Cancer Sequencing Service
Getting Started Guide

Cancer Pipeline 2.0

This document provides an overview of Complete Genomics data and references to additional documentation, tools, and resources that will familiarize you with Complete Genomics data and maximize the value of your sequencing project.

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5 Things You Need to Know

If you haven't worked with Complete Genomics data before, we want to do everything we can to make sure you are off to a good start. This short checklist will help you get the most from your sequencing data:

- ☐ Schedule a local Workshop Training if you haven't yet done so.
- ☐ Backup your data and perform a data integrity check.
- ☐ Familiarize yourself with the structure and content of the data.
- ☐ Prepare yourself for downstream analysis by becoming acquainted with Complete Genomics open-source software tools package CGA™ Tools and our Tool Repository.
- ☐ Make sure you know where to get help: learn about Complete Genomics support resources.

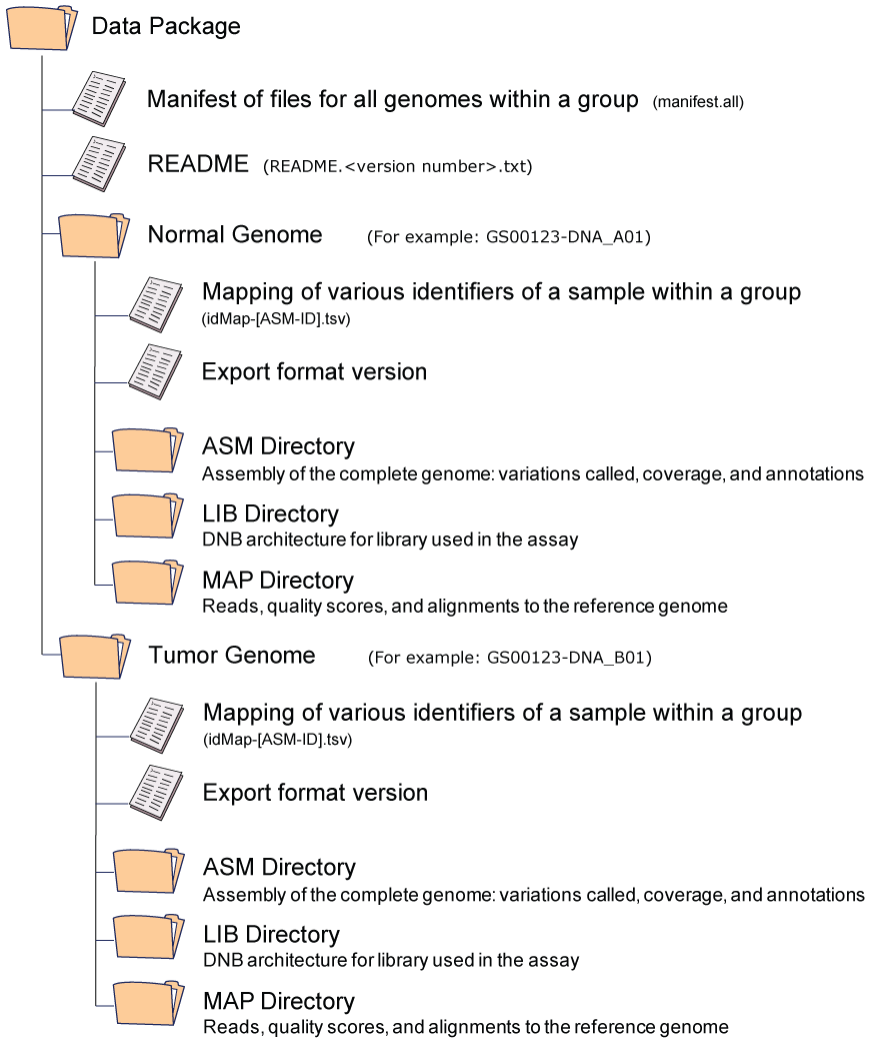
Understanding the Data Structure and Content

Data Structure

The data delivered by Complete Genomics include sequenced reads, their mappings to a reference human genome, annotated variants from the human reference, and somatic variants present in a tumor sample but absent from its matched normal sample. The data files are organized according to the directory structures shown in Figures 1 through 3. Most files are provided in tab-delimited text format (.tsv) or in a compressed version of this format (tsv.bz2). Detailed descriptions of Complete Genomics data files can be found in our *Data File Formats* document.

