GDC WGS Variant Calling Workflow Updates

31 March 2025

Bill Wysocki, Ph.D – GDC Director of User Services
Zhenyu Zhang, Ph.D – GDC Director of Bioinformatics
Center for Translational Data Science
University of Chicago

NIH NATIONAL CANCER INSTITUTE

Agenda

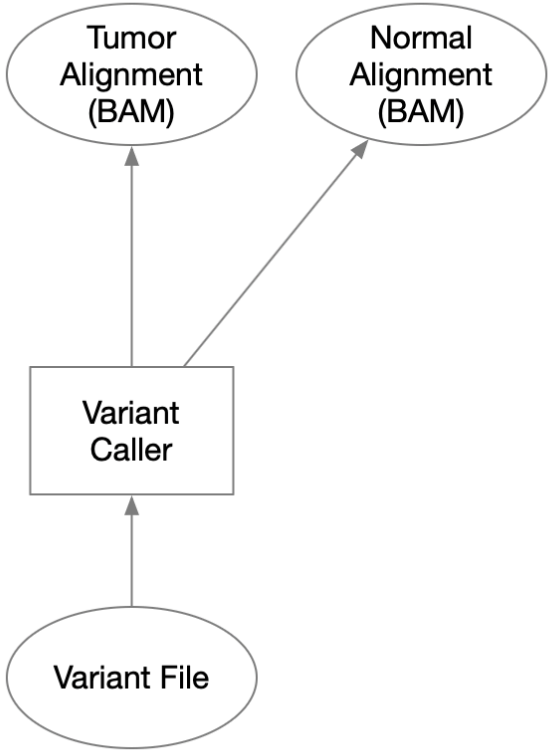
1. *Overview of Whole Genome Variants in the GDC*
2. *Available WGS Data*
3. *WGS Pipeline Overview*
4. *Downloading WGS Data*
5. *Documentation and Repositories*
6. *Questions*

Overview of Whole Genome Variants in the GDC

Somatic Mutations

WGS Data in the GDC

- Tumor and normal samples are aligned
- The resulting alignment are processed with variant caller workflows of different types:
  - Point mutations/ Indels (SSM)
  - Copy number variants
  - Structural variants



- There are two WGS workflow sets -

Existing Sanger WGS Workflow Set

1. *Point Mutation: CaVEMan*
2. *Indel: Pindel*
3. *CNV: ascatNGS*
4. *SV: BRASS*

New GDC WGS Workflow Set

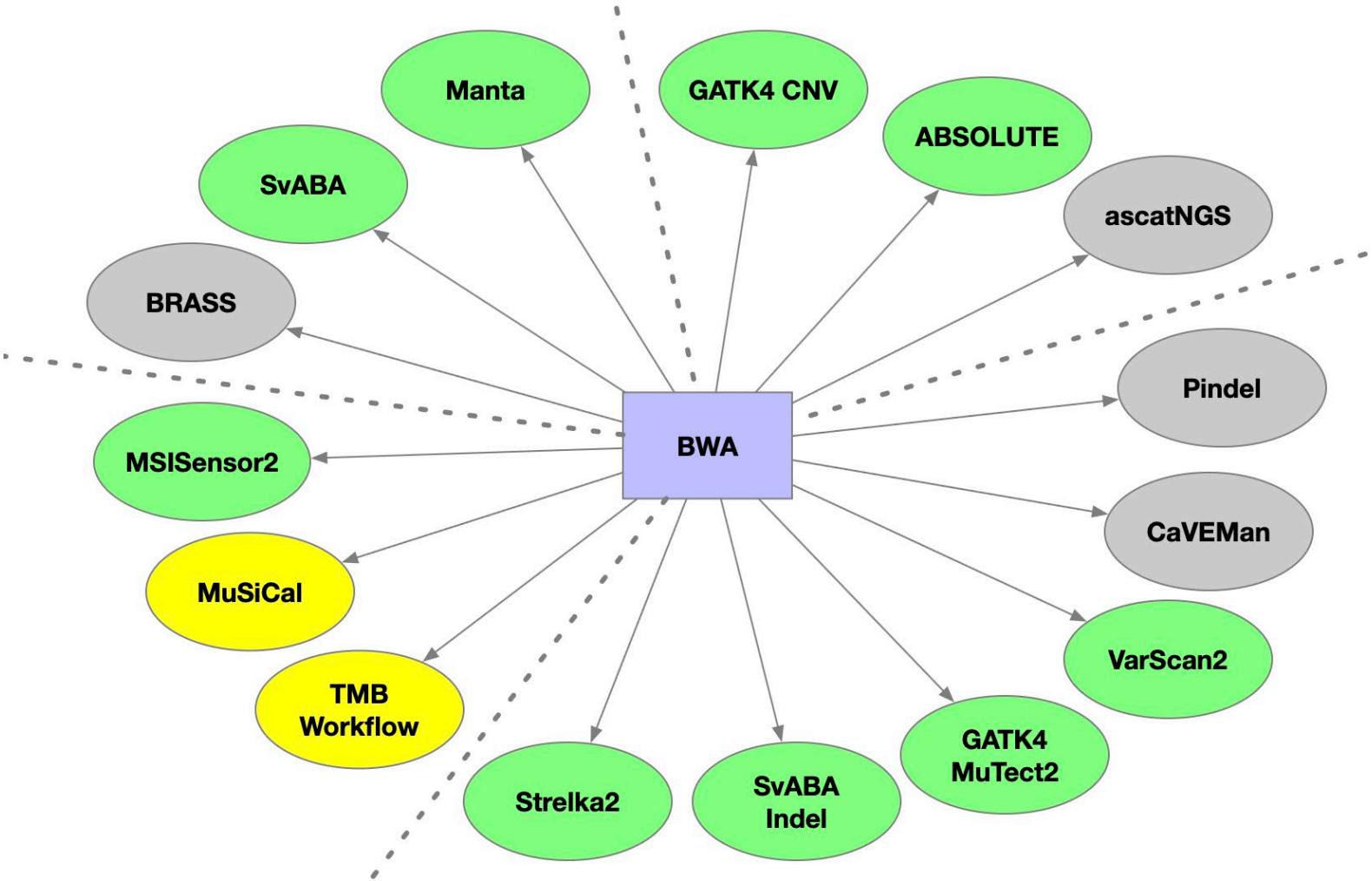
1. *Simple Somatic Mutation (SSM):*
   - *GATK4 MuTect2*
   - *VarScan2*
   - *SvABA Indel*
   - *Strelka2*
2. *Copy Number Variation (CNV):*
   - *GATK4 CNV + ABSOLUTE*
3. *Structural Variation (SV):*
   - *Manta*
   - *SvABA*

