Introduction to Sequencing Data Analysis

Lecture 7

October 17, 2019

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FRED HUTCH
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Overview

I. Sequence data

II. Tools for analyzing and visualizing sequencing data

III. Genome variant analysis

Overview: Learning Objectives

1. Sequence data
    - Databases and online resources for sequence data
    - Learn the common sequence data file formats
2. Tools for sequencing data
    - Tools to query, inspect, visualize an aligned sequence file
    - Learn the contents of sequence data files
    - Learn to generate sequencing metrics and to process sequence data
    - Learn about Python and R libraries/packages to read sequence data
3. Genome variant analysis
    - Types of genomic variation
    - Tools to predict genomic variations
    - Learn the common file formats for variation data
    - Databases and online resources for human variation data

Sequence Data: International Consortia and Projects

1. What is DNA/RNA sequencing?
    - Types of nucleic acids
    - Types of sequencing platforms

Slides on sequencing projects and consortia

Disclaimer about lecture:

Focused on short read sequencing data

Variant analysis on genomes and less about transcriptomes

No single-cell sequencing data

Sequence Data: Databases and Online Resources

Repositories/Databases for sequence data

1. NCBI Sequence Read Archive (SRA)

- Publicly available data submitted from studies (e.g. Gene Expression Omnibus [GEO])
  - https://www.ncbi.nlm.nih.gov/gds/
  - Controlled access (e.g. dbGaP)

2. European Genome Phenome Archive (EGA)

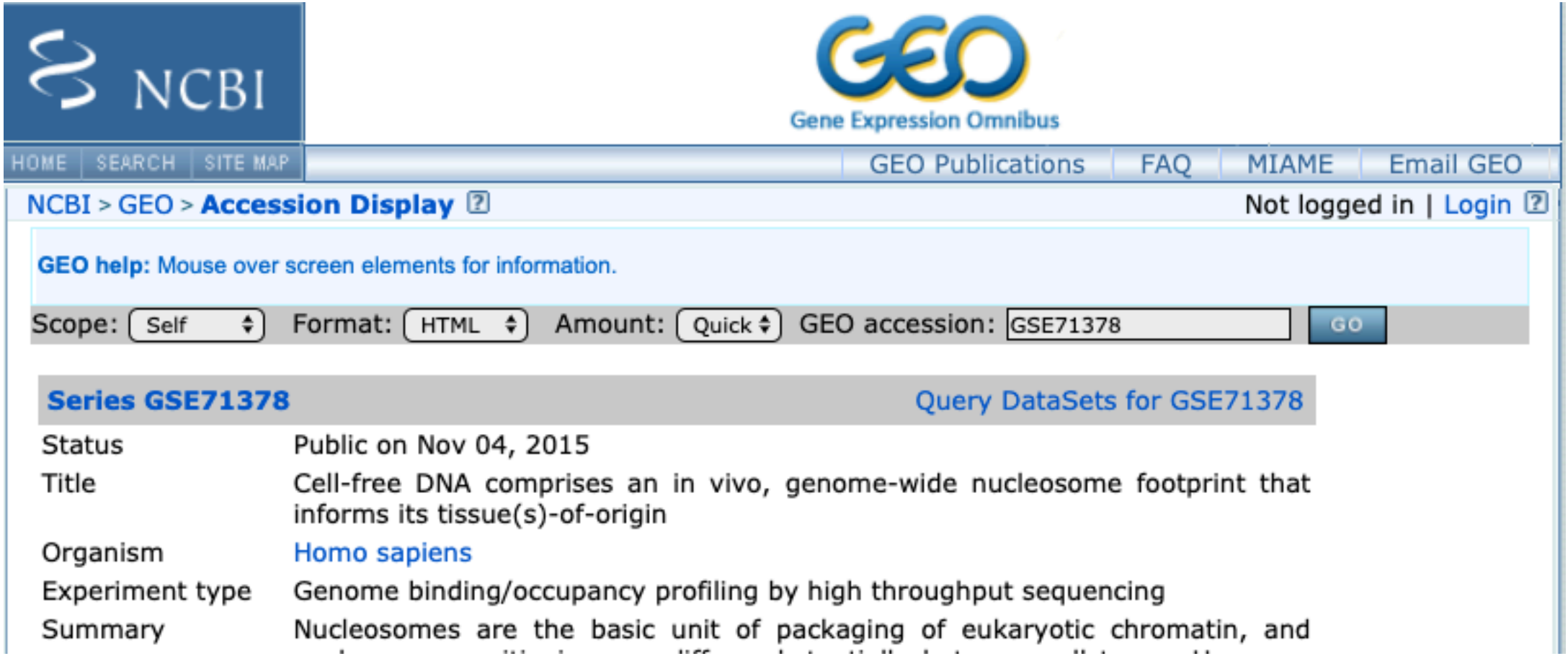
- https://www.ebi.ac.uk/ega/home

3. NIH NCI Genomic Data Commons (GDC) Data Portal

- https://portal.gdc.cancer.gov/
- Harmonized Cancer Datasets

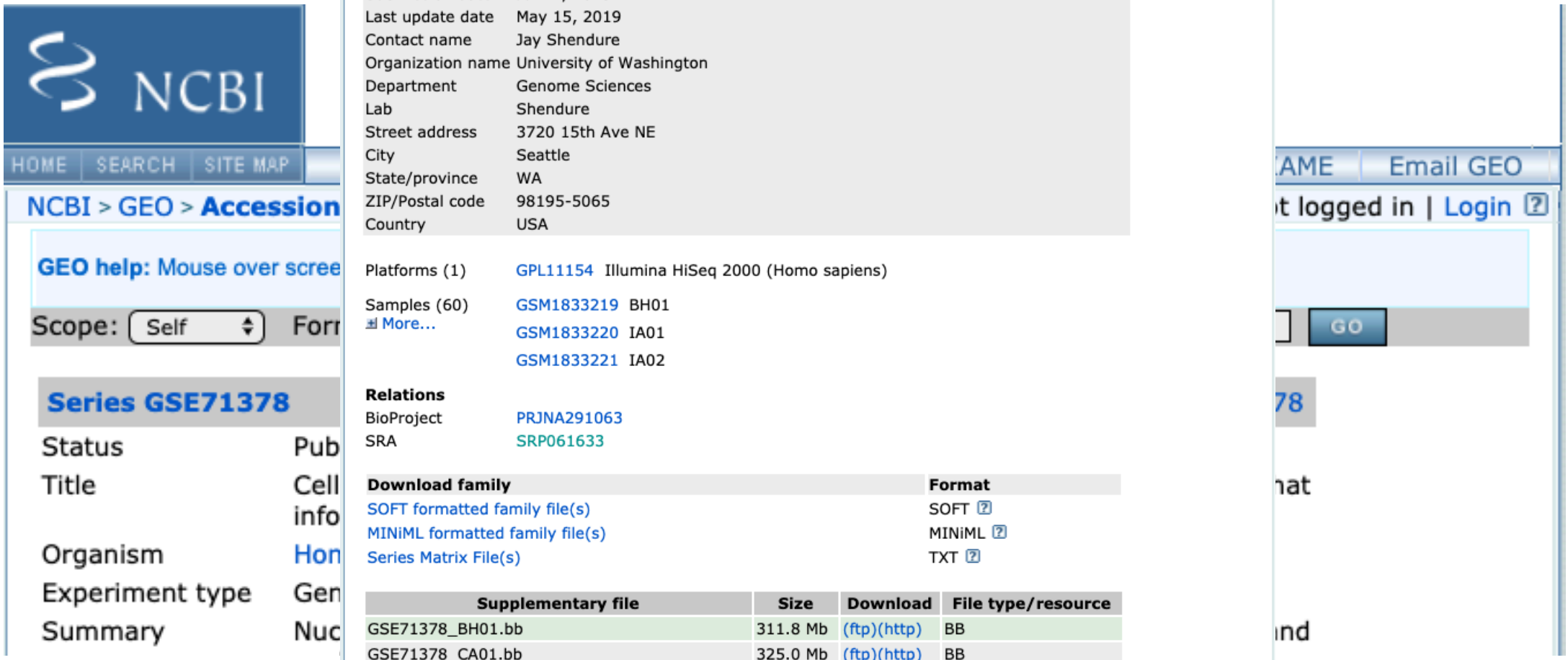
Sequence Data: Databases and Online Resources