As shown in Figure 1, genomes that are sequenced and analyzed together using Complete Genomics Cancer Sequencing Service are delivered as a group, either a pair or trio, in a single package. The ***idMap-[ASM-ID].tsv*** file within each individual genome directory provides a map of the sample identifiers in the multi-genome dataset.

Figure 1: Complete Genomics Genome Data Structure



As shown in Figure 2 and 3, the data delivered for the tumor and matched normal genomes are slightly different. Each genome directory contains variant calls relative to the reference genome and an EVIDENCE directory containing read support for the called variants. Both genomes also have an EVIDENCE directory containing read support for the variants present in the other genome (described below). Unique to the tumor genome directory are additional files containing somatic variant calls.

Figure 2: ASM Directory Structure for Normal Genome

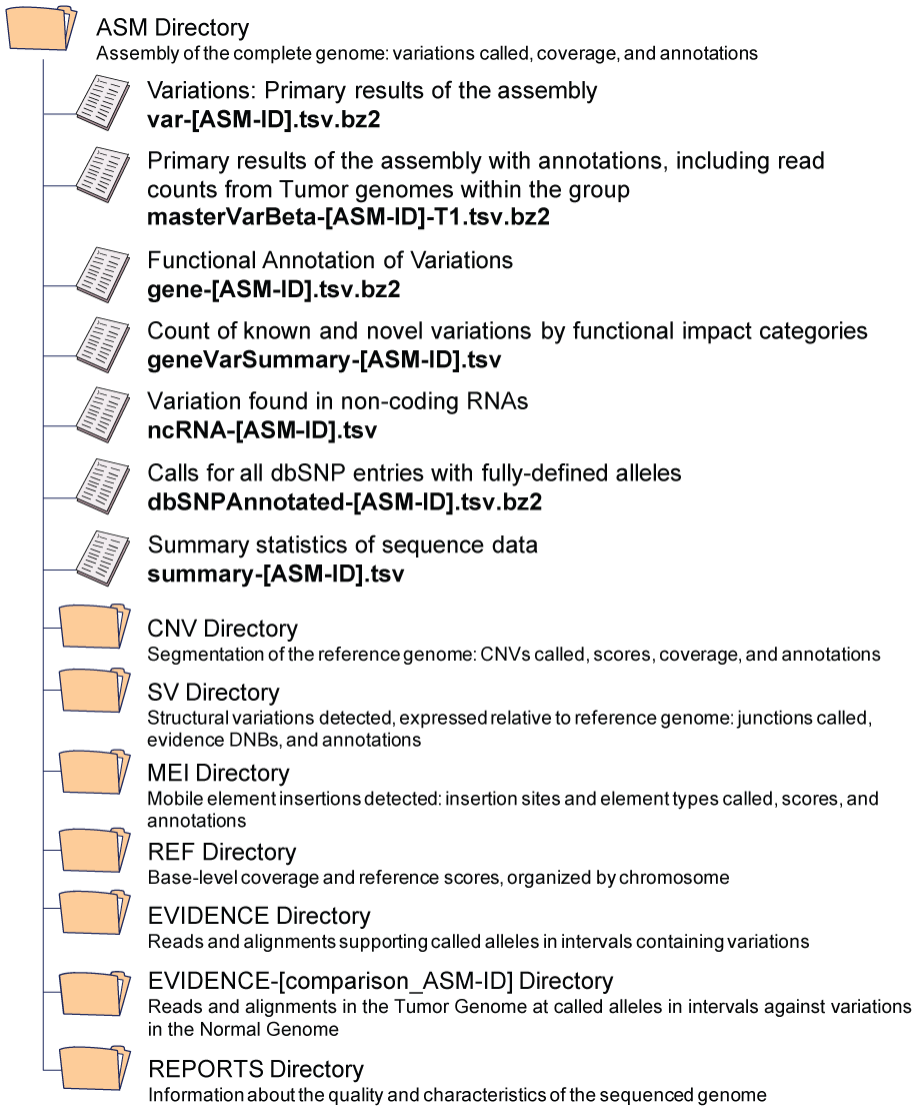
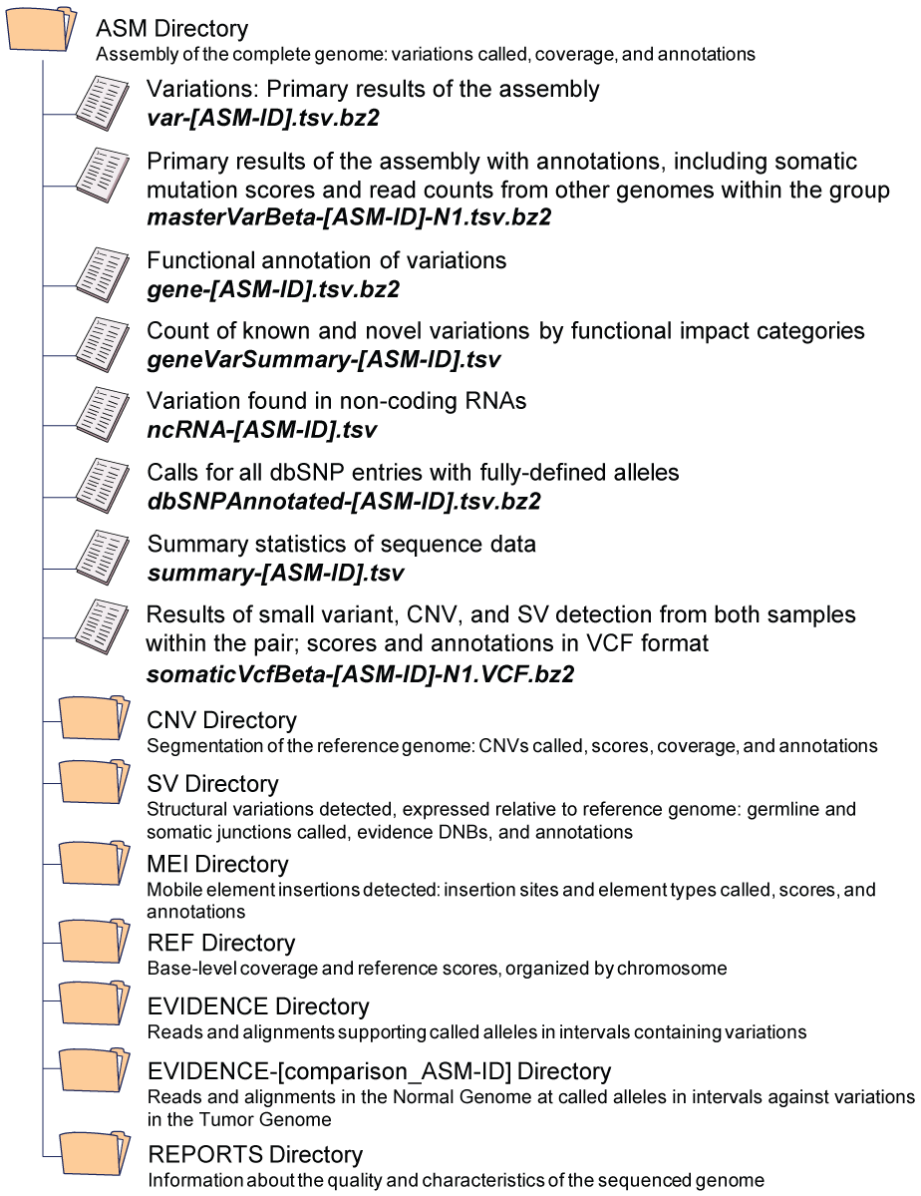


Figure 3: ASM Directory Structure for Tumor Genome



Data Content

Each individual genome directory within the data package contains three sub-directories:

- ASM — contains information on coverage, annotated called variants, and the reads and mappings that support the variant calls.
- LIB — contains information on the library of clones (DNBs) used for sequencing.
- MAP — contains the raw reads, their quality scores, and their initial mappings to the reference genome. The mappings in this directory support homozygous reference calls across the genome.

