



Sample Submission Instructions

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About this Document

This document details the following information:

- Instructions for preparing samples to be sent to Complete Genomics
- Instructions on how to complete the Sample Manifest
- Instructions for packaging and shipping DNA samples to Complete Genomics

Contact the Complete Genomics Project Manager (PM) for support at:

projectmgr@completegenomics.com

Sample Preparation Instructions

Terminology

This document uses the following terms to describe samples and their preparation and shipment:

- **Analysis Pipeline Version** — Version of Analysis Pipeline software used to map reads, call variants, and apply annotations.
- **Batch** — Sample grouping for processing through the Complete Genomics lab.
- **Cancer Pair** — A matched set of two samples submitted under a single part number for the Cancer Sequencing Service. Generally these samples represent a tumor-normal matched set.
- **Cancer Trio** — A matched set of three samples submitted under a single part number for the Cancer Sequencing Service. Generally these samples represent a tumor-tumor-normal matched set.
- **IDQC** — Identity Quality Control (a SNP genotyping assay).
- **Library Preparation Version** — Version of the Library Preparation standard operating procedure.
- **PI** — Principal Investigator.
- **PM** — Complete Genomics Project Manager, the primary contact for each project.
- **PO** — Purchase Order.
- **Replacement Sample** — An additional sample sent after an original sample failed quality control. This sample replaces the original sample.
- **Sample Manifest** — Complete Genomics Microsoft® Excel® form used to obtain detailed sample information from the customer.
- **Sample QC** — Sample Quality Control.
- **Samples** — Customer-provided genomic DNA.
- **SO** — Sales Order.
- **SOP** — Standard Operating Procedure.
- **T&Cs** — Standard Terms and Conditions.
- **Top-Off Sample** — Sample that will be added directly to a sample previously submitted, to raise the quantity or adjust the concentration to the required range. The top-off sample differs from a replacement sample in that it will be combined with the earlier sample, rather than replacing it completely.

DNA Requirements

DNA samples submitted to Complete Genomics for sequencing must meet the criteria listed in Table 1.

Note that if any sample fails to meet these requirements, it can delay sequencing for the remaining samples in the shipment.

Table 1- Sample Requirements

Characteristic	Sample Requirement	Top-Off Sample Requirement
Quality	High-molecular weight double stranded genomic DNA (> 20 kb single band, see Appendix B)	High-molecular weight double stranded genomic DNA (> 20 kb single band, see Appendix B)
Minimum Amount	7.5 µg (10 µg recommended)	Enough DNA to bring the total amount up to the required 7.5 µg. Minimum of 2.25 µg.
Concentration range	75 to 400 ng / µl	Sufficiently concentrated to bring the DNA concentration up to the required range of 75 to 400 ng/µl. Maximum of 400 ng/µl.
Buffer	1xTE, pH 8.0 <ul style="list-style-type: none"> • 10 mM Tris, 1 mM EDTA • H₂O is not recommended 	1xTE, pH 8.0
Volume Range	50 to 200 µl	Minimum of 30 µl. The combined top-off and original samples have a maximum of 200 µl.
Amplification	None	None

DNA Preparation

Follow the recommendations of the commercial DNA extraction and preparation kit being used, without deviation from their protocols. For example, do not overload columns in an attempt to get a higher yield or reduce the volume of column wash solution, as it can result in increased contamination. Proteinase K and RNase treatments are strongly recommended, but avoid incubating samples above 45°C at any time. If using phenol-chloroform for DNA extraction, include a purification step to remove potential contaminants. Refer to the [Sample Prep FAQ](#) for more information on sample preparation, including a list of recommended extraction kits.

Quantity Assessment

DNA should be quantified after dilution to the range specified in Table 1 by a PicoGreen assay (preferably with the Quant-iT™ PicoGreen® dsDNA kit from Invitrogen). The [Sample Quality Control Protocol](#) outlines the quantitation performed during Sample QC. Note that using the correct standards when creating the standard curve is essential to accurate quantitation. Spectrophotometric quantification (such as using a NanoDrop™) is not recommended because contaminating protein and RNA can result in an overestimation of DNA concentration (see "[Appendix A: Effects of Contamination on Quantitation](#)"). Improper DNA quantification can result in sample failure during Sample Quality Control (QC) due to insufficient amounts.

Quality Assessment

Verify DNA integrity through gel electrophoresis, as described in the [Sample Quality Control Protocol](#). A digital image of the gel should be included when sending the Sample Manifest for review, prior to shipping the DNA. See "[Appendix B: Examples of Agarose Gels from Passing and Failing](#)" for examples.

Some agarose gels reveal a smear or band migrating above the main band of DNA, indicating that the sample contains some DNA with abnormally slow mobility (potentially single stranded DNA or protein-DNA complexes). The effect of this slow-migrating DNA is still under investigation, but it is possible that we will not be able to deliver CNV and SV data for these samples. It is also possible that these samples will have increased error rates for small variants. For this reason, we highly recommend providing a replacement sample that does not include slow-migrating DNA.