Sequence Read Archive (SRA) & GEO example (GSE71378)



Sequence Data: Databases and Online Resources

Sequence Read



1378)

Contributor(s)	Shendure J
Citation(s)	Snyder MW, Kircher M, Hill AJ, Daza RM et al. Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin. *Cell* 2016 Jan 14;164(1-2):57-68. PMID: 26771485
Submission date	Jul 27, 2015
Last update date	May 15, 2019
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State/province	WA
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Country	USA

Platforms (1)	GPL11154 Illumina HiSeq 2000 (Homo sapiens)
Samples (60)	GSM1833219 BH01
More...	GSM1833220 IA01
	GSM1833221 IA02

Relations

BioProject	PRJNA291063
SRA	SRP061633

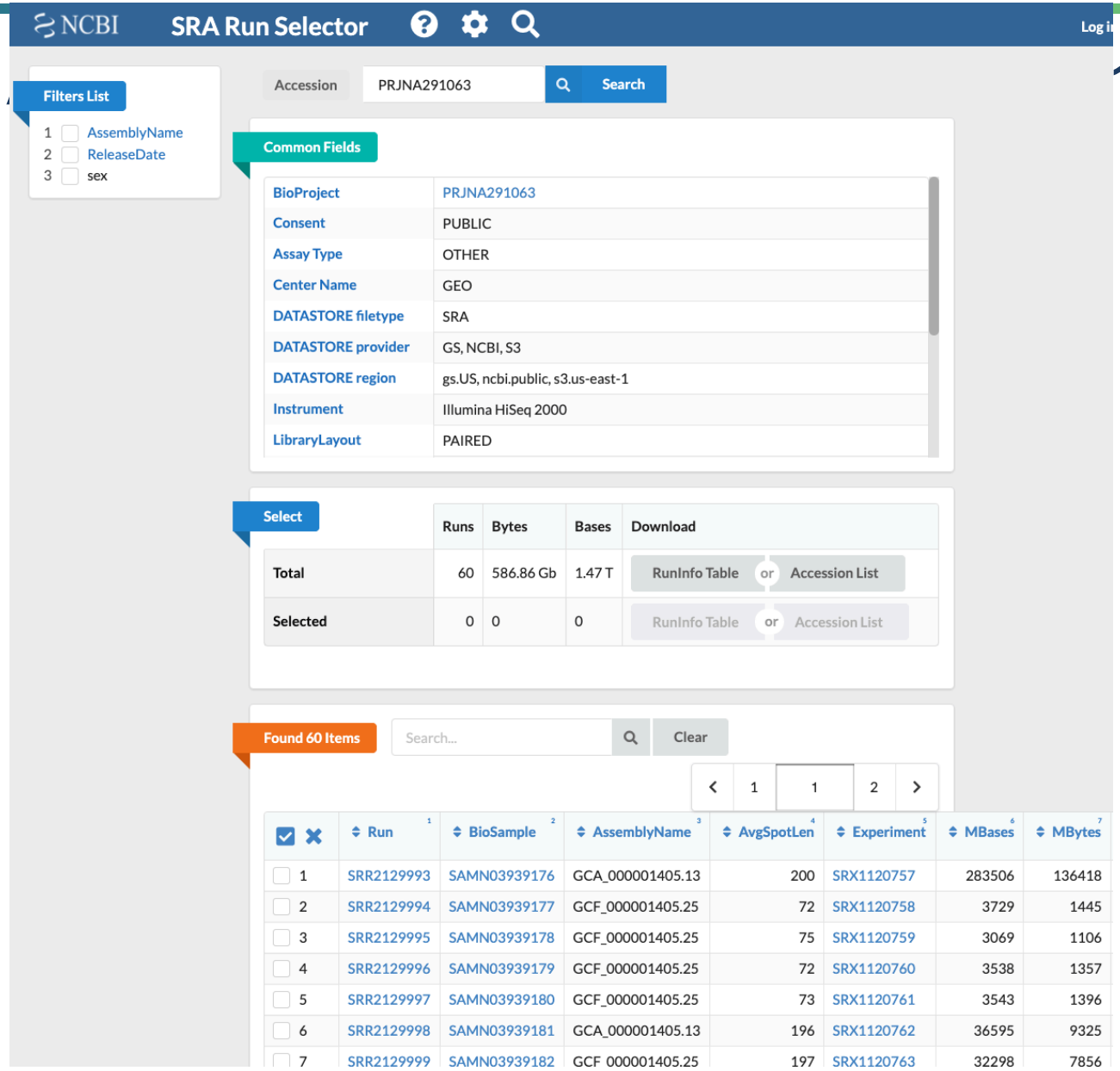
Download family	Format
SOFT formatted family file(s)	SOFT
MINiML formatted family file(s)	MINiML
Series Matrix File(s)	TXT

Supplementary file	Size	Download	File type/resource
GSE71378_BH01.bb	311.8 Mb	(ftp)(http)	BB
GSE71378_CA01.bb	325.0 Mb	(ftp)(http)	BB
GSE71378_CH01.bb	319.7 Mb	(ftp)(http)	BB
GSE71378_IH01.bb	296.6 Mb	(ftp)(http)	BB
GSE71378_IH02.bb	248.3 Mb	(ftp)(http)	BB

SRA Run Selector

Sequence Data: Databases and Online Resources

Sequence Read … 1378)



Sequence Data: Databases and Online Resources

Sequence Read Archive (SRA) & GEO example (GSE71378)

SRA Toolkit required to download and extract **`.sra`** files

- Download .sra file

```
prefetch SRR2130004
```

- Convert `.sra` file to fastq

```
fastq-dump SRR2130004 # use accession
fastq-dump SRR2130004.sra # use file if already downloaded
```

- Convert `.sra` file to SAM/BAM file

```
# will write data to a SAM file
sam-dump --header SRR2130004.sra > SAMN03160688.sam
# will write data to a BAM file
sam-dump --header SRR2130004.sra | samtools view -bS - > BRCA_IDC_cfDNA.bam
```

Sequence Data: File formats

Sequences

- Genome sequences - **FASTA** (.fasta or .fa)
- Sequenced reads - **FASTQ** (.fastq or .fq)

Sequence Alignment/Map Format

- https://samtools.github.io/hts-specs/SAMv1.pdf
- Sequence Alignment - **SAM** (.sam)
- Binary Alignment - **BAM** (.bam)

Sequence Data: Sequence alignment

Burrows-Wheeler Aligner, bwa (http://bio-bwa.sourceforge.net/)

- aln - for 35bp to 100bp reads
- mem - for reads with length 70bp to 1Mb (Recommended for most)

```
# If two fastq files, one for each mate of paired-end reads
bwa mem -M reference.fa BRCA_IDC_cfDNA_R1.fq BRCA_IDC_cfDNA_R2.fq > BRCA_IDC_cfDNA.bam


# If single fastq file with paired-end reads interleaved
bwa mem -M -p reference.fa BRCA_IDC_cfDNA.fq > BRCA_IDC_cfDNA.bam
```

Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60. [PMID: 19451168]

Sequence Data: Inspecting and Reading SAM/BAM Files

SAMtools (http://www.htslib.org/)

- Indexing

```
samtools index BRCA_IDC_cfDNA.bam #required for all BAM files
```

- File operations

```
samtools sort BRCA_IDC_cfDNA.bam #sort by coordinate
```

- Statistics

```
samtools flagstat BRCA_IDC_cfDNA.bam #get general alignment metrics
```

- Viewing

```
# view header information
samtools view -H BRCA_IDC_cfDNA.bam

# view aligned reads at chr17:25,000,000
samtools view BRCA_IDC_cfDNA.bam 17:37844393
```

Sequence Data: SAM Format

https://samtools.github.io/hts-specs/SAMv1.pdf

A. Header information

```
samtools view -H BRCA_IDC_cfDNA.bam

@HD     VN:1.2  SO:coordinate
@SQ     SN:1    LN:249250621
@SQ     SN:2    LN:243199373
@SQ     SN:3    LN:198022430
@SQ     SN:4    LN:191154276
@SQ     SN:5    LN:180915260
@SQ     SN:6    LN:171115067
@SQ     SN:7    LN:159138663
@SQ     SN:8    LN:146364022
@SQ     SN:9    LN:141213431
...
@RG     ID:P12.17.7_Breast
```