Quality Information

Information on the quality of the genome sequence can be found in two locations:

- The ***summary-[ASM-ID].tsv*** file (located in the ASM directory) contains summary statistics of sequencing metrics and variations. This file provides information about coverage, call rate, the number of variants (including small variations, copy number variations, and structural variations), and the transition-to-transversion ratio.
- The REPORTS directory contains additional files that support the statistics described in the summary file and also contains files describing the distribution of coverage, GC bias, and the size of small indels across the genome. These files can be used to plot the genome-wide or exome-wide coverage distribution or to plot coverage as a function of GC content.

Small Variations

Small variant calls (SNPs, substitutions, insertions and deletions of ≤ 50 bp) across the genome are provided in two formats. The ***masterVarBeta*** files present variants in a single line per locus format, whereas the ***var*** file presents variants in a multiline format. Both files are located in ASM directory, with the ***masterVarBeta-[ASM-ID]-N1.tsv.bz2*** and ***masterVarBeta-[ASM-ID]-T1.tsv.bz2*** files located in the ASM directories of the tumor and normal genomes, respectively. ***masterVarBeta*** contains a rich set of annotations, including:

- Read count support for each variation in the genome and read counts supporting the same variation in the other samples within the cancer group
- Variation scores (*varScoreVAF* and *varScoreEAF*) and confidence category (*varQuality*) that measure the confidence that the called variation is true
- Variety of somatic scores that enable identification of somatic variants (***masterVarBeta-[ASM-ID]-N1.tsv.bz2*** only)
- Matching dbSNP and COSMIC record IDs
- RefSeq transcript, PFAM, and functional annotations
- Overlapping miRNAs
- Copy number information
- Somatic CNV calls and estimates of the lesser allele fraction of the CNV segment in the tumor compared to the normal sample (***masterVarBeta-[ASM-ID]-N1.tsv.bz2*** only)

The tumor genome ASM directory also contains the ***somaticVcfBeta-[ASM-ID]-N1.VCF.bz2*** file which includes all of the variant calls and annotations present in the ***masterVarBeta*** file for a given tumor genome **and** its matched normal. See "Somatic Variant VCF File" for more information.

Customers interested in the exome can refer to the ***gene-[ASM-ID].tsv.bz2*** file, which lists all variants identified in each RefSeq transcript. It provides the location of the variant within the transcript, COSMIC and dbSNP annotations, PFAM information, and functional annotations, including information on protein coding alterations. The ***geneVarSummary-[ASM-ID].tsv*** file summarizes this information, distilling it down to the number of known and novel variants predicted to affect protein function for each RefSeq transcript.

The EVIDENCE directories contain the reads and mappings that support small variant calls. Genomes sequenced using the Complete Genomics Cancer Sequencing Service contain two such directories for each genome. The EVIDENCE directory contains the reads and mappings supporting variants identified within the given genome. Because of the presence of the variant sequence, some of these reads may not have aligned to the reference genome during the initial mapping, but will be present here. Conversely, some reads mapped to this location during initial mapping will be absent from the EVIDENCE directory, because the more precise and stringent local de novo assembly will exclude them.

For tumor genomes, the EVIDENCE-[comparison_ASM-ID] directory (for example, `EVIDENCE-GSxxxxxxxxx-ASM-N1`) contains the reads and mappings from the matched normal genome that support the variants called in the tumor genome. For the normal genome, the EVIDENCE-[comparison_ASM-ID] directory (for example, `EVIDENCE-GSxxxxxxxxx-ASM-T1`) contains the reads and mappings from the designated tumor genome that support the variants called in the normal genome. Unlike the reads and mappings in the EVIDENCE directory, the mappings in these comparative directories are not generated by local *de novo* assembly. Instead, they are produced by realignment of reads from one genome to the alleles detected in the other genome (either tumor or normal).

