North American Genomics Services Guide

Table of Contents

SERVICES PROCESS OVERVIEW

North American Genomics is a facility that offers affordable and fast services for extraction procedures. North American Genomics state-of-the-art facilities embrace lean concepts to improve both efficiency and quality of data delivered. When customer data is generated, we ensure the safety of this information by real-time data migration to secured servers. Our short turnaround times allow our customers to conduct their data analyses rapidly, improving their time to publication and grant submission.

There are 3 steps involved in the services process:

- **Project Initiation**: Customer communicates with North American Genomics to discuss the project. A project manager will send an email to the customer providing a quote alongside guidance on sample submission. Customer will send a purchase order to North American Genomics prior to sample submission.

- **Sample Submission**: Customer sends samples to North American Genomics and laboratory reviews the sample integrity.

- **Sample Processing, Analysis and Data Delivery:** North American Genomics processes customer's samples and generates data analysis files and a report with QC results for final delivery to customer.

1. PROJECT INITIATION

1.1. Customer Call

As the first step of each services project, the customer's needs will be discussed in a conference call between the customer's team and an assigned project manager from North American Genomics. This includes the type of project, product to be used, number of samples, timeline, sample return, and an overview of the services process. This call will help North American Genomics understand the customer's requirements. The project manager will follow up email to confirm all project details covered on the call.

1.2. Documentation

Several pieces of documentation are needed to initiate a service project. Please see the sections below for details on each of these documents.

1.2.1. *Service Project Information Form*

A Service Project Information Form allows North American Genomics to document the details of the customer's project. The project manager will complete the form and assign a project number to track the project throughout the process. The following information will be included in the form.

Appropriate contact information ensures proper communication during the project.

Specific project details such as the service requested, number of samples, sample source (species), sample type (i.e., blood), nucleic acid target, anticipated delivery date, requested downstream applications, etc. allows North American Genomics to generate an appropriate quote for the project, prepare in advance of sample receipt, and minimize turnaround times. Any additional instructions requested by the customer will be documented.

1.2.2. Quote

After the project manager has collected all the details regarding the customer's project, North American Genomics will review the project details. A quote will be generated and sent to the client by the project manager outlining the services that will be provided and the service costs associated with the project.

1.2.3. Purchase Order

Once an offered quote has been accepted by the customer, a purchase order (PO) must be emailed to the project manager at North American Genomics. A PO is a commitment to pay for services provided by North American Genomics. Services will not commence until a PO has been received.

2. SAMPLE SUBMISSION

North American Genomics will begin working on the project after the purchase order has been confirmed. At that point, samples should be shipped to North American Genomics according to the specific sample type and services requested. Please read section 2.1 for more detail on sample submission.

Once samples are shipped to North American Genomics, an email must be sent to the project manager providing courier and tracking number so the shipment can be monitored for safe receipt. This same email should also include the sample manifest. Upon receipt of samples, a visual inspection is conducted, samples are accessioned, and the customer is notified of sample receipt. Once the samples have been accepted, samples are queued for gene expression services.

2.1. Sample Amount

All samples should be submitted according to service type. Please contact the North American Genomics project manager with any questions.

2.1.1. *Sample Amount for Gene Expression Services*

Extracted samples should be shipped to North American Genomics in a sealed 96 well plate. Samples will not be accepted in individual tubes. Please refer to the table below for the quantity of extracted RNA that is required for the GeneChip™ WT PLUS, GeneChip™ WT Pico, and FlashTagTM Biotin HSR RNA gene expression protocols.

If North American Genomics is performing extraction services prior to downstream gene expression services, North American Genomics will quantify and plate the samples for the client.

Assay Type	RNA Input	Recommended Concentration (ng/uL)	Recommended Volume (uL)	Min OD260/OD230	Min OD260/OD280
Standard Assays	Total RNA from fresh, fresh-frozen tissue, cells and whole-blood (no globin mRNA reduction required).	50	10	1.5	1.95
Pico Assays	Total RNA from fresh, fresh-frozen tissue, cells and whole-blood (no globin mRNA reduction required).	2			1.95
	Total RNA from formalin-fixed paraffin-embedded tissues	7			1.95
miRNA Assays (miRNA 3.0 higher)	Total RNA from fresh, fresh-frozen tissue, cells and whole-blood, serum, exosomes	16.25 - 125	25	1.5	1.95

2.2. *Sample Quality and Treatment*

Samples must not be contaminated with other genomic sources. Samples must be from a single source; no mixing of samples should be done even if they are the same species or strain. It is the responsibility of the customer that the samples are collected, stored, and shipped appropriately to prevent nucleic acid degradation.