Sample Sources and Numbers

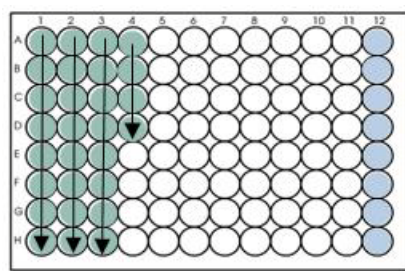
DNA samples may be derived from blood, frozen tissue, cell lines, or saliva. Complete Genomics does not currently accept DNA from whole genome amplified (WGA) samples, formalin-fixed, paraffin-embedded (FFPE) samples, buccal swabs, Guthrie cards, or pooled samples. The minimum number of samples per plate that Complete Genomics accepts is eight samples.

Preparing Samples for Shipment

Complete Genomics provides bar-coded 96-well plates and sealing mats, which ensure compatibility with Complete Genomics' Laboratory Information Management System (LIMS) and automation. Only these plates and sealing mats may be used for shipping samples to Complete Genomics, and all samples on each plate must be intended for sequencing. Please note that top-off samples may not be mixed with other samples on the same plate. Use a separate plate if sending top-off samples.

Samples must be organized by columns, as shown in Figure 1. For example, if sending 28 samples, they should be entered into wells A01 – H01, A02 – H02, A03 – H03, and A04 – D04.

Figure 1 - Example Plate Layout



In this example, green wells are samples provided by the customer, white wells are open/blank wells, and blue wells are reserved for Complete Genomics in-house controls. The maximum number of samples per plate is 88, as the last column (A12 – H 12) is reserved for in-house controls. Consult the PM for all plate layout questions.



Complete Genomics has process controls to minimize variability between samples processed through separate sample and library preparations. Nevertheless, we recommend that you include related samples—such as tumor and normal pairs—on the same plate. This arrangement increases the probability that related samples will be included in the same processing batch. Complete Genomics is available for consultation to help determine the optimal plate layout and shipment schedule to minimize differences in data between related samples.

Sample Receipt at Complete Genomics

When Samples Arrive at Complete Genomics

When samples arrive, the PM confirms receipt of samples and lets you know if there are any issues with the shipment of samples. Issues that can cause problems and delay processing include:

- The use of a non-Complete Genomics sample plate or seals.
- Incorrect placement of samples into the plate (such as placing samples in column 12).
- Issues with the Sample Manifest form, such as:
 - Sample Manifest is missing.
 - Hard-copy of Sample Manifest does not match electronic version.
 - Sample Manifest is improperly completed.
- Samples have thawed in transit.
- Samples do not arrive with a valid Purchase Order.

Complete Genomics Sample Quality Control

Sample QC is performed within two weeks of sample delivery to Complete Genomics' facility. Sample QC includes DNA quantification using PicoGreen®, confirmation of the volume provided, and visual inspection for degradation by running the DNA sample on an agarose gel. This QC check ensures that samples have sufficient quantity and are sufficiently intact (not fragmented) for whole genome sequencing. It is not a functional QC that guarantees the sequencing success of the sample. Our experience is, however, that > 99% of samples that pass this QC result in data that passes our rigorous metrics for final data QC.

Identity QC (IDQC) is also performed for each sample by genotyping a panel of 96 SNPs. Results of the IDQC are used to:

- Provide data to confirm that the sequencing data generated matches the sample received.
- Confirm that the gender detected matches the gender reported.
- Identify any unexpected sample duplications on the plate.
- Verify that samples submitted as a Cancer Pair or a Cancer Trio are derived from the same subject.



Complete Genomics provides a comprehensive QC report indicating which samples have passed QC and reasons for any sample failures.

When Samples Fail QC

Sample failures are communicated via the QC report provided to the customer. The PM will highlight the failures in the QC Report and discuss options. Complete Genomics holds all samples on the plate until the customer indicates how they would like to proceed. Therefore, QC failures can cause a delay in getting sequencing results for the remaining samples.

- **Replacement sample:** It is possible to send a replacement for a failed sample. The replacement sample does not have to come from the same sample as the original DNA submitted.
- **Top-off sample:** When a sample fails due to insufficient DNA amounts, it is possible to send more DNA to raise the sample's DNA amounts above the specified requirement. Failures due to incorrect concentration can be corrected by sending supplemental DNA that would be added directly to the failed sample. Complete Genomics combines the original sample with the top-off sample only after using IDQC to confirm that they consist of the same DNA.

When a sample fails that was submitted as part of a Cancer Pair or a Cancer Trio, the rest of the samples within the group are held until the failed sample is replaced. If it is not possible to replace a specific failed sample, you may choose to replace the entire group.

Why Samples Fail QC

Sample failure statistics from 2011 are as follows:

- 83% of failures were due to low quantity, or because the concentration was out of range (also usually on the low side).
- 6% of failures were due to degradation, as visualized by gel electrophoresis.
- 10% of failures were due to gender mismatching. Two thirds of these were determined to be the wrong sample shipped. The remaining third were due to customer reporting errors and so were accepted based on the customers' explanations.
- Remaining failures were due to other reasons such as being outside of the specified volume range or receiving unexpected sample duplications.

IMPORTANT:

The most common mode of sample failure is low quantity. To avoid delays in sample sequencing due to the need to replace a failed sample, Complete Genomics highly recommends sending more DNA than the minimum requirement. Only the required amount of DNA will be used, and if requested, all remaining DNA will be returned after sequencing and analysis are complete.