Summary of Major Changes

1. *Increase SSM calling from 2 callers to 4, which enables proper variant ensemble.*
2. *Increase SV calling from 1 caller to 2 callers.*
3. *Add ABSOLUTE for better CNV inference.*
4. *Stop Sanger workflow production, and run new WGS workflows across the GDC.*

WGS Data Releases in the GDC

- Data Release 27 (2020):
  - WGS variants were first released from the Sanger workflow set
- Data Release 42 (2025):
  - WGS variants were first released from the new GDC workflow set
- **Transition:**
  - All data that was processed with the Sanger workflow set will be processed with the new GDC workflow set
  - WGS processing is in currently in progress, any pipeline that is completed will be released in the subsequent data release
  - For SSMs (point + indels), this means that an annotated VCF is available


Manta
GATK4 CNV
ABSOLUTE
SvABA
ascatNGS
BRASS
Pindel
BWA
MSISensor2
CaVEMan
MuSiCal
VarScan2
TMB
Workflow
GATK4
MuTect2
Strelka2
SvABA
Indel

Available WGS Data

Currently Available in the GDC Data Portal

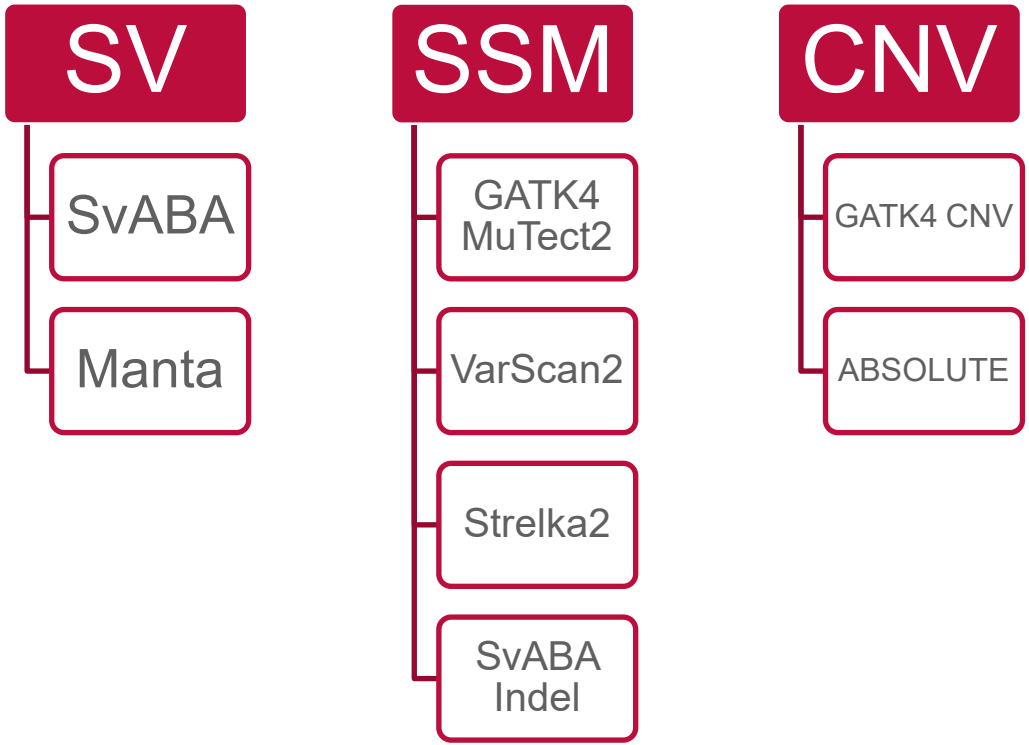
1. *More than 15,000 cases with aligned WGS BAMs*
2. *5,000+ cases with Sanger calls. We no longer use Sanger for production and will gradually release the rest of the Sanger calls that have already been completed.*
3. *Data from <14,000 cases of Manta, GATK4 CNV, GATK4 MuTect2, SvABA Indel callers have been released. We will release more in future releases and hopefully cover all WGS data in the GDC soon.*

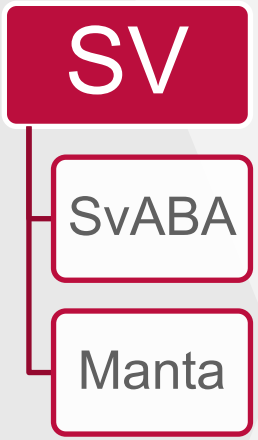
WGS Pipeline Overview

Why Switch Pipelines?

1. *SSM calling: increased from 2 callers to 4, that enables multi-caller variant ensemble.*
2. *SV calling: increased from 1 caller to 2, that enables users to derive all confidence SV set.*
3. *CNV calling: added ABSOLUTE for better CNV inference.*
4. *Efficiency: the new workflows, particularly modern tools like Strelka2 and Manta, require significantly fewer compute resources than the existing Sanger workflows.*

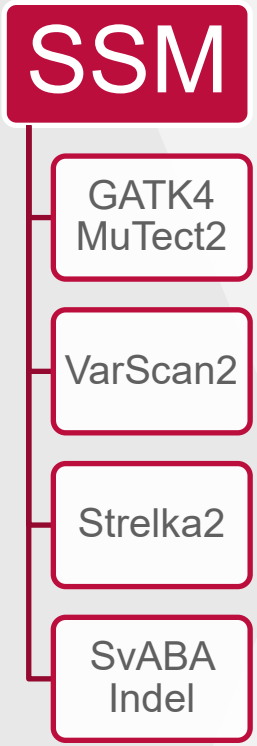
WGS Workflows





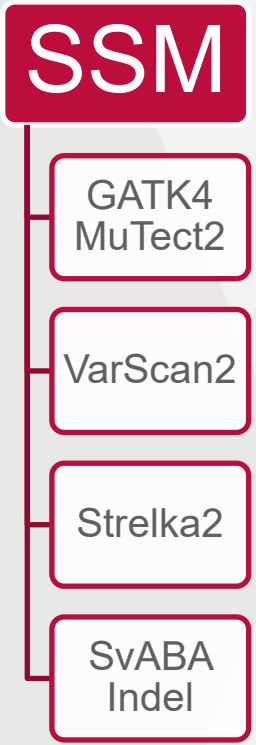
Output: VCF and BedPE

1. *SvABA SV Caller*
   - *Generate both VCF and BedPE.*
   - *Also generate an Indel VCF for SSM ensemble.*
2. *Manta SV Caller*
   - *Generate both VCF and BedPE.*
   - *Also generate a candidate SSM VCF. This VCF is then fed into Strelka2 calling to enhance the quality and coverage of Strelka2.*