Show description
One field per slide

Sequence Data: SAM Format

https://samtools.github.io/hts-specs/SAMv1.pdf

A. Header information

- `@HD`: Header line
  - `SO`: Sorting order of alignments (`unknown, unsorted, coordinate, queryname`)
- `@SD`: Reference sequence dictionary
  - `SN`: Reference sequence name - typically, one row for each chromosome
  - `LN`: Length of reference sequence
- `@RG`: Read group
  - `ID`: Read group identifier (must be unique)
  - `PL`: Platform or technology used (e.g. ILLUMINA)
  - `SM`: Sample ID and/or pool being sequenced
- `@PG`: Program/tool information
  - `ID`: Unique name, `PN`: Program name; `CL`: Command line

Sequence Data: SAM Format

https://samtools.github.io/hts-specs/SAMv1.pdf

B. Alignment information

```
samtools view BRCA_IDC_cfDNA.bam 17:37844393

41976164        83      17      37845717        60      39M     =       37845620
-136    AATACTGTTTTTTTTTCTTTTTTCATTTCATTTTGTCCT
FFFFFFFF7FFFFFFFFFFFFAFFFFFFFFFAFFAAAAA RG:Z:P12.17.7_Breast    NH:i:1  NM:i:0

41976166        99      17      37845724        29      33M6S   =       37845842
157     TTTTTTTTTTATTTTTACTTTTCATTTTGTCCTTAATGTG AAAAAFFF..FFFFF)F)FFAA)FFFF.
7F)FF.).)F. RG:Z:P12.17.7_Breast    NH:i:1  NM:i:3
```

Show description
One field per slide

Sequence Data: SAM Format

https://samtools.github.io/hts-specs/SAMv1.pdf

B. Alignment Format

1. QNAME: query (read) template name
2. FLAG: bitwise value describing the alignment
   - e.g. 0x4 (=4) - read is unmapped; 0x2 (=2) - proper pair; 0x400 (=1024) - PCR duplicate
3. RNAME: reference sequence name (i.e. chr1 or 1)
4. POS: position of aligned read (leftmost; 1-based)
5. MAPQ: Mapping quality
6. CIGAR: Code string to describe read alignment sequence match to reference
7. RNEXT: reference sequence name of mate read
8. PNEXT: position of mate read
9. TLEN: template (read) length; 0 if mates on different chromosomes
10. SEQ: sequence of mapped reads on forward genomic strand
11. QUAL: base qualities (Phred-scale)

Sequence Data: SAM Format

https://samtools.github.io/hts-specs/SAMv1.pdf

B. Alignment Format: CIGAR string (common operators)

M	alignment match (sequence match or mismatch)
I	insertion relative to reference
D	deletion relative to reference
S	soft clipping (mismatch bases included in SEQ)
H	hard clipping (mismatch bases excluded in SEQ)
N	skipped sequence from reference
=	sequence match
X	sequence mismatch

```
Reference: GACCTTACTTCATCTTGTG--CTTACTATCAAGTGATTA
     Read:     TTACTT----TTCTGAACTTACTGCTCCTA     What is the CIGAR?
```

Tools for Sequencing Data: Overview

1. Inspecting and Reading SAM/BAM files

- SAMtools

2. Interactive Visualization

- Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv)
- BioViz (https://bioviz.org/)
- Table (https://ics.hutton.ac.uk/tablet/)

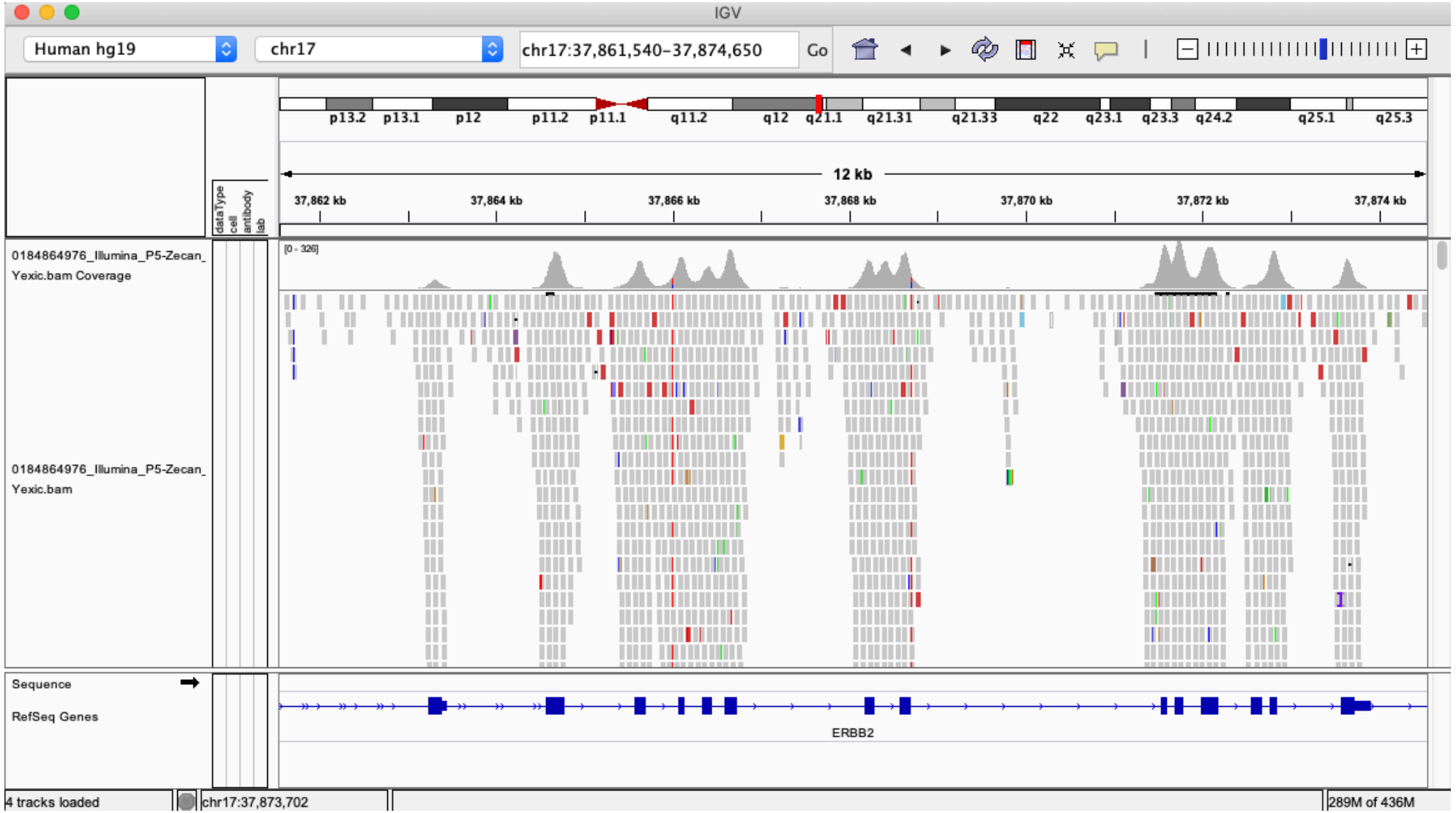
3. Sequencing metrics and Processing

- SAMtools
- Picard Tools
- Genomic Analysis Toolkit (GATK)