How to Fill Out the Sample Manifest

The Sample Manifest is a Microsoft® Excel® form that collects the information Complete Genomics requires to process a genome sequencing request. This form should be completed and reviewed with the PM prior to shipment.

The PM will provide the Sample Manifest, pre-populated with a Project Code. This code should be referenced in all communications. Please contact the PM for any questions. These instructions apply to Sample Manifest version 4.4.

IMPORTANT:

You must complete the Sample Submission Manifest and have it reviewed and approved by the PM *before* shipping samples to Complete Genomics.

Step 1: Contact Information and Plate Information

Enter Contact and Shipment information in rows 7 through 25 as follows:

1. Enter the complete contact information of the person responsible for sending the samples.
2. Enter the anticipated Shipping Date (MM/DD/YYYY). Notify the PM of the actual shipping date via email.
3. If you have a Purchase Order (PO) number, enter it in row 21.
4. Enter the total number of samples on the plate in row 23.
5. Indicate whether or not these samples are Top-off Samples by selecting "Yes" or "No" in row 24. For more information on Top-off Samples, please see [When Samples Fail QC](#).
6. Enter the total number of plates in the shipment in row 25.

NOTE ABOUT TOP-OFF SAMPLES:

If any sample is a top-off sample, all samples on the plate must be top-off samples. If sending additional samples that are not top-off samples, send them on a separate plate.

After "Yes" is selected in row 24, indicating that all samples on the plate are top-off samples, columns L, M, and N of the Sample Information Table (see below) are grayed out. The information will be inherited from the original sample submission.

If "No" is selected in row 24, column O will be grayed out.

Step 2: Plate Barcode

1. Enter the barcode in row 31 of the manifest.

The plate containing the samples to be listed in this manifest should include a barcode in the form of "GSxxxxx-DNA".

IMPORTANT:

Make sure that you match the plate bar code number to the appropriate plate when entering your samples. By entering the barcodes, the Complete Genomics Sample ID will be automatically assigned and entered in the sample information table.

Step 3: Sample Information

Enter the genome data information into the Sample Information Table as shown in three sections in Figure 2. The gray-shaded rows represent example entries.

Figure 2 - Example of a Sample Manifest

Sample Information Table

IMPORTANT: Make sure that you enter the plate bar code number (e.g GSXXXXX-DNA) in row 31 before entering samples. Also ensure that the answer
The first rows represent the following examples: (row 84) standard sample, (row 85) tumor sample within a tumor-normal pair, (row 86) normal sample within a tumor-normal pair, (row 87) replacement sample for a tumor submitted as part of a tumor-normal pair, and (row 88) a top-off sample.

Blue columns will be filled out by Complete Genomics.

* Fields are optional.

Sample Information										
A	B	C	D	E	F	G	H	I	J	K
Complete Genomics Sample ID	Well	Customer Initials	Customer Sample ID	Customer Subject ID	Gender	Sample Source	Tumor Status	Group Size	Coverage	Part Number
GS10101-DNA_A01	A01	DB	StandardSample	NA19240	Female	Blood	Non-Tumor	Individual	Standard	ST001RM
GS10101-DNA_B01	B01	DB	TumorWithinPair	1394	Female	Cell line	Tumor	Cancer Pair	High	T2H001RNM
GS10101-DNA_C01	C01	DB	NormalWithinPair	1394	Female	Cell line	Non-Tumor	Cancer Pair	High	T2H001RNM
GS10101-DNA_D01	D01	DB	Replacement4 TumorInPair	ColCR144	Male	Frozen Tissue	Tumor	Cancer Pair	High	T2H001RNM
GS10101-DNA_E01	E01	DB	TopOffSample	ASRR	Male	Frozen Tissue	Non-Tumor	Cancer Trio	High	T3H001RNM
_A01	A01									
_B01	B01									

Cancer Pairs & Trios ONLY		For Replacements ONLY		DNA Measurements			CG Use Only
L	M	N	O	P	Q	R	S
Group ID	Is Baseline Sample?	"Complete Genomics Sample ID" of Sample Being Replaced	"Complete Genomics Sample ID" of Sample Getting Topped Off	Volume (µl)	Conc. (ng/µl)	Quantity (µg)	Sales Order
				100	100	✓ 10	
GS10101-GRP_01	No			100	100	✓ 10	
GS10101-GRP_01	Yes			100	100	✓ 10	
		GS10090-DNA_C02		100	100	✓ 10	
			GS10089-DNA_B01	100	100	✓ 10	
						✗ 0	
						✗ 0	

Optional Information							
T	U	V	W	X	Y	Z	AA
Reference Build *	DNA Extraction Method *	DNA Quantitation Method *	Mother's Subject ID *	Father's Subject ID *	Ethnicity *	Phenotype *	Comments *
GRCh37 (hg19)	QIAGEN DNeasy Blood and Tissue Kit	PicoGreen	NA19238	NA19239	YORUBA	unaffected	HapMap Sample
GRCh37 (hg19)	QIAGEN Genra Puregene Cell Kit	PicoGreen	N/A	N/A	caucasian	breast cancer cell line	Her2-; p53+; EPG2+, ER-, PR-
GRCh37 (hg19)	QIAGEN Genra Puregene Cell Kit	PicoGreen	N/A	N/A	caucasian	Normal match for HCC1395	not truly diploid (cell line)
NCBI build 36 (hg18)	QIAGEN DNeasy Blood and Tissue Kit	PicoGreen	N/A	N/A	unknown	colon cancer	
GRCh37 (hg19)	QIAGEN DNeasy Blood and Tissue Kit	PicoGreen	N/A	N/A	unknown	colon cancer	This is a Top-off Sample.

