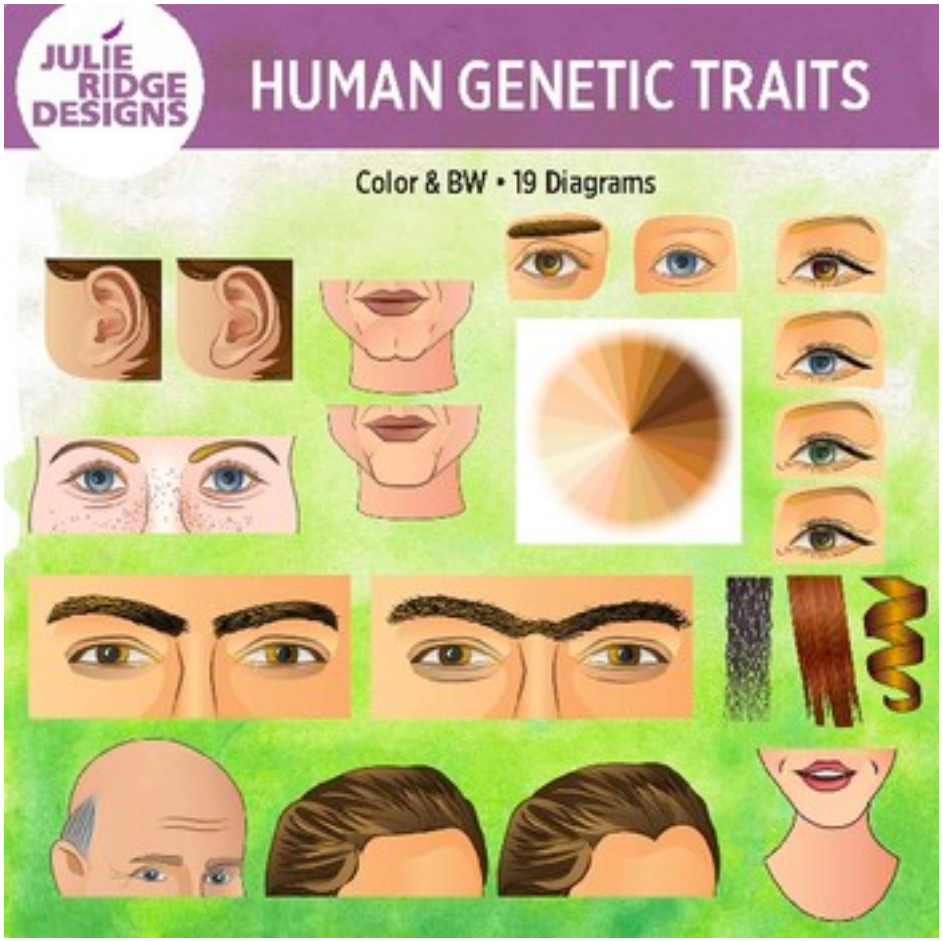
Mutation Identification in Genomics

- What is a genetic disease?
- What is a genome, exome and Gene Panel
- What is Variation
  - Somatic vs Germline
  - SNVs, Indels and Structural Variation

- Is there an easy way to run all those command line programs?
  - BioHPC Astrocyte

Genetic Traits

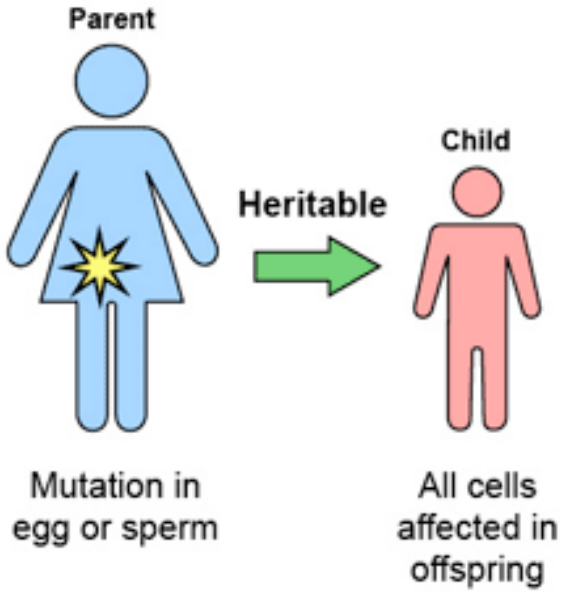
- A phenotype is an individual's observable traits, such as height, eye color, and blood type.
- The genetic contribution to the phenotype is called the genotype.
- Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors.



Genetic Disease

- A genetic disorder is a genetic problem caused by one or more abnormalities in the genome.
- A single-gene disorder is the result of a single mutated gene.
- Autosomal dominant disorders occur with only one mutated copy of the gene.
- Recessive disorders require both copies are mutated.
- X-linked dominant disorders are caused by mutations in genes on the X chromosome.
- Mitochondrial disease, also known as maternal inheritance, applies to genes encoded by mitochondrial DNA.
- Genetic disorders may also be complex, multifactorial, or polygenic, meaning they are associated with the effects of multiple genes in combination with lifestyles and environmental factors.

Acquired vs Inherited Variation

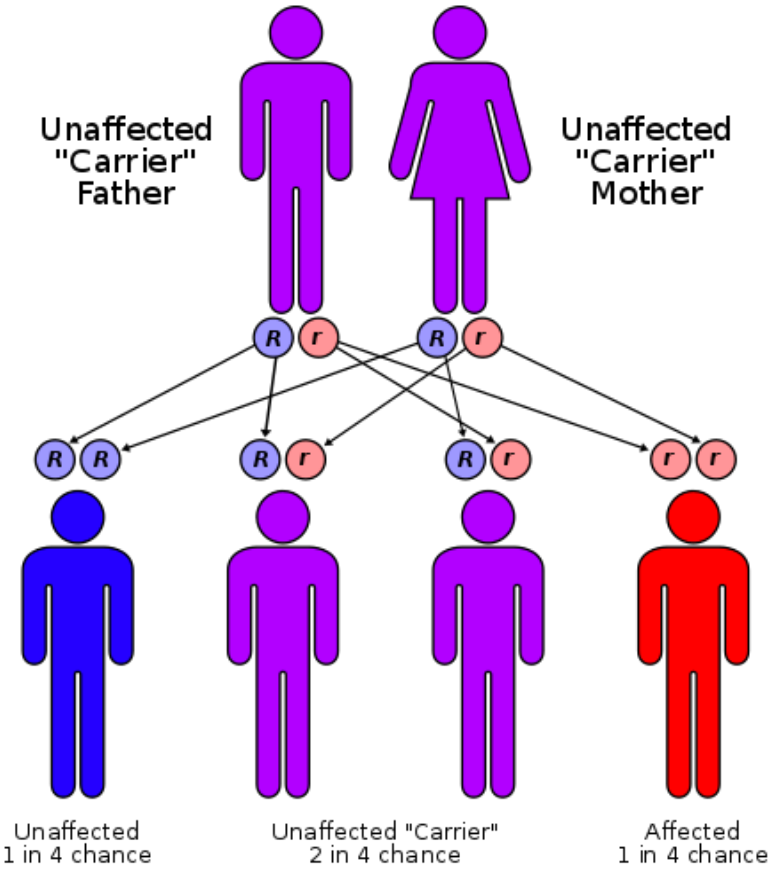


Germline

Somatic

Mendelian Disease

Disorder prevalence (approximate)	
Autosomal dominant	
Familial hypercholesterolemia	1 in 500
Polycystic kidney disease	1 in 1250
Neurofibromatosis type I	1 in 2,500
Hereditary spherocytosis	1 in 5,000
Marfan syndrome	1 in 4,000
Huntington's disease	1 in 15,000
Autosomal recessive	
Sickle cell anaemia	1 in 625
Cystic fibrosis	1 in 2,000
Tay-Sachs disease	1 in 3,000
Phenylketonuria	1 in 12,000
Mucopolysaccharidoses	1 in 25,000
Lysosomal acid lipase deficiency	1 in 40,000
Glycogen storage diseases	1 in 50,000
Galactosemia	1 in 57,000
X-linked	
Duchenne muscular dystrophy	1 in 7,000
Hemophilia	1 in 10,000



Somatic/Mosaic Disease

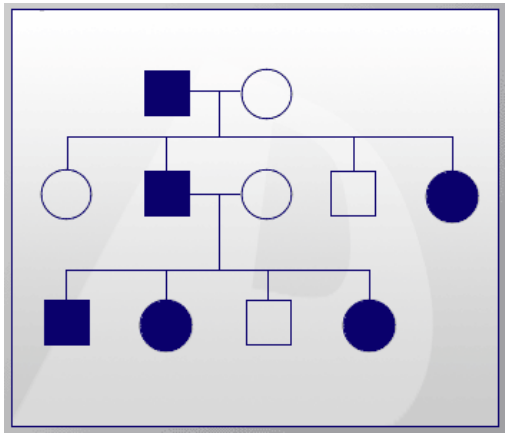
- Acquired diseases are caused by acquired mutations in a gene or group of genes that occur during a person's life.
- These include many cancers, as well as some forms of neurofibromatosis.
- Mosaicism, involves the presence of two or more populations of cells with different genotypes in one individual, who has developed from a single fertilized egg.
- Intersex conditions can be caused by mosaicism where some cells in the body have XX and others XY chromosomes
- Other endogenous factors can also lead to mosaicism including mobile elements, DNA polymerase slippage, and unbalanced chromosomal segregation.
- Exogenous factors include nicotine and UV radiation

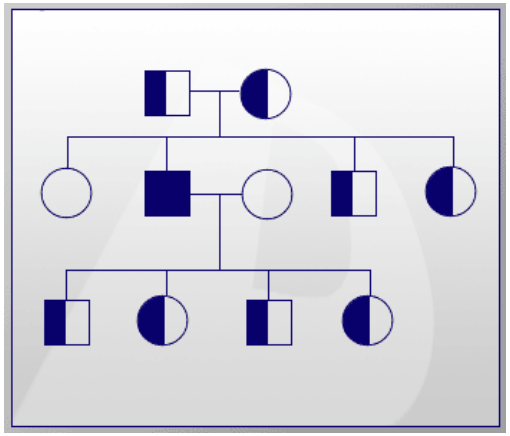
Complex Disease

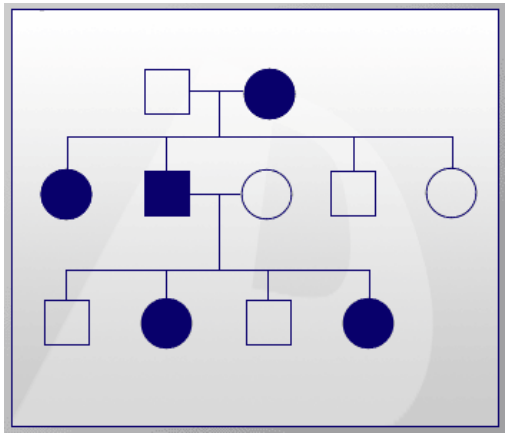
- Complex diseases are caused by a combination of genetic, environmental, and lifestyle factors, most of which have not yet been identified.
- Some examples include Alzheimer's disease, scleroderma, asthma, Parkinson's disease, multiple sclerosis, osteoporosis, connective tissue diseases, kidney diseases, autoimmune diseases, etc

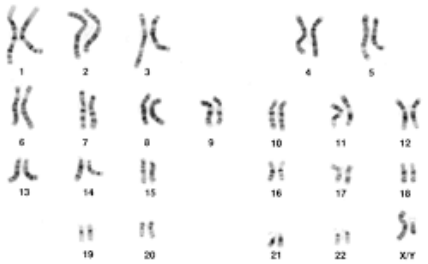
Pedigrees

- Identification of disease causing variation was originally done using pedigrees (multigenerational family studies)

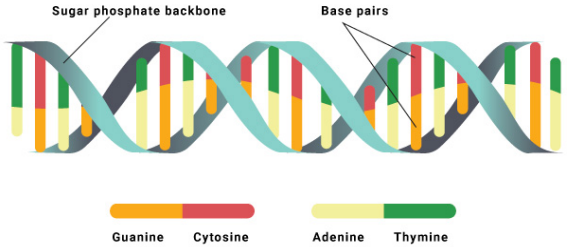






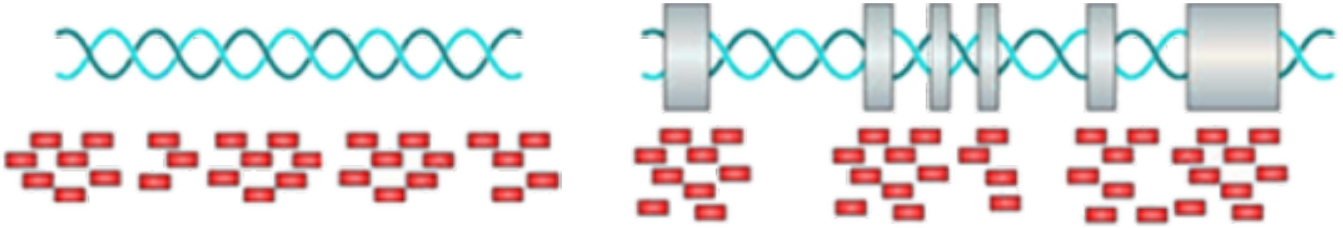


Genome



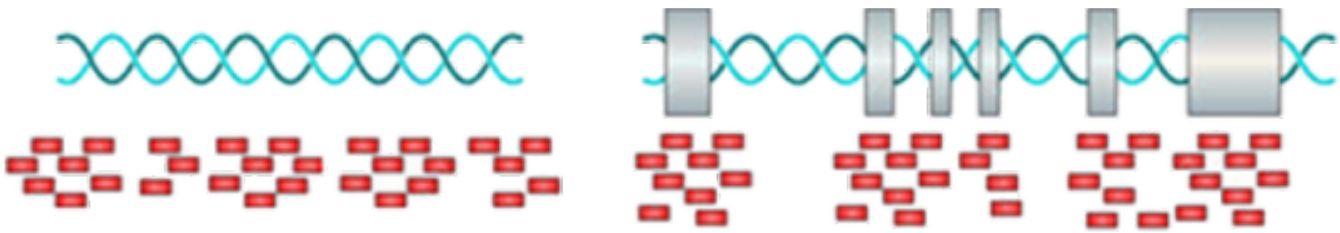
- A genome is the entire set of genetic material for an organism.
- The human genome consists of about 3 billion base pairs of DNA across 23 pairs of chromosomes.
- More than 99 percent of the human genome is the same in all people.
- That means that differences in less than 1 percent of our genome accounts for the vast diversity of humans across the globe.

Exome



- The exome is a subset of the genome that contains protein coding genes.
- Exons are also referred to as the coding region of a gene
- The exons of all our genes make up approximately 1.5% of our genome and are collectively referred to as the “exome”.
- There are some important DNA sequences that are not contained within the exome in noncoding DNA that have important biological functions, such as regulating the coding regions of the genome.