Somatic Variant VCF File

The ***somaticVcfBeta-[ASM-ID]-N1.vcf.bz2*** file is located in the ASM directory of each tumor genome and contains the annotated small variant, structural variation, and copy number variation calls for the given tumor and its matched normal. It also contains information about discordances between the two. Small variant annotations are similar to those provided in the ***masterVarBeta*** file (see "Small Variations"). CNV annotations are similar to those provided in the ***cnvSegment*** files, and structural variant annotations are similar to those provided in the ***highConfidenceJunctionsBeta*** files (see below). The ***somaticVcfBeta*** file conforms to the VCF 4.1 specification, and includes cancer-specific extensions for structural variants. Currently there is no corresponding file comparing the normal genome to the tumor genome.

Coverage

The ***coverageRefScore-[chr]-[ASM-ID].tsv.bz2*** file, located in the ASM/REF directory, provides various measurements of coverage for each position in the reference genome. These measurements are based on the initial mappings of the reads to the reference genome and can be used to plot coverage across the genome.

Copy Number Variations (CNVs)

Both diploid and nondiploid models are used to analyze coverage information and segment the genome into regions with distinct coverage levels. For normal genomes and tumor genomes with little copy number aberration, Complete Genomics recommends analyzing segmentation calls generated using the diploid model reported in the ***cnvSegmentsDiploidBeta-[ASM-ID].tsv*** file, located in the ASM/CNV directory. This file includes coverage, confidence scores, segment ploidy and annotations for regions of abnormal ploidy with information about genes that overlap or are contained within the CNV and links to known CNVs and repetitive elements. The related ***cnvDetailsDiploidBeta-[ASM-ID].tsv*** file provides ploidy and coverage information for 2kb windows across the reference genome and can be used to visualize CNVs.

For tumor genomes with significant coverage variation, Complete Genomics recommends analyzing segmentation calls generated using the nondiploid model reported in the ***cnvSegmentsNondiploidBeta-[ASM-ID].tsv*** file, located in the ASM/CNV directory. This file is analogous to the diploid model file but calls segment coverage levels instead of ploidy. ***cnvDetailsNondiploidBeta-[ASM-ID].tsv*** is similarly analogous to the diploid version of the same file, except that the window size is expanded to 100 kb.

Somatic CNV calls are provided in additional "somatic" versions of the files described above in the CNV directory of the tumor genome. Somatic CNV calls are generated by comparing the tumor genome to its matched normal. In addition to the annotations described above, the somatic CNV segments files also contain estimates of the lesser allele fraction, which can be used to identify loss-of-heterozygosity regions in the tumor genome.

Structural Variations (SVs)

Discordant mate-pair analysis is used to identify junctions, adjacent regions in the genome that are not adjacent in the reference genome. Where possible, reads that straddle the junction are assembled

to provide the sequence of the junction. The ***highConfidenceJunctionsBeta-[ASM-ID]-ASM.tsv*** file, located in the ASM/SV directory, lists high-quality junctions in the sample genome that have been filtered using a variety of quality metrics to enhance specificity. Junction information includes the junction location, the assembled junction sequence, the number of reads supporting a given junction, and the size of the insertion or deletion for intrachromosomal junctions. The file is also annotated with information on repeats and transcripts that overlap the junction as well as known structural variations. In tumor genomes, a similar ***somaticHighConfidenceJunctionsBeta-[ASM-ID]-N1.tsv*** file lists high-confidence somatic junctions detected in tumor genome that were absent in the normal genome.

In addition to providing a list of junctions, Complete Genomics rationalizes those junctions into the structural rearrangement events from which they derive, including insertions, deletions, translocations, and inversions. The ***highConfidenceSvEventsBeta-[ASM-ID].tsv*** file lists all SV events predicted by rationalizing high confidence junctions and annotates each putative event with information including event type, genomic location, and any gene fusions or disruptions predicted by the event.