2.2.1. *Sample Quality for Gene Expression Services*

RNA samples should be free of genomic DNA and we recommend including a DNase treatment or genomic DNA removal step with the RNA purification method. The contaminating genomic DNA may be amplified along with the RNA, which will lead to inaccurate measurement of whole transcriptome expression. In addition, the contaminating genomic DNA could cause over0estimation of the RNA amount.

RNA quality affects how efficiently an RNA is amplified. High-quality RNA is free of contaminating proteins, DNA, phenol, ethanol and salts. To evaluate RNA quality, determine its $A_{260/280}$ ratio. Quality recommendations can be found in the above table.

Any kit for purification of total RNA or LMW RNA will be compatible with FlashTag Biotin HSR. Elute or resuspend the RNA in nuclease-free-water. Ensure that the purification method retains low molecular weight species.

The quality of RNA from FFPE samples can impact the success of gene expression analyses due to chemical modifications of RNA, cross-links of RNA with other molecules, degradation of RNA, and the limited amounts of sample usually available. Using real-time RT-PCR, quality of RNA from FFPE samples can be reliably and reproducibly assessed by measuring levels of abundance gene such as 18S ribosomal RNA prior to performing microarray experiments.

If North American Genomics is performing extraction services prior to downstream Gene expression services, North American Genomics will determine the quality of your samples post extraction.

2.3. *Plate Layout for Gene Expression Services*

When plating nucleic acids into a plate, researcher should use good lab technique ensuring the outside of the plate is clean. Plates when received will be treated in a clean fashion (BSL1) and assumed to be clean of biohazards. RNA that was derived from specimens know to have contagious disease shall be documented in the manifest. No Bovine samples known to originate from animals with BSE will be accepted. Animals that show signs or are part of a herd know to have BSE will not be accepted. As well, no samples derived from humans or other animals know to have forms of prion disease will be accepted.

While you may place your own controls on your sample plate, we recommend that you omit negative controls from your plate, such as water. North American Genomics generally adds positive controls to each customer sample plate as appropriate for the project type to ensure correct processing of the samples.

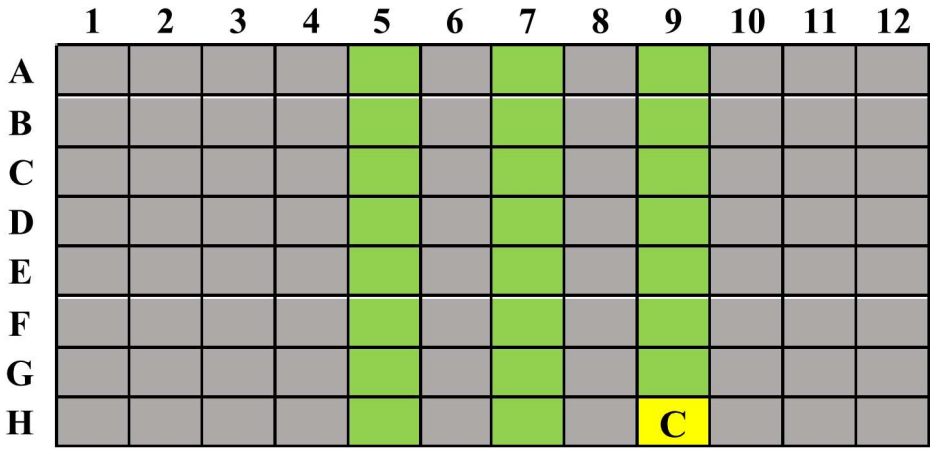
Specific wells should be left blank for North American Genomics internal controls depending on sample species and array format, as shown below. Green indicates wells where samples can be included, yellow (with the letter "C") indicates the wells reserved for North American Genomics controls, and grey indicates locations that should be left empty.

All plates should contain the number of samples indicated below, unless other arrangements are agreed upon between both parties.