Gene Panels

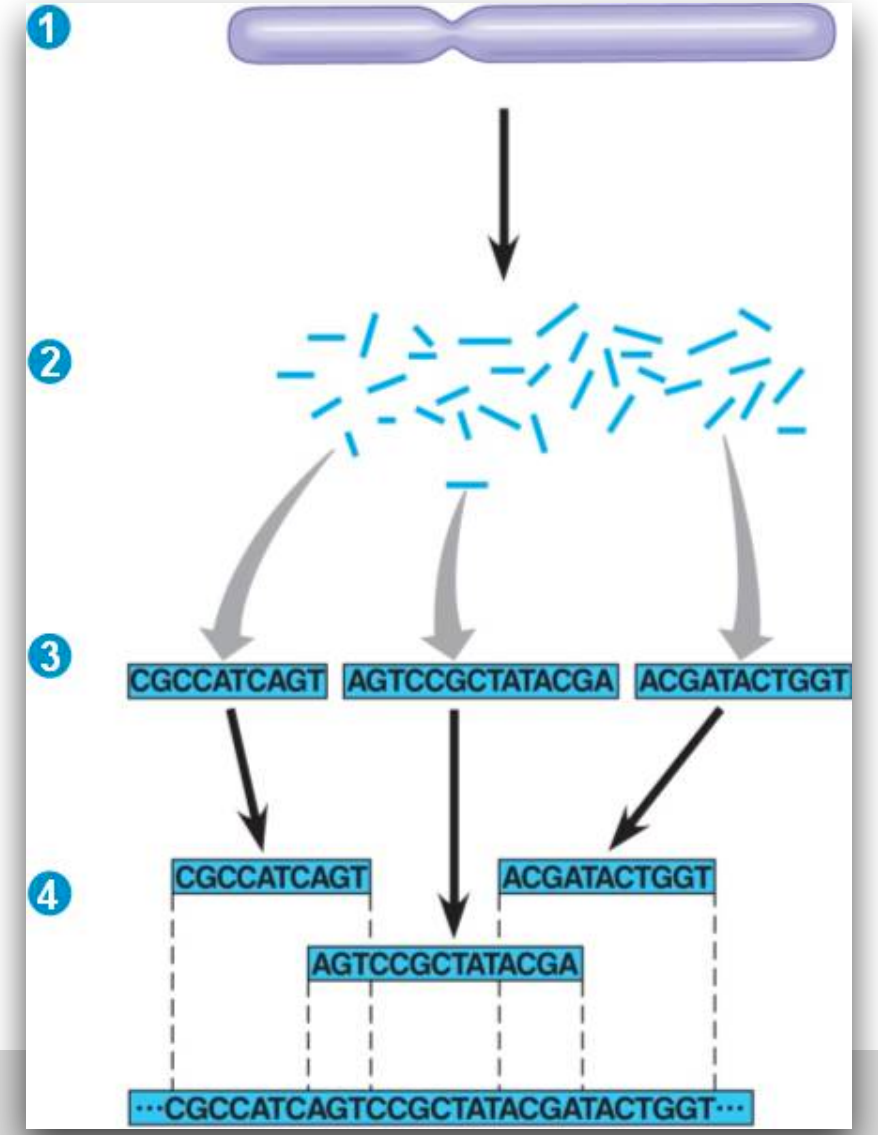


- A gene panel is a gene subset of the exome
- It contains a subset of exons for a select group of genes
- Gene Panels are useful if you need to do deep sequencing > 1000X
- Many clinical tumor tests use gene panels.

Sequencing Applications

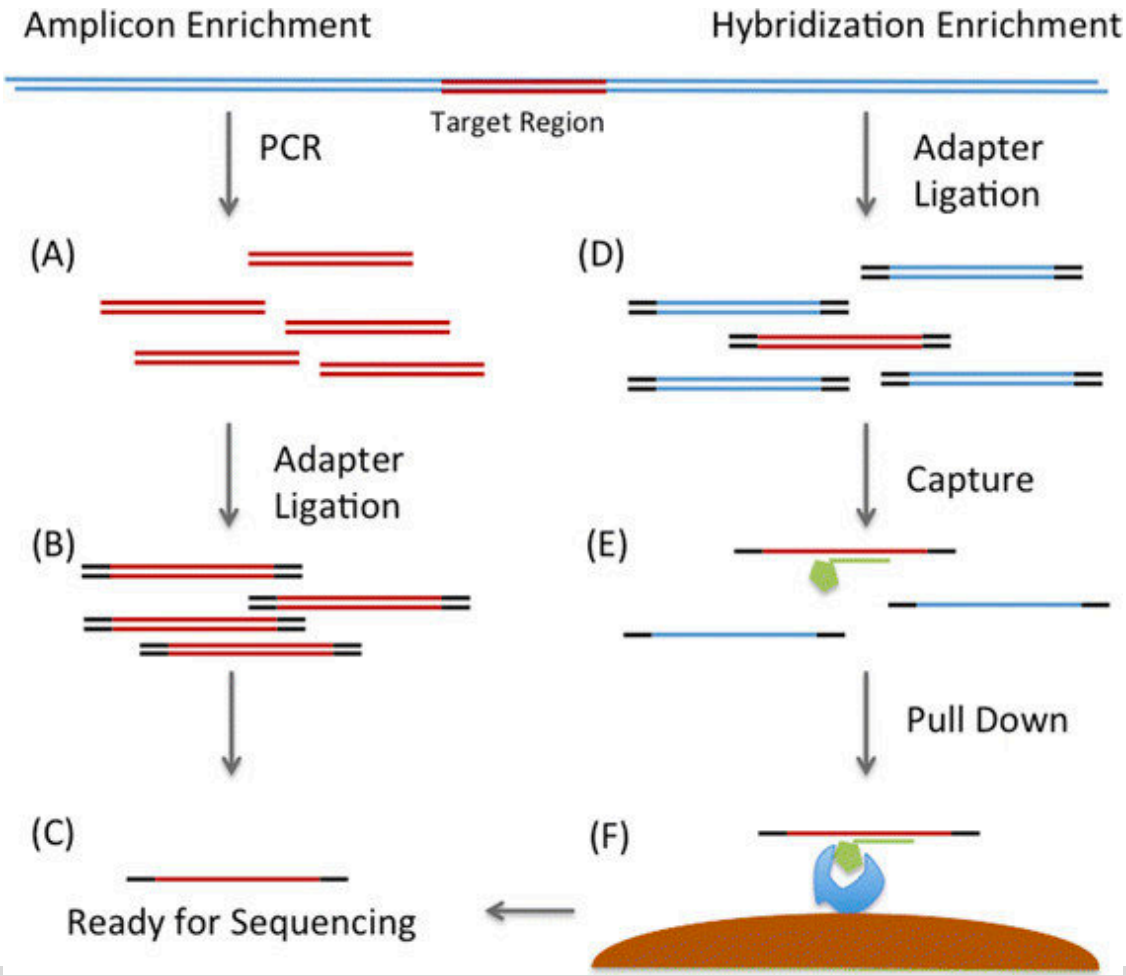
- Sanger Sequencing (~1kb)
  - Used to identify the common pathogenic driver mutations in a few genes: KRAS, EGFR, etc
  - 1-100 amplicon targets
  - Microsatellite or STR analysis
- Illumina Short Read Sequencing (50bp-300bp)
  - “Next-generation Sequencing”
  - Whole Genome Sequencing (WGS)
  - Amplicon-based Sequencing
    - uses primers
    - feasible for ~ 100 genes
  - Hybridization-based Sequencing
    - uses baits
    - feasible for any size gene panel from whole exome sequencing (WES) to small gene-panels

Whole Genome Shotgun



- The Genome is fragmented
- Sequence library is created with the dna fragments
- For model organism, reads are mapped to a reference genome
- For non-model organism, reads are assembled into contigs

Targeted Capture Hybridization and Amplicon Based

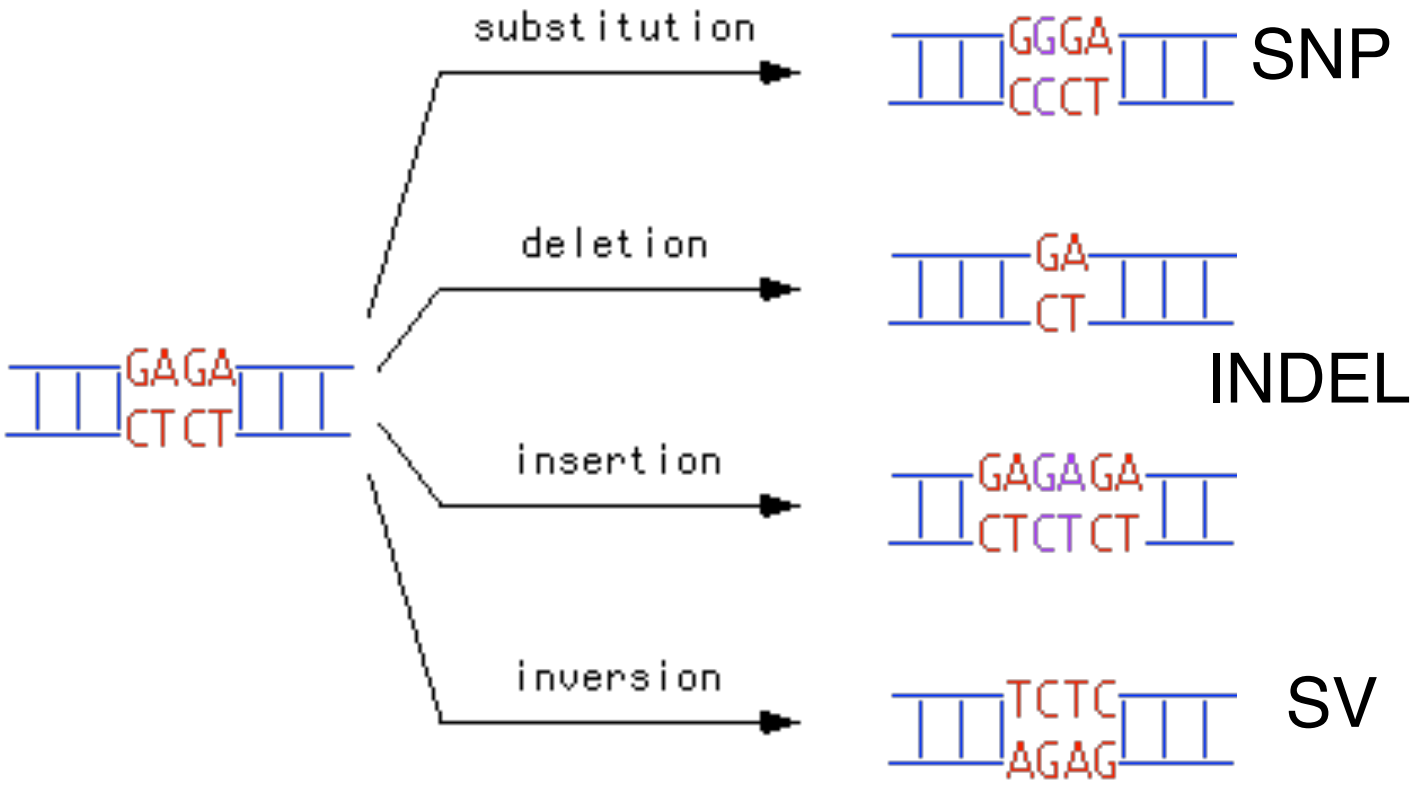


Pros and Cons of WGS vs Targeted Panels

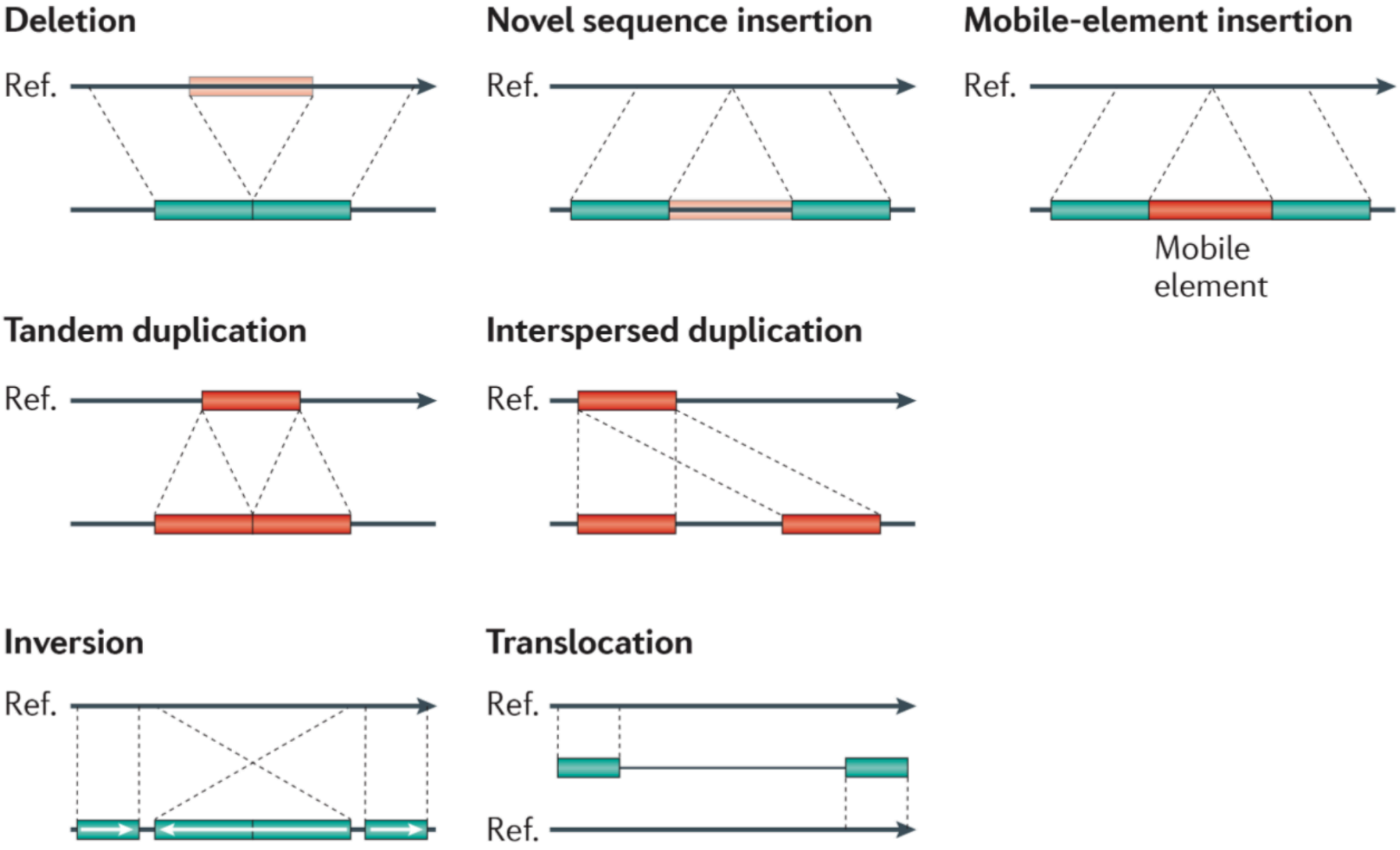
- Whole Genome Sequence can better predict large structural changes including CNV, large Indels, etc
- Whole Genome has more uniform coverage of the protein coding regions
- ~$1300 30-40X coverage

- Targeted panes are cheaper
- Whole Exome Sequencing costs ~$500 for 100X coverage
- In somatic/mosaic conditions you might need > 1000X coverage.
- Generate less data to store and analyze

Types of Variation



Types of Structural Variation



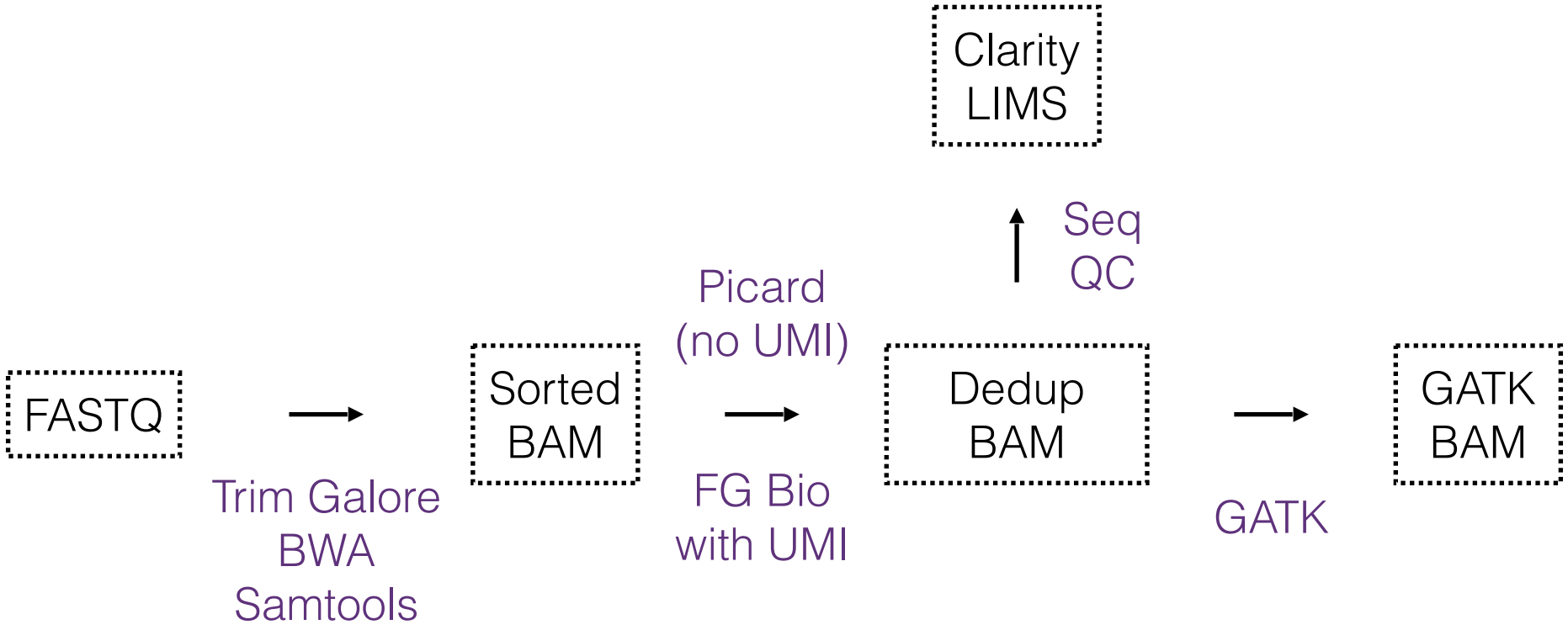
Large Reference Populations

- HapMap
  - The International HapMap Project was an organization that aimed to develop a haplotype map (HapMap) of the human genome using SNP genotyping arrays
- 1000G
  - The 1000 Genomes project aimed to sequence using NGS > 1000 genomes in “pure” and “ad-mixture” human populations to identify human variation across the genome
- ExAC
  - ExAC collected the SNP and Indel calls in ~ 26K genomes/exomes to accumulation prevelence in the population studied in many genomes projects
- gnomAD
  - The Genome Aggregation Database (gnomAD) is a resource of aggregate genomes and aimed to harmonize both exome and genome sequencing data from over 120K exomes and 15K genomes.

