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GeoMx NGS Readout Library Preparation & Sequencing

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We use this protocol and it's working

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Abstract

This protocol outlines the GeoMx Digital Spatial Profiling NGS readout workflow, including library preparation, quality control, NovaSeq 6000 sequencing, and conversion of FASTQ data into Digital Count Conversion (DCC) files for downstream spatial transcriptomic analysis.

Guidelines

- Thoroughly treat all surfaces 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.
- A **12-channel pipette** is recommended to streamline liquid handling, as DSP aspirates are deposited in row-wise format.
- Ensure that AMPure XP beads are at room temperature and thoroughly vortexed before adding to the pooled PCR product.

Materials

Equipment

Vortex
Picofuge
Plate spinner
Thermal Cycler (BioRad cat # C1000 Touch with 96–Deep Well Reaction Module); others okay
Qubit fluorometer (or similar device for library quantification)
Agilent Bioanalyzer (or similar capillary electrophoresis device for nucleic acid analysis)
Illumina NovaSeq 6000 (other Illumina NGS instruments okay)

Materials/reagents

GeoMx Seq Code Pack A - H (Bruker cat # various depending on number of indexes required)
Pipettes, including 12-channel P10 or P20
Filter tips (DNase/RNase-free)
Microcentrifuge tubes (DNase/RNase-free)
DNA LoBind tubes (for library storage before sequencing) (Eppendorf cat # 022431021)
96-well PCR plates (DNase/RNase free) to match thermal cycler specs
Magnetic stand
AeraSeal (Sigma cat # A9224-50EA)
Adhesive PCR foil sheets (Fisher cat # AB-0626)
PCR 12-strip tubes (Fisher cat # AB-1112)
PCR domed strip caps (Fisher cat # AB-0852)
RNase AWAY (Fisher cat # 21-402-178)
Nuclease-free water
100% ethanol
AMPure XP Beads (Beckman Coulter cat # A63880)
Elution buffer (Tris-HCl 10 mM with 0.05% Tween-20, pH 8.0) (Teknova cat # T1485)
Agilent DNA High Sensitivity Kit (Agilent cat # 5067-4626)
Qubit dsDNA High Sensitivity (HS) Assay Kit (Fisher cat # Q32851)
Illumina Sequencing Reagent Kit

Sample Reconstitution

1h 10m

1 If DSP aspirates are already **dry**, skip to Step 2. Otherwise, seal the plate with a breathable (permeable) membrane and dry at 65 °C in a thermocycler with the **lid open** for up to 01:00:00. Monitor periodically; remove immediately once wells are completely dry.

1h

2 **Rehydrate** samples with 10 µL nuclease-free water. Pipette mix 5 times and incubate at Room temperature for 00:10:00 to ensure full recovery. Perform a quick spin to collect the volume at the bottom of the wells.

10m

Library Preparation & Quality Control

5h

3 Library preparation should be performed according to the manufacturer's instructions. The **GeoMx DSP NGS Readout Quick Guide** is posted here for reference.

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Consult the **LabWorksheet.txt** from the relevant Readout Package to verify the correct mapping of **GeoMx Seq Code index plates** to the corresponding DSP collection plates.

Assess **library quality** using the Agilent Bioanalyzer High Sensitivity DNA assay. The library should appear as a discrete, narrow peak at ~150 bp, without the presence of primer dimers (<100 bp) or high-molecular-weight artifacts.

Determine final **library concentration** using the Qubit dsDNA High Sensitivity (HS) assay.

Illumina sequencing

4 **Sequencing Depth Calculation:** Calculate the required reads by identifying the **Total Area** (μm^2) profiled in the **LabWorksheet.txt** and multiplying by a factor of 100.

Sample Sheet Generation: Populate the Illumina SampleSheet.csv using the specific i5 and i7 index sequences provided in the **SeqCodeIndices.csv** file.

NovaSeq 6000 S4 Loading: Load at [M] 250 picomolar (pM) with a 1-5% PhiX spike-in.

Run Parameters:

- Paired-end
- Minimal Read Lengths: Read 1 = 27 bp; Read 2 = 27 bp.

- Index Lengths: Index 1 (i7) = 8 bp; Index 2 (i5) = 8 bp.

Note: While 27 bp is the minimum requirement, standard **100 bp PE** configurations are compatible and frequently utilized by core facilities to align with routine flow cell partitioning.

Data Processing & Analysis

5 Once the sequencing run is complete, the raw FASTQ files must be processed to generate digital count data.

1. **GeoMx NGS Pipeline:** Process the FASTQ files using the GeoMx NGS Pipeline. This requires the **config.ini** file from the relevant Readout Package to map the sequences back to the AOIs.
2. **DCC Generation:** The pipeline will output **Digital Count Conversion (DCC)** files. These files contain the quantified expression counts for each segment.
3. **Data Import:** Import the DCC files back into the **GeoMx DSP Control Center**. This reconnects the count data with the high-resolution images and metadata (ROI/AOI selection) for integrated spatial analysis.

For advanced analysis, data can be exported from the Control Center or processed directly using specialized R-based computational frameworks such as **GeomxTools** and **GeoMxWorkflows**.

Protocol references

The quick guide was obtained from the NanoString University website (https://university.nanostring.com). The latest guidance and complete manual is also available on the site, and certain procedures and descriptions are quoted directly from the original manual.