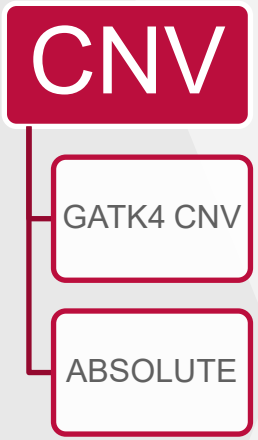
Output: raw VCF, annotated VCF, aliquot-level MAF, 4-caller ensemble MAF

1. *GATK4 MuTect2*
   - *Different from the MuTect2 used in WXS calling (GATK3).*
   - *Known issue: Missing variants on chr10 and chr20 in currently released VCFs. We expect to fix in later data releases.*
2. *VarScan2*
   - *The same caller used in WXS calling.*
   - *Not released yet.*



Output: raw VCF, annotated VCF, aliquot-level MAF, 4-caller ensemble MAF

3. *Strelka2*
   - *Implemented as Strelka2 – Manta joint calling. Manta candidate VCFs is further filtered by Strelka2 calling to enhance the quality and coverage of Strelka2.*
4. *SvABA Indel*
   - *Indel output from the SvABA SV calling.*



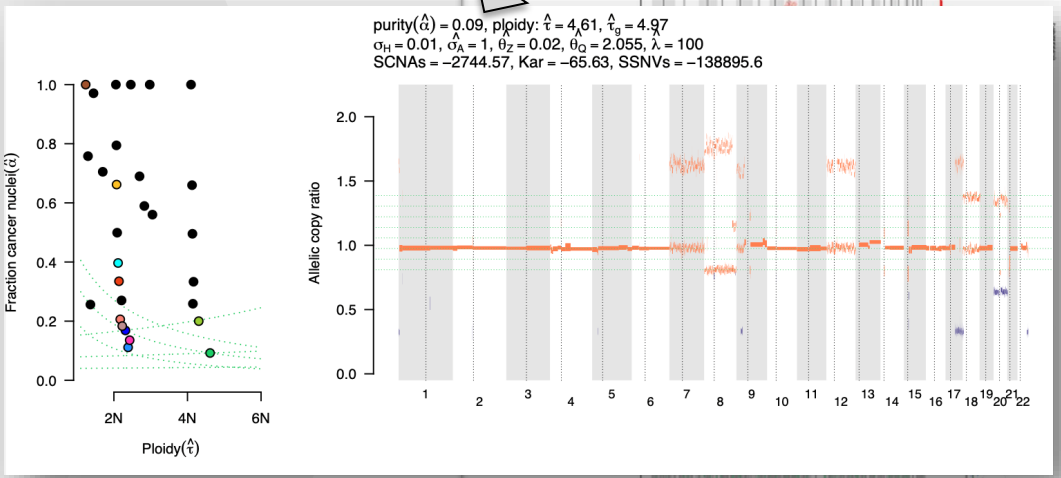
Output: Segmentation, gene-level CNV, purity/ploidy estimation

1. *GATK4 CNV caller*
   - *Non-integer copy number segmentation file (segmean).*
   - *Per request by genomics analysis groups, we also provide a controlled-access intermediate analysis archive tarball for expert manual review purpose.*
2. *ABSOLUTE CNV Caller*
   - *Use GATK4 CNV and GATK4 MuTect2 input to derive integer-level copy number segmentation, model PDF, and gene-level copy numbers.*
   - *Not released yet.*

Output: Segmentation, gene-level CNV, purity/ploidy estimation

- *More on ABSOLUTE*
  - *ABSOLUTE is run in two steps: 1) "model generation" that generates multiple possible CNV models (purity and ploidy combinations); 2) "model extraction" that extracts the segmentations from one selected model.*
  - *We plan to provide a PDF report that contains all models from a run, and also automatic model extraction of the first model called "ABSOLUTE GATK4 CNV Auto". With curation provided by community experts, we may later release "ABSOLUTE GATK4 CNV Curated" when available.*

ABSOLUTE Model PDF Report



Other WGS Data and Workflows

1. *BAM metrics, including coverage and other information. They are on the AlignedReads node, and available via API query, various facet filtering in GDC portal, and single BAM entity page.*
2. *Microsatellite Instability (MSI) by MSISensor2, at the same place as other BAM metrics, but only available in Tumor BAMs.*
3. *Tumor Purity and Ploidy. They are on the segmentation file node from ascatNGS and ABSOLUTE workflows, available via API.*

Development in Progress

1. *Tumor Mutational Burden (TMB)*
2. *Tumor Mutational Signature (MuSiCal)*

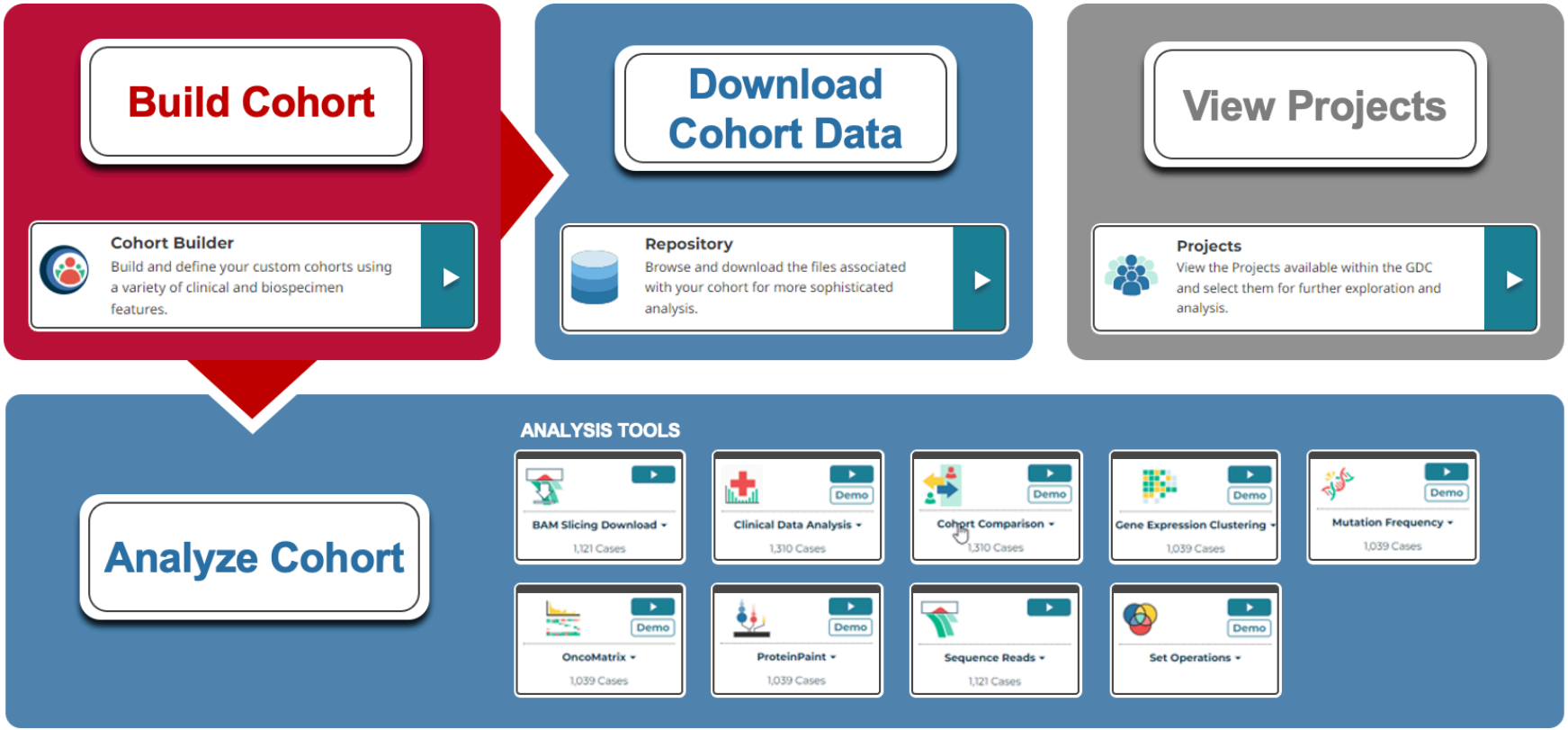
Downloading WGS Data (Demo)