NOTE: Columns P, Q, and R will alert you if your samples do not meet the minimum volume, concentration and quantity guidelines.

- Column A Complete Genomics Sample ID This ID is pre-populated. Its format is "GSxxxxx-DNA_Ao1".
- Column B Well The well location of the sample. It is denoted as A01, B01, C01, etc., and is pre-populated.
- Column C Customer Initials The initials of the PI for the given project.
- Column D Customer Sample ID Unique identifier assigned by the customer to each sample. It that can be used to facilitate communication about sample and data issues. No samples should share the same Customer Sample ID. This ID should not contain any patient identifiers.
- Column E Customer Subject ID Unique identifier assigned by the customer based on the individual from which the sample was derived. In cases where multiple samples are provided from the same individual (for example, Matched Tumor and Matched Normal), these samples should be assigned the same Customer Subject ID. This ID should not contain any patient-identifying information.
- Column F Gender Select from the drop-down menu. Options include Male, Female, or Unknown.
- Column G Sample Source Source from which the DNA was obtained. Select from the drop-down menu, or enter your own. Options include Blood, Cell line, Frozen tissue, Saliva, or Other.
- Column H Tumor Status Select from the drop-down menu. Options include Tumor or Non-Tumor.

Column I	Group Size	Select from the drop-down menu. Options include Individual, Cancer Pair, or Cancer Trio. Cancer Pairs and Cancer Trios represent samples submitted as a group under a single part number through the Cancer Sequencing Service. All other samples are Individuals.
Column J	Coverage	Amount of coverage purchased for the sample. Select from the drop-down menu. Options include Standard or High Coverage. High Coverage results in double the amount of sequencing output as compared to the Standard Coverage option.
Column K	Part Number	Automatically populated service offering identifier generated from the entries in Group Size, Sample Source, and Coverage. The Part Number will only appear after the relevant information has been entered. Check that the Part Numbers that appear are included as part of your order for these samples. If the resulting combination is not supported by an existing service offering, the cell will be left blank.
Column L	Group ID	For Cancer Pairs and Cancer Trios ONLY. Indicate which samples are members of a Cancer Pair or a Cancer Trio by assigning them the same, unique Group ID. Select from the drop-down menu if creating a new pair or trio. If the group spans across multiple Sample Manifests, it is possible to enter the Group ID manually. It is essential that you provide the correct Group ID to ensure that samples are properly matched throughout sample processing, analysis, and delivery.
Column M	Is Baseline Sample?	For Cancer Pairs and Cancer Trios ONLY. Select from the drop-down menu. Select Yes if this is the baseline sample and No if it is not. For any pair or trio, there must be only one sample with a Yes in this column. For more information, see "Column M: Is Sample Baseline?" .
Column N	"Complete Genomics Sample ID" of Sample Being Replaced	Complete this column ONLY if the sample submitted is intended to completely replace an earlier sample submission. Enter the Complete Genomics Sample ID of the sample that is to be replaced with this new sample (from column A of the original manifest). Otherwise, leave blank or enter "N/A".

Column O	“Complete Genomics Sample ID” of Sample Getting Topped Off	Complete this column ONLY if the sample submitted is intended to be directly combined with an earlier sample submission (to raise the quantity and/or adjust the concentration). Enter the Complete Genomics Sample ID of the sample that is to be supplemented with this new sample (column A of the original manifest). Otherwise, leave blank or enter “N/A”. Note that top-off samples cannot be included on the same plate as samples that are not intended for top-off.
Column P	Volume (µl)	Sample volume reported in microliters (µl). This value must be between 50 and 200 µl. If the value is outside of the required range, the cell will be shaded red. For top-off samples, the required range is between 30 and 200 µl.
Column Q	Concentration (ng/µl)	Sample concentration (as measured by a PicoGreen assay) reported as nanograms per microliter (ng/µl). Concentration must be between 75 – 400 ng/µl. If the concentration is outside of this range, the cell will be shaded red. For top-off samples, the required range is between 30 and 500 ng/µl.
Column R	Quantity (µg)	Amount of DNA in micrograms. This value is automatically computed based on the volume and concentration values entered into columns P and Q. Complete Genomics recommends providing a minimum of 10 µg, and even more when available. If the quantity is below the 7.5 µg minimum requirement (or, 2.25 µg for top-off samples), the cell will be marked with a red X.
Column S	Sales Order	For Complete Genomics Use ONLY. The sales order for each genome will be auto-populated if only one exists for all genomes on the plate. If multiple sales orders are available for the samples sent on the plate, Complete Genomics will fill this column in after receiving the manifest.
Column T	Reference Build (optional)	The human reference build that will be used for mapping reads and annotating variants. Select from the drop-down menu. Options include: <ul style="list-style-type: none"> ▪ GRCH37: for alignment (using dbSNP version 132 for annotating known variations and NCBI annotation build 37.2 for RefSeq annotations) or ▪ NCI Build 36: (using dbSNP version 130 for annotating known variations and NCBI annotation build 36.3 for RefSeq annotations).
Column U	DNA Extraction	The method that was used to extract DNA from its source.