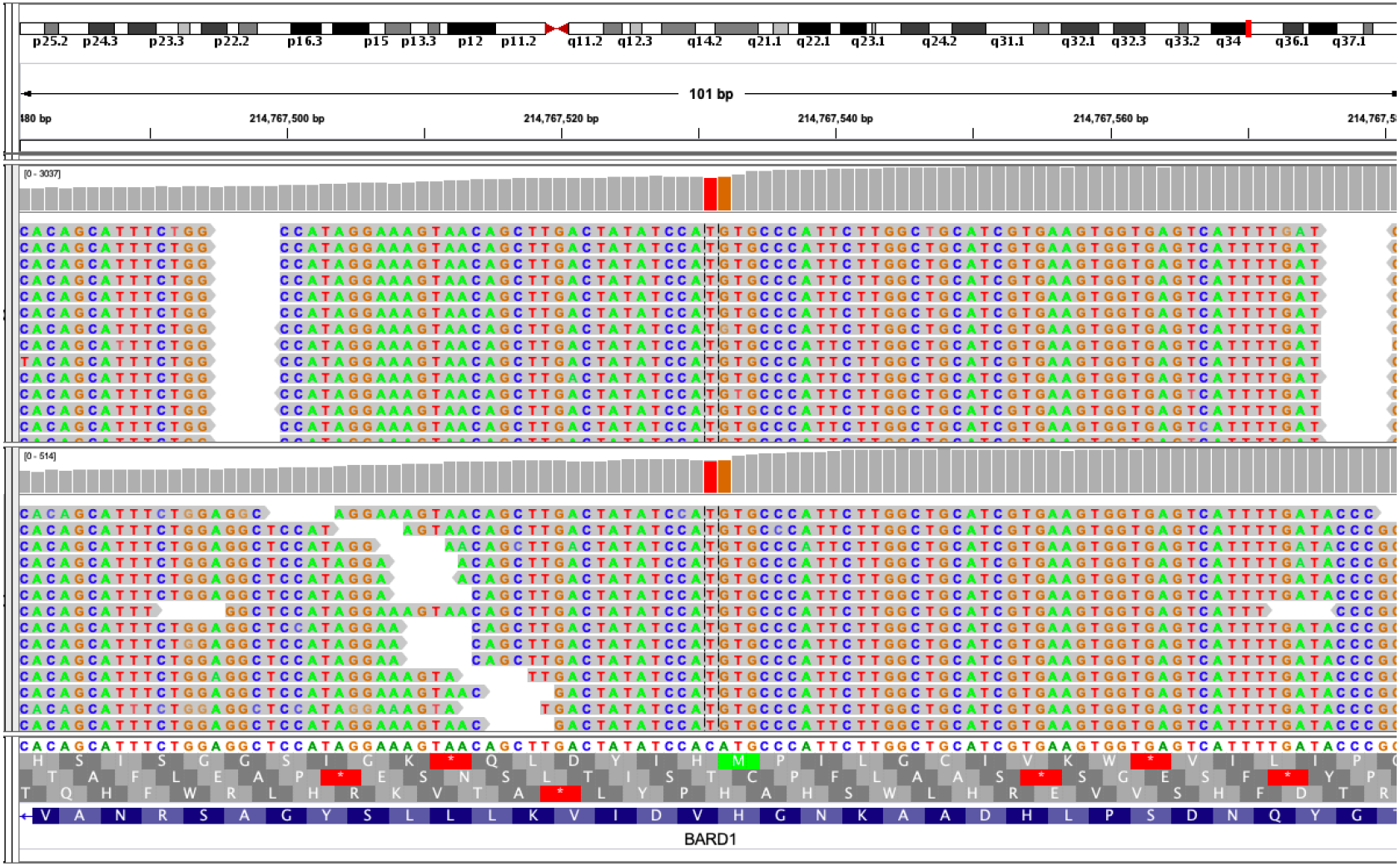
How do we turn alignments into genetic mutations?

- Short Read Alignment (mapping) onto a reference genome
  - Short read aligners assume that the read came from “intact” from the reference
  - Mutation or variation is assumed when reads deviate from the reference
- Identification of differences between the reference genome and the genome of interest
  - hg19 reference genome is largely based on 5 individuals
  - hg38 includes population specific alternative chromosomes based on 1000G populations

Alignment Workflows



Alignment



Mismatches and Gaps can indicate a genetic variation (naturally occurring in a population) or mutation (an error in replication)

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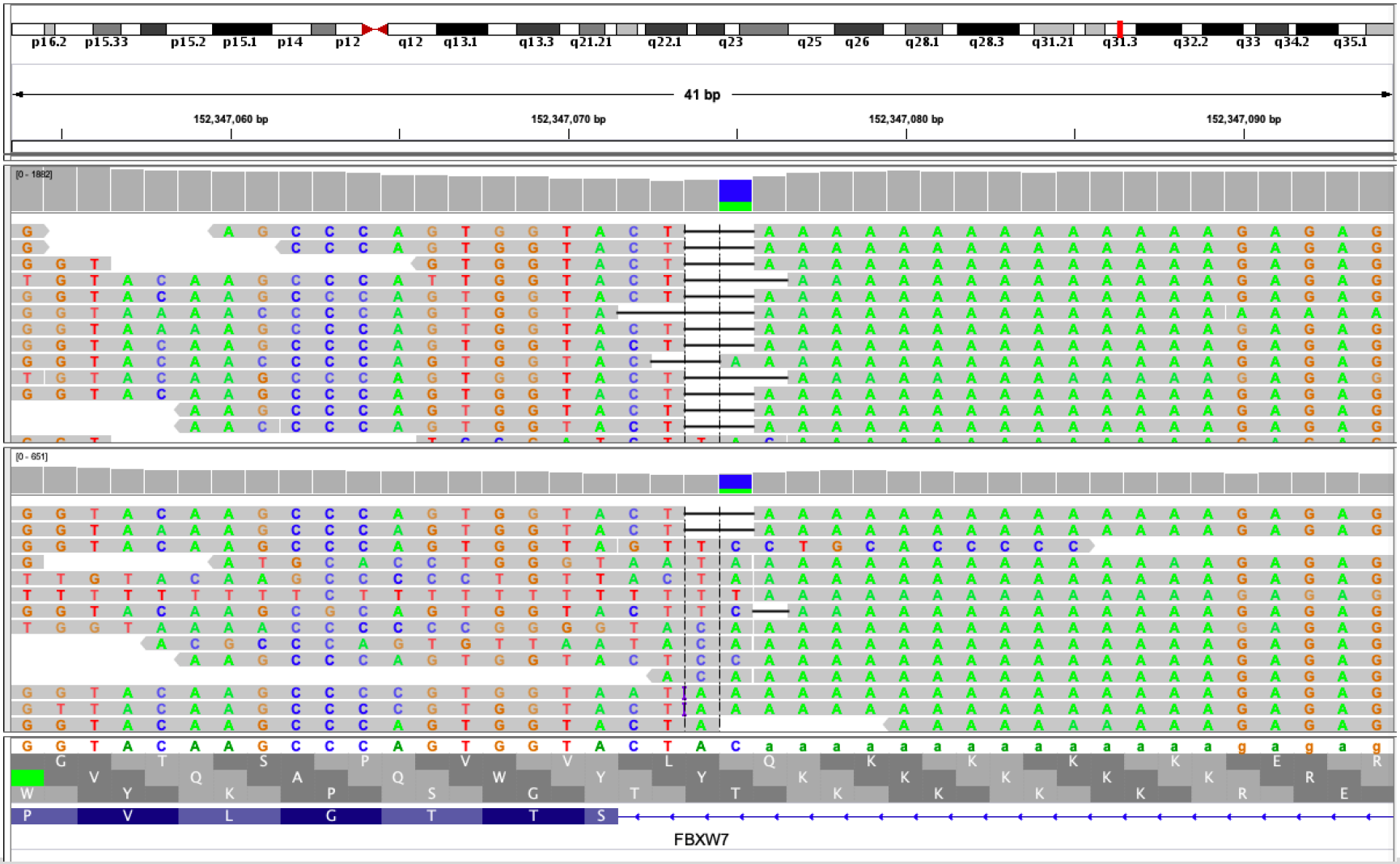
Alignment



Mismatches and Gaps can indicate a genetic variation (naturally occurring in a population) or mutation (an error in replication)

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Segmental Duplication can be the cause of Alignment Errors in Alignments

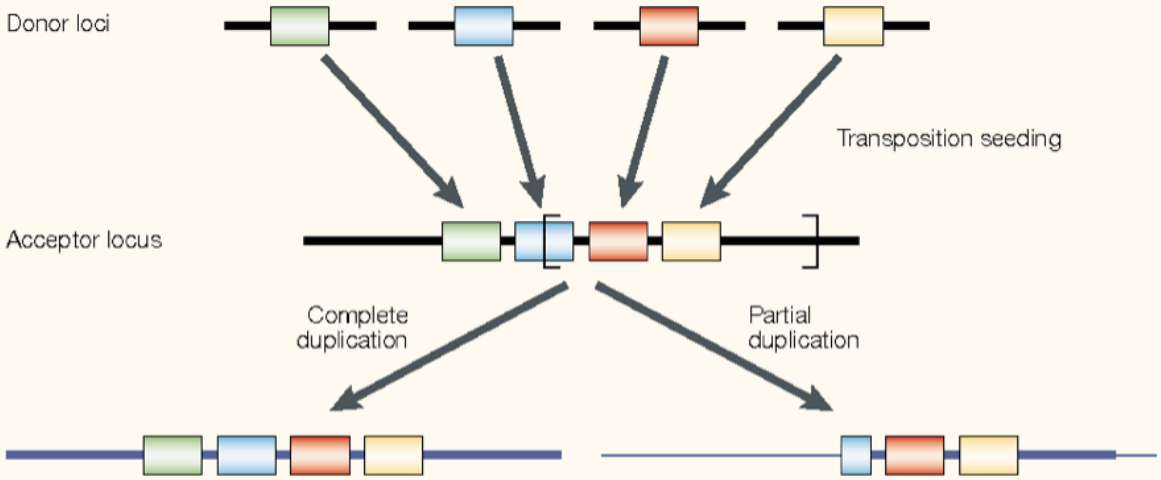


Figure 1 | **Model of segmental duplication.** Acceptor regions of the genome acquire segments of genomic material that range from 1–200 kb from disparate regions (donor loci) through a process of duplicative transposition. Events occur independently over time, which results in the formation of larger blocks of duplicated sequence that are mosaic in structure. Secondary events duplicate portions of this mosaic structure to other regions of the genome. Rearrangements (deletions and inversions) subsequently alter the structure of these regions.

Figure 1 | Model of segmental duplication. Acceptor regions of the genome acquire segments of genomic material that range from 1–200 kb from disparate regions (donor loci) through a process of duplicative transposition... Continue Reading

Published in Nature Reviews Genetics 2002

Segmental duplications and the evolution of the primate genome

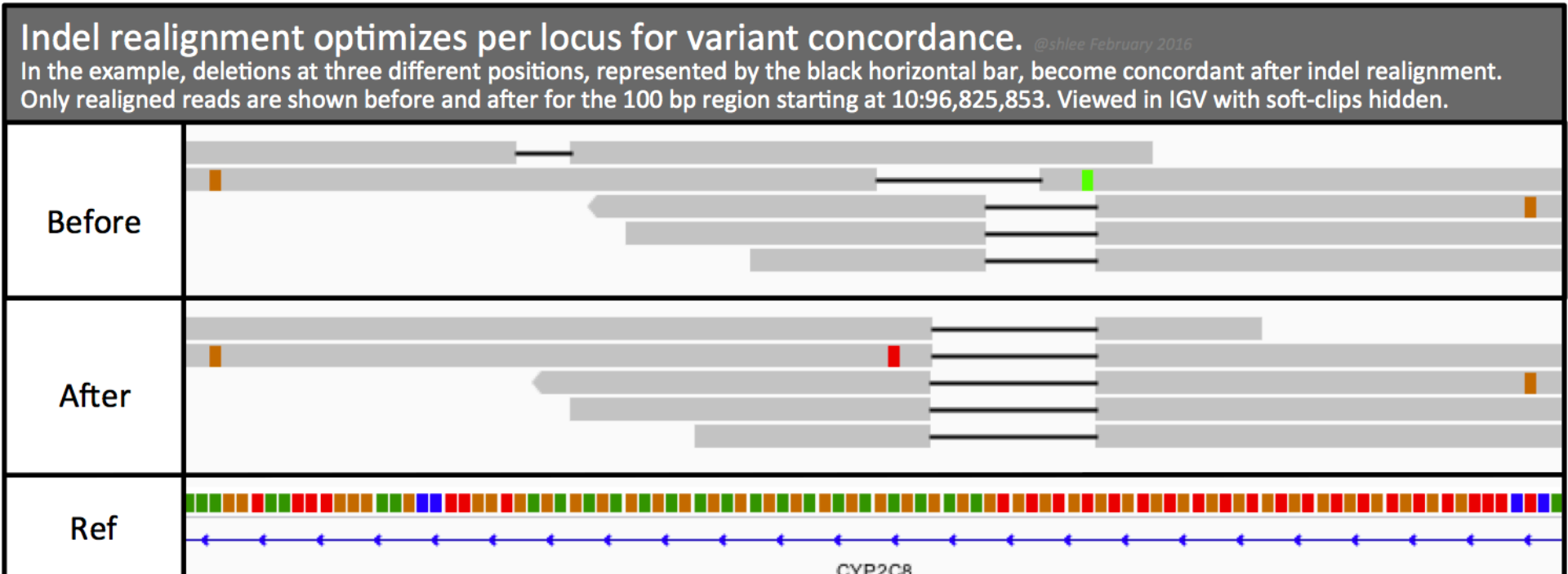
R. V. Samonte, E. Eichler

Why are we so worried about sequence duplication?