4. Genome Variation Analysis

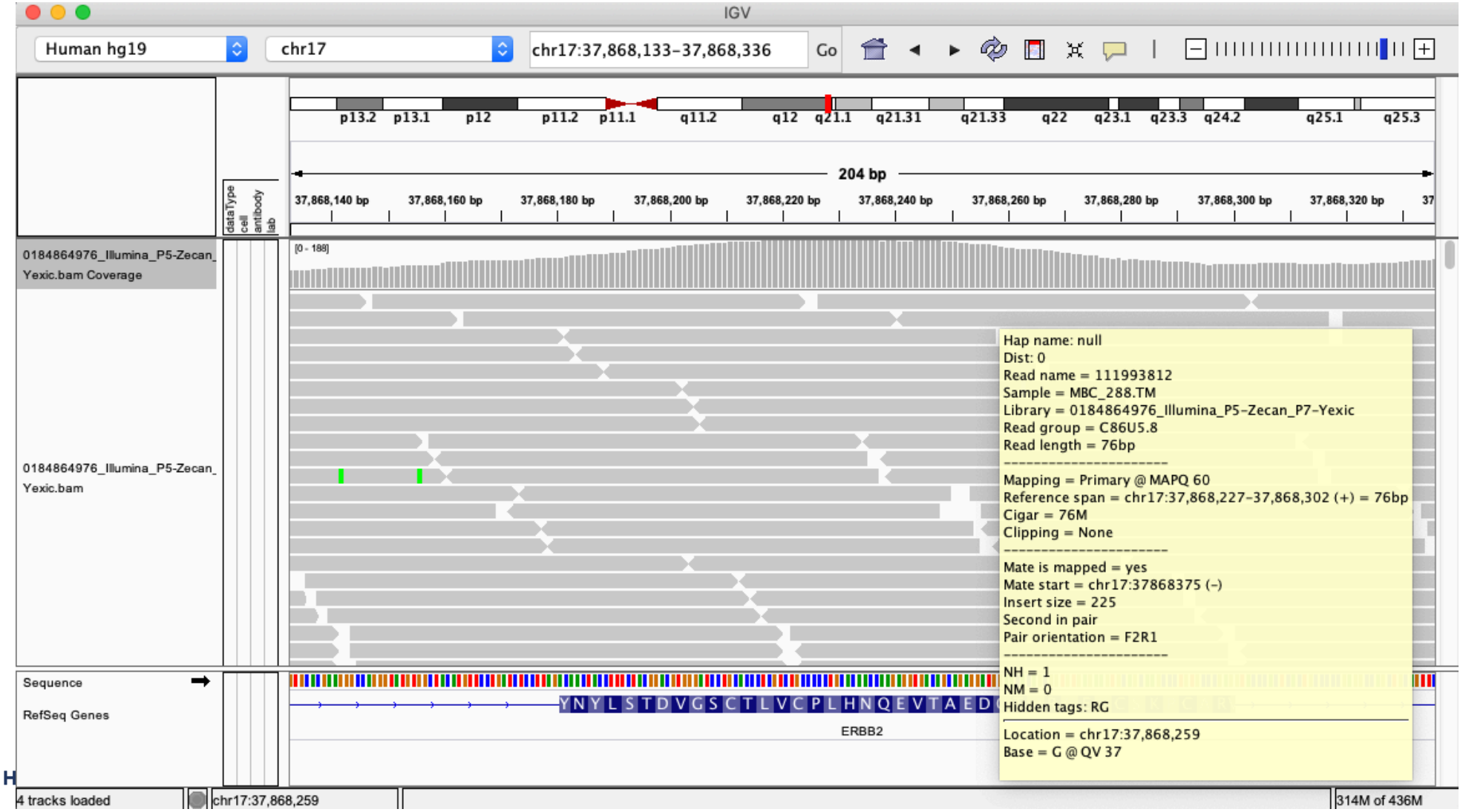
Tools for Sequencing Data: Interactive Visualization

Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv)



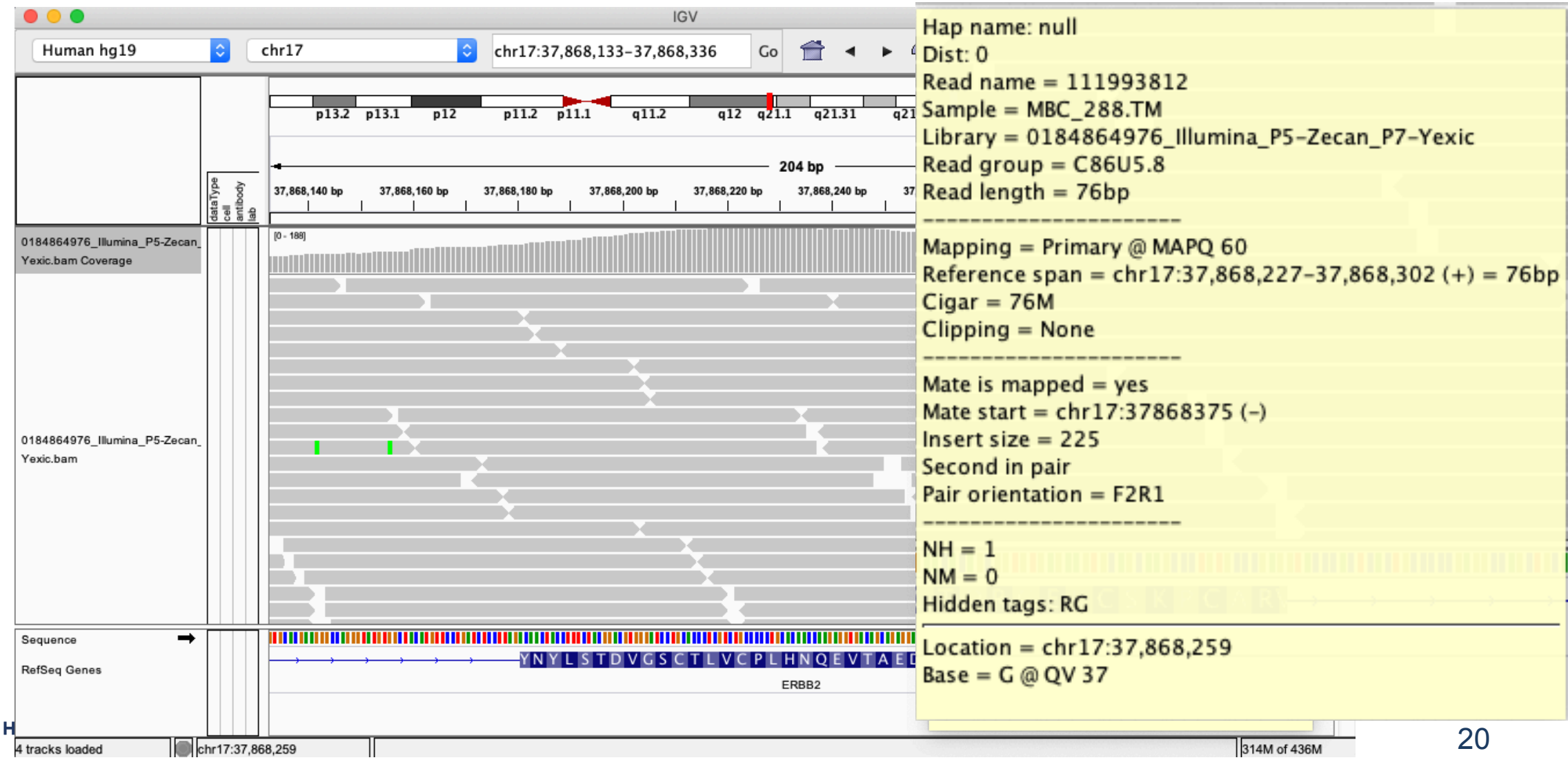
Tools for Sequencing Data: Interactive Visualization

Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv)



Tools for Sequencing Data: Interactive Visualization

Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv)