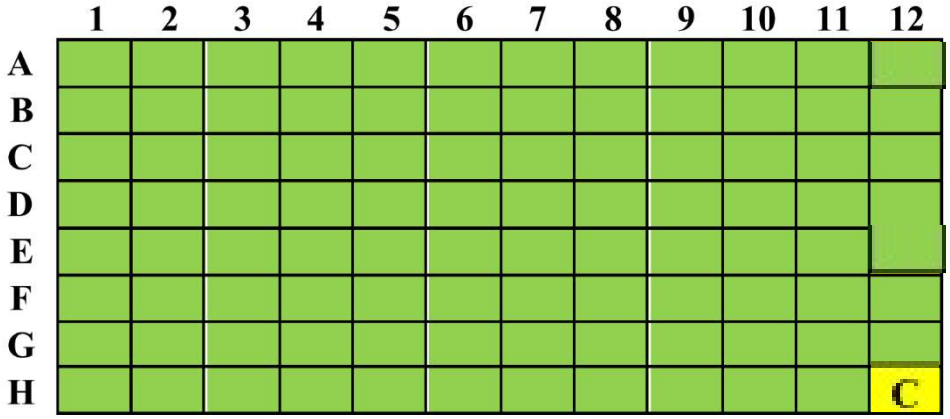
Blank and water samples included in sample areas of submitted plates, as well as samples not meeting minimum concentration requirements, will be counted as samples and processed. If trios are included as part of your sample submission, members of the same family should be submitted in the same batch. Please note that RNA samples will not be accepted in individual tubes.

Plate Layouts

24 Array Format



96 Array Format



2.4. Sample Manifest

A Sample Manifest is required for sample submission. The project manager will provide a template for the Sample Manifest. This is a tab-delimited text file with a row for each sample being submitted. Please see Appendix A for details about the requested fields. Not all columns are applicable to all projects. Additional columns may be added by the customer after these columns, as needed. An electronic copy must be emailed to the project manager and a paper copy must be shipped with the samples.

2.5. Sample Packaging and Shipment

To ensure proper packaging of samples, please follow the guidelines associated with the service type.

2.5.1. Sample Packaging for Gene Expression Services

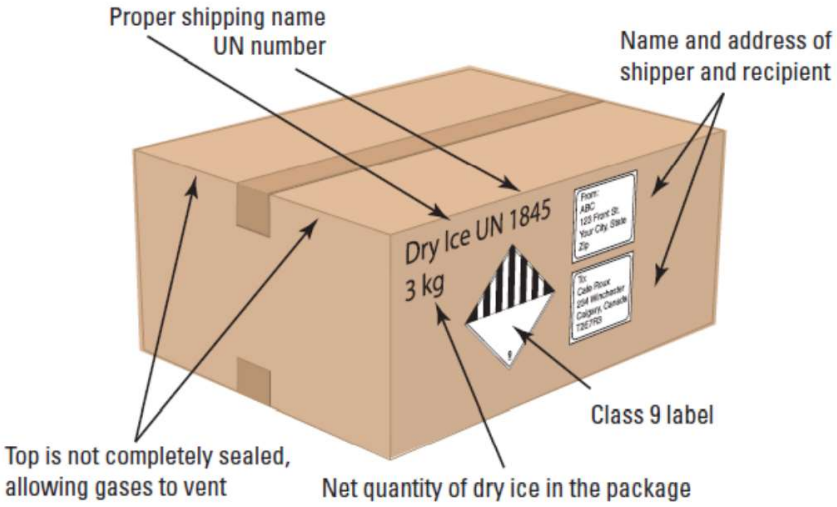
DNA samples must be submitted to North American Genomics in the 96 well plate format. We strongly recommend that the plate be heat sealed using heat seals to prevent possible contamination during transit. If you do not have the equipment to heat seal the plate, please hand seal using an adhesive foil seal followed by a clear seal. Please see Appendix B: Plate Sealing Procedure for instructions on how to properly seal the plate using the foil and adhesive seals.

Place sealed plates in a waterproof container such as a plastic re-closable bag (e.g. Ziploc bag). We recommend only 2 plates be packed per bag. Wrap plate with a layer of extra padding (e.g. bubble wrap or foam sheets) for protection and set in granular dry ice in a Styrofoam box. Ensure that granular dry ice is used on top, bottom, and both sides of the plates, and that there is enough dry ice to last for TWO days shipping for domestic projects. Please DO NOT use dry ice blocks or slabs, use only dry ice pellets – this may cause cracking of the plates and possibly loss or contamination of your samples.

In the Shipper's Declaration for Dangerous Goods form, please indicate that "The shipment consists of purified DNA that is not infectious, non-hazardous, non-pathogenic, not an etiologic agent, and not for human consumption."

