High quality nCounter® data is achieved with high quality starting material. The guidelines set forth in this document will assist you in preparing your samples for your NanoString® run. 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, please contact the core or NanoSring directly at ***support@nanostring.com*****.**

Sample Preparation Reference Chart

NanoString Assay	Sample Type	Assay Input Amount	Required Sample Amount/Concentration Per Replicate*	See Page...
Gene Expression and *Elements*	Total RNA	> 100ng	no less than 150ng, normalized to 20ng/µL	3
	Cell Lysate	10,000 cells	no less than 15,000 cells (2,000 - 10,000 cells/µL) or minimum 3,300 cells/ul for *Elements*	
	FFPE RNA	≥ 100ng	no less than 150ng, normalized to 20ng/µL	
Single Cell and *Elements*	Single cells, low input RNA	varied (amplification)	≥ 1 cell or 10pg RNA	
miRNA	Total Purified RNA	> 100ng	no less than 150ng, normalized to 33ng/µL	4
	FFPE RNA	≥ 100ng	no less than 150ng, normalized to 33ng/µL	
	Plasma, Serum, biofluid	1-3ul	equivalent of > 200ul plasma or serum	
CNV and *Elements*	Purified Genomic DNA *only*	600ng	no less than 650ng, normalized to 85ng/µL	5
ChIP and *Elements*	Purified Genomic DNA *only*	varied (5-10µL)	no less than 10µL	5

***** *Please provide 1.5X the required assay input amount. If you cannot meet these sample requirements, please contact the core.*

Sample Submission Instructions

We request a minimum of 1.5X the required assay input amount per each technical replicate. This ensures ample material for additional QC testing (if necessary), and dead volume for shipment. Material should be supplied in RNAse-free sample tubes (1.5mL 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 Parafilm use. 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 and submitted to the core, detailing your project information. If you have sample QC information (Bioanalyzer traces, UV absorbance readings), please include this data on the Sample Manifest form. Please include a hard copy of the manifest with your samples.

Shipping Considerations

- Sample tubes or 96 well microtiter plates should be packed in a freezer box with a lid securely attached and placed on a sufficient amount of dry ice in a shipping container.
- All samples should be normalized to a single concentration as stated in the sample preparation reference chart and verified by standard quantitation methods (*e.g.* Nanodrop™ absorbance). Sample concentration and integrity are major factors in achieving a successful project in a short period of time. Please make sample concentrations as accurate as possible. Additional sample manipulation may delay project conclusion.
- Please send no less than 5µl volume per sample to avoid evaporation during storage.
- Samples should be shipped overnight ***on dry ice***.
- Sample material ***will not be returned*** and all samples received by the core which are not exhausted during the course of the project will be destroyed.
- ***If any sample requirements cannot be met, please contact the core or your NanoString representatives.***
- ***nCounter Elements Projects***: Elements customers will receive Probe A and B oligos directly from IDT. Please ship Probe A and Probe B pools together with samples in one package on dry ice. Please fill in required Elements information on the Sample Manifest.

Submit to: UCI GHTF
340 Sprague Hall
Irvine, CA 92697
949-824-6023

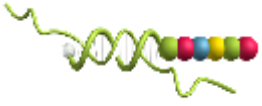
Data Analysis & Delivery Project Data

Your 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 request a copy: www.nanostring.com/lifesciences. For data analysis assistance, please contact your NanoString representative: Shauna Gerold, sgerold@nanostring.com.

Gene Expression



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 **20ng/µl** and dispensed into RNAse free tubes or microtiter plates.

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

Sample QC

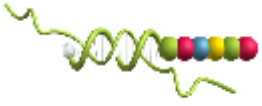
Purified RNA sample quality should be evaluated via a spectrophotometer by measuring absorbance at 230 nm (A230), 260 nm (A260) and 280 nm (A280). The A260/ A280 ratio can help identify contamination with proteins, whereas the A260/A230 ratio can help identify contamination with organic compounds, such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

Cell lysates

Cell lysates should be prepared in a Guanidinium-based (GITC) lysis buffer such as Qiagen$_{TM}$ buffer RLT. Cell concentration should be approximately 2,000 – 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 no less than 15,000 cells in a volume of 5µl. **Note:** for *Elements* chemistry, the maximum lysate sample volume is **3ul**. Please ensure lysates are at a minimum concentration of 3,300 cells/ ul for Elements assays.

Single Cell for Gene Expression

The nCounter Single Cell Gene Expression Assay allows nCounter technology to be applied to single cell and low RNA input analysis. Samples are amplified at a given number of cycles based on initial input amount ranging from 10pg (~1 cell) up to 10ng (~1000 cells). It is critical to state on the sample manifest the approximate number of cells or RNA concentration per sample tube to ensure the appropriate number of amplification cycles is performed.
To avoid loss of starting material, NanoString recommends carrying out the entire Single-Cell protocol in the same tube in which sample is provided. Therefore, cells or RNA should be provided in PCR-ready 200µl strip tubes or in 96 well PCR plates.



miRNA & miRGE

Purified Total RNA & FFPE RNA

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

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

Unpurified lysates may not be used with the nCounter miRNA 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.

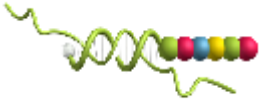
NanoString recommends the following commercially available miRNA purification kits:

- miRNeasy mini kit, Qiagen, catalog #217004
- mirVana PARIS, Ambion, catalog #AM1556
- Plasma/Serum Circulating RNA Purification Kit, Norgen Biotek, catalog #30000

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. These sample types should be discussed with a NanoString scientist prior to shipping (***support@nanostring.com***).



CNV

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 7µl at 85ng/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.

ChIP (Chromatin Immunoprecipitation)

The nCounter assay is compatible with DNA samples derived from Chromatin Immunoprecipitation. The amount of DNA input required will depend on several factors including chromatin input amount, antibody efficiency and Immunoprecipitation protocol. For an example ChIP-NanoString protocol, see reference below. Please consult your NanoString Field Application Scientist (or email ***support@nanostring.com***) for more details.

Ram, et.al. *Combinatorial Patterning of Chromatin Regulators Uncovered by Genome-wide Location Analysis in Human Cells. Cell. 2011.*