Goal: Download the following:

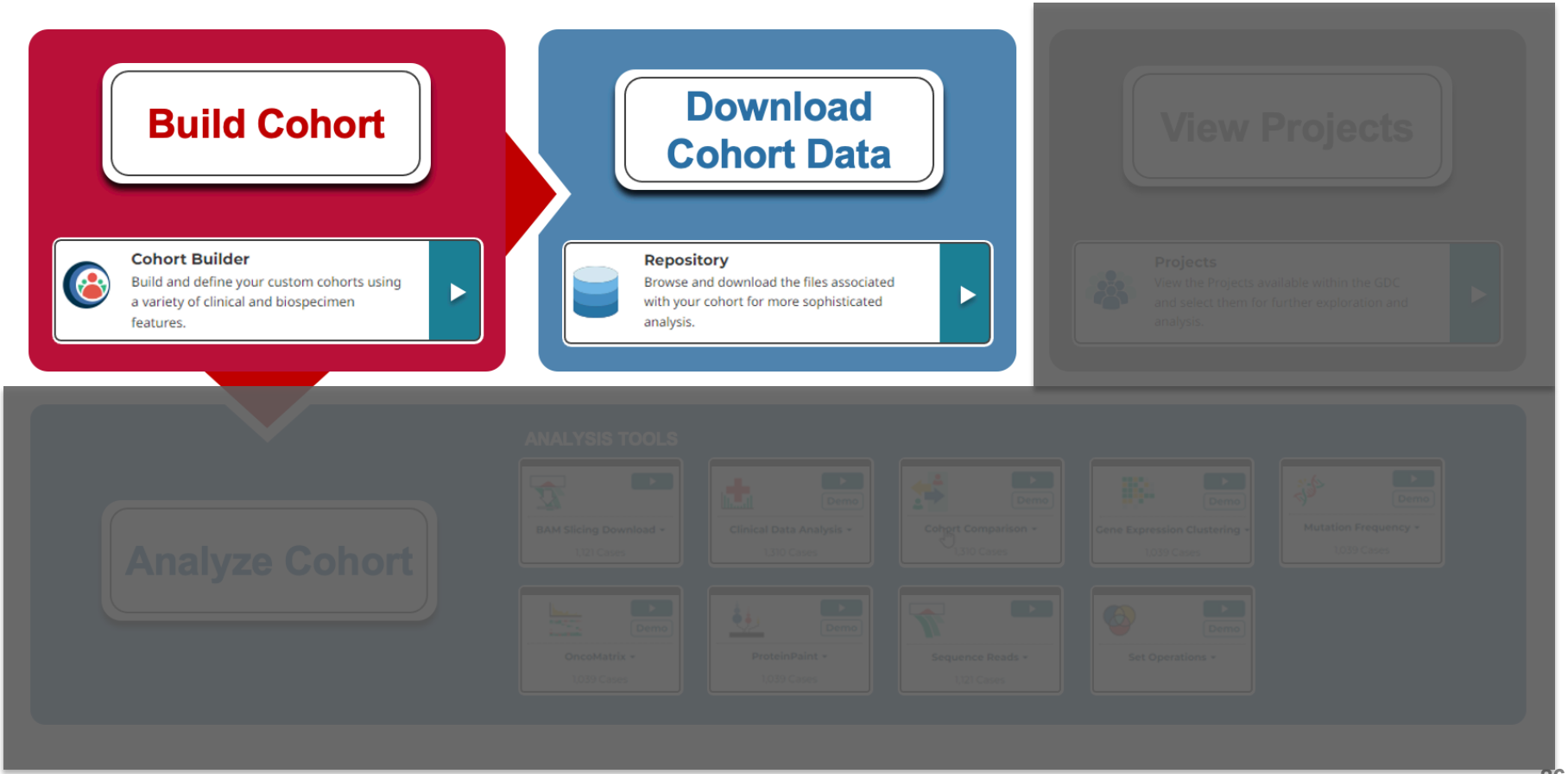
- Annotated Somatic Mutation VCFs (SNVs + Indels)
- Manta BEDPE files