- When DNA is sequenced, PCR is used to amplify sequence library to ensure that only DNA with “a known adapter” is sequenced.
- Since PCR has a small error rate, “early errors” can be amplified and could skew your results
- We remove duplicates to remove potential noise.
- Although in my experience in deep sequencing removing duplicates doesn’t really change downstream results
- Some PCR errors can be corrected using UMI sequences, where consensus sequences can be used with UMIs

Indel Realignment is Not Necessary for Current Variant calling Methods

- Sometimes, alignment algorithms align reads inconsistently, adding the alignment gaps to different places.
- Indel Realignment uses “known” gold standard indels to realign these gaps

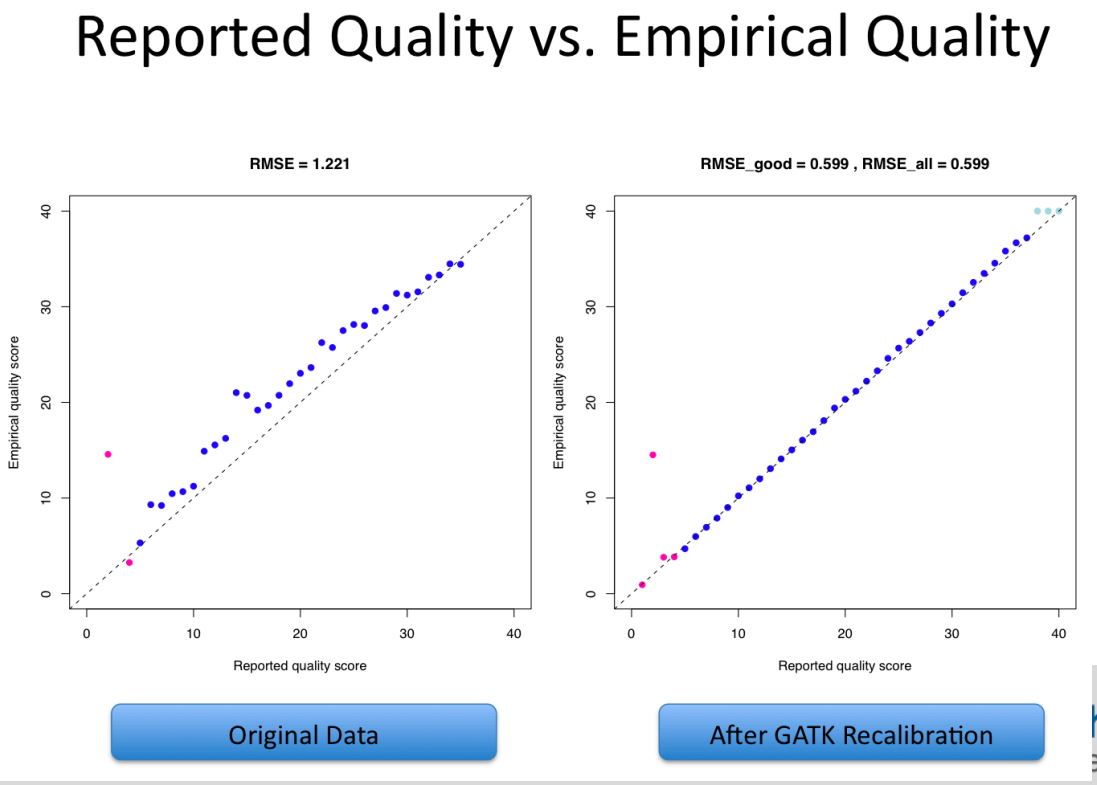


Indel Realignment Might Confuse Modern Callers

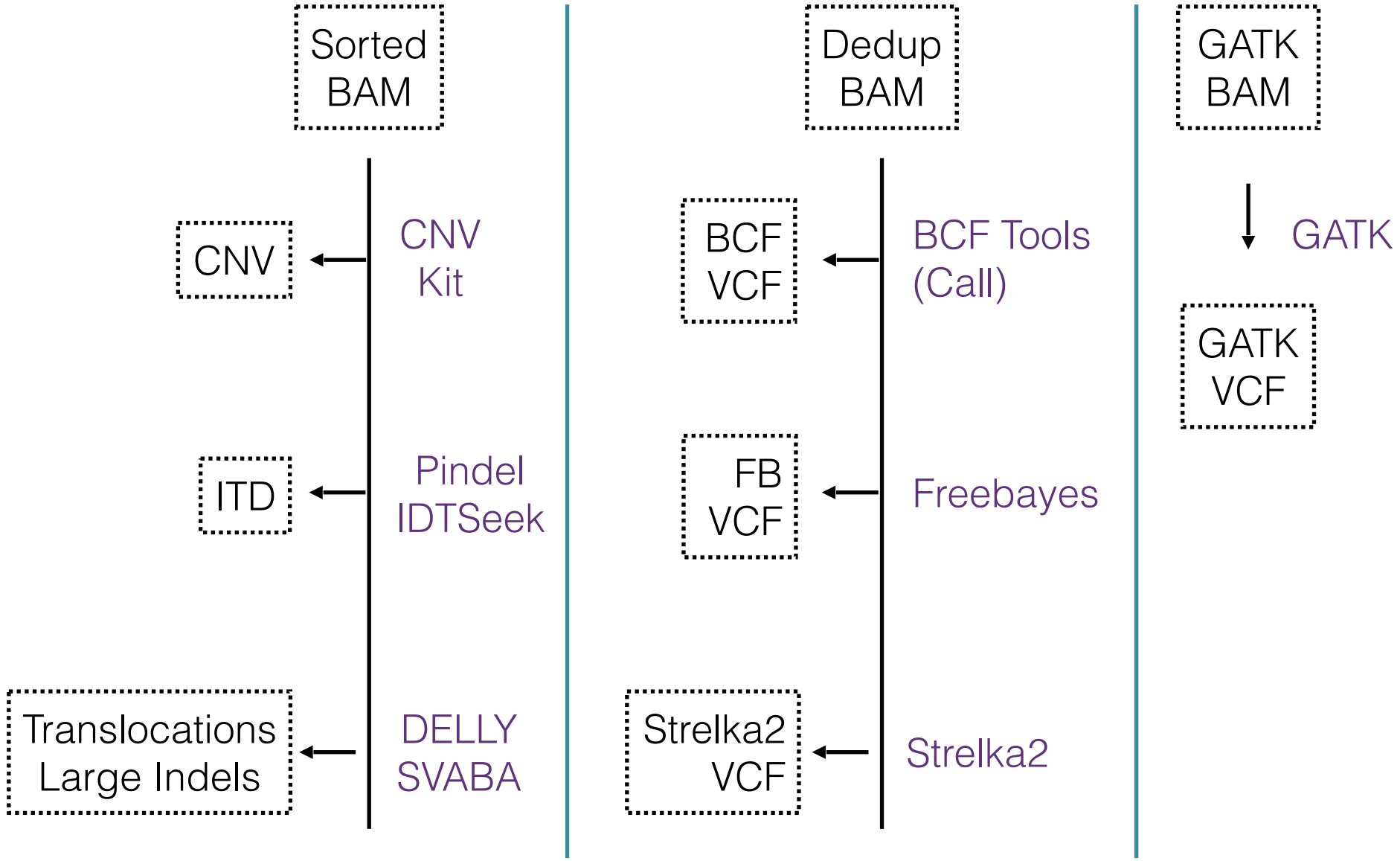
SampleID	Method	InDel TP	InDel FP	InDel FN	InDel SN	InDel PPV
w/o ABRA2	fb	15	182	5	75	7.6
with ABRA2	fb	16	243	4	80	6.2
w/o ABRA2	mutect	18	212	2	90	7.8
with ABRA2	mutect	18	221	2	90	7.5
w/o ABRA2	pindel	20	0	0	100	100
with ABRA2	pindel	15	4	5	75	78.9
w/o ABRA2	strelka2	19	1	1	95	95
with ABRA2	strelka2	19	8	1	95	70.4

Why does GATK need Base Recalibration?

- Base recalibration detects systematic errors made by the sequencer when it estimates the quality score of each base call



Germline Workflow

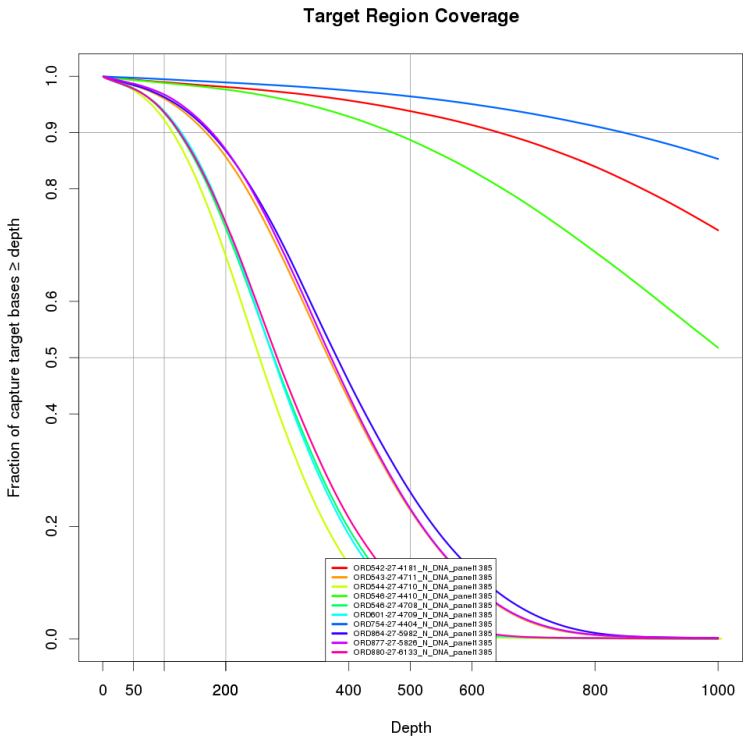


Differences in Results between Callers?

Sample	Caller	SNV TP	SNV FP	SNV FN	Indel TP	Indel FP	Indel FN	SNV SN	Indel SN	SN	SP
FFPE control, 40 ng	gatk	1238	36	21	34	1	3	98.3	91.9	98.1%	97.2
FFPE control, 40 ng	strelka2	1238	2	21	34	1	3	98.3	91.9	98.1%	99.8
FFPE control, 40 ng	sam	1238	2	21	33	5	4	98.3	89.2	98.1%	99.5
FFPE control, 40 ng	FB	1224	2	35	34	0	3	97.2	91.9	97.1%	99.8
FFPE control, 40 ng	platypus	1215	7	44	34	0	3	96.5	91.9	96.4%	99.4
FRESH sample, 200 ng	gatk	1252	36	6	37	4	1	99.5	97.4	99.5%	97
FRESH sample, 200 ng	strelka2	1237	0	20	34	6	3	98.4	91.9	98.2%	99.5
FRESH sample, 200 ng	sam	1237	0	21	17	0	21	98.3	44.7	96.8%	100
FRESH sample, 200 ng	DB	1236	1	22	36	0	2	98.3	94.7	98.1%	99.9
FRESH sample, 200 ng	platypus	1215	5	43	34	1	4	96.6	89.5	96.4%	99.5

What is sequence coverage and depth?

- Base depth is the number of reads that cover a particular base
- Coverage is “how much” of your target did you cover
- Depth of Coverage is how deep was that coverage?

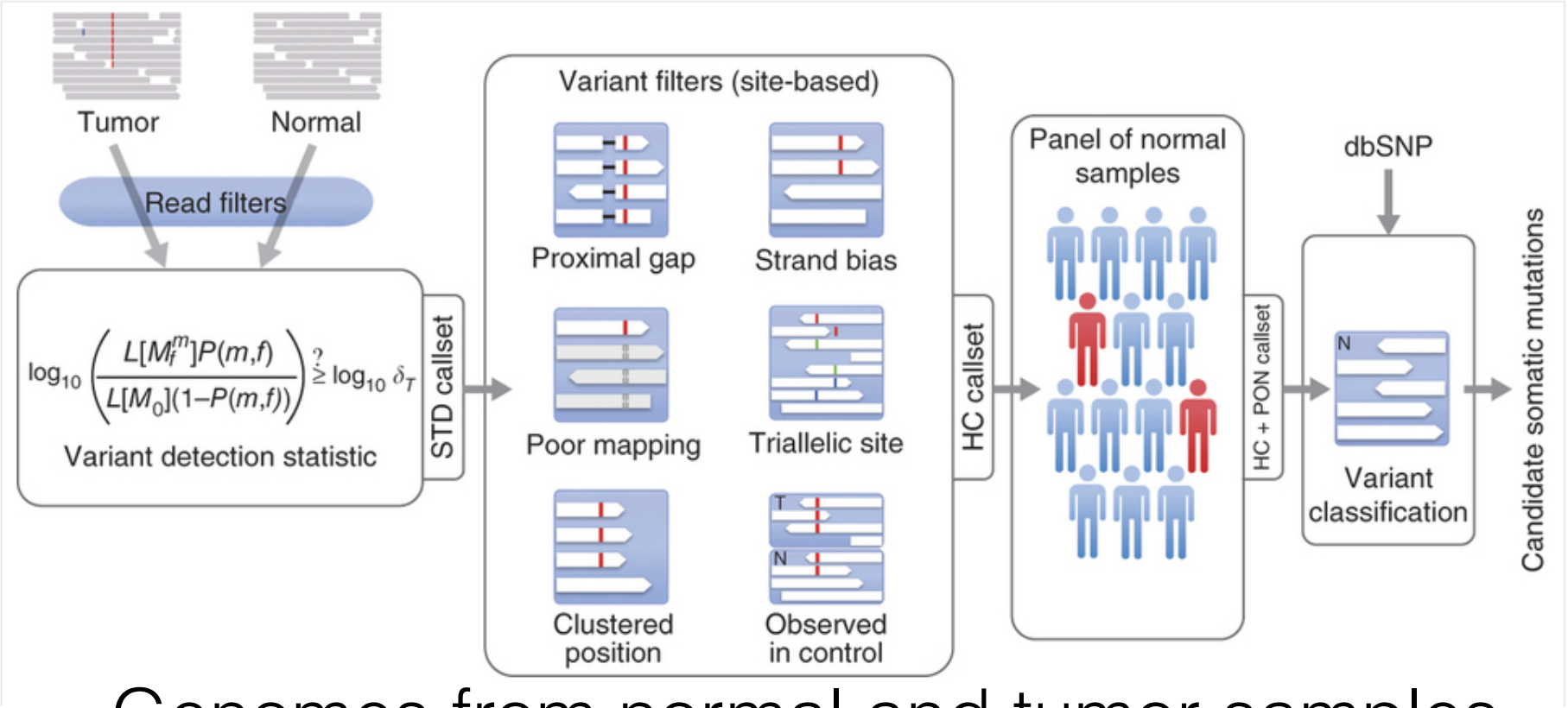


Effect	Impact
3_prime_UTR_truncation +exon_loss	M
3_prime_UTR_variant	NC
5_prime_UTR_premature start_codon_gain_variant	L
5_prime_UTR_truncation + exon_loss_variant	M
5_prime_UTR_variant	NC
bidirectional_gene_fusion	H
chromosome	H
coding_sequence_variant	NC
coding_sequence_variant	LOW
conserved_intergenic_variant	NC
conserved_intron_variant	NC
disruptive_inframe_deletion	M
disruptive_inframe_insertion	M
downstream_gene_variant	NC
duplication	H
duplication	H
duplication	H
duplication	M
exon_loss_variant	H
exon_loss_variant	H
exon_variant	NC
feature_ablation	H
feature_ablation	H
frameshift_variant	H
gene_fusion	H
gene_fusion	H
gene_variant	NC
inframe_deletion	M
inframe_insertion	M
initiator_codon_variant	L
intergenic_region	NC
intragenic_variant	NC
intron_variant	NC
inversion	H
inversion	H
inversion	H
miRNA	NC
missense_variant	M
protein_protein_contact	H
rare_amino_acid_variant	H
rearranged_at_DNA_level	H
regulatory_region_variant	NC
sequence_feature + exon_loss_variant	NC
splice_acceptor_variant	H
splice_donor_variant	H
splice_region_variant	L
splice_region_variant	L
splice_region_variant	M
start_lost	H
start_retained	L
stop_gained	H
stop_lost	H
stop_retained_variant	L
stop_retained_variant	L
structural_interaction_variant	H
synonymous_variant	L
transcript_variant	NC
upstream_gene_variant	NC

Recommended Filtering for Germline Testing

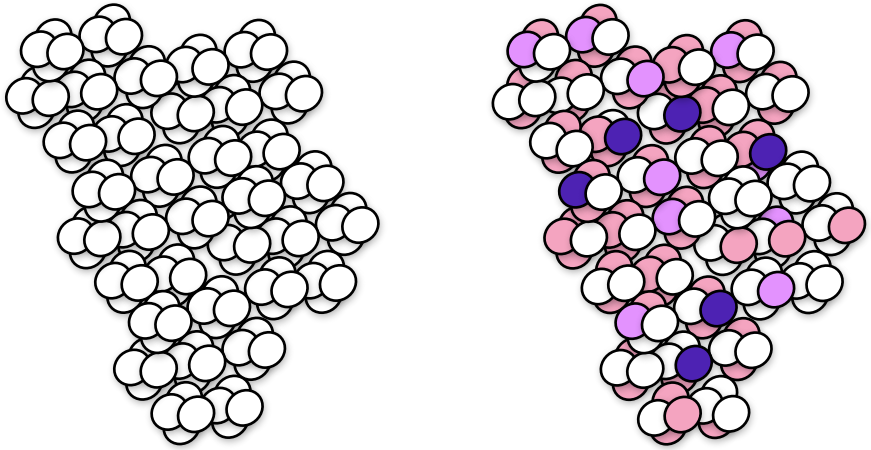
- Depth >10
- LOF or Misssense (Coding Changes)
- Alt Read Ct > 3
- Mutation Allele Frequency (MAF) > 0.15
- If novel:
  - Called by 2+ callers

Somatic Mutation Identification



Genomes from normal and tumor samples from the same patient are compared.

Tumors are Heterogeneous



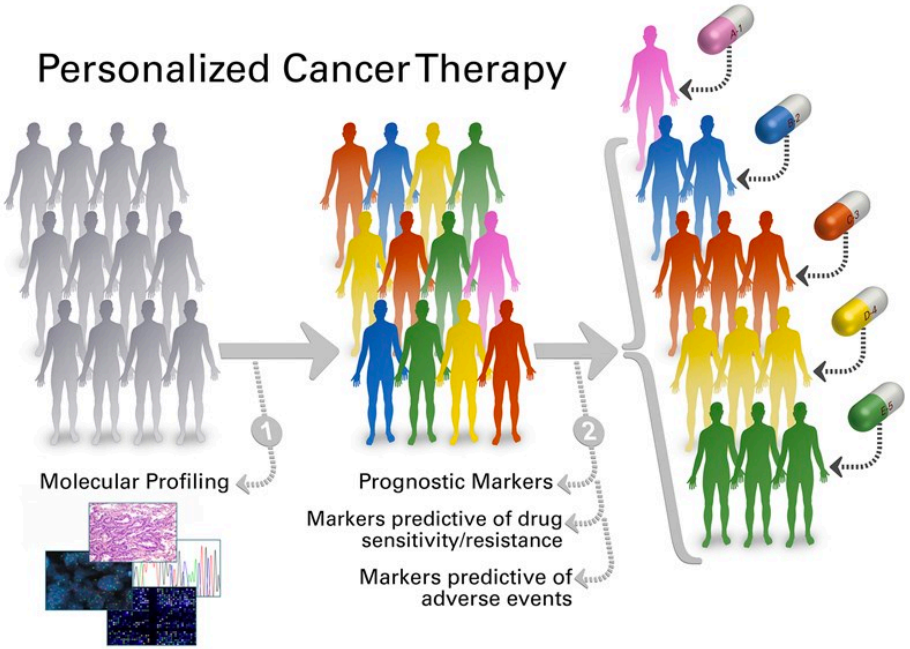
Normal Tumor

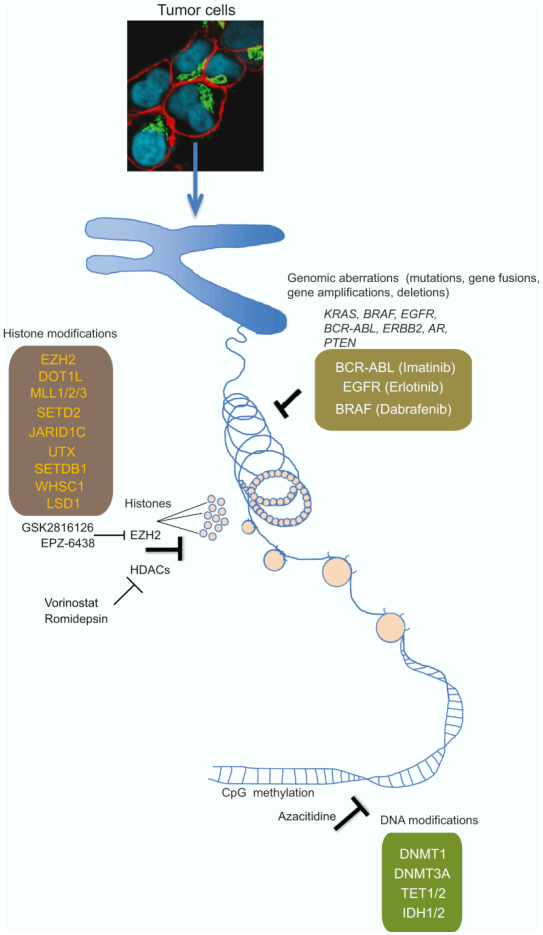
Somatic Mutation Calling Compares the Tumor and Normal samples to identify low frequency mutations.

Tumor DNA Sequencing

- Each person's cancer has a unique combination of genetic changes
- In some cases, knowledge of the genetic alterations in your cancer can help determine a treatment plan
- Genetic tests do not benefit every patient.
  - they might not identify the DNA alteration that is driving the growth of your tumor.
  - they might find such an alteration but it cannot be targeted by existing therapies.

Tumor DNA Sequencing





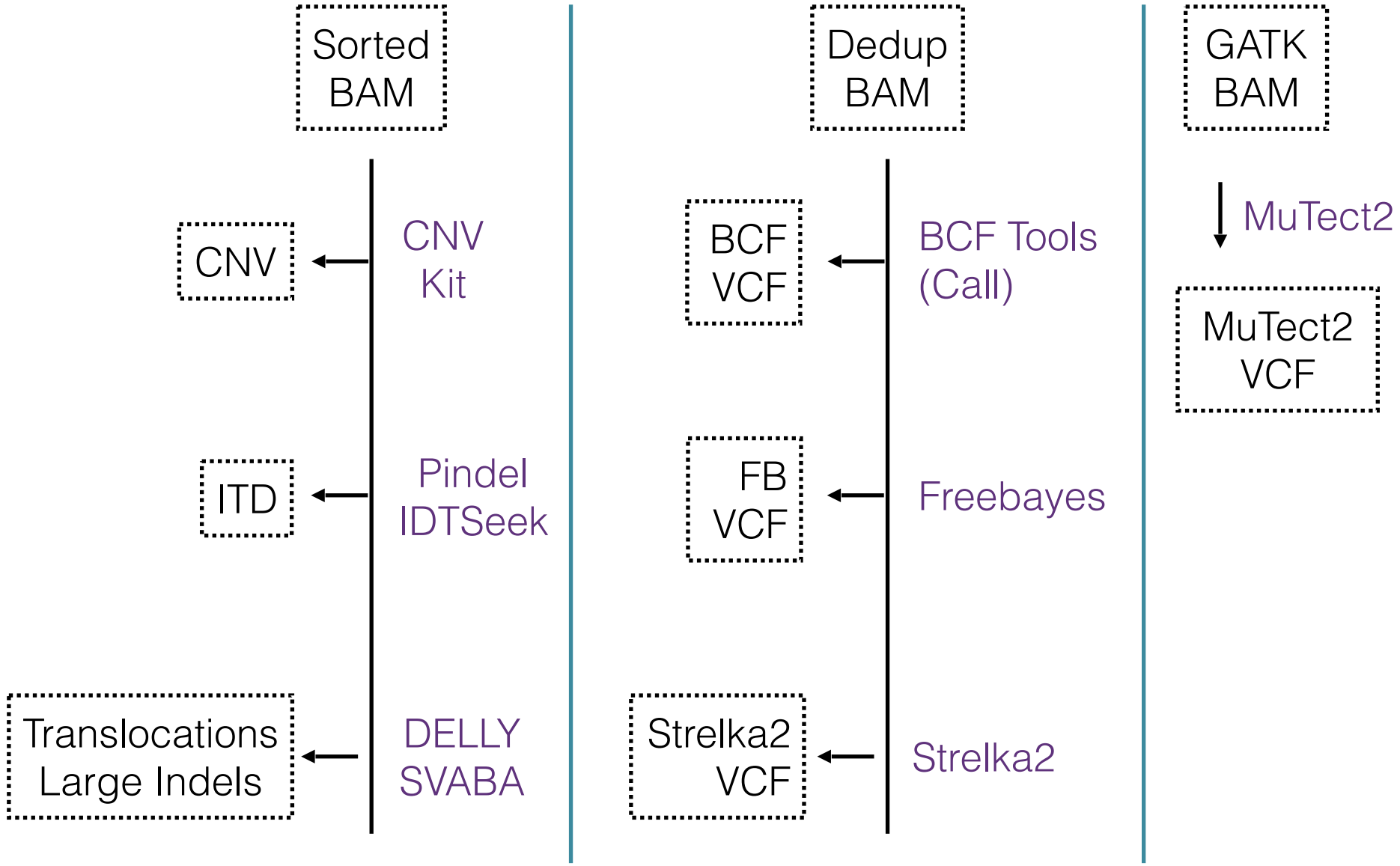
Limitations of Software Designed to Identify Somatic Mutations

- Variant detection software needs specialized training in order to:
  - Install -- must know unix and be able to install dependent software
  - Use -- must know unix and work through any “bugs” when the data isn’t exactly as software is expecting
- The variant “scores” are hard to interpret, so weeding out errors (FP) is hard.
- SNP calling methods often don’t agree very much given the same data

Tumor Only DNA Sequencing

Gene	AA	Type	E AF	Freebayes	BCF Tools	LoFreq	Platypus	GATK	MuTect	Strelka2	Vscan	Samtools	Scapel	Pindel
FLT3	ITD300	INS	5											1.3%
NRAS	Q61L	SNP	10	9.1%	8.4%	9.0%	9.2%		9.8%					
DNMT3A	R882C	SNP	5	4.4%	4.3%	4.4%			4.7%					
SF3B1	G740E	SNP	5	4.9%	4.7%	5.0%			4.7%					
IDH1	R132C	SNP	5	3.2%	3.1%	3.2%			4.4%					
GATA2	G200fs*18	DEL	35	32.8%			28.0%	34.2%	35.5%	34.2%	32.2%			33.5%
TET2	R1261H	SNP	5	4.3%	4.1%	4.4%			4.0%					
NPM1	W288fs*12	INS	5	2.7%	1.8%				4.5%				4.6%	
EZH2	R418Q	SNP	5	3.6%	3.3%	3.6%			4.0%					
JAK2	F537-K539>L	DEL	5	2.3%					3.4%				3.3%	
JAK2	V617F	SNP	5	3.4%	3.3%	3.4%			3.9%					
ABL1	T315I	SNP	5	4.0%	3.8%	3.9%			3.6%					
CBL	S403F	SNP	5	4.3%	4.3%	4.3%			5.1%					
KRAS	G13D	SNP	40	32.7%	32.0%	32.8%	32.8%	32.9%	35.9%	32.8%	31.3%	31.3%		
FLT3	D835Y	SNP	5	3.7%	3.6%	3.8%			3.6%					
IDH2	R172K	SNP	5	4.5%	4.4%	4.5%			5.0%					
TP53	S241F	SNP	5	5.3%	5.3%	5.4%			5.3%					
ASXL1	G646fs*12	INS	40	31.5%			31.1%	37.2%	5.3%	39.2%	32.0%			31.1%
ASXL1	W796C	SNP	5	4.9%	4.8%	5.1%								
RUNX1	M267I	SNP	35	33.5%	32.7%	33.4%	33.0%	33.0%	32.4%	33.2%	32.3%	32.4%		
BCOR	Q1174fs*8	INS	70	63.4%			52.4%	65.1%	67.3%	67.2%	56.5%		47.1%	
GATA1	Q119*		10	9.1%		9.1%	9.0%	9.5%	9.9%					

Somatic Workflows



Recommended Filtering for Somatic Mutations

- Depth < 20
- LOF or Misssense
- MAF (Normal) * 10.< MAF (Tumor)
- In COSMIC > 5 Subject
  - Tumor: Alt Read Ct < 3
  - Tumor: MAF < 0.01
- Others
  - Tumor: Alt Read CT < 8
  - Tumor: MAF < 0.05
  - Tumor: Called by 2+ callers

Effect of Variation in Genes

- snpEff
  - Changes affecting genes
  - Changes affecting regulatory regions
  - ENCODE
  - Epigenome Roadmap
  - NextProt
    - proteomic annotations
  - Motifs
- VEP
  - Changes affecting genes
  - Changes affecting regulatory regions
  - Integrated with downstream tools like cBioporal and GenVisR

Variant Functional Classification

- Pathogenic - a sequence variant that is previously reported and is a recognized cause of the disorder.
- Likely Pathogenic – a sequence variant that is previously unreported and is of the type which is expected to cause the disorder.
- VUS (Variant of Unknown Significance) – a sequence variant that is previously unreported and is of the type which may or may not be causative of the disorder.
- Likely Benign – a sequence variant that is previously unreported and is probably not causative of disease.
- Benign – a sequence variant is previously reported and is a recognized neutral variant.
- A sequence variant that is previously not known or expected to be causative of disease, but is found to exist in people with a particular disease or disorder.

Disease Studies

- ClinVar
  - ClinVar is a freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence
- GWAS Catalog
  - The Catalog is a quality controlled, manually curated, literature-derived collection of all published genome-wide association studies assaying at least 100,000 SNPs and all SNP-trait associations with p-values < 1.0 x 10-5
- Decipher
  - The DECIPHER database contains data from 20305 patients who have given consent for broad data-sharing; DECIPHER also supports more limited sharing via consortia.

Cancer Datasets and Annotation

- Clinical Interpretation of Variants in Cancer (CIVIC)
- Catalog of Somatic Mutation in Cancer (COSMIC)
  - Gene Fusions
  - Gene Census
  - Curated Genes
  - Drug Resistance (so far 9 genes)
  - Genome Wide Screens
- The Cancer Genome Atlas (TCGA)
  - Tons of Data, RNASeq, CNV, WES, WGS, etc

Annotating Genomic Variation

- Gene Annotation (Genes, Regulation and TFBS)
- dbSNP, ExAC, gnomAD
- clinvar, gwas catalog
- cosmic
- dbNSFP
  - SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, VEST3, CADD, MetaLR, MetaSVM, PROVEAN, DANN, fathmm-MKL, fitCons
  - PhyloP x 2, phastCons x 2, GERP++ and SiPhy
  - Allele frequencies in 1000 Genomes Project phase 3 data, UK10K cohorts data, ExAC consortium data and the NHLBI Exome Sequencing Project ESP6500 data
- genesets (MSigDB)
- CIVIC
- BROAD Target

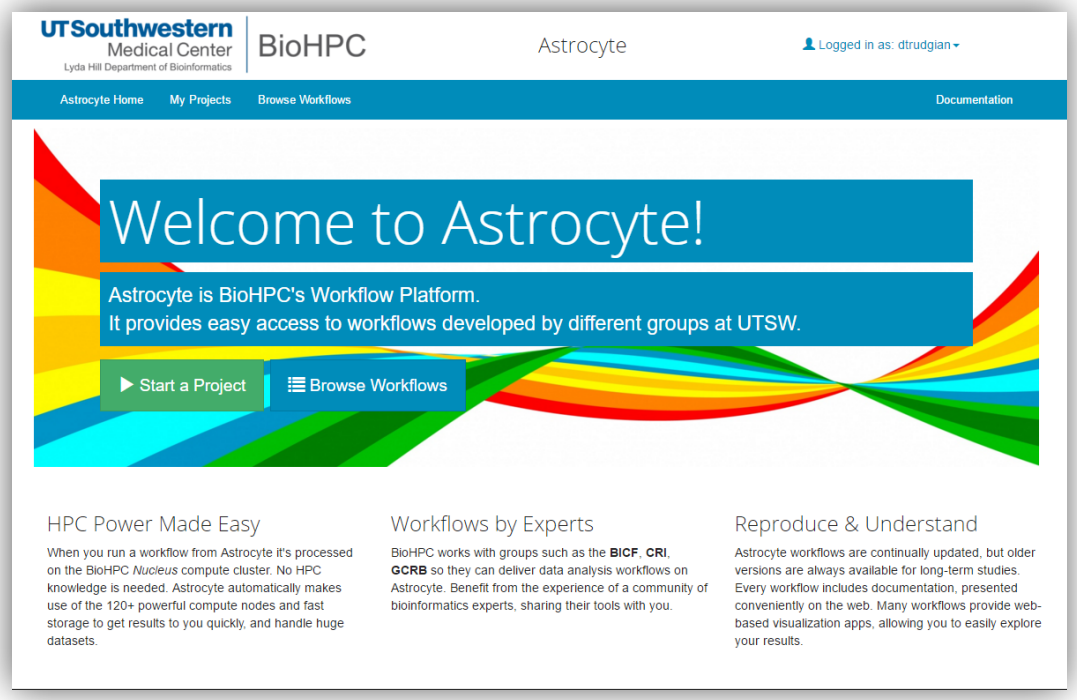
- What is Variation
  - Somatic vs Germline
  - SNVs, Indels and Structural Variation

- Is there an easy way to run all those command line programs?
  - BioHPC Astrocyte

Point and Click Analysis Tools from the BioHPC and BICF

Astrocyte – BioHPC Workflow Platform

Allows groups to give easy-access to their analysis pipelines via the web



Standardized Workflows

Simple Web Forms

Online documentation & results visualization*

Workflows run on HPC cluster without developer or user needing cluster knowledge

Bioinformatics Core Facility (BICF)

BICF provides bioinformatics, statistics and data management support for researchers on campus.

BICF functions as the conduit between bioinformatics research programs and the clinical- and basic-science research community at UTSW.

Please email bicf@utsouthwestern.edu with questions or comments about these workflows.

BICF ChIP-seq Analysis Workflow This is a workflow package for the BioHPC/BICF ChIP-seq workflow system. It implements a simple ChIP-seq analysis workflow using deepTools, Diffbind, ChipSeeker and MEME-ChIP, visualization application.	**Current Version:** chipseq_analysis_bicf - 0.0.12 **Author:** Beibei Chen **Contact:** biohpc-help@utsouthwestern.edu	▶ Run Workflow Documentation View Versions
BICF RNASeq Analysis Workflow This is a workflow package for the BioHPC/BICF RNASeq workflow system. It implements differential expression analysis, gene set enrichment analysis, gene fusion analysis and variant identification using RNASeq data.	**Current Version:** rnaseq_bicf - 0.3.3 **Author:** Brandi Cantarel **Contact:** biohpc-help@utsouthwestern.edu	▶ Run Workflow Documentation View Versions
BICF RNASeq Variant Analysis Workflow THIS WORKFLOW IS OBSOLETE! The Main BICF workflow includes variant analysis and differential expression analysis as one easy to use workflow.	**Current Version:** rnaseq_variant_bicf - 0.0.11 **Author:** Brandi Cantarel **Contact:** biohpc-help@utsouthwestern.edu	▶ Run Workflow Documentation View Versions
BICF Somatic Mutation Calling This is a workflow package for the BioHPC/BICF Somatic Mutation workflow system. It implements a simple Somatic Mutation analysis workflow.	**Current Version:** somatic_bicf - 0.0.3 **Author:** Brandi Cantarel **Contact:** biohpc-help@utsouthwestern.edu	▶ Run Workflow Documentation View Versions
BICF Germline Variant Analysis Workflow This is a workflow package for the BioHPC/BICF Germline Variant workflow system. It implements a simple germline variant analysis workflow using TrimGalore, BWA, Speedseq, GATK, Samtools and Platypus. SNPs and Indels are integrated using BAYSIC; then annotated using SNPEFF and SnpSift.	**Current Version:** germline_bicf - 0.0.10 **Author:** Brandi Cantarel **Contact:** biohpc-help@utsouthwestern.edu	▶ Run Workflow Documentation View Versions

https://astrocyte.biohpc.swmed.edu/brand/bicf/browse/

UTSouthwestern Medical Center | BICF

Create a new project

My Projects

In Astrocyte **projects** are used to organize your work. You upload **input data** into a project, and can then run **workflows** against this input data. Try to separate your work into natural projects, so that you can easily share them with other users if required.

+ Start a New Project

Project Name | Create New Project

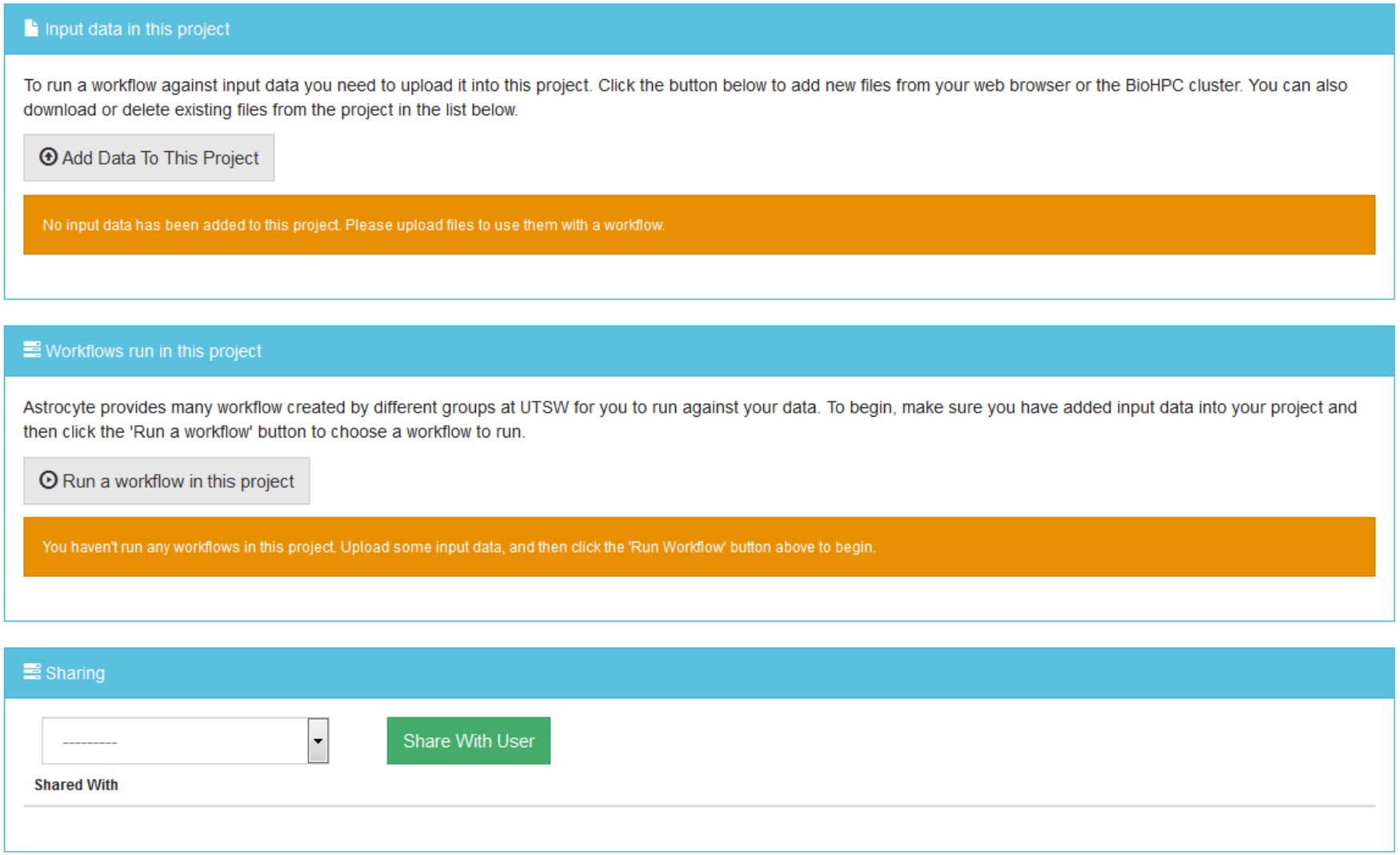
Existing Projects

ID	Name	Created	Workflows Run	Input Files	Size	Actions
PRJ21	RNAseq_test	Aug. 23, 2016, 3:03 p.m.	0	0	0 bytes	

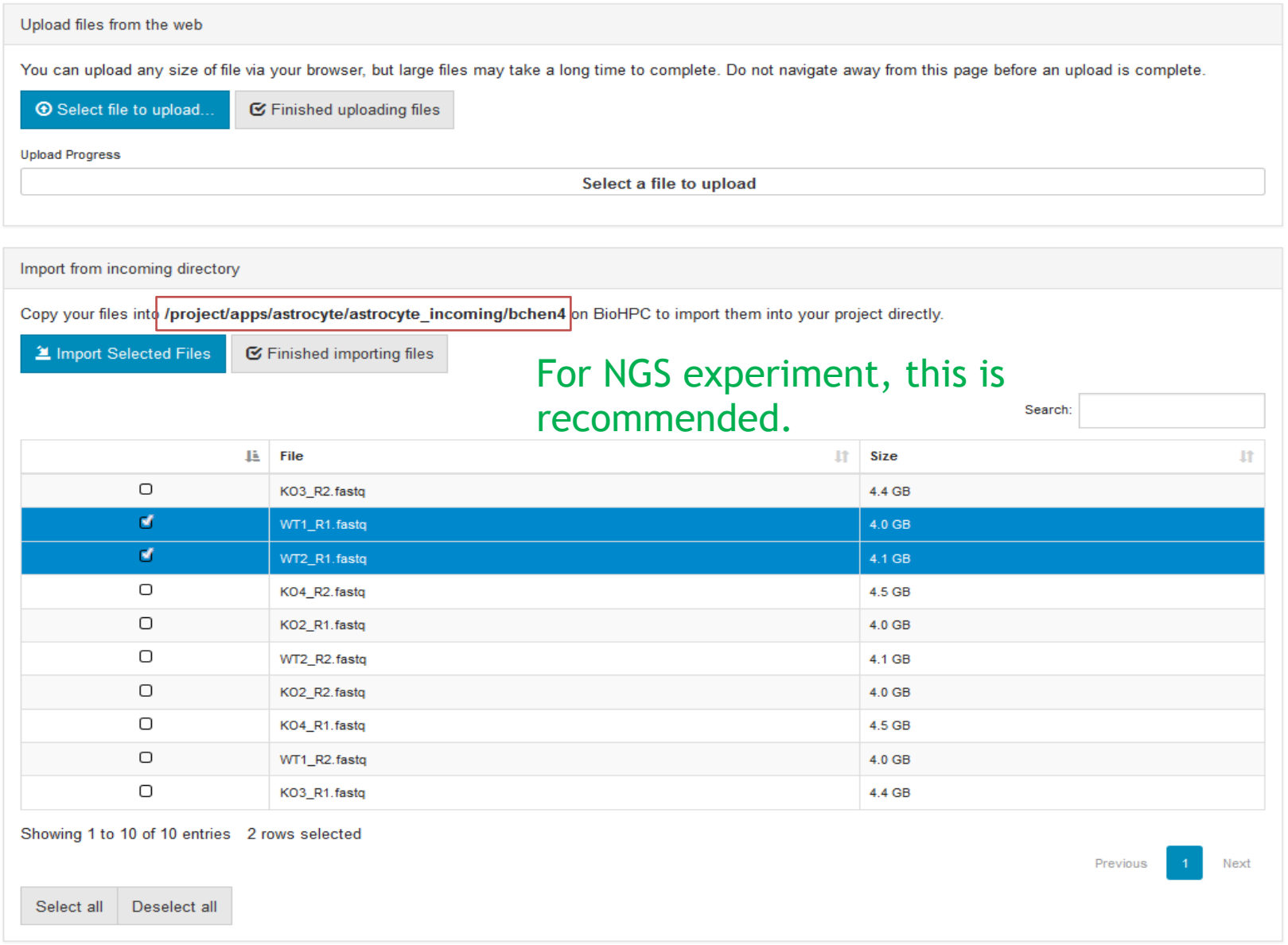
Projects Shared with Me

ID	Name	Created	Workflows Run	Input Files	Size	Actions
PRJ10	test	June 1, 2016, 5:02 p.m. by Brandi Cantarel	4	10	218.5 GB	

Add Data To Your Project



Add Data To Your Project



Make your design file

```
FamilyID
This ID will be used to call samples in batch
SampleID
This ID will be used to name all workflow produced files ie S0001 will produce S0001.bam
FullPathToFqR1
Name of the fastq file R1 (not the full path)
FullPathToFqR2
Name of the fastq file R2 (not the full path)
```

FamilyID	SampleID	FqR1	FqR2
F1	GM12877	GM12877.R1_001.fastq.gz	GM12877_S124_R2_001.fastq.gz
F1	GM12878	GM12878.R1_001.fastq.gz	GM12878_S124_R2_001.fastq.gz
F1	GM12879	GM12879.R1_001.fastq.gz	GM12879_S124_R2_001.fastq.gz
F2	GM12887	GM12887.R1_001.fastq.gz	GM12887.R2_001.fastq.gz
F2	GM12888	GM12888.R1_001.fastq.gz	GM12888.R2_001.fastq.gz
F2	GM12889	GM12889.R1_001.fastq.gz	GM12889.R2_001.fastq.gz

Make your design file

- Use tab as delimiter
  - Excel save as “Text (tab delimited)”
- If no SubjectID, use same number/character for all rows
- SampleID and SampleName
- If no FqR2, leave them empty
- For all contents, no “-”
- For all contents, no spaces
- Columns names MUST be exactly the same as documented

Select your data files and set up workflow and submit

Parameters

Project

Project 47: panel_utswv2

Name for this run

temp

One or more input paired-end FASTQ files from a RNASeq experiment and a design file with the link between the same name and the sample group regex: ".*(fastq|fq)*" min: 1

panel_utswv2.design.txt
utswv2_H2_AP14-924.R2.fastq.gz
utswv2_H2_AP14-924.R1.fastq.gz
utswv2_H2_33.R2.fastq.gz

SELECT YOUR FILES

In single-end sequencing, the sequencer reads a fragment from only one end to the other, generating the sequence of base pairs. In paired-end reading it starts at one read, finishes this direction at the specified read length, and then starts another round of reading from the opposite end of the fragment.

Paired End

A design file listing sample names, fastq files, and additional information about the sample

panel_utswv2.design.txt

A capture bed file is a bed file of the targeting panel or exome capture used for the sequencing, this file is used to assess capture efficiency and to limit variants to capture region

UTSWV2.bed

Reference genome for alignment

Human GRCh38

Run Workflow

Project is running

Run 'temp' in Project 'panel_utswv2'

Run Information

Running Workflow	BICF Germline Variant Analysis Workflow brandi.cantarel/variant_germline.git / 0.0.10
Status	RUNNING
Created	Sept. 13, 2017, 8:39 p.m. by s166458
Size	116.0 KB

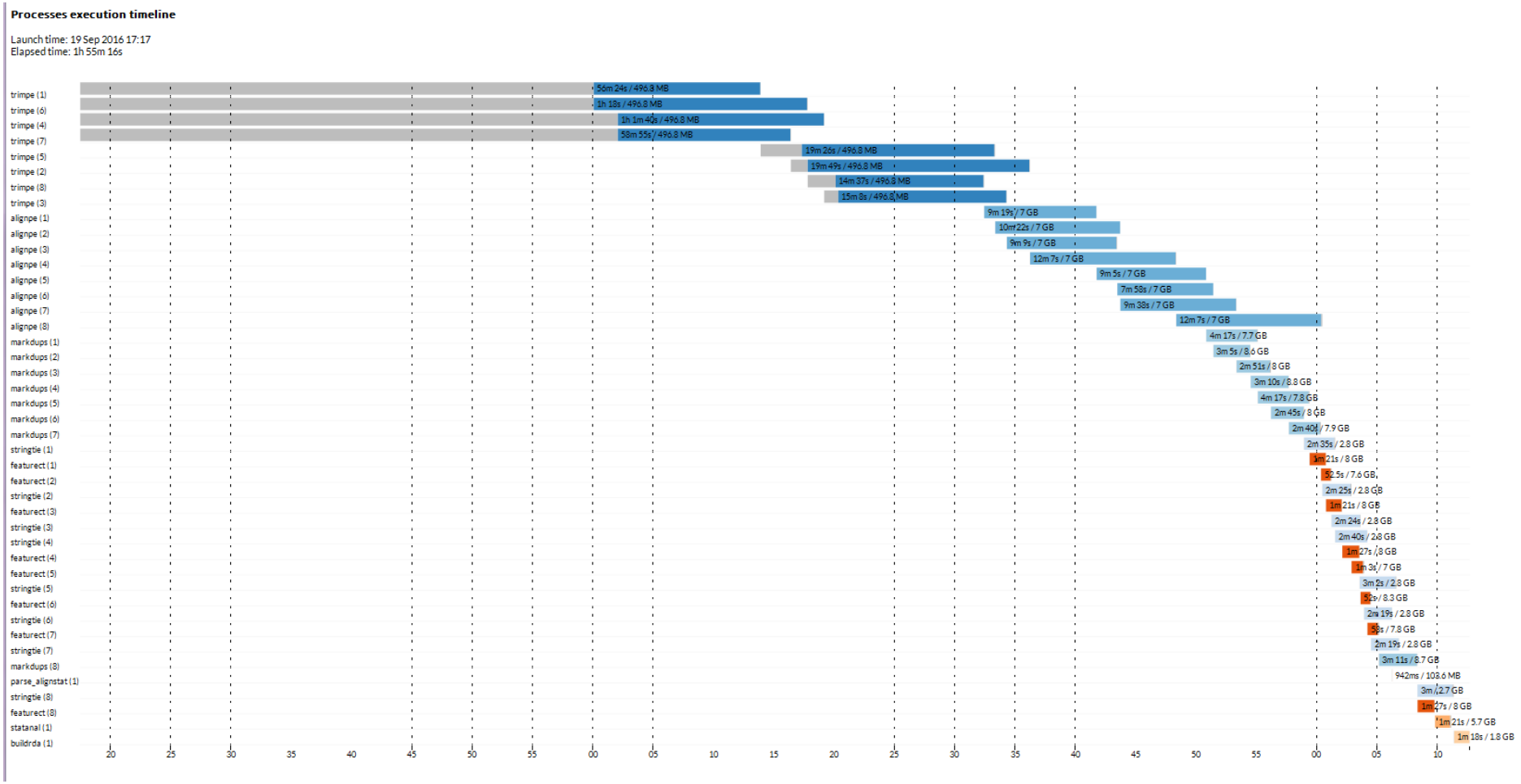
Parameters

Parameter	Value
design	panel_utswv2.design.txt
genome	/project/shared/bicf_workflow_ref/GRCh38
pairs	pe
fastqs	utswv2_H2_AP14-924.R2.fastq.gz
fastqs	utswv2_H2_AP14-924.R1.fastq.gz
capture	UTSWV2.bed

Input Files

Filename	Size
panel_utswv2.design.txt	1.3 KB
utswv2_H2_AP14-924.R2.fastq.gz	1.6 GB
utswv2_H2_AP14-924.R1.fastq.gz	1.5 GB
UTSWV2.bed	486.3 KB

Timeline of the whole run



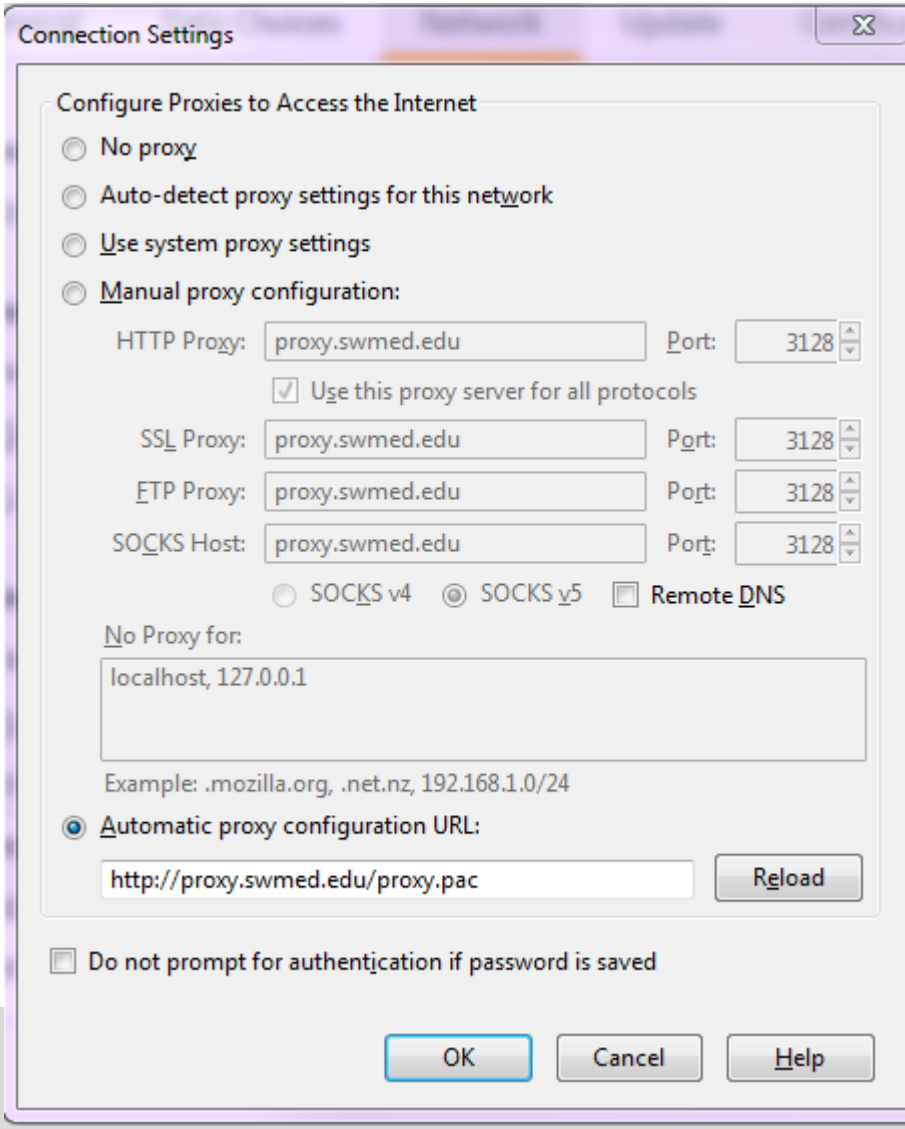
Common errors and solutions

```
        Error running workflow. Diagnostic output

N E X T F L O W  ~  version 0.20.1
Launching main.nf
Didn't match any input files with entries in the design file

 -- Check script 'main.nf' at line: 49 or see '.nextflow.log' file for more details
```

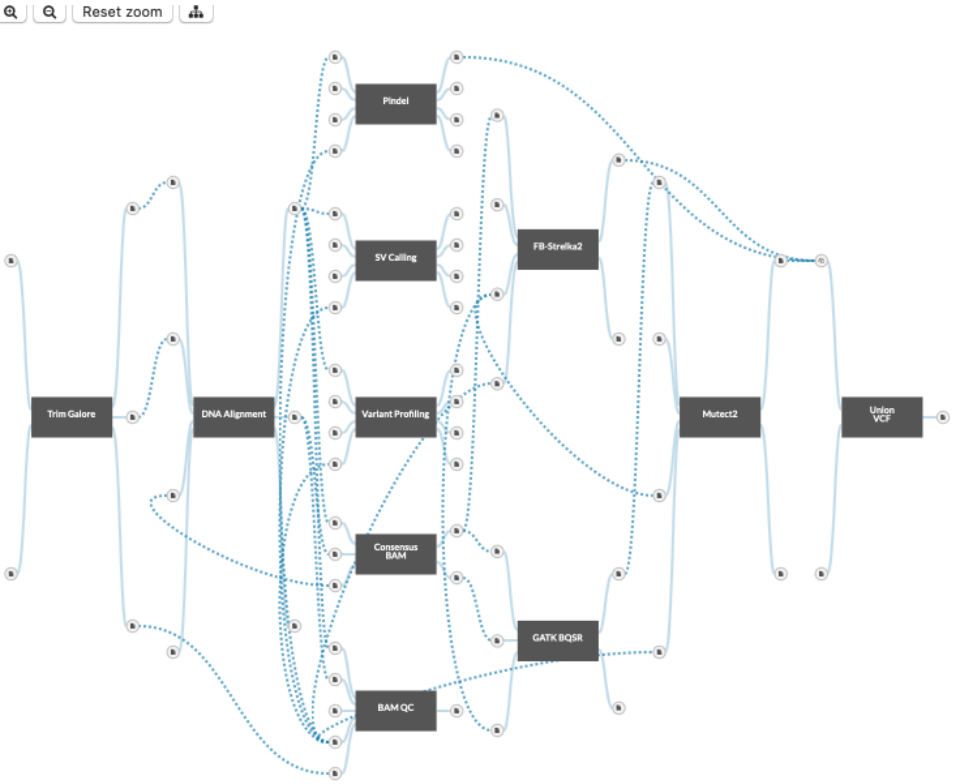
- Make sure the delimiter is tab
- Make sure the column name are the same as mentioned in documentation
- Make sure the file names match

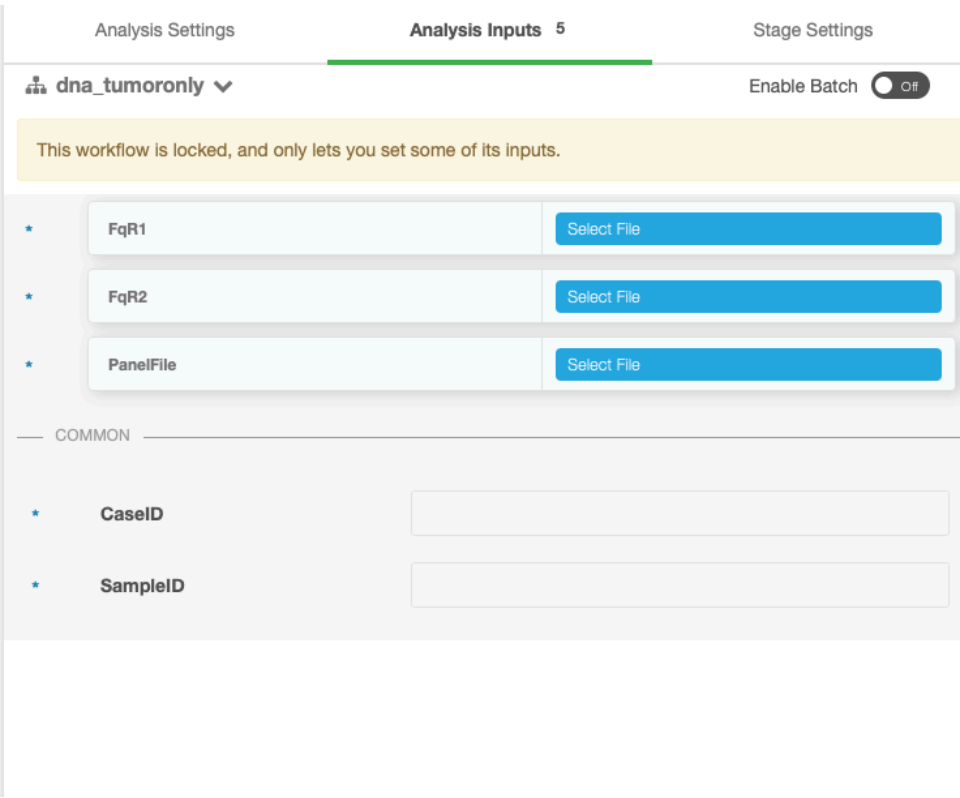
Common errors and solutions

- Not all files are uploaded
- It's about the proxy setting
- Use auto-detect proxy



Alternatives to BioHPC





https://platform.dnanexus.com/

DNAnexus®

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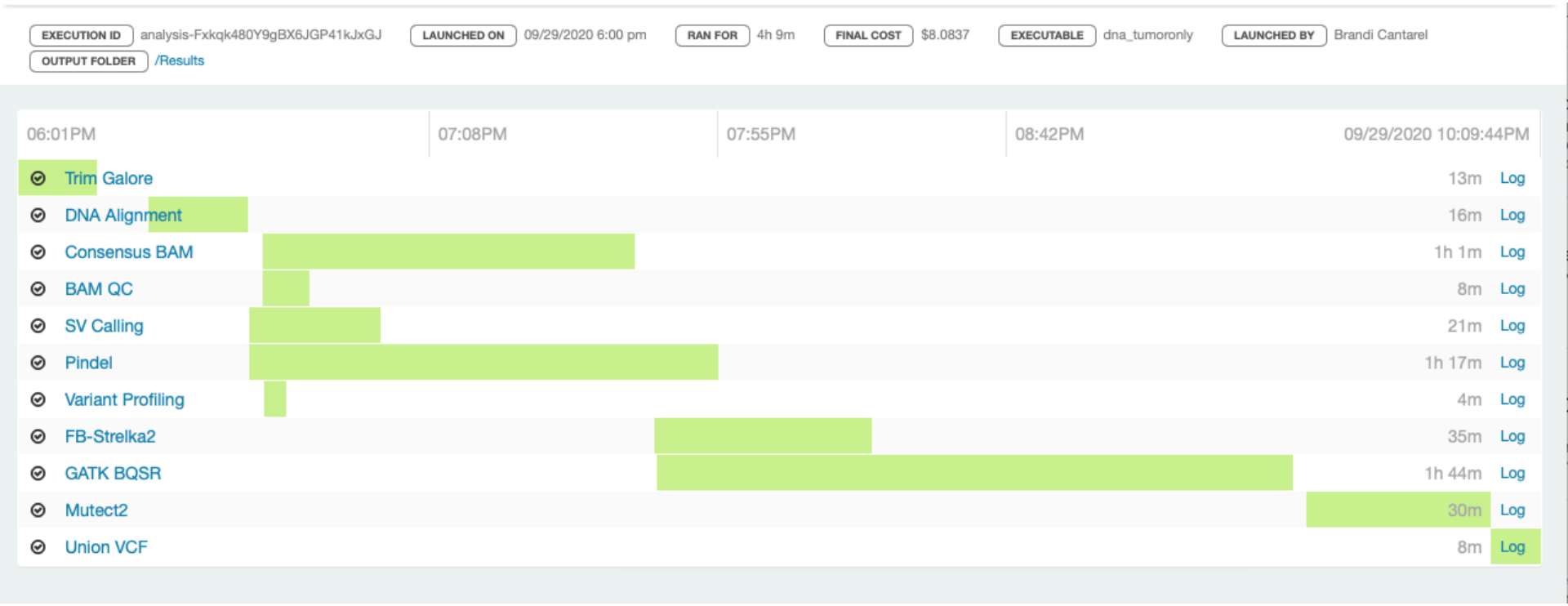
Alternatives to BioHPC



DNAnexus®

UT Southwestern Medical Center | BICF

Prices

Pipeline	Time (m)	DNANexus Price ($)
Gene Panel ~200	3-6h	~7
Gene Panel ~1000	7-9h	~12
Exome	10-12h	~20
RNASeq	< 1 h	1

DX ToolKit

```
    for fq in "${!fqfiles[@]}"
    do
        read="${inputdir}/${fqfiles[$fq]}"
        Fq=$(dx upload $read --destination /$RunID/$CaseID/ --brief)
        opts="$opts -i${fq}=$Fq"
    done
    runwkflow=$(dx run $wkflow $opts --destination /$RunID/$CaseID -y
--brief)
    echo $runwkflow >> ${outdir}/${RunID}.${CaseID}.joblist.txt
```

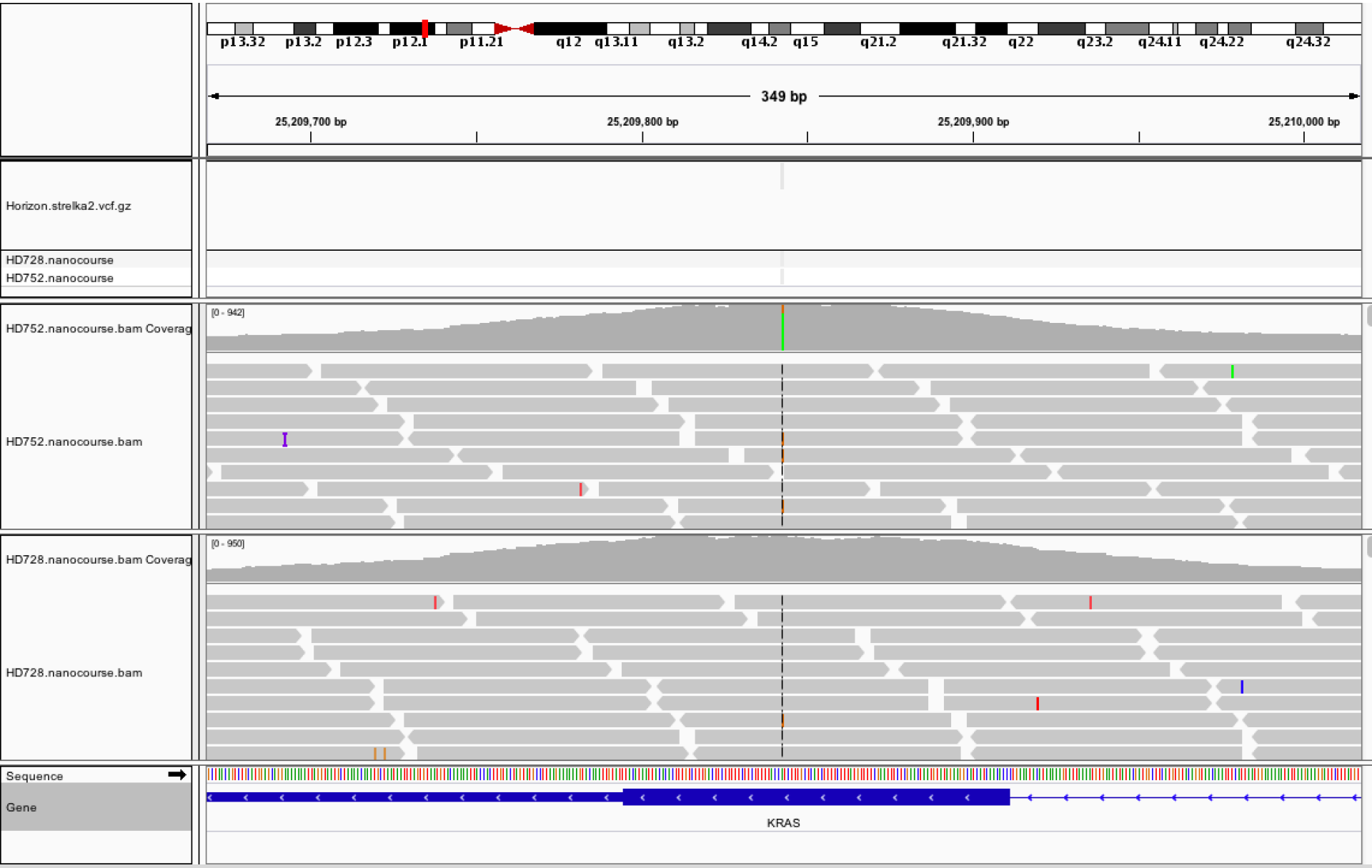
Key Files Germline Pipeline

- VCF file — SNPs/Indels for each sample
  - SampleID.annot.vcf.gz
- Coverage Histogram for each sample
  - SampleID.coverage_histogram.png
- Cumulative Distribution Plot for all samples
  - coverage_cdf.png
- QC for all samples
  - sequence.stats.txt
- Structural Variants (unfiltered)
  - SampleID.sssv.sv.vcf.gz.annot.txt

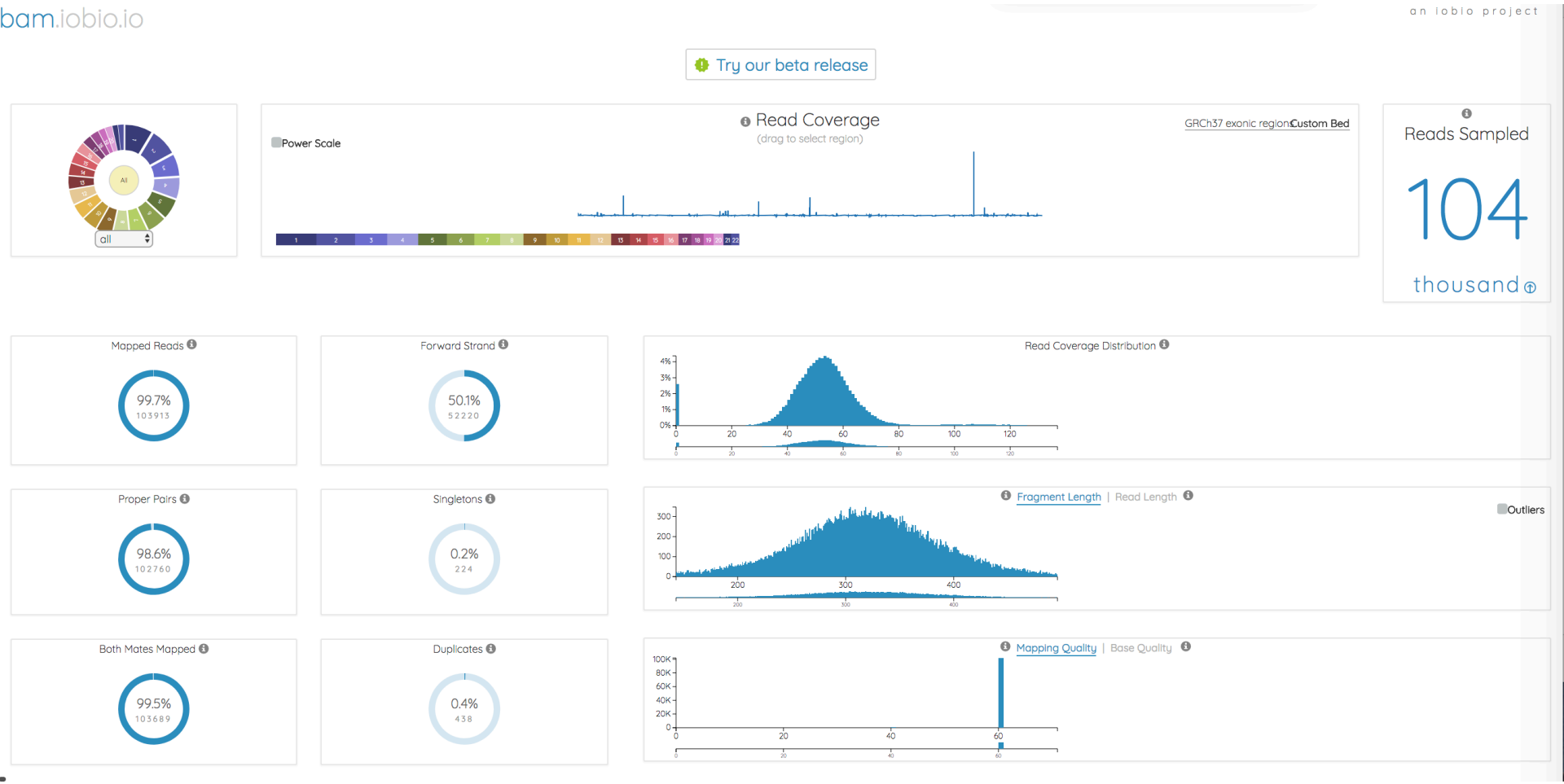
Key Files Somatic Mutation Pipeline

- VCF file — SNPs/Indels for each sample
  - TumorID_NormalID.annot.vcf.gz
- Match Check File
  - TumorID_NormalID_matched.txt

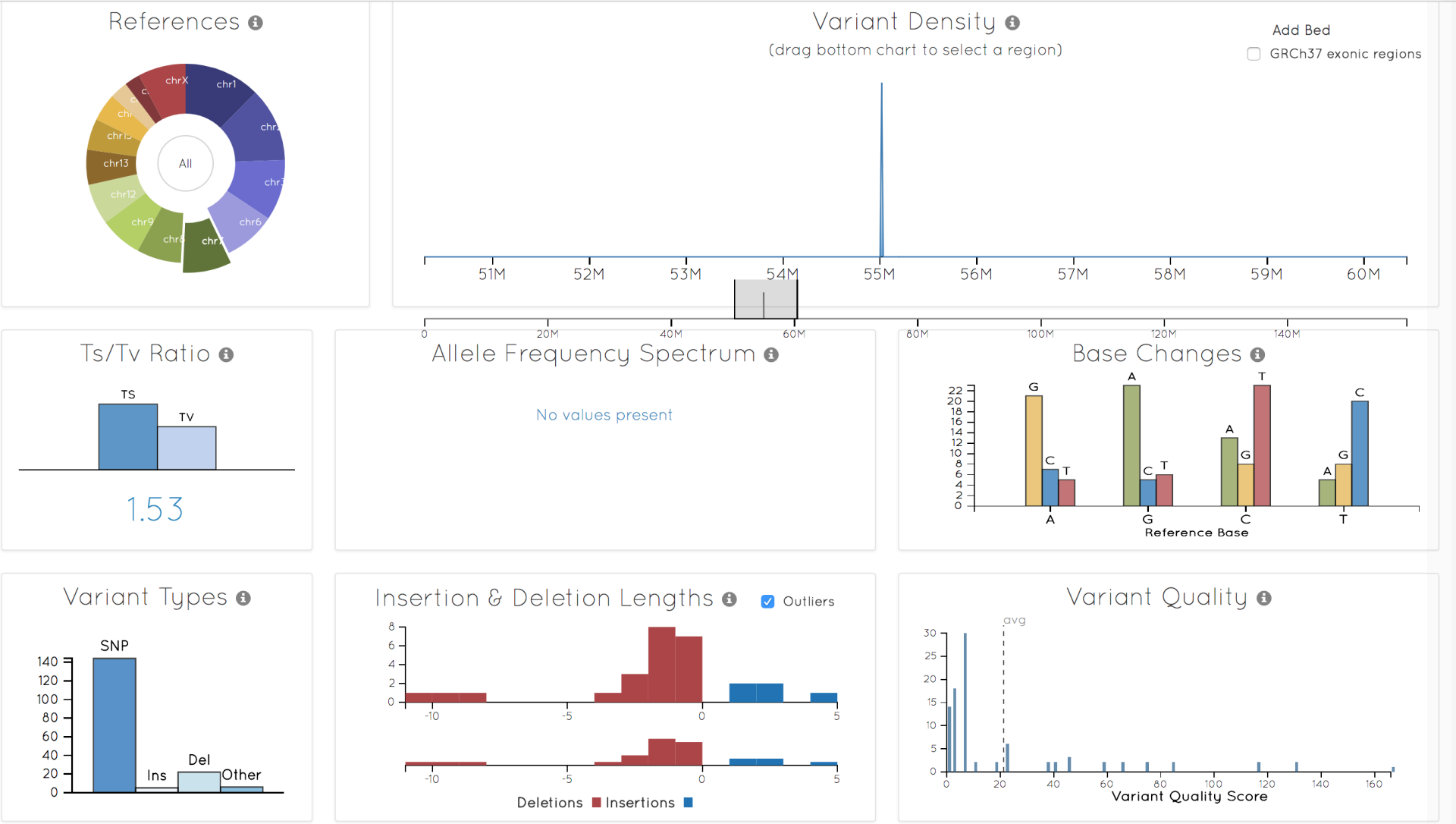
IGV Viewer


p13.32 p13.2 p12.3 p12.1 p11.21 q12 q13.11 q13.2 q14.2 q15 q21.2 q21.32 q22 q23.2 q24.11 q24.22 q24.32
349 bp
25,209,700 bp
25,209,800 bp
25,209,900 bp
25,210,000 bp
Horizon.strelka2.vcf.gz
HD728.nanocourse
HD752.nanocourse
HD752.nanocourse.bam Coverag
[0 - 942]
HD752.nanocourse.bam
HD728.nanocourse.bam Coverag
[0 - 950]
HD728.nanocourse.bam
Sequence
Gene
KRAS

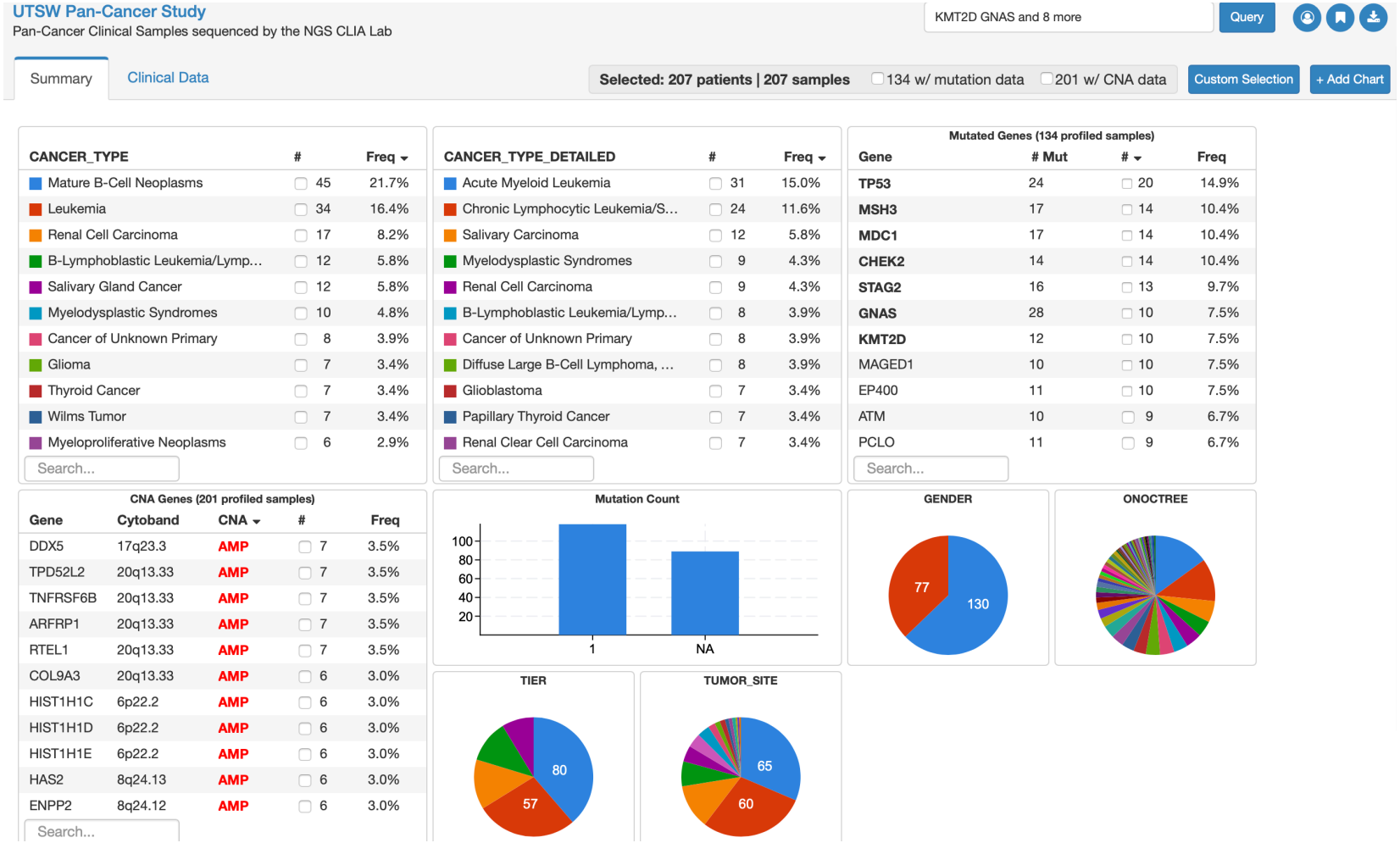
BAM ioBIO


bam.iobio.io
an iobio project
Try our beta release
all
Read Coverage
(drag to select region)
Power Scale
GRCh37 exonic region
Custom Bed
Reads Sampled
104
thousand
Mapped Reads
99.7%
103913
Forward Strand
50.1%
52220
Read Coverage Distribution
Proper Pairs
98.6%
102760
Singletons
0.2%
224
Fragment Length | Read Length
Outliers
Both Mates Mapped
99.5%
103689
Duplicates
0.4%
438
Mapping Quality | Base Quality

VCF ioBIO


References
All
chrX
chr1
chr13
chr12
chr9
chr6
Variant Density
(drag bottom chart to select a region)
Add Bed
GRCh37 exonic regions
51M
52M
53M
54M
55M
56M
57M
58M
59M
60M
0
20M
40M
60M
80M
100M
120M
140M
Ts/Tv Ratio
TS
TV
1.53
Allele Frequency Spectrum
No values present
Base Changes
Reference Base
Variant Types
SNP
Ins
Del
Other
Insertion & Deletion Lengths
Outliers
Deletions
Insertions
Variant Quality
avg
Variant Quality Score

Local Cbioporal



Questions?