University of Hawaii Cancer Center - Sample Submission Guide

High quality nCounter® data is achieved with samples that meet NanoString® assay requirements. The guidelines set forth in this document will assist you in preparing your samples prior to submission. Please adhere to the recommendations for your assay and sample type as outlined below. Doing so will result in the highest quality data possible for your project. If you have further questions regarding your project submission, please contact:

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808.564.5967

NanoString Contact:
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Sample Preparation Reference Chart for NanoString nCounter MAX or FLEX Instrument

NanoString Assay	Sample Type	Assay Input Amount	Assay Input
nCounter XT Gene Expression and *Elements*	Total RNA	>100ng	no less than 150 ng, normalized to 20 ng/µl
	Cell Lysate	10,000 cells	no less than 15,000 cells. Minimum 6,500 cells/µl, or minimum 3,300 cells/µl for Elements
	FFPE RNA	≥ 100 ng	no less than 150 ng, normalized to 20 ng/µl
nCounter Low Input Kit and *Elements*	Low Input RNA	Up to 5 µl of amplified sample from Low Input Kit assay.	≥ 1ng unamplified. Please contact your NanoString team for more information.
miRNA	Total Purified RNA	> 100 ng	no less than 150 ng, normalized to 33 ng/µl
	FFPE RNA	≥ 100 ng	no less than 150 ng, normalized to 33 ng/µl
	Plasma, Serum, Biofluid	1-3 µl	purified RNA equivalent of > 200 µl plasma or serum
nCounter XT CNV and *Elements*	Purified Genomic DNA only	600 ng	no less than 650 ng, normalized to 85 ng/µl
nCounter RNA:Protein assays	Cell lysates or FFPE tissue	Please contact your NanoString team.	Please contact your NanoString team.

[1] Please provide 1.5X the required assay input amount. If you cannot meet these sample requirements, please contact **support@nanostring.com** to discuss your project with a NanoString Scientist.

Sample Submission Instructions

Your NanoString service lab requests a minimum of 1.5X the required assay input amount. This ensures ample material for additional QC testing (if necessary), and dead volume for dry-ice shipment (if shipping). Material should be supplied in RNAse-free sample tubes (1.5 mL Eppendorf). Each sample tube should be clearly labeled with a sample identifier on the top and side of the tube. Ensure tube lids are secure but please refrain from use of Parafilm. When sending >24 samples, we recommend submitting samples in a 96-well microtiter plate sealed with an adhesive backed plate cover. A sample map indicating sample/well locations should be provided for the plate.

The sample manifest form should be filled out completely (all fields required) and submitted with your samples, detailing your project information. If you have sample QC information (Bioanalyzer traces, UV absorbance readings), please submit this data with the nCounter Sample Manifest form, and include a hard copy of the manifest with the samples.

Please coordinate and schedule your NanoString nCounter run with:

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Annette Jones
annette@cc.hawaii.edu
808.564.5967

NanoString location: UH Cancer Center Bldg A Rm:120

Data Analysis & Delivery of Project Data

Your NanoString nCounter data will be delivered to you via email. The data package will include:

1. Compressed folder containing .rcc files for upload into NanoString nSolver® software for analysis

To receive a copy of nSolver software, register on the NanoString website and download a copy here: **https://nanostring.com/products/analysis-software/nsolver**

For data analysis assistance, please see the nSolver Data Analysis Support Webpage here: **https://nanostring.com/support/data-analysis/nsolver-data-analysis-support**, contact **support@nanostring.com**, or your local NanoString representative.

Gene Expression Sample Preparation – additional details

Total RNA & FFPE RNA

For purification of total RNA and FFPE RNA, standard commercially available kits are recommended, such as Ambion and Qiagen. Samples should be resuspended in purification kit buffers, RNAse free water, TE, or Tris buffer at a normalized concentration of at least 20 ng/µl and dispensed into RNAse free tubes or microtiter plates.

Minimum of 100ng of sample material is required for the Gene Expression Assay per technical replicate (ideally 5 µl at 20 ng/µl). Please provide at least 150 ng of intact total RNA. Due to the significant degradation of FFPE RNA samples, we recommend running >200 ng per sample if additional material is available.

Sample QC

Purified RNA sample quality should be evaluated via a spectrophotometer by measuring absorbance at 230 nm (A230), 260 nm (A260) and 280 nm (A280). The A260/A280 ratio can help identify contamination with proteins, whereas the A260/A230 ratio can help identify contamination with organic compounds, such as phenol, and guanidinium salts. We recommend a 260/280 ratio of 1.9 or greater and a 260/230 ratio of 1.8 or greater for optimal results.