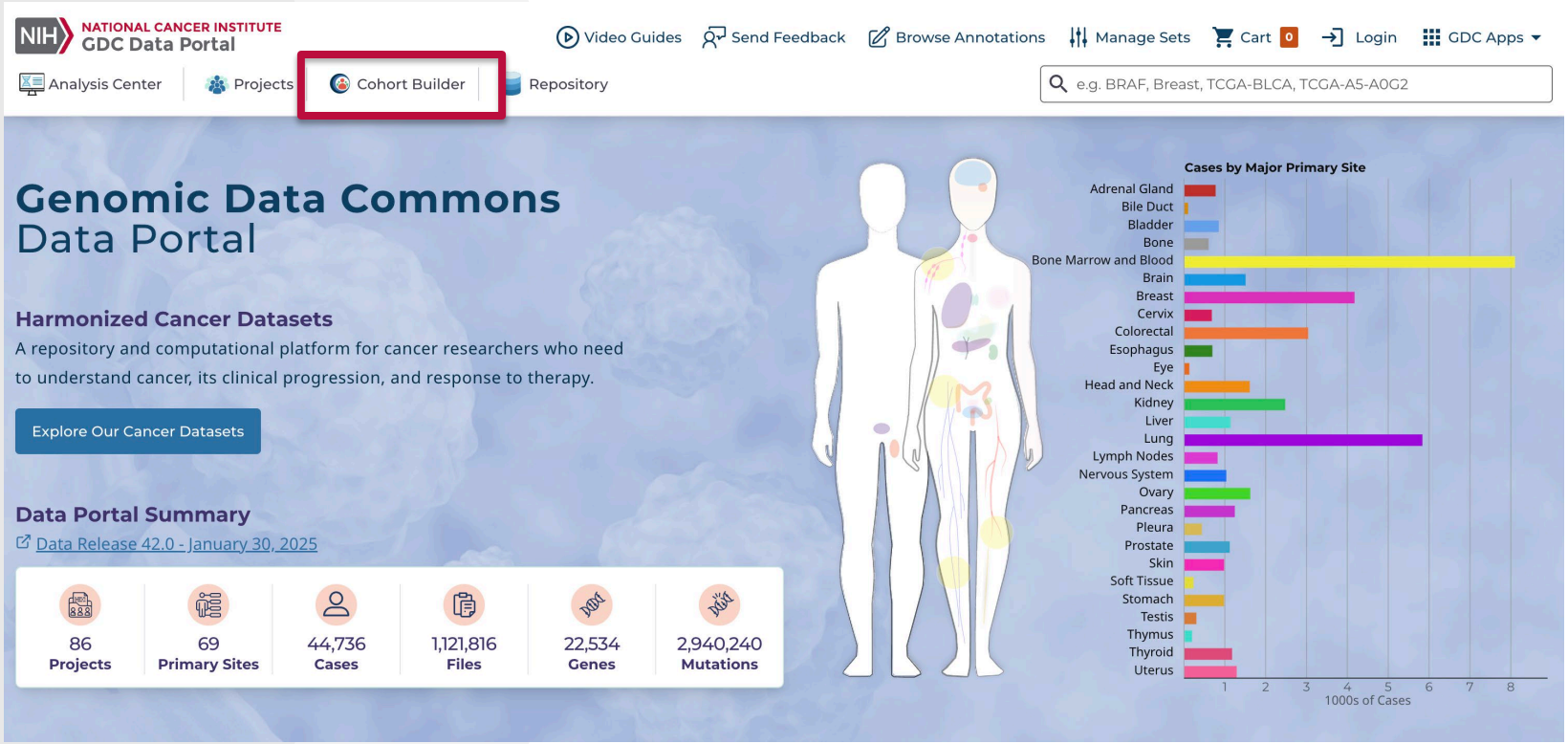
from cases with lung cancer

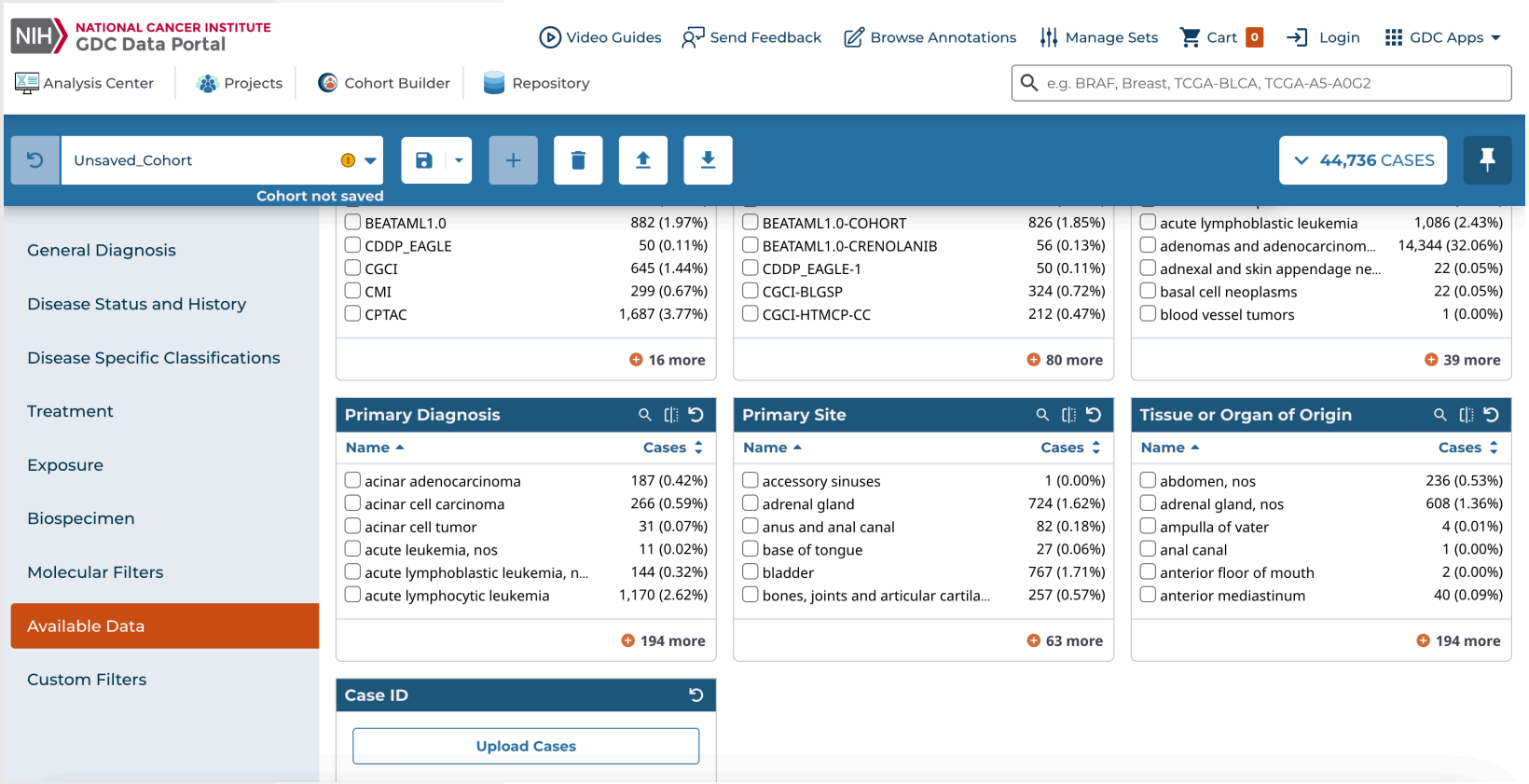
GDC 2.0 Workflow

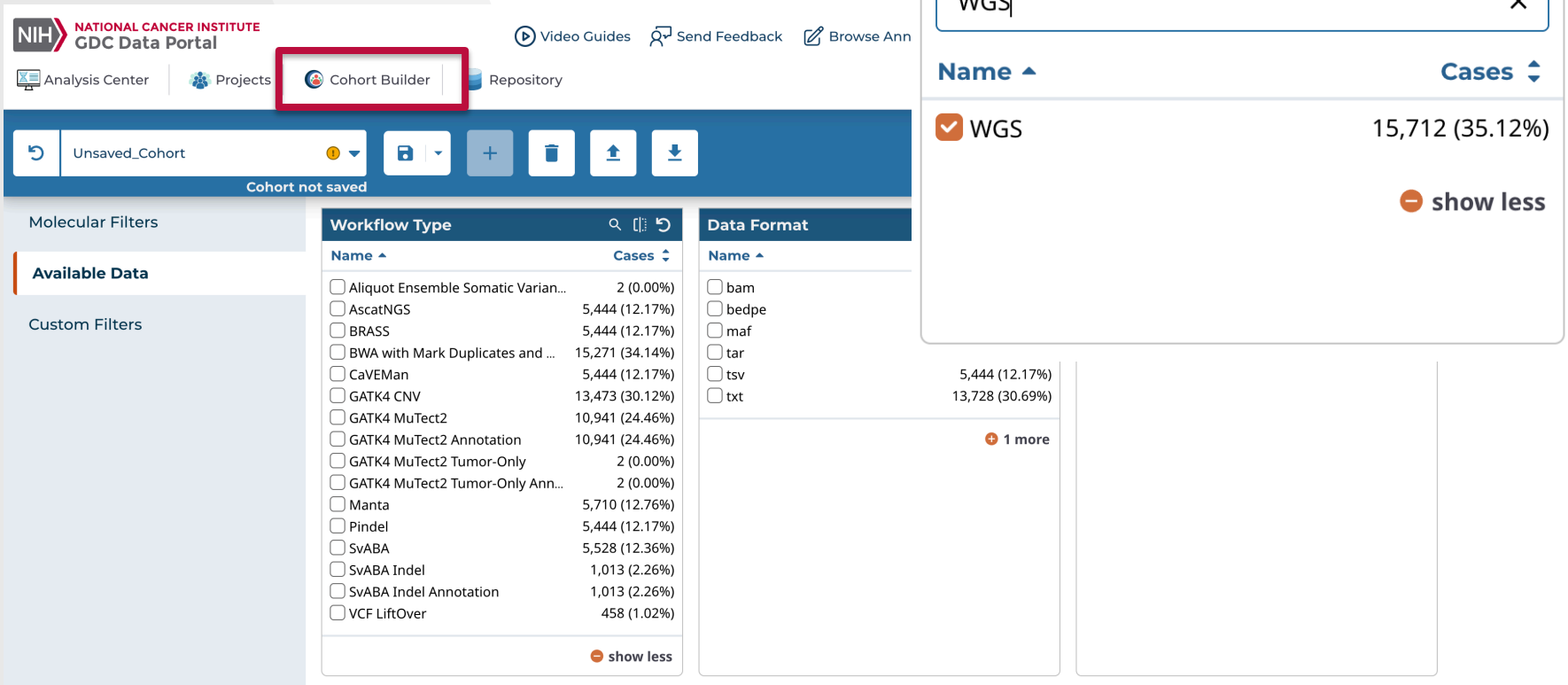


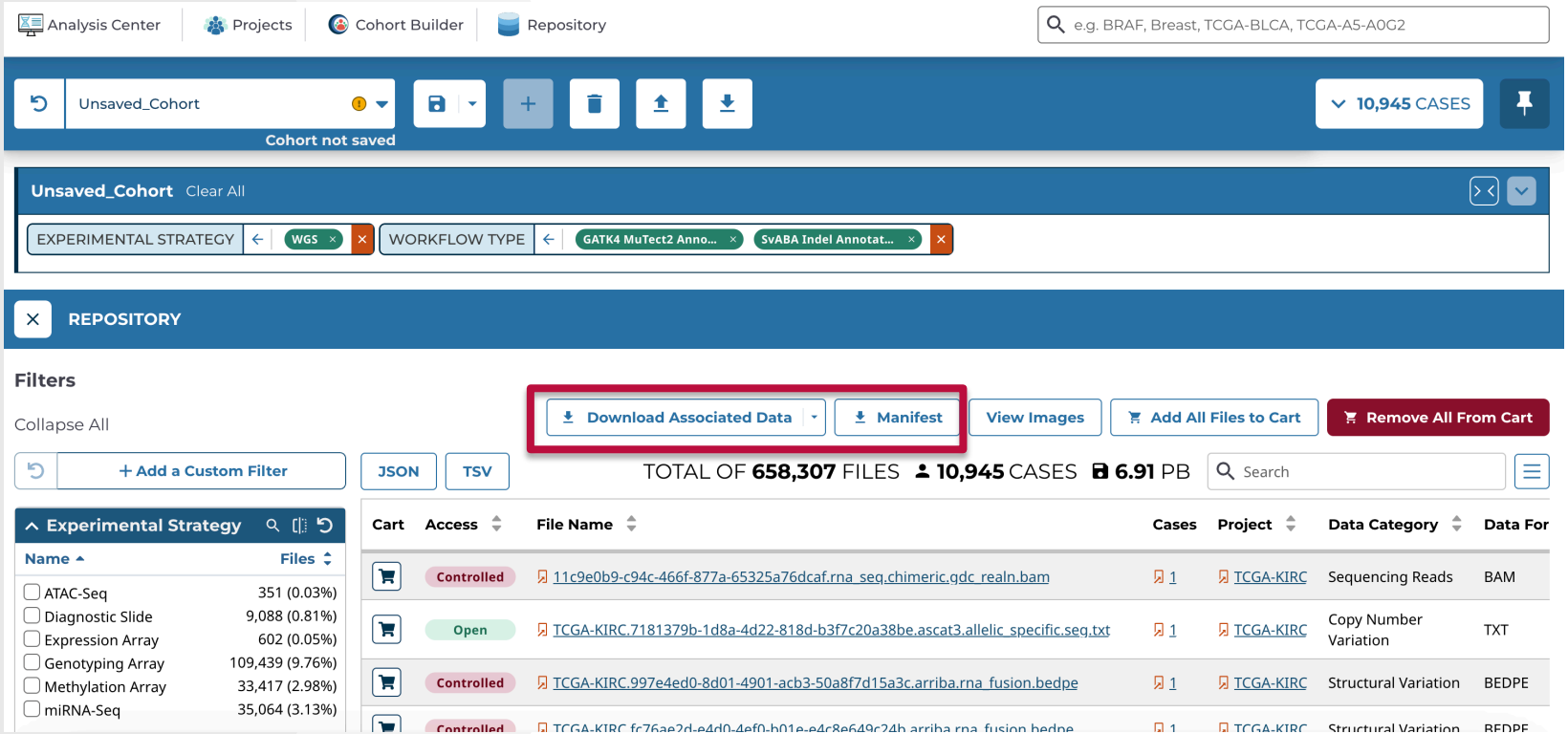
GDC 2.0 Workflow – Downloading Files




NIH NATIONAL CANCER INSTITUTE
GDC Data Portal
Video Guides
Send Feedback
Browse Annotations
Manage Sets
Cart 0
Login
GDC Apps
Analysis Center
Projects
Cohort Builder
Repository
e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2
Genomic Data Commons
Data Portal
Harmonized Cancer Datasets
A repository and computational platform for cancer researchers who need to understand cancer, its clinical progression, and response to therapy.
Explore Our Cancer Datasets
Data Portal Summary
Data Release 42.0 - January 30, 2025
86 Projects
69 Primary Sites
44,736 Cases
1,121,816 Files
22,534 Genes
2,940,240 Mutations
Cases by Major Primary Site
Adrenal Gland
Bile Duct
Bladder
Bone