	Method (optional)	Select from the drop-down menu, or enter an alternate approach.
Column V	DNA Quantitation Method (optional)	The method that was used to quantify the concentration of the DNA sample. PicoGreen should be used for DNA quantitation, but in cases where it is unavailable, please enter the alternate approach that was taken (options are available in a drop-down menu).
Column W	Mother's Subject ID (optional)	A unique subject ID assigned by the customer to the mother of subject.
Column X	Father's Subject ID (optional)	A unique subject ID assigned by the customer to the father of subject.
Column Y	Ethnicity (optional)	Ethnicity of individual.
Column Z	Phenotype (optional)	The sample phenotype/disease status.
Column AA	Comments (optional)	Additional information relevant to sample.

Column M: Is Sample Baseline?

All of the genomes sequenced by Complete Genomics are compared to the human reference genome. In a Cancer Pair, one genome is also compared to the other genome to identify which events are somatic. In a Cancer Trio, two genomes are compared to a third genome. Therefore, for either group type, one sample within the group is the baseline sample (the sample getting compared to), while any remaining samples are not.

In general, a non-tumor sample should be selected as the baseline. Note that assigning the tumor sample as the baseline can adversely impact the identification of somatic events. The following caveats should be weighed if the study design calls for assigning a tumor sample as baseline within a Cancer Pair or Trio:

- Somatic output summarizing small variants, copy number variation, and structural variations, is unidirectional. It is produced by comparing the non-baseline sample to baseline sample only. A comparison in the reverse direction is not performed.
- CNV calling will work best when the baseline genomes are diploid/euploid.

Step 4: Submit Manifest

Submit a hard-copy of the completed Sample Manifest along with the sample shipment and e-mail an electronic copy to projectmgr@completegenomics.com at least one day before planning to ship.

Packaging Details and Shipping Instructions

IMPORTANT:

The information contained in this document is intended to serve as a quick reference and does not provide detailed guidelines for packaging and shipping of biological and/or hazardous material. Refer to the IATA “Dangerous Goods Regulations” and your shipping courier for detailed shipping guidelines. It is the responsibility of the shipper to ensure that proper packaging and shipping procedures are followed.

Pre-shipment Checklist

Prior to shipping samples to Complete Genomics, please confirm that each of the following requirements has been met:

- Sample Manifest has been completed and an electronic copy has been sent the Complete Genomics Project Manager (PM).
- The PM has reviewed and approved the Sample Manifest.
- Plates and samples comply with the specifications outlined in this document.
- A hard copy of the Sample Manifest has been included in the shipment.

To ensure sample acceptance, see below for details on shipping.

Packaging Details

Samples or shipments sent that do not adhere to the following guidelines will be rejected and returned.

1. All samples should be sent on sufficient amounts of dry ice to keep samples frozen through delivery. The following table indicates how much dry ice is necessary.

Country of Origin	Transit Time	Dry Ice Weight
U.S. and Canada	2 days	10 lbs / 5 kg
All other countries	3-5 days	15-25 lbs / 7.5-12.5 kg, depending on transit time

2. Sample plates should be securely sealed using the plate mats provided by Complete Genomics. This prevents accidental spillage and potential cross-contamination. **Ensure that the plate mats are firmly pressed onto each individual well.** Do not use adhesive plate seals, as they tend to peel away at low temperature.



3. Place the sealed plates inside the resealable plastic bag provided with the plate, and seal the bag. This is to avoid accidental exposure of the DNA sample to carbon dioxide from the dry ice.
4. Sample plates should be frozen at -20°C *prior* to packaging into the shipping box.
5. Include a hard copy of the Sample Manifest in the package. The manifest should be outside of the insulated container.
6. Sample plates, containing frozen DNA, are to be placed upright in an insulated container (such as a Styrofoam cooler) and surrounded by dry ice. We recommend that you use an excess of dry ice in the package to safeguard against unanticipated shipping delays. Do not ship samples on wet ice or at ambient temperature.
7. When multiple plates are packaged in the same insulated container, they should be individually wrapped, such as with bubble-wrap, to prevent damage.
8. Place the insulated container inside a cardboard box to comply with courier requirements. The package should permit the release of carbon dioxide gas.
9. Include the Project Code (provided by Complete Genomics on the Sample Manifest) on the mailing label.
10. Place the "Class 9, UN1845" Identifier Label supplied by CGI on the outside of the shipping package.
11. Complete an airway bill with the following information:
 - Classification (Dry Ice, Class 9, UN1845)
 - Number of packages
 - The net quantity of dry ice per package

Place the completed airway bill in a clear plastic pouch and affix it to the outer packaging.