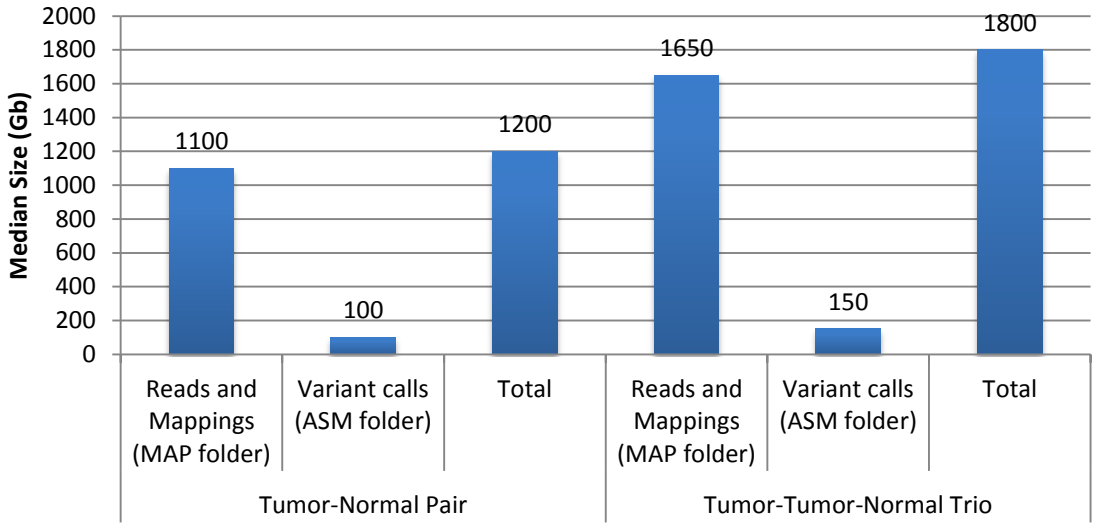
Mobile Element Insertions (MEIs)

Novel MEIs (those not present in the reference genome) are identified by locating clusters of mate pairs where one end maps unexpectedly to a mobile element and the other maps to unique reference sequence. The presence, rather than the precise location or complete sequence of the novel MEI is recorded. The ***mobileElementInsertionsBeta-[ASM-ID].tsv*** file provides information on each insertion, including location, mobile element type, number of supporting reads, and overlapping genes. ***mobileElementInsertionsRefCountsBeta-[ASM-ID].png*** graphs the number of DNBs supporting the reference sequence for each MEI and can be used to predict MEI zygosity. Finally, the ***mobileElementInsertionsROCBeta-[ASM-ID].png*** graph shows the relationship between a specific MEI confidence score and the predicted frequency of false negatives and false positives, and should be used to select a score cutoff to balance the needs of specificity and sensitivity.

How to Manage Sequencing Data

Data Volume

This chart shows an overview of the sizes of the data packages produced using Complete Genomics Cancer Sequencing Service at high coverage:



For more information about data management, including receiving, checking, and working with Complete Genomics data see the frequently asked questions in Managing Data FAQ.

Data Integrity

IMPORTANT

You ***must*** ensure the security and completeness of your data within the first 30 days after receipt.

As indicated in the Complete Genomics Service contract, Complete Genomics will begin deleting customer data 30 days after shipment to the customer.

Complete Genomics recommends taking the following steps to ensure and maintain data integrity:

- Step 1: Make Backup Copies of Your Data
- Step 2: Check that the Data Package is Complete

Step 1: Make Backup Copies of Your Data

Complete Genomics strongly recommends that you make backup copies of the data. If you receive data on a hard disk drive, we do not recommend using the delivered hard drives as the primary backup location. This storage method provides no redundancy in case of drive failure.

Step 2: Check that the Data Package is Complete

After receiving Complete Genomics data, one of your first actions should be to verify that all data files are present and uncorrupted. Data verification should be performed using the ***manifest.all*** file provided for each multigenome assembly. The ***manifest.all*** file contains SHA-256 checksums of each

file in the data package (except for the ***manifest.all*** and ***manifest.all.sig***), and is suitable for use with the sha256sum tool present on Linux operating systems (also available for other platforms).

Assuming the data is copied to another system immediately upon receipt (both to provide working storage and as a backup), customers should check the SHA-256 sums on the copy made, and if any problems arise, check the SHA-256 sums on the original hard disk drive. If any data appears to be missing or corrupted you should contact support@completegenomics.com immediately.

To check the integrity of the data package:

On Linux:

```
sha256sum -c /path/to/manifest.all
```

On Mac OS X, from the terminal window:

```
shasum -a 256 -c /path/to/manifest.all
```

If no errors are reported, the verification was successful.