Bone Marrow and Blood
Brain
Breast
Cervix
Colorectal
Esophagus
Eye
Head and Neck
Kidney
Liver
Lung
Lymph Nodes
Nervous System
Ovary
Pancreas
Pleura
Prostate
Skin
Soft Tissue
Stomach
Testis
Thymus
Thyroid
Uterus
1 2 3 4 5 6 7 8
1000s of Cases

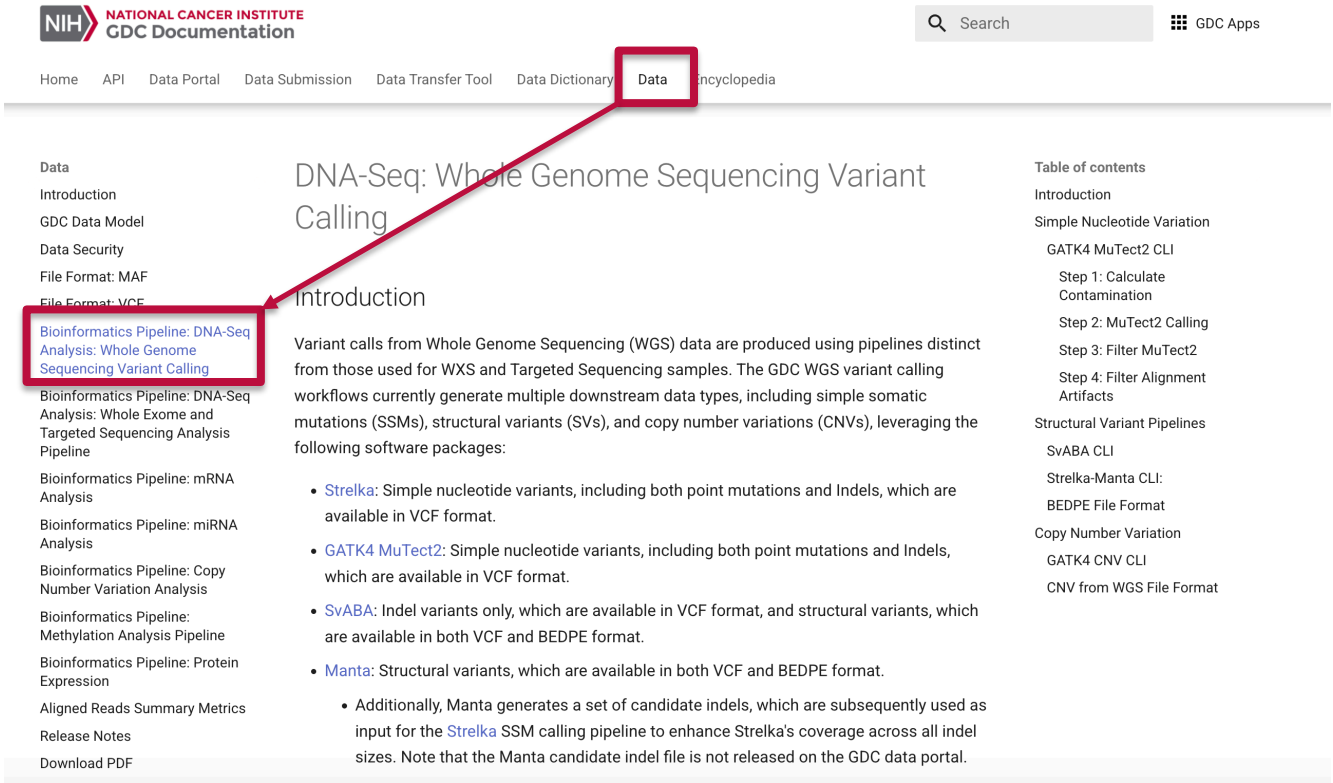

NIH NATIONAL CANCER INSTITUTE
GDC Data Portal
Video Guides
Send Feedback
Browse Annotations
Manage Sets
Cart 0
Login
GDC Apps
Analysis Center
Projects
Cohort Builder
Repository
e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2
Unsaved_Cohort
Cohort not saved
44,736 CASES
General Diagnosis
Disease Status and History
Disease Specific Classifications
Treatment
Exposure
Biospecimen
Molecular Filters
Available Data
Custom Filters
BEATAML1.0 882 (1.97%)
CDDP_EAGLE 50 (0.11%)
CGCI 645 (1.44%)
CMI 299 (0.67%)
CPTAC 1,687 (3.77%)
16 more
BEATAML1.0-COHORT 826 (1.85%)
BEATAML1.0-CRENOLANIB 56 (0.13%)
CDDP_EAGLE-1 50 (0.11%)
CGCI-BLGSP 324 (0.72%)