Shipping Details

The following requirements ensure that samples arrive safely and timely to Complete Genomics:

1. Ship DNA samples by overnight courier.
2. Ship samples on Monday, Tuesday, or Wednesday.

Complete Genomics accepts deliveries Monday through Friday between 8:00 am and 5:00 pm Pacific Time (excluding U.S. public holidays). Note that holidays observed at Complete Genomics in 2012 include: January 16, February 20, May 28, July 4, September 3, November 22 and 23, December 24, 25, and 31.



3. Address the shipment to:
Complete Genomics, Inc.
Attn: Project Manager (enter the Complete Genomics PM name here)
2071 Stierlin Court
Mountain View, CA 94043
4. Include the following email address in the courier's electronic shipment notification update:
SamplesReceiving@completegenomics.com
5. Inform the PM by email when the shipment has been dispatched. The email should include the following information:
 - Complete Genomics Project Code
 - Contact Name
 - Contact Phone
 - Shipping company and Tracking Number
 - Shipping Date

Quick Shipping Checklist

- Sample Manifest is complete.
- The Complete Genomics PM has approved the electronic version of the manifest.
- DNA meets the following criteria:
 - $\geq 7.5 \mu\text{g}$ of unamplified high molecular weight DNA (or $2.25 \mu\text{g}$ for top-off samples), preferably $\geq 10 \mu\text{g}$
 - Concentration is 75 to 400 ng/ μl , verified by PicoGreen
 - Volume is 50 to 200 μl (or 30 to 200 μl for top-off samples)
 - DNA is intact, of high molecular weight
 - Top-off samples are not included on the same plate as other samples
 - DNA is frozen prior to shipment
- Samples are in a bar-coded plate, sealed with a Complete Genomics supplied plate mat.
- Sample plates are frozen prior to packing.
- Plates are individually wrapped (for multi-plate shipments).
- Frozen sample plates are placed upright in a Styrofoam cooler and are surrounded by sufficient amounts of dry ice
 - Domestic: 10 lbs or 5 kg
 - International: 15-25 lbs or 7.5-12.5 kg
- Insulated container is placed in a cardboard shipping box.
- Cardboard box has the following labels on the outside:
 - Mailing label indicating Project Code
 - "Class 9, UN1845" identifier label provided by Complete Genomics
- Completed airway bill indicating
 - Classification ("Dry Ice, Class 9, UN1845")
 - Number of packages
 - The net quantity of dry ice per package
- A hard-copy of the Sample Manifest is enclosed with the shipment.
- Samples are shipped by overnight courier (specifying next-day delivery).
- Samples are shipped Monday, Tuesday, or Wednesday, with consideration of U.S. holidays.
- Shipment is addressed to the PM and the shipping address has been verified.
- Courier has been informed of Complete Genomics' shipment notification email address.
- PM has been emailed the shipment tracking information.

Appendix A: Effects of Contamination on Quantitation

Complete Genomics uses PicoGreen to quantitate DNA in each sample because it allows specific and sensitive measurement of DNA amounts independent of contamination. We strongly recommend that DNA is quantitated by the PicoGreen method. This method is not adversely affected by RNA or protein, which are common contaminants of DNA samples. It is also specific for double-stranded DNA and does not detect single-stranded DNA.

If spectrophotometry is used (including the NanoDrop) to measure DNA concentration by determining the absorbance of a sample at 260 nm (A_{260}), please be aware that concentrations may differ from those determined by PicoGreen. In particular, spectrophotometric measurements of DNA concentration are prone to the following issues:

- DNA, RNA, and proteins absorb light at 260 nm (the peak absorbance for DNA and RNA is 260 nm, while the peak absorbance for protein is 280 nm). Therefore, any contamination of RNA or protein in the sample will result in an overestimation of DNA concentration.
- DNA concentration via spectrophotometry does not distinguish between double-stranded or single-stranded DNA, whereas Complete Genomics sequencing process requires double-stranded DNA molecules.
- Phenol also absorbs light at 260 nm (the peak absorbance is at 270-275 nm). DNA samples extracted using methods including phenol may include contamination that also results in the overestimation of DNA concentration.
- Conversion of absorbance readings to concentration assumes that an A_{260} of 1 equals 50 $\mu\text{g/ml}$. This assumption is only correct at neutral pH.

Absorbance Spectrums and Ratios

Exploring beyond the A_{260} value for a DNA sample is important, as there are many molecules that can absorb at that specific wavelength. It is useful to observe the full absorption spectrum from 230 to 320 nm. The sample should have a clear peak at 260 nm and a clear trough at 230 nm, as described in the NanoDrop [To42-Technical Bulletin](#) and the Qiagen newsletter "[Quantitation of DNA](#)". A shifted peak, a shifted trough, or additional peaks are indicative of contaminants in the sample that would result in overestimation of DNA concentration.

An A_{260}/A_{280} ratio is often recorded, where a ratio of 1.8 suggests sample purity. A higher ratio may indicate RNA contamination. A lower ratio may indicate contamination with protein, phenol, or other contaminants, or that there is limited DNA present.

Similarly, an A_{260}/A_{230} ratio should be above 2.0. A higher ratio may indicate that the wrong blank was used. A lower ratio may indicate contamination, including phenol or guanidine.