Complete Genomics also provides a security certificate that can be used to ensure that the data provided to the customer was shipped from Complete Genomics. Please see "How do I verify that the data files are present and uncorrupted?" in the Managing Data FAQ for more information.

Resources for Interpreting the Data

Analysis Tools and Resources

Several tools are available for downstream analyses of Complete Genomics data:

- CGA Tools — A repertoire of file conversion and analysis modules developed by Complete Genomics.
- Complete Genomics Tool Repository — The Tool Repository on the Complete Genomics User Community website contains a collection of Complete Genomics authored scripts for downstream analysis.
- Complete Genomics Public Genome Data Repository — Complete Genomics offers whole human genome sequence data sets on its FTP server for free download and general use. These data result from the sequencing of 69 standard, non-diseased samples as well as two matched tumor and normal sample pairs. This large data set may be used for many things including filtering out non-disease causing variants. Resources available include:
  - Overview of the Public Genomes
  - Public Genome Data Repository Service Note — detailed description of the public genome sequence data.
  - Summary Analysis Readme — Overview of additional files containing summary statistics across the standard genomes and lists of the variants found in each genome.
- Software Partners — Complete Genomics has partnered with some of the leading developers of software for downstream analysis. The tools offered by our partners have been tested to ensure compatibility with Complete Genomics data.
- Third-Party Tools — A list of tools that can be used to analyze Complete Genomics data.

Documentation

Complete Genomics provides an abundance of documentation on our website:

http://www.completegenomics.com/customer-support/documentation/

The following documents may be particularly helpful to new users of our services.

- *Complete Genomics Technology Whitepaper* — A concise description of the Complete Genomics sequencing technology, including the library construction process and the ligation-based sequencing.
- *Complete Genomics Data File Formats Cancer Pipeline 2.0* — A description of the organization and content of the format for complete genome sequencing data delivered by Complete Genomics.
- *Complete Genomics Analysis Pipeline Release Notes* — Indicates new features and enhancements by release.
- *Complete Genomics FAQs* — Important questions and answers related to data management, variant calling, and other topics.

Getting Support

Complete Genomics Data Analysis Training

Complete Genomics Field Application Science team provides bioinformatics training to assist its customers in the analysis of Complete Genomics data. These free training programs are organized regionally and scheduled based on customers' needs. Complete Genomics highly recommends you and your team attend a workshop to maximize your knowledge of Complete Genomics sequencing platform, data files, and best practices for downstream analysis. For more information, or to arrange training, contact your Complete Genomics Account Representative or email Complete Genomics support at support@completegenomics.com.

Call Center

Complete Genomics Call Center is available to answer technical questions during regular business hours (8am-6pm Pacific Time). The call center aims to respond to all questions within 24 business hours.

- support@completegenomics.com
- Toll-free in the US or Canada: 1-855-267-5383
- International: 1-650-943-2600

Questions about Complete Genomics Data Files and Analysis

When contacting the call center for questions about data and downstream analysis, provide the following information from the header of the ***summary-[ASM-ID].tsv*** file located in the ASM directory of the data package:

Header Field	**Description**
#SAMPLE	Sample ID for the genome(s) in question. For example, "GS11111-DNA_A01".
#SOFTWARE_VERSION	Version of the Complete Genomics Analysis Pipeline software used to generate the data.
#GENOME_REFERENCE	Version of the haploid human reference used for genome assembly.

Questions about CGA Tools

When contacting the call center about CGA Tools, provide the following information:

- Version of CGA Tools (run `cgatools` from the command line with no arguments)
- Operating system (run `uname -a` on the command line)
- Was CGA Tools installed using the provided binaries or recompiled from source?

Field Application Science Team

Complete Genomics Field Application Scientists (FAS) are experienced bioinformaticians who can help you maximize utility of your Complete Genomics data. Contact support@completegenomucs.com to obtain the contact information for your local FAS.

Complete Genomics User Community

Complete Genomics User Community provides a forum where customers can interact, collaborate, and share knowledge with each other. The Tools Repository is also located in the User Community.