Gene Expression Sample Preparation – additional details (continued)

Cell Lysates

Cell lysates should be prepared in a Guanidinium-based (GITC) lysis buffer such as Qiagen buffer RLT, although detergent based lysis buffers not containing chaotropic salts are also fully compatible with the nCounter assay. Cell concentration should be approximately 6,500 – 10,000 cells/µl. At higher cell/buffer ratios, cell lysis and denaturation is inhibited and the solution too viscous to pipette effectively. Please provide at least 5ul sample volume in tubes or PCR plates.

Note: for Elements chemistry, the maximum lysate sample volume is 3 µl. Please ensure lysates are at a minimum concentration of 3,300 cells/µl for Elements assays.

For mammalian cell lines grown in tissue culture, a simple method for making cell lysates is:

1. Harvest appropriate number of cells, and pellet by centrifugation for 5 minutes at 300 x g in a microcentrifuge tube. Carefully remove all supernatant by aspiration. Failure to remove all supernatant may dilute lysis buffer and result in incomplete lysis or RNA degradation.
2. Disrupt cells by adding appropriate volume of Qiagen Buffer RLT. Addition of βmercaptoethanol to RLT is optional, but may improve RNase inactivation in cell types expressing high levels of nucleases. Use 10 µl β-mercaptoethanol per 1ml RLT.
3. Homogenize cells by vortexing for 1 minute. Centrifuge briefly to recover all material to bottom of tube. It is not necessary to pellet cell debris, hybridization can be performed using the complete lysate.
4. Immediately freeze lysate at -80°C for storage or shipping.

Low Input RNA

Please reference manual for the nCounter Low RNA Input Kit: **https://nanostring.com/low-RNA-input**

miRNA & miRGE – additional details

Purified Total RNA & FFPE RNA

The nCounter miRNA Expression Assay requires purified total RNA as input material. The maximum sample input volume is 3µl for the miRNA Assay. Samples should be resuspended in purification kit buffers, RNAse free water, or Tris pH 8.0 at a normalized concentration of 33 ng/µl and dispensed into RNAse free tubes or microtiter plates.

Approximately 100 ng of sample material is input into the miRNA Expression Assay (ideally 3 µl at 33 ng/µl). Please provide at least 150 ng of intact total RNA. Due to the significant degradation of FFPE RNA samples, NanoString recommends running >200 ng per sample if additional material is available.

Unpurified lysates may not be used with the nCounter miRNA or miRGE™ Expression assay, as the denaturants in the lysis buffer will inhibit the sample preparation reaction.

The quality of the purified RNA is critically important for the nCounter miRNA assay as residual contaminants left over from lysis and RNA extraction impact assay performance by inhibiting the enzymatic ligation and purification steps.

NanoString recommends the following commercially available miRNA purification kits: miRNeasy mini kit, Qiagen, catalog #217004; mirVana PARIS, Ambion, catalog #AM1556; Plasma/Serum Circulating RNA Purification Kit, Norgen Biotek, catalog #30000.

miRNA & miRGE – additional details (continued)

Sample QC for miRNA

Purified RNA sample quality should be evaluated via a spectrophotometer by measuring absorbance at 230 nm (A230), 260 nm (A260) and 280 nm (A280). The A260/A280 ratio can help identify contamination with proteins, whereas the A260/A230 ratio can help identify contamination with organic compounds, such as phenol, and guanidinium salts. NanoString recommends a 260/280 ratio of 1.9 or greater and a 260/230 ratio of 1.8 or greater for optimal results.

Please note: for miRNA derived from biological fluids (serum/plasma) low RNA yields make quantitative absorbance measurements difficult. In order to ensure highly pure miRNA from these samples types, we recommend a secondary purification and concentration step using either of the following products:

1. Amicon ultra-0.5 centrifugal filter (Millipore, cat # UFC500396)
2. RNA Clean & Concentrator-5 (Zymo Research, cat # R1015)

CNV – additional details

Purified Genomic DNA

The nCounter Custom CNV Assay requires purified double stranded genomic DNA as input material. Samples may be purified with one of several commercially available DNA purification kits. Samples should be resuspended in purification kit buffers, RNAse free water, or Tris pH 8.0 at a normalized concentration of 85ng/µl and dispensed into RNAse free tubes or microtiter plates. Approximately 600ng of sample material is input into the CNV Assay (ideally 7 µl at 85 ng/µl).

Accurate quantitation of genomic DNA is important. Some DNA purification methods may leave significant amounts of residual RNA which can result in over-estimation of DNA concentration when measured by UV absorbance and lower counts in the CNV assay. For pure DNA preparations, NanoString recommends A260/280 ratios between 1.7 and 1.9 and A260/230 ratios between 1.3 and 2.0. DNA specific, fluorescence-based assays will provide the most accurate concentration measurements.

RNA:Protein and Protein Only – additional details

Purified Genomic DNA

NanoString offers multiple assay protocols for Protein, RNA:Protein, and DNA:RNA:Protein products. Please review the appropriate protocol before preparing samples for shipment and contact **support@nanostring.com** or your local NanoString contact for additional information.

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