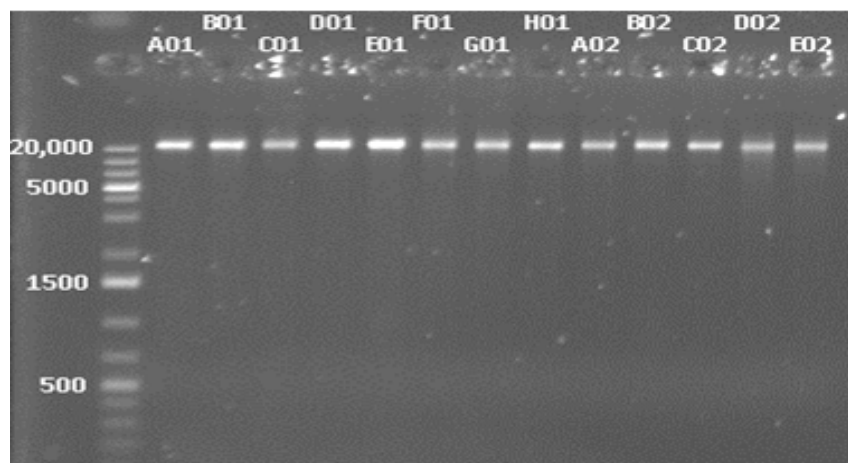
Note that when a sample contains contamination from multiple sources, interpretation of absorbance ratios would be complicated.

Appendix B: Examples of Agarose Gels from Passing and Failing Samples

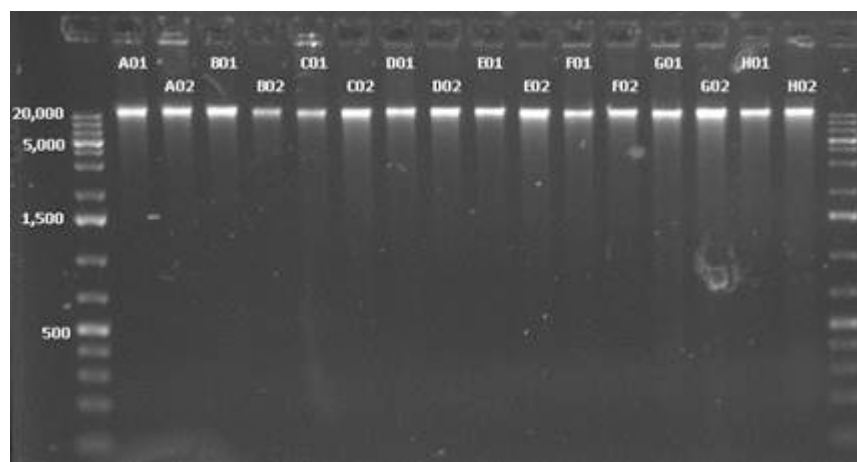
During Sample QC, DNA samples are diluted and aliquots for each sample are run on a 0.8% agarose gel. The following are examples of the agarose gels from DNA samples that either pass or fail our QC, with explanations of why the sample was or was not accepted for sequencing.

Samples that Pass QC

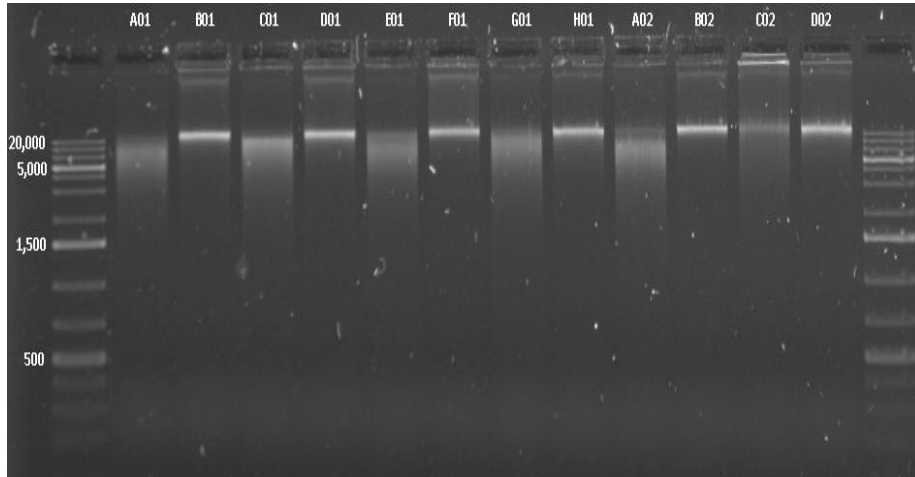
Example 1: Gel results indicate that the sample DNA is > 20 kb.



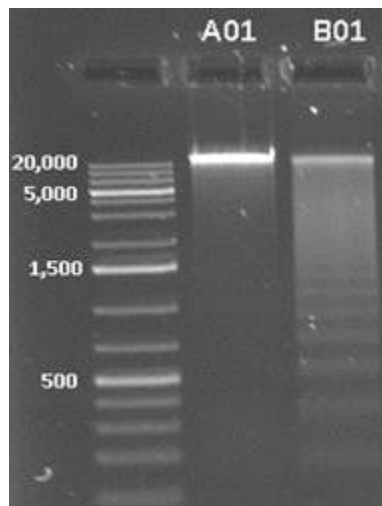
Example 2: Gel results indicate that the majority of sample DNA is > 20 kb, with minimal smearing.