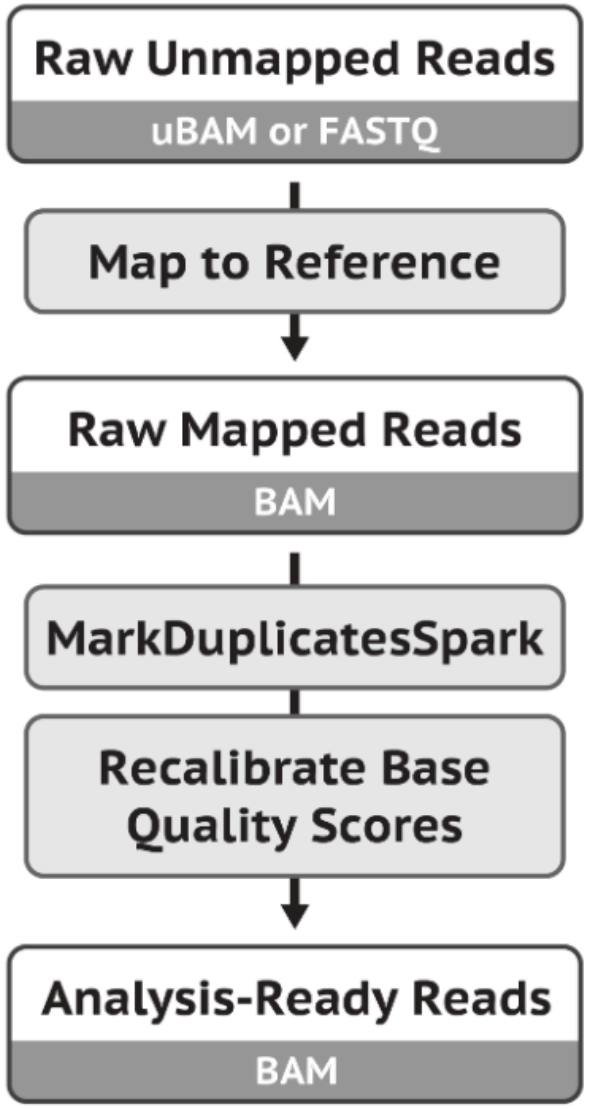


Tools for Sequencing Data: Processing

Picard Tools & GATK4: Best practices

1. Mark Duplicates
    1. MarkDuplicates + SortSam (Picard)
2. Base Quality Score Recalibration (BQSR)
    1. BaseRecalibrator (GATK4)
    2. ApplyBQSR (GATK4)

```
java -jar picard.jar \
MarkDuplicates \
INPUT=BRCA_IDC_cfDNA.bam \
REMOVE_DUPLICATES=false \
OUTPUT=BRCA_IDC_cfDNA.marked_duplicates.bam \
METRIC_FILE=BRCA_IDC_cfDNA.markDupMetrics.txt
```



https://software.broadinstitute.org/gatk/best-practices/

Tools for Sequencing Data: Sequencing Metrics

Picard Tools & GATK4: Best practices

3. Generate alignment metrics
    a. `CollectMultipleMetrics`
        - `CollectAlignmentSummaryMetrics`
        - `CollectInsertSizeMetrics`
    b. Collect assay-specific metrics
        - `CollectWgsMetrics` - Whole genome sequencing
        - `CollectHsMetrics` - Hybrid Selection (i.e. whole exome)
        - `CollectRnaSeqMetrics` - RNA-seq
        - `CollectTargetedPcrMetrics` - Targeted PCR amplicon sequencing
    c. `EstimateLibraryComplexity`
        a. Estimates the number of unique molecules in the library

https://broadinstitute.github.io/picard/command-line-overview.html

http://broadinstitute.github.io/picard/picard-metric-definitions.html

Tools for Sequencing Data: Sequencing Metrics

Picard Tools & GATK4: Best practices

3. Generate alignment metrics: (a) `CollectAlignmentSummaryMetrics`

```
java -jar picard.jar CollectAlignmentSummaryMetrics \
INPUT=BRCA_IDC_cfDNA.bam \
OUTPUT=BRCA_IDC_cfDNA.alignMetrics.txt \
REFERENCE_SEQUENCE=hs37d5.fa \
```

CATEGORY	TOTAL_READS	PF_READS	PCT_PF_READS	PF_READS_ALIGNED	PCT_PF_READS_ALIGNED	PF_ALIGNED_BASES	PF_HQ_ALIGNED_READS	PF_HQ_ALIGNED_BASES	MEAN_READ_LENGTH	STRAND_BALANCE	PCT_CHIMERAS
FIRST_OF_PAIR	49333221	49333218	1	49333218	1	1920603633	42832799	1667902478	39	0.50011	0.019043
SECOND_OF_PAIR	49333221	49333218	1	49333218	1	1918882942	42822426	1665977301	39	0.500715	0.019904
PAIR	98666442	98666436	1	98666436	1	3839486575	85655225	3333879779	39	0.500412	0.019474
UNPAIRED	3371706	3349869	0.993523	3349869	1	106596546	2050309	69312874	31.872292	0.501895	0

http://broadinstitute.github.io/picard/picard-metric-definitions.html#AlignmentSummaryMetrics

Tools for Sequencing Data: Sequencing Metrics

Picard Tools & GATK4: Best practices

3. Generate alignment metrics: (a) `CollectWgsMetrics`

```
java -jar picard.jar CollectWgsMetrics \
INPUT=BRCA_IDC_cfDNA.bam \
OUTPUT=BRCA_IDC_cfDNA.alignMetrics.txt \
REFERENCE_SEQUENCE=hs37d5.fa \
```

GENOME_TERRITORY	MEAN_COVERAGE	SD_COVERAGE	MEDIAN_COVERAGE	PCT_EXC_MAPQ	PCT_EXC_DUPE	PCT_1X	PCT_5X
2900340137	1.053882	1.383867	1	0.137741	0	0.578236	0.015963

```
coverage        high_quality_coverage_count
0       1223257622
1       854276028
2       475072046
3       215728575
4       85708030
5       30916117
6       10376403
7       3318514
8       1041100
9       329830
10      111513
```

https://broadinstitute.github.io/picard/picard-metric-definitions.html#CollectWgsMetrics.WgsMetrics

Tools for Sequencing Data: Accessing BAM files in R & Python

Python

- PySam

  https://pysam.readthedocs.io/en/latest/api.html

R and Bioconductor

- **Rsamtools**
  - Import BAM files into R
  - View the header information
  - Accessing read sequences, aligned positions, CIGAR, read names, etc
  - Large BAM files can be read in chunks to optimize memory
  - Create new BAM files using “Views” of a subset of reads

https://bioconductor.org/packages/release/bioc/vignettes/Rsamtools/inst/doc/Rsamtools-Overview.pdf

Genome Variant Analysis: Overview

1. Types of genomic variation

2. Visualization using IGV

3. Tools for Predicting Genome Variation

4. File Formats for Variation Data

5. Variant Annotation Tools

6. Variant databases

Genome Variant Analysis: Types of Genomic Variation

Variant or Mutation or Alteration or Polymorphism)

- Changes in the genome sequence of a sample compared to a reference sequence
- Chromosomes: 22 autosomal pairs + 1 sex pair
  - Each set inherited from maternal and paternal germline cells

Germline Variant

- Variant inherited from one or both parental chromosomes
- Source of genetic differences between ancestral populations and individuals
- Polymorphism: >1% frequency in a population

Somatic Variant

- Mutation acquired during individual's lifetime
- Important to identify in sporadic cancers and other non-familial diseases

Genome Variant Analysis: Types of Genomic Variation

a. **Single nucleotide base substitutions**
   - Germline single nucleotide polymorphism (SNP)
   - Somatic single nucleotide variant (SNV)

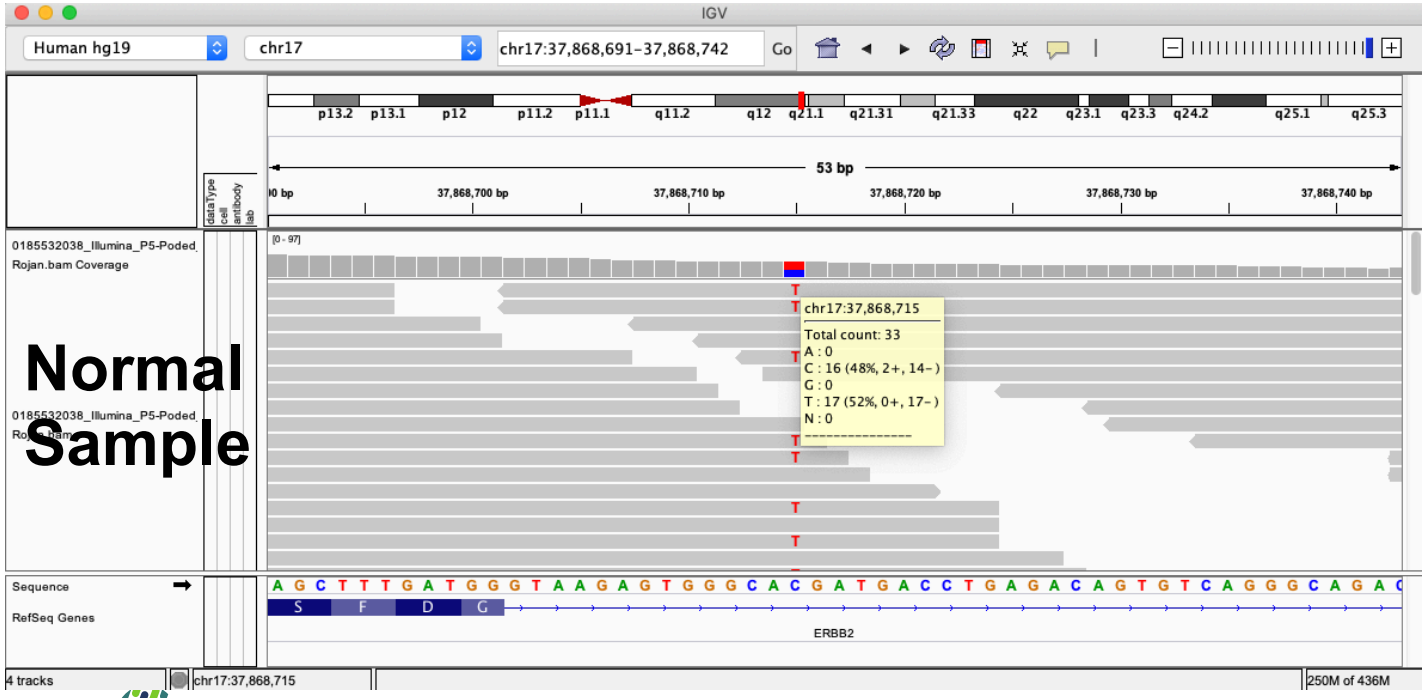
b. **Small insertions or deletions**
   - Germline or somatic insertion or deletion (INDEL)

