (actual size)** to use as a physical guide for verifying correct tissue placement during mounting.

2 Expel residual water trapped under the paraffin section by carefully incising the wax with a razor blade and dabbing with a Kimwipe, taking care not to touch the tissue.

3 Air dry at Room temperature Overnight For long-term storage, slide may be kept at 4 °C or Room temperature in a sealed container with a desiccant pouch.

Manual slide preparation

2d

4 Slides should be processed according to the manufacturer's instructions. The **GeoMx Manual Slide Preparation: RNA FFPE Quick Guide** is posted here for reference.

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Placenta-optimized parameters are as follows:

- **Baking:** 60 °C for 01:30:00 .
- **Target retrieval:** 100 °C in a vegetable steamer for 00:17:30 .
- **Proteinase K treatment:** [M] 1 µg/mL for 00:15:00 at 37 °C .
- **Hybridization:** 18:00:00 overnight at 37 °C .
- **Morphology marker staining:** see table below

GeoMx channel	Antibody/stain	Target	Final concentration
FITC	SYTO13	DNA	NanoString proprietary
Cy3	Pan-Ck-AF532	Pan-trophoblast	2 µg/ml
Texas Red	HLA-G-AF594	Mature extravillous trophoblast	2 µg/ml
Cy5	CD31-AF647	Endothelial cells	4 µg/ml

After the final 2x SSC wash, proceed to loading onto the instrument. Alternatively, slides can be stored in 2x SSC for up to 06:00:00 at Room temperature , protected from light; or in 2x SSC for up to 21 days at 4 °C , protected from light.

Instrument operation and ROI selection

1d

5 Slides should be loaded and scanned according to the manufacturer's instructions, and by following the onboard system prompts. The **GeoMx DSP Instrument Quick Guide** is posted here for reference.

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Scan settings:

GeoMx channel	Exposure time
FITC*	100 ms
Cy3	300 ms
Texas Red	300 ms
Cy5	300 ms

**FITC channel is selected for Auto Focus.*

Following the scan, review the channel intensities. Use the software's scaling tools to manually fine-tune the display settings if the default automated levels do not provide

sufficient contrast.

Initialize or assign a **Readout Group**, which will be the same for all collection plates in a planned sequencing run (max 8 collection plates per group).

6 **Regions of Interest (ROIs)** are selected based on morphology and antibody staining patterns. ROIs can be segmented into constituent **Areas of Illumination (AOIs)** using cell-type-specific segmentation rules based on antibody signal intensity. Once defined, **segmentation rules apply globally to all ROIs on a given slide**; however, channel thresholds can be adjusted locally to account for staining variability.

Segmentation Symbols & Rules:

When defining rules in the DSP software, each channel is assigned one of three logic gates to determine which pixels are included in an Area of Illumination (AOI):

- **Plus (+):** The segment includes only areas where expression is **above** the set threshold.
- **Minus (-):** The segment includes only areas where expression is **below** the set threshold
- **Theta (ø):** The channel is **ignored** for the purposes of generating that specific segment.

By fine-tuning these thresholds for each channel within individual ROIs, the resulting AOIs can be precisely optimized.

Placental Segment Definitions:

Segment Definitions:	DNA	Pan-Ck	HLA-G	CD31	
Segment	FITC 525nm	Cy3 568nm	Texas Red 615nm	Cy5 666nm	Color
Endothelial	ø	-	-	+	
Trophoblast	ø	+	-	ø	
Others	ø	ø	ø	ø	

Note: all three segments are automatically generated for each ROI, but individual segments may be deleted if not relevant.

Villus ROIs are segmented into:

- **Capillary Endothelial Cells:** Defined by the "Endothelial" rule. **Note:** Because red blood cells (RBCs) exhibit broad-spectrum autofluorescence - particularly in the Texas Red and Cy5 channels - a Texas Red (-) requirement is imposed. This effectively "gates out" RBCs in close proximity to the capillary lining, ensuring segment purity.
- **Syncytiotrophoblast:** Defined by the "Trophoblast" rule.
- **Villous Stroma:** Defined by the "Others" rule; a "catch-all" segment, generated last, that is neither trophoblast, nor endothelial. **Note:** When profiling villous stroma, the segment may overlay portions of the intervillous space. This is acceptable and does not compromise data quality, as the intervillous space is acellular and will not contribute RNA to the collection.

Extravillous trophoblast (EVT) ROIs: Selected based on morphology and location, and defined by the "Trophoblast" rule. While EVT express HLA-G, the intensity varies significantly between mature (strong) and immature (weak) populations. To ensure an unbiased collection of all EVT subtypes, the HLA-G threshold is set to the **maximum value (255)**. This effectively overrides the HLA-G (-) requirement, rendering the channel non-selective for this segment definition.