2.5.2. Sample Shipment

For shipments containing dry ice, you will need to ensure that the outside of your box has the appropriate hazard stickers prior to shipment. The proper shipping name, namely Dry Ice (or Carbon Dioxide Solid), must be on the same surface of the package as the hazard label or Class 9 label, when package dimensions are adequate. As shown in image below, please also include UN 1845 and the net quantity of dry ice in kilograms (1 kg = 2 lb) on the package.



All self-adhesive labels must be affixed directly to the package.
Do not place labels in, or on, a plastic pouch.

For all services and sample types, a paper copy of the "Sample Manifest" (section 2.2.4) must be enclosed in a waterproof bag and shipped with the samples. Please include the project number at the top of the document and add any additional information that is necessary to maintain secure and safe receipt.

Please ship samples to:

North American Genomics
4280 Memorial Drive, Suite A
Decatur, GA 30032, USA
(470) 650-6048

Samples on dry ice should be shipped as Next Day Air with morning delivery to ensure their expedient arrive. We prefer FedEx Priority Overnight.

Please notify the project manager by email when the samples have been shipped and include a delivery date and associated tracking information. Please attach an electronic copy of the Sample Manifest to this email.

North American Genomics is happy to accept samples during business hours on any weekday (Monday through Friday), except on U. S. national holidays. It is recommended that delivery of a shipment be scheduled for Tuesday, Wednesday, or Thursday to account for any delays in transit. North American Genomics does not accept sample deliveries on Saturday or Sunday.

The customer will be emailed to acknowledge receipt of samples and report on the arrival condition of the samples. The project manager should be contacted for additional questions or further clarification.

2.6. Sample Acceptance

2.6.1. Sample Acceptance for Gene Expression Services

Following visual inspection of your RNA samples and sample accessioning into our LIMS, North American Genomics will perform Quant-iT RiboGreen RNA Assay analysis to determine the concentration of your samples to ensure that we have a sufficient amount for the experiments (see section 2.1.1 Sample Amount for detail).

We strongly encourage that you perform your own sample QC after sample preparation to ensure the proper concentration and volume requirements are meet for the shipped samples. This will help to expedite your project. Please note that the Quant-iT RiboGreen RNA assay and OD readings can differ by up to 10-fold; if customer uses NanoDrop or OD readings, please note this 10X variance to overestimate your sample submission.

If North American Genomics is performing extraction services prior to downstream gene expression services, North American Genomics will re-extract any failed samples for the client assuming there is sufficient sample for a second extraction.

If more than 20% (e.g. 20 out of 96) of the samples fail the Quant-iT RiboGreen RNA assay threshold based on North American Genomics readings, you will be notified and you will have the option to re-submit failing samples. If you would like to resubmit, please notify us within 5 business days. If not, we reserve the right to move forward with processing all samples on that plate. Please note that you may resubmit samples only once. After the resubmission, every sample received will be processed, regardless of passing the QC requirements.

Once we are ready to move forward to genotyping your samples, you will receive a "Samples Accepted" message, which officially starts the timeline. This message is sent when one of the following events occurs:

- All of the sample plates have failure rate <20%
- You have already resubmitted samples once for the failed plate(s)
- You choose not to resubmit and approve processing "as is".

Once samples are accepted, the project timeline begins. Every sample received and processed will be invoiced.

3. Sample Processing, Analysis, and Data Delivery

3.1. Processing

Once samples are accepted, the laboratory will start processing the samples.

3.1.1. Processing for Gene Expression Services

Quant-iT RiboGreen RNA assays will be performed on each sample to determine accurate nucleic acid concentrations and normalized plates will be prepared for all samples. The appropriate library preparation assays will be completed. All prepared

plates will undergo hybridization and scanning using the GeneTitan Multi-Channel (MC) instrument. At the end of this process, CEL files are available for data analysis.

3.2. Analysis

3.2.1. Analysis for Gene Expression Services

Once North American Genomics has completed wet lab processing of your samples and raw data is available, we will start analyzing the data collected and determining which samples pass or fail.

Furthermore, we will examine the data to ensure that samples are tracked correctly. Depending on your project type, different standard files will be delivered to you. The standard deliverables are outlined below. Should you need any other file(s), please discuss with your project manager and we will do our best to accommodate your request.