c. **Copy number changes**
   - Germline copy number variant (CNV) or polymorphism (CNP)
   - Somatic copy number variant (CNV) or alterations (CNA)

d. **Structural rearrangements**
   - Germline or Somatic structural variant (SV)

Genome Variant Analysis: Single Nucleotide Polymorphism

- ~1.5 to 2 million **SNPs** per individual
- Identify SNPs from normal peripheral blood mononuclear cells (PBMC)

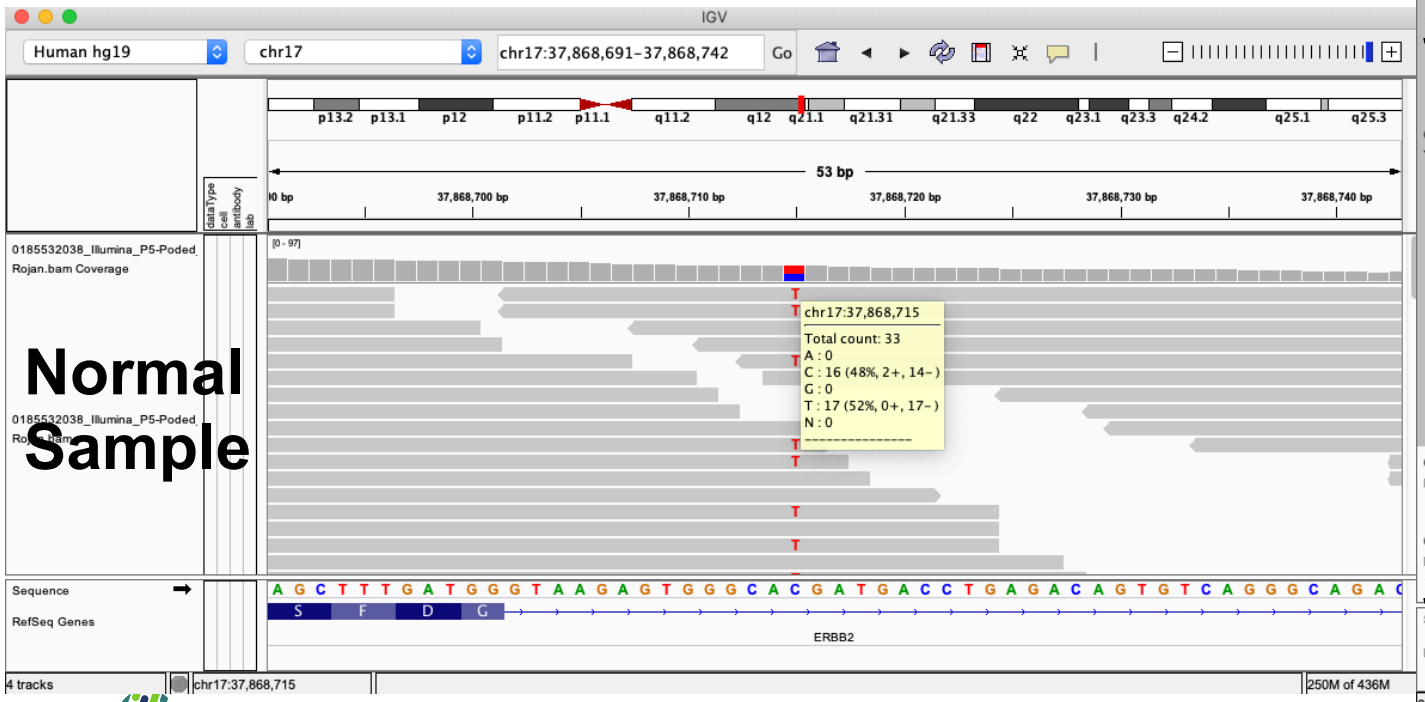


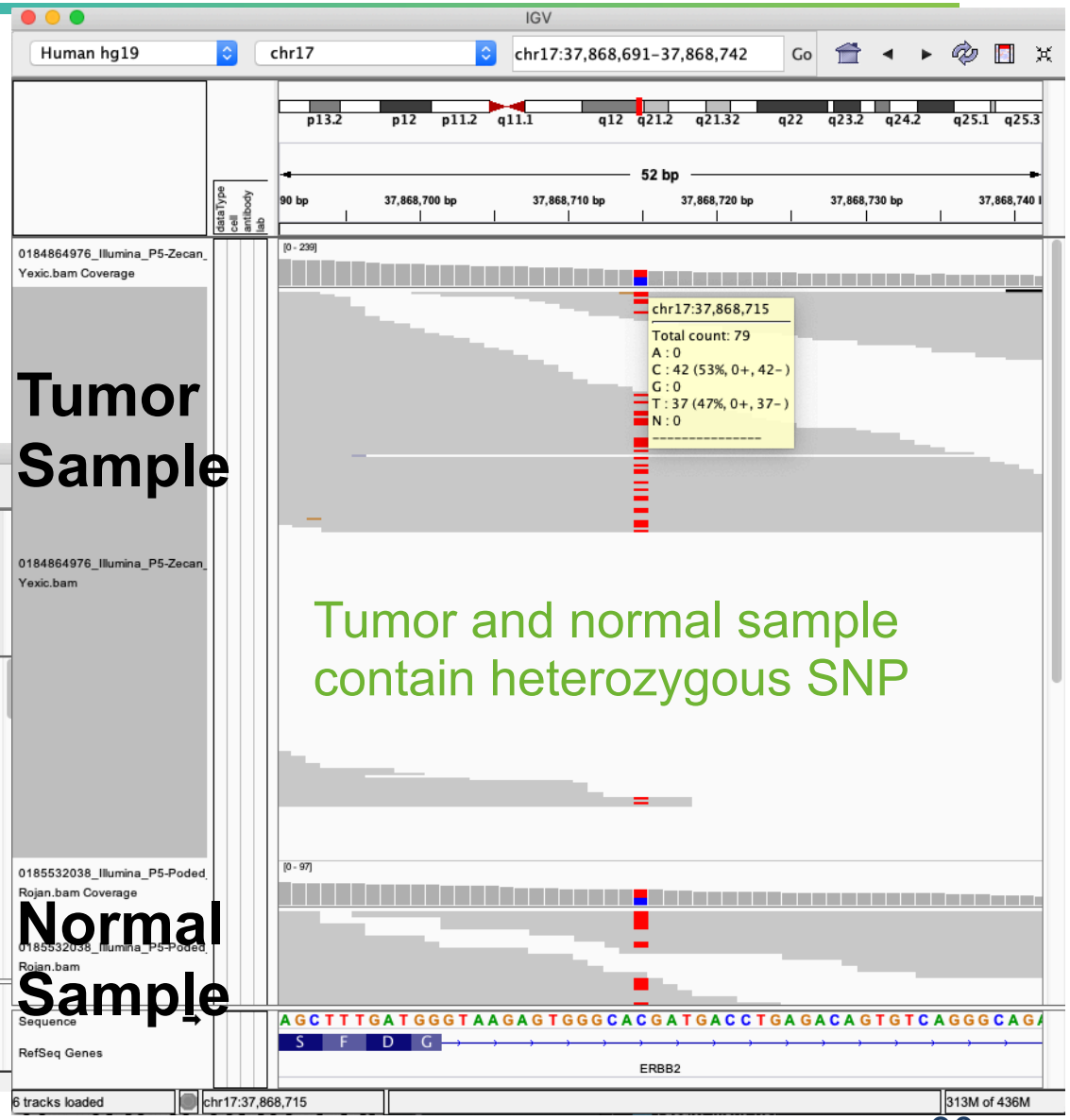
Heterozygous SNP with 37 reads containing the variant and having depth 79 reads