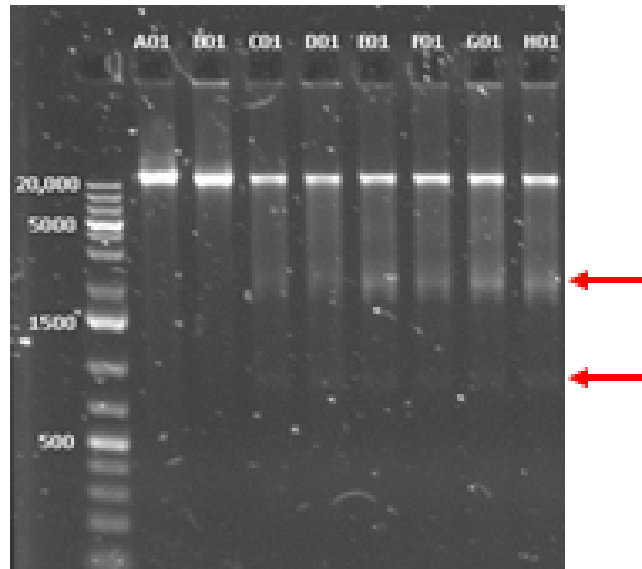
Example 3: This set includes tumor-normal pairs. The tumors do not have a clear band above 20 kb, but the DNA is still clearly above 5 kb in size.



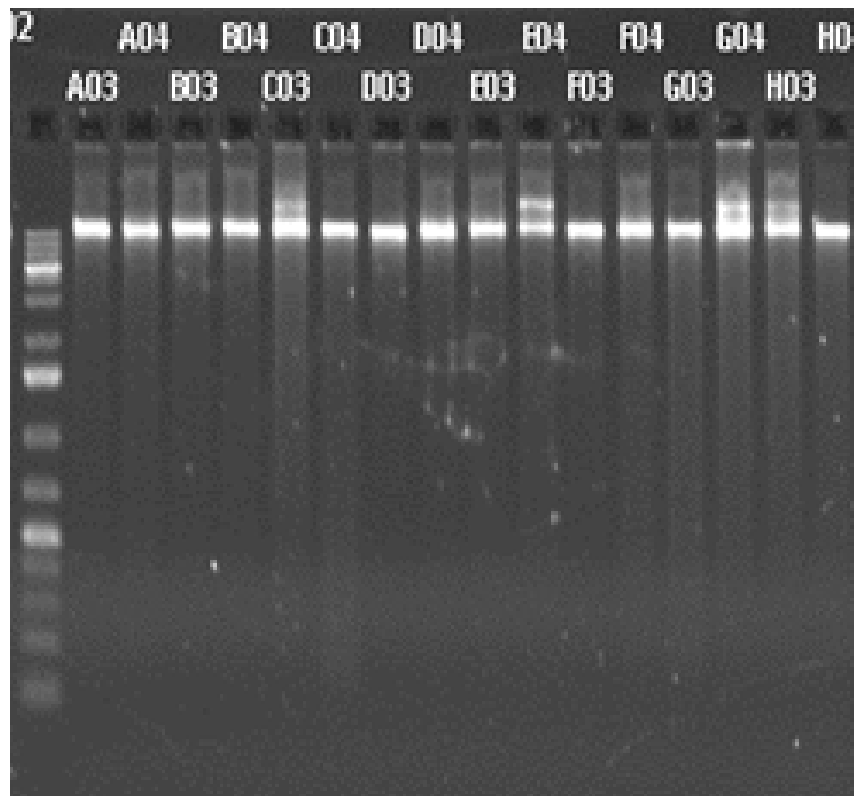
Example 4: Sample B01 shows signs of low molecular weight banding indicative of nucleosomal degradation. There is currently no evidence of nucleosomal banding impacting sequence data quality, and so such samples are accepted, so long as the bulk of the DNA is larger than 5 kb.



Example 6: Most of these samples contain contaminating ribosomal RNA (indicated by bands marked with red arrows). No evidence exists that RNA impacts sequence data quality, but it can result in overestimation of DNA quantity (see [Appendix A: Effects of Contamination on Quantitation](#)).



Example 7: All samples indicate a strong band > 20 kb of double-stranded DNA. However, a second, high molecular weight band is present for samples C03, E04, G04, and H03. This slower mobility DNA can suggest the presence of single-stranded DNA or protein contamination. There is a possibility that we will not be able to deliver CNV and SV data for these samples, and that there will be increased error rates for small variants. For this reason, **we highly recommend that you provide a replacement sample that does not include slow-migrating DNA.** Many of the other samples (such as A03 – B04) show some smearing of DNA above the >20 kb band, which indicates lesser effected samples or sub-optimal electrophoretic conditions.



Samples that Fail QC

Example 8: Samples C01, F01, and G01 fail due to degraded or absent DNA. The remaining samples pass with clear bands > 20 kb.

