GeoMx® Custom RNA Panels for NGS Readout: Guideline for Use as a Standalone Panel

SEV-00181-01

Introduction

NanoString's RNA Barcoding Service provides custom probes for GeoMx® DSP assays, enabling researchers to measure gene expression of their specific targets of interest. With GeoMx instrument software v2.2 and later, Custom RNA Panels for NGS readout may be run as "standalone", without an Atlas panel like Cancer Transcriptome Atlas or Whole Transcriptome Atlas.

To run a standalone Custom RNA-NGS Panel, certain modifications to the traditional GeoMx protocol are needed. These modifications are outlined in this Guideline.

Custom RNA-NGS Panels include 32 negative probe controls and 2 targets designated by the customer as housekeepers or positive controls, based on their tissue and experimental design. If running as a standalone panel, customers should include more than 2 housekeeper or positive control targets, to enable more options in normalizing the data based on housekeepers' concordance.

Probe formulations for manual/half-automated slide preparation methods are different from probe formulations for the fully automated slide preparation method and are optimized for specific sample preparation conditions. They may *not* be used interchangeably or modified to substitute one for the other. The designation for your Custom RNA-NGS Panel should have been made during design with NanoString Bioinformatics.

Data analysis considerations for custom standalone panels are discussed in the white paper "Selection and Optimization of Custom Probes in GeoMx RNA assays" (MK3582).

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I. Considerations during Slide Preparation for Standalone Custom RNA-NGS Panels

A. FFPE Samples with Manual Slide Preparation or Leica BOND Half-Automated Slide Preparation

- Be sure to use the Custom RNA-NGS Panel Probe Mix intended for the manual/half-automated slide preparation method, indicated by a white tube label and an amber cap (Figure 1). The formulation is optimized for these slide preparation conditions and may not be used interchangeably with formulations intended for fully automated slide preparation method. The designation for your Custom RNA-NGS Panel should have been made during design with NanoString Bioinformatics.

Figure 1. Custom probe mix formulated for manual/half-automated slide preparation.

- Refer to "GeoMx-NGS Slide Preparation User Manual" (MAN-10115-05) for the slide preparation protocol. There are no changes to the workflow steps Prepare Reagents, Prepare Tissue Samples, Deparaffinize and Rehydrate FFPE Tissue Sections, Perform Target Retrieval, Expose RNA Targets, Postfix, Stringent washes, Morphology marker staining, and Slide storage.

- At "Step 7. In situ Hybridization", (Table 16 in MAN-10115-05) the Hybridization solution equation is modified to reflect Custom RNA-NGS Panel(s) without an Atlas (CTA, WTA) panel:

Table 1.
In situ Hybridization Solution for Standalone Custom RNA-NGS Panels (n = number of slides)

Panel Configuration	Buffer R	Atlas Panel	Custom Panel 1	Custom Panel 2	DEPC-treated H_2O	Final volume
1 RNA custom panel (No Atlas)	200 µL x n	NA	12.5 µL x n	NA	37.5 µL x n	250 µL x n
2 RNA custom panels (No Atlas)	200 µL x n	NA	12.5 µL x n	12.5 µL x n	25 µL x n	250 µL x n

B. FFPE Samples with Leica BOND Fully Automated Slide Preparation

- Be sure to use the Custom RNA-NGS Panel Probe Mix intended for the fully automated slide preparation method, indicated by a yellow tube label and a red cap (Figure 2). The formulation is optimized for these slide preparation conditions and may not be used interchangeably with formulations intended for manual/half-automated slide preparation method. The designation for your Custom RNA-NGS Panel should have been made during the design process with NanoString Bioinformatics.

Figure 2. Custom probe mix formulated for fully automated slide preparation.

- Refer to the user manual "GeoMx DSP Fully Automated Slide Preparation for FFPE RNA Assays using Leica BOND RX" (SEV-00180) for the slide preparation protocol.

II. Considerations during Instrument Workflow for Standalone Custom RNA-NGS Panels

Refer to GeoMx-NGS Instrument User Manual (SEV-00087) for the instrument workflow, with attention to these deviations:

A. Scan Set-Up

- NanoString's bioinformatics department will provide a Probe Kit Configuration or *.pkc* file to accompany your Custom RNA-NGS Probe Mix. Upload this file to the GeoMx instrument **Kit Management** tab using a USB drive or by accessing the user interface over Chrome remote connection. When setting up Scan Configuration, select this .pkc file from the **Probe Reagent Kit** drop-down menu.
- If using two Custom RNA-NGS Probe Mixes on a slide, two custom *.pkc* files need to be uploaded and selected from the Probe Reagent Kit drop-down menu in Scan Configuration.
- No other *.pkc* file is needed, as long as you are following all guidance to run the Custom RNA-NGS Panel as a standalone panel.

B. ROI Selection

- If running a standalone Custom RNA-NGS Panel, special consideration should be given to the minimum ROI size (surface area) and number of ROIs selected.
  - If the combination of plex, ROI surface area and ROI number is too low, there is the risk of little-to-no signal on the Bioanalyzer (or alternative) trace of the NGS library, making it difficult to qualify the library for sequencing.
  - A general guideline for a standalone Custom RNA-NGS Panel of 20 targets is a minimum collection area of 425,000 μm^2 (equal to, for example, 54 circular ROI of 100µm diameter), per pooled library.

III. Considerations during NGS Library Preparation for Standalone Custom RNA-NGS Panels

Refer to "GeoMx-NGS Library Prep User Manual" (SEV-00088) for the NGS Library Preparation workflow, with attention to these deviations:

A. AMPure Cleanup and Bioanalyzer Trace

- At "AMPure Cleanup, Step 28. Resuspend beads in Elution Buffer", reduce the Elution Buffer volume for standalone Custom RNA-NGS Panels. NanoString recommends eluting in 12ul when preparing a low-plex library for the first time. You may increase to 25ul at your discretion or in subsequent library preparations.
- For all standalone Custom RNA-NGS Panel experiments, we recommend running the undiluted purified library and a 1:4 dilution of the purified library on the Bioanalyzer.

B. Sequencing Depth

- Bear in mind that the sequencing depth needed for a given NGS library relies on many factors including the number of targets, expression levels of the targets, and total ROI area sampled. A general guideline to ensure enough sequencing depth:

  Minimum number of sequenced read-pairs =

  (total collection area in μm^2) X (number of targets in Custom RNA-NGS Panel) X 0.05

Please contact GeoMxSupport@nanostring.com with questions.