CGCI-HTMCP-CC 212 (0.47%)
80 more
acute lymphoblastic leukemia 1,086 (2.43%)
adenomas and adenocarcinom... 14,344 (32.06%)
adnexal and skin appendage ne... 22 (0.05%)
basal cell neoplasms 22 (0.05%)
blood vessel tumors 1 (0.00%)
39 more
Primary Diagnosis
Name
Cases
acinar adenocarcinoma 187 (0.42%)
acinar cell carcinoma 266 (0.59%)
acinar cell tumor 31 (0.07%)
acute leukemia, nos 11 (0.02%)
acute lymphoblastic leukemia, n... 144 (0.32%)
acute lymphocytic leukemia 1,170 (2.62%)
194 more
Primary Site
Name
Cases
accessory sinuses 1 (0.00%)
adrenal gland 724 (1.62%)
anus and anal canal 82 (0.18%)
base of tongue 27 (0.06%)
bladder 767 (1.71%)
bones, joints and articular cartila... 257 (0.57%)
63 more
Tissue or Organ of Origin
Name
Cases
abdomen, nos 236 (0.53%)
adrenal gland, nos 608 (1.36%)
ampulla of vater 4 (0.01%)
anal canal 1 (0.00%)
anterior floor of mouth 2 (0.00%)
anterior mediastinum 40 (0.09%)
194 more
Case ID
Upload Cases