37/79 (47%) variant allele fraction (VAF)

Genome Variant Analysis: Single Nucleotide Polymorphism

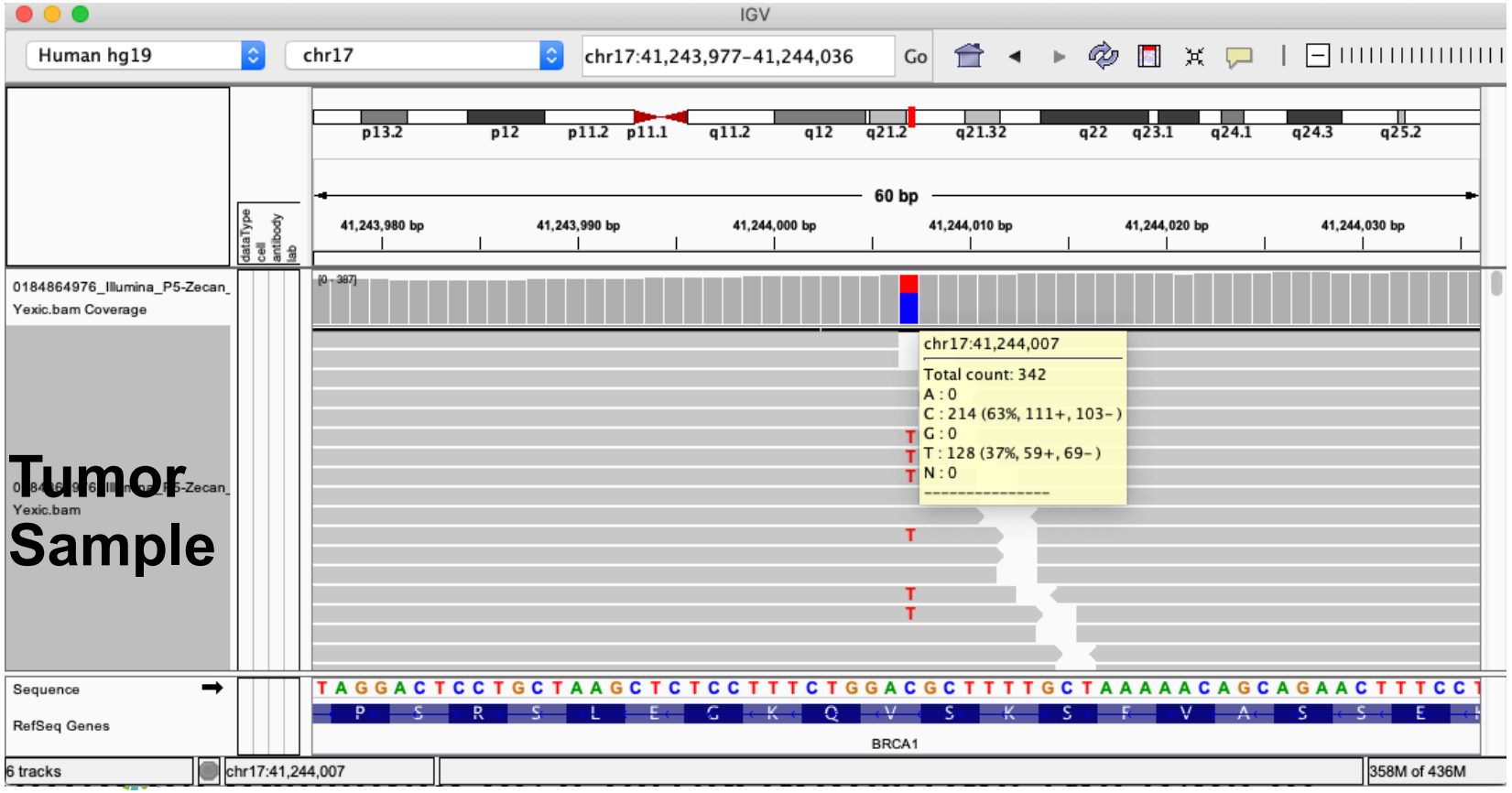
- ~1.5 to 2 million **SNPs** per individual
- Identify SNPs from normal peripheral blood mononuclear cells (PBMC)





Genome Variant Analysis: Single Nucleotide Variant (SNV)

- Somatic **SNV** requires comparing case (tumor) with control (PBMC)