Vascular endothelial ROIs: Large vessels within stem villi are selected based on location, and defined by the "Endothelial" rule. These ROIs often require aggregation of multiple neighboring vessels to meet the minimum recommended area and nuclei count.

Advanced Parameter Tuning:

To further refine the AOIs, the following parameters are applied:

Advanced Parameter	Purpose	Segment Settings			Rationale
		Endo	Troph	Others	
Erode	The border width to be eroded from each segment; this effectively increases the boundary between segments. Increasing erode decreases segment size.	1 μm	1 μm	2 μm	A 2 μm erode for villous stroma ensures higher purity by avoiding "bleed" from neighboring cells.
Hole Size	Holes smaller than this size in the detected segment area will be filled and included in the segment. Increasing hole size increases total segment area.	1 μm^2	160 μm^2	160 μm^2	1 μm^2 preserves the thin, delicate structure of capillaries; 160 μm^2 maximizes area for bulkier segments.
Particle Size	Any small segment areas (particles) less than this size will be removed (despeckled). Increasing minimum particle size decreases total segment area.	5 μm^2	25 μm^2	25 μm^2	5 μm^2 captures fragmented capillary signals; 25 μm^2 despeckles other AOIs to ensure only robust cell populations are collected.
Collection Order	The default order in which segments will be illuminated and collected, starting with 1. This will be the same as the segment generation order but can be changed if desired. Generation order should be set in the order of increasing overlap. This means that more specific segments should be generated first and less specific segments last.	1	2	3	Prioritizes specific populations to minimize signal overlap. The villous stroma segment is generated last since there is no specific antibody included for fibroblasts.

N-Dilate: Adds segment area around detected nuclei. *This setting is only applicable for nuclear-tagged segments, and is not used here.*

Collection & Plate Storage

3h

7 Upon final approval of the ROIs, initiate the automated collection sequence.

- **AOI Collection:** Each individual segment or standalone ROI is defined as an Area of Illumination (AOI) and is aspirated into a unique well of a 96-well collection plate.
- **No Template Control:** If configured during run setup, a No Template Control (NTC) will be assigned to a dedicated well. This is helpful for assessing background noise levels and potential reagent contamination during the library preparation and sequencing workflow.
- **Plate Management:** The system supports collection of up to 95 AOIs, plus one NTC, per plate; the software will prompt for a plate change if the collection exceeds this limit.

Post-Collection Processing

Plates must be dried before library preparation. Use one of the following methods:

- **Overnight:** Allow plates to dry within the instrument.

- **Accelerated:** Seal the plate with a breathable (permeable) membrane and dry at 65 °C in a thermocycler with the **lid open** for up to an hour. Check regularly and remove when DSP aspirates are dried.

Once processed, proceed to NGS library preparation and sequencing.

Storage Guidelines

If library preparation is not performed immediately, seal the plate with adhesive foil to prevent evaporation or contamination, and store according to the following intervals:

- ≤ 24 Hours: Store at 4 °C.
- 24 Hours – 30 Days: Store at -20 °C.
- > 30 Days: Store at -80 °C.

Finalizing the Readout Group

8 A Readout Group should be finalized once an experiment is complete or when the maximum capacity of 8 collection plates (corresponding to the 8 unique GeoMx Seq Code index plates) has been reached.

Lookup Readout Group: From the DSP Control Center, click the plate icon (bottom right), and search for the target Readout Group.
Configure NGS Readout: Enter the following parameters for a NovaSeq 6000 run:
Counting Device Model: NovaSeq 6000
Counting Platform: Illumina
Read Strategy: Paired
Read Length 1: 27
Read Length 2: 27
i5 Sequence Orientation: Reverse
Index Assignment: For each collection plate, assign one of the eight GeoMx Seq Code Index Plates (A–H). Ensure these assignments match the physical plates to be used during library preparation.
Export: Click "Finalize & Download Readout Package."

Readout Package Contents:

The downloaded ZIP folder contains three critical files required for downstream processing:

- **config.ini:** The configuration file required by the GeoMx NGS Pipeline to process the FASTQ files.
- **LabWorksheet.txt:** A metadata manifest for each AOI, documenting the specific mapping of index plates to collection plates.
- **SeqCodeIndices.csv:** A reference file containing the i5 and i7 index sequences necessary for sample demultiplexing.

Upon finalization, proceed to library preparation and sequencing.

Protocol

NAME

GeoMx NGS Readout Library Preparation & Sequencing

CREATED BY

Scott Lindsay-Hewett

Preview

Protocol references

Quick guides were obtained from the NanoString University website (https://university.nanostring.com). The latest guidance and complete manuals are also available on the site, and certain procedures and descriptions are quoted directly from the original manuals.