NIH NATIONAL CANCER INSTITUTE
GDC Data Portal
Video Guides
Send Feedback
Browse Ann
Analysis Center
Projects
Cohort Builder
Repository
Unsaved_Cohort
Cohort not saved
Molecular Filters
Available Data
Custom Filters
Workflow Type
Name
Cases
Aliquot Ensemble Somatic Varian... 2 (0.00%)
AscatNGS 5,444 (12.17%)
BRASS 5,444 (12.17%)
BWA with Mark Duplicates and ... 15,271 (34.14%)
CaVEMan 5,444 (12.17%)
GATK4 CNV 13,473 (30.12%)
GATK4 MuTect2 10,941 (24.46%)
GATK4 MuTect2 Annotation 10,941 (24.46%)
GATK4 MuTect2 Tumor-Only 2 (0.00%)
GATK4 MuTect2 Tumor-Only Ann... 2 (0.00%)
Manta 5,710 (12.76%)
Pindel 5,444 (12.17%)
SvABA 5,528 (12.36%)
SvABA Indel 1,013 (2.26%)
SvABA Indel Annotation 1,013 (2.26%)
VCF LiftOver 458 (1.02%)
show less
Data Format
Name
bam
bedpe
maf
tar
tsv 5,444 (12.17%)
txt 13,728 (30.69%)
1 more
Experimental Strategy
WGS
Name
Cases
WGS 15,712 (35.12%)
show less


Analysis Center
Projects
Cohort Builder
Repository
e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2
Unsaved_Cohort
Cohort not saved
10,945 CASES
Unsaved_Cohort
Clear All
EXPERIMENTAL STRATEGY
WGS
WORKFLOW TYPE
GATK4 MuTect2 Anno...
SvABA Indel Annotat...
REPOSITORY
Filters
Collapse All
Download Associated Data
Manifest
View Images
Add All Files to Cart
Remove All From Cart
+ Add a Custom Filter
JSON
TSV
TOTAL OF 658,307 FILES 10,945 CASES 6.91 PB
Search
Experimental Strategy
Name
Files
ATAC-Seq 351 (0.03%)
Diagnostic Slide 9,088 (0.81%)
Expression Array 602 (0.05%)
Genotyping Array 109,439 (9.76%)
Methylation Array 33,417 (2.98%)
miRNA-Seq 35,064 (3.13%)
Cart
Access
File Name
Cases
Project
Data Category
Data For
Controlled
11c9e0b9-c94c-466f-877a-65325a76dcaf.rna_seq.chimeric.gdc_realn.bam
1
TCGA-KIRC
Sequencing Reads
BAM
Open
TCGA-KIRC.7181379b-1d8a-4d22-818d-b3f7c20a38be.ascat3.allelic_specific.seg.txt
1
TCGA-KIRC
Copy Number Variation
TXT
Controlled
TCGA-KIRC.997e4ed0-8d01-4901-acb3-50a8f7d15a3c.arriba.rna_fusion.bedpe
1
TCGA-KIRC
Structural Variation
BEDPE

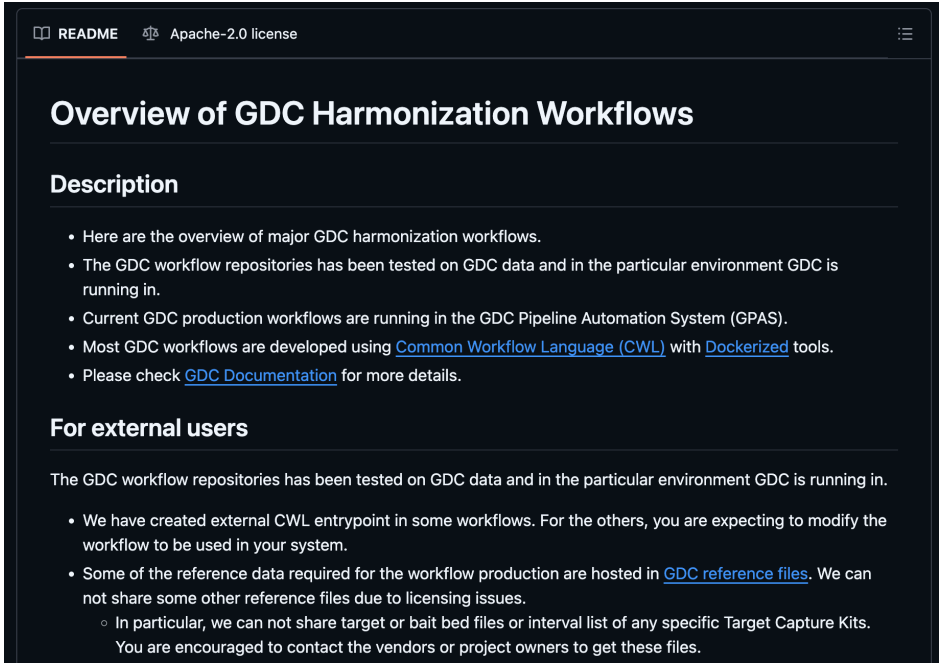
Documentation and Repos

Documentation – https://docs.gdc.cancer.gov



Bioinformatics Pipeline Documentation and Resources:

https://github.com/NCI-GDC/gdc-workflow-overview


README Apache-2.0 license

Overview of GDC Harmonization Workflows

Description

- Here are the overview of major GDC harmonization workflows.
- The GDC workflow repositories has been tested on GDC data and in the particular environment GDC is running in.
- Current GDC production workflows are running in the GDC Pipeline Automation System (GPAS).
- Most GDC workflows are developed using Common Workflow Language (CWL) with Dockerized tools.
- Please check GDC Documentation for more details.

For external users

The GDC workflow repositories has been tested on GDC data and in the particular environment GDC is running in.

- We have created external CWL entrypoint in some workflows. For the others, you are expecting to modify the workflow to be used in your system.
- Some of the reference data required for the workflow production are hosted in GDC reference files. We can not share some other reference files due to licensing issues.
  - In particular, we can not share target or bait bed files or interval list of any specific Target Capture Kits. You are encouraged to contact the vendors or project owners to get these files.

Questions?

U.S. Department of Health & Human Services
National Institutes of Health | National Cancer Institute

https://www.cancer.gov/

1-800-4-CANCER

Produced April 2024