Category	File Description
Standard product	Read Me
	Annotation Files
	Library Files
Raw	.CEL
	.ARR
Processed	Axiom Analysis Suite – Data Analysis Files

3.3. Data and Sample Delivery

3.3.1. Data and Sample Delivery for Gene Expression Services

Data deliverables will be generated and provided to the customer via a secured Box account. Once the data is delivered, you will receive a "Project Complete" email from North American Genomics informing you of the completion of your project. North American Genomics will then move forward with invoicing of your project. Any sample leftover at the completion of the project will be destroyed unless requested to have the samples shipped to an address specified during project initiation.

Should you have any questions, please do not hesitate to contact your project manager. We look forward to working with you on many successful projects.

APPENDIX A: SAMPLE MANIFEST FIELDS

Column Name	Description
Sample Unique Identifier	Name of the sample. Each sample should have a unique name. Repeated samples should have the same name but ending in a different character. (e.g. 191106001, 191106001A, 191106001B)
Plate Unique Identifier	If sending more than one plate containing samples, please label each plate with a unique identifier.
Well Location (if plated)	Match the sample unique identifier to the well location in the plate that it is found. (e.g. Sample 191106001 is in A1)
Sample Species	Provide the species or subspecies of the sample.
Sample Type	Provide the type of sample type. (e.g. Blood, Swab, Extracted DNA)
Ploidy	For non-human, ploidy of the sample.
Gender	Gender of the sample. Accepted values are “Male”, “Female”, “Unknown”, “M”, “F”, or “U”. This column is important in troubleshooting for potential sample tracking issues and the gender for normal samples is used to correctly set the copy number baseline for the sex-linked chromosomes.
Family	If trios are present, value for the family ID (pedigree ID).
Relationship	If trios are present, enter one of the following for relationship: P01, 01, Father P02, 02, Mother Off , 03, Offspring
Inbred	For non-human, indicate whether the sample is inbred. Accepted values are “Yes”, “No”, “Unknown”, “Y”, “N”, or “U”.
Sample Origin	For non-human, the geographical origin of the sample.
WGA	Indicates whether the sample has been Whole Genome Amplified. Accepted values are “yes” or “true” if the sample is WGA and “no”, “false” or blank if the sample is genomic.
Other Sample Info	Other sample attribute that is useful for genotyping analysis.

APPENDIX B: PLATE SEALING PROCEDURE

I. Hand Seal Method

Items for sealing a plate include a foil seal and a clear adhesive seal.

1. Place the foil seal on the plate and press firmly to ensure adherence to plate.

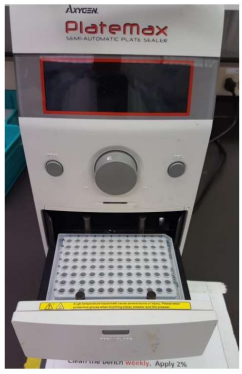
2. Rub the top, the edges, and the corners of the plate. Seal around each well by pressing firmly between each row and column, making sure there is an indentation around and in between each well. **Do not use any object with sharp edges as it will tear the foil seal.**

3. Apply the clear adhesive seal on top of the foil seal and rub so it is fully in contact with the foil seal and plate around all edges of the plate. This is to help protect the top of the plate from any punctures.

Upon completion of the steps above, the plate should have criss-cross indentations between each well.

II. Heat Seal Method

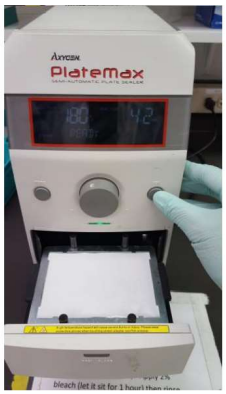
Items for heat sealing a plate include a heat sealer (Axygen PlateMax pictured here), heat seal and a metal block for cooling.



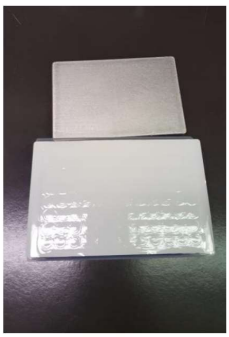
1. Place the sample plate onto the 96-well plate holder of the heat sealer.

2. Align the heat seal and place on top of the plate.

3. Align and place the square metal ring on top of the plate to hold the heat seal in place.



4. Set heat sealer at 180°C for 4.2 seconds and push the button to close and seal.



5. Take the plate out of the heat sealer and place on a benchtop.

6. Place a cool metal block on top of the warm plate.

7. Remove the metal block and ensure that all individual wells have a complete compressed outer ring indicating closure.