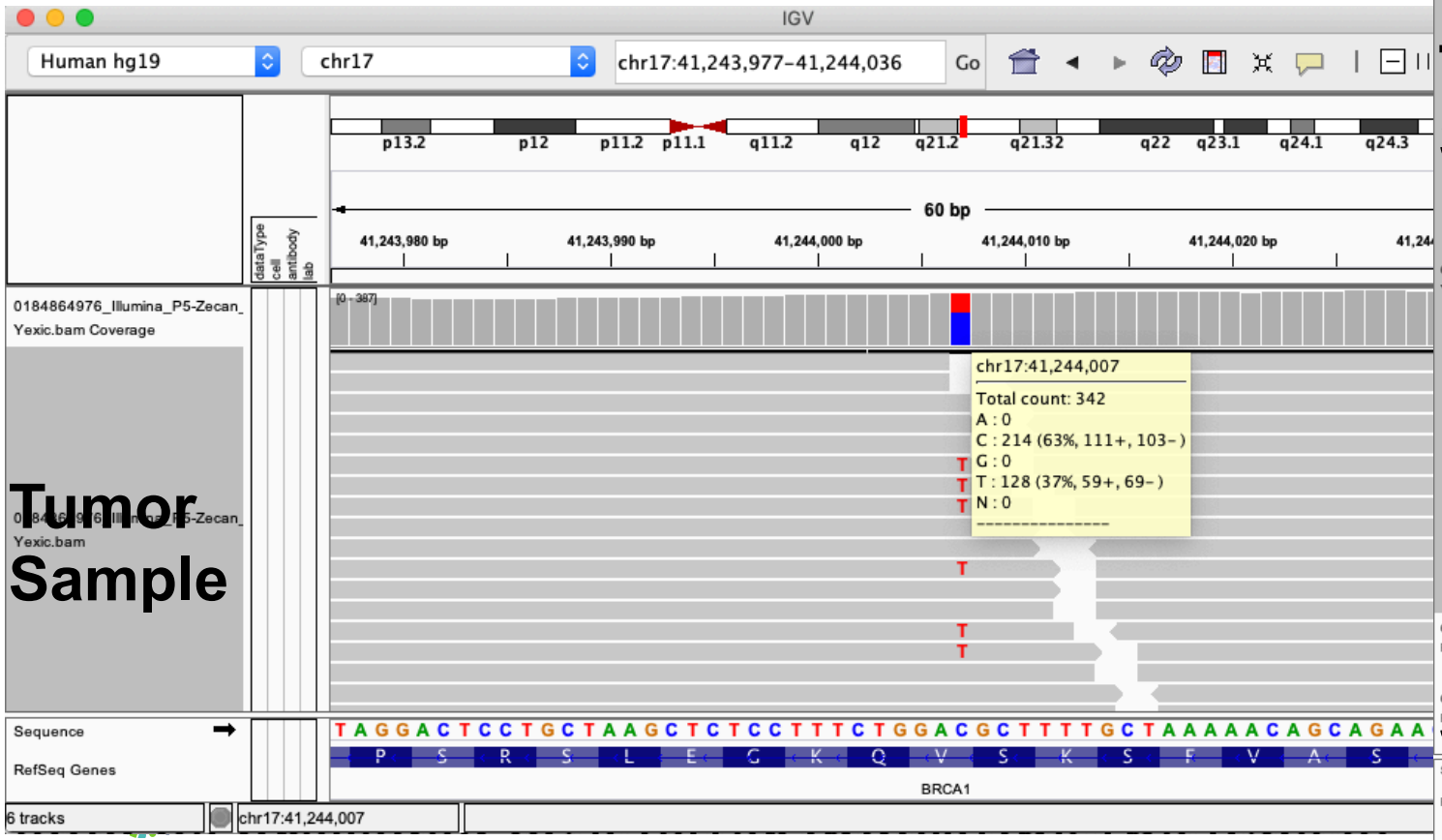


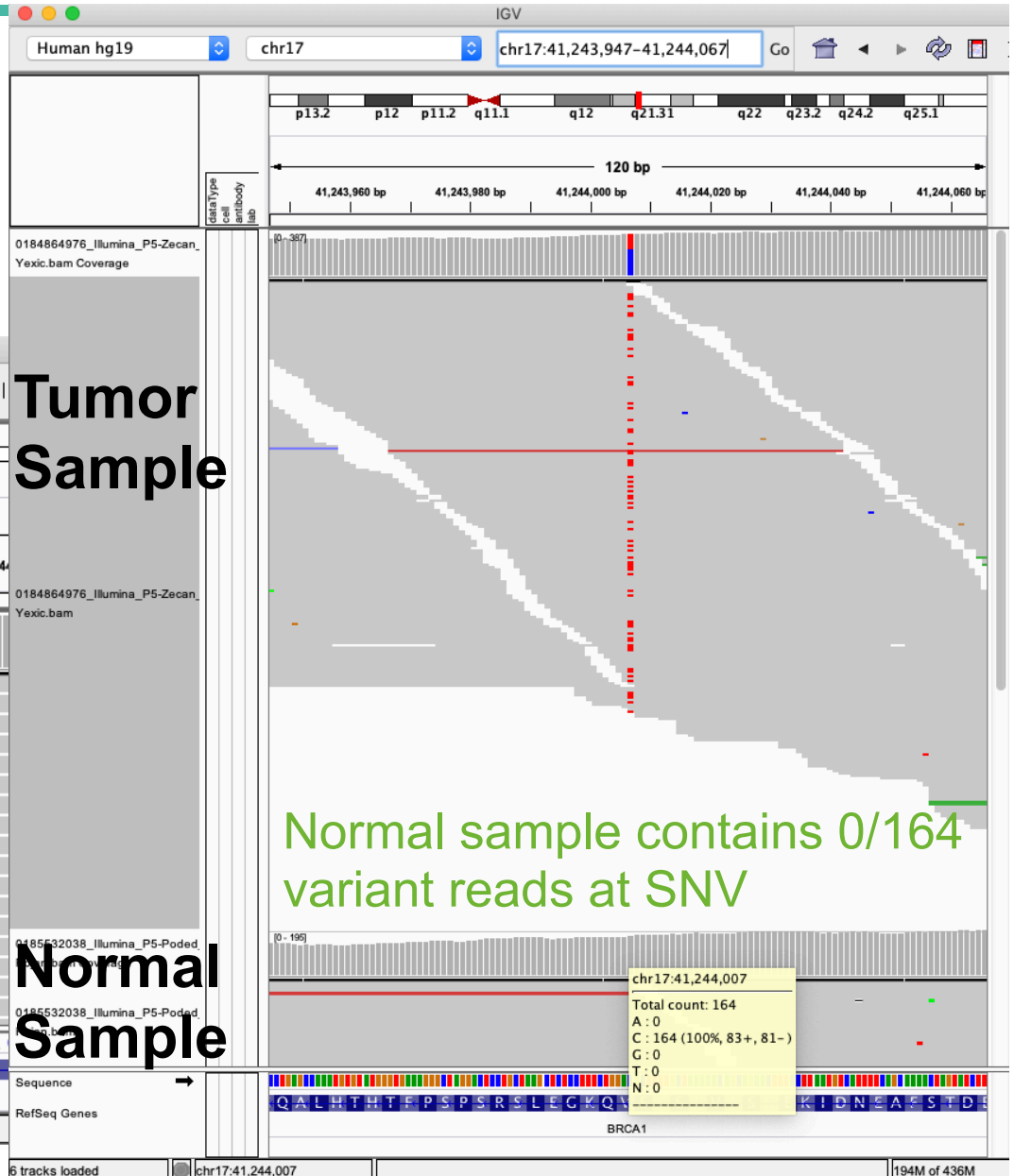
Potential SNV with 128/342 (37%) VAF

p.V1181I

Genome Variant Analysis: Single Nucleotide Variant (SNV)

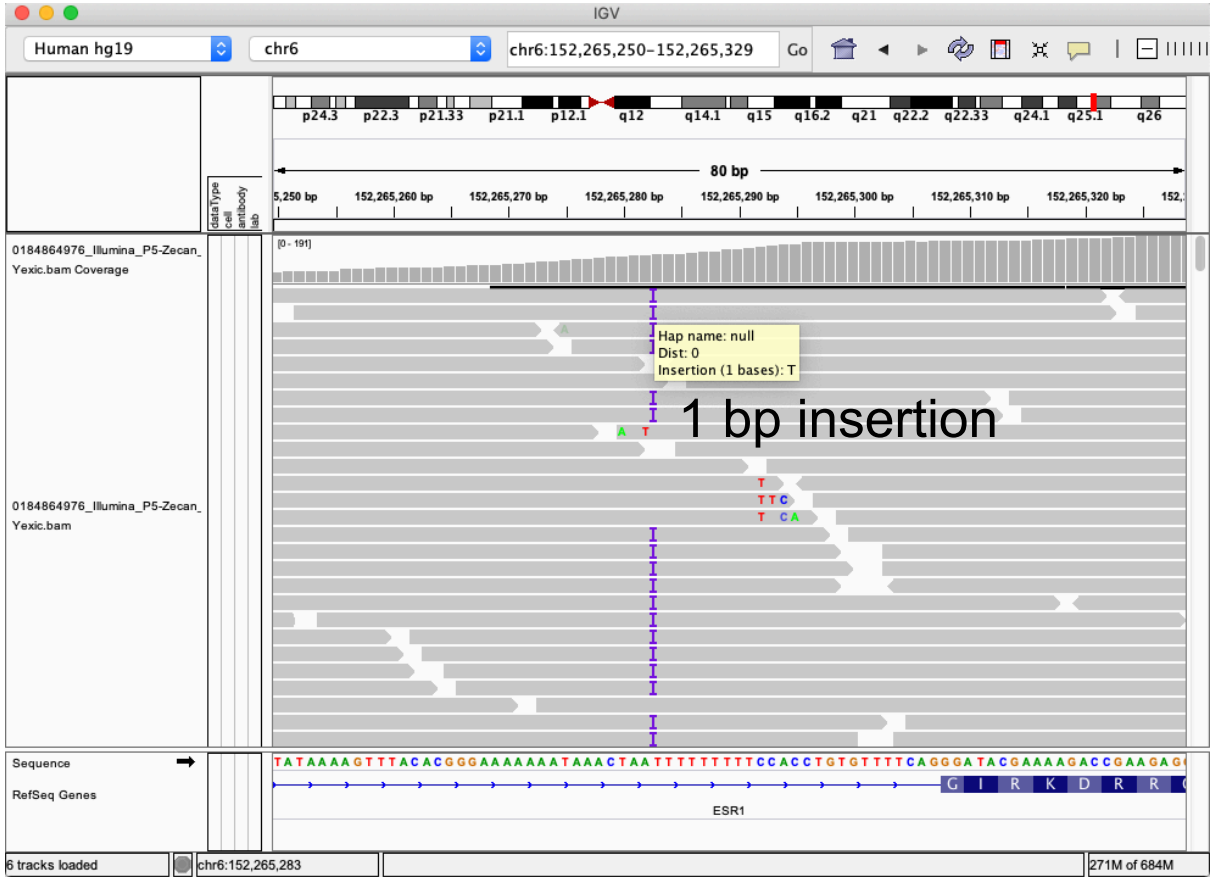
- Somatic **SNV** requires comparing case (tumor) with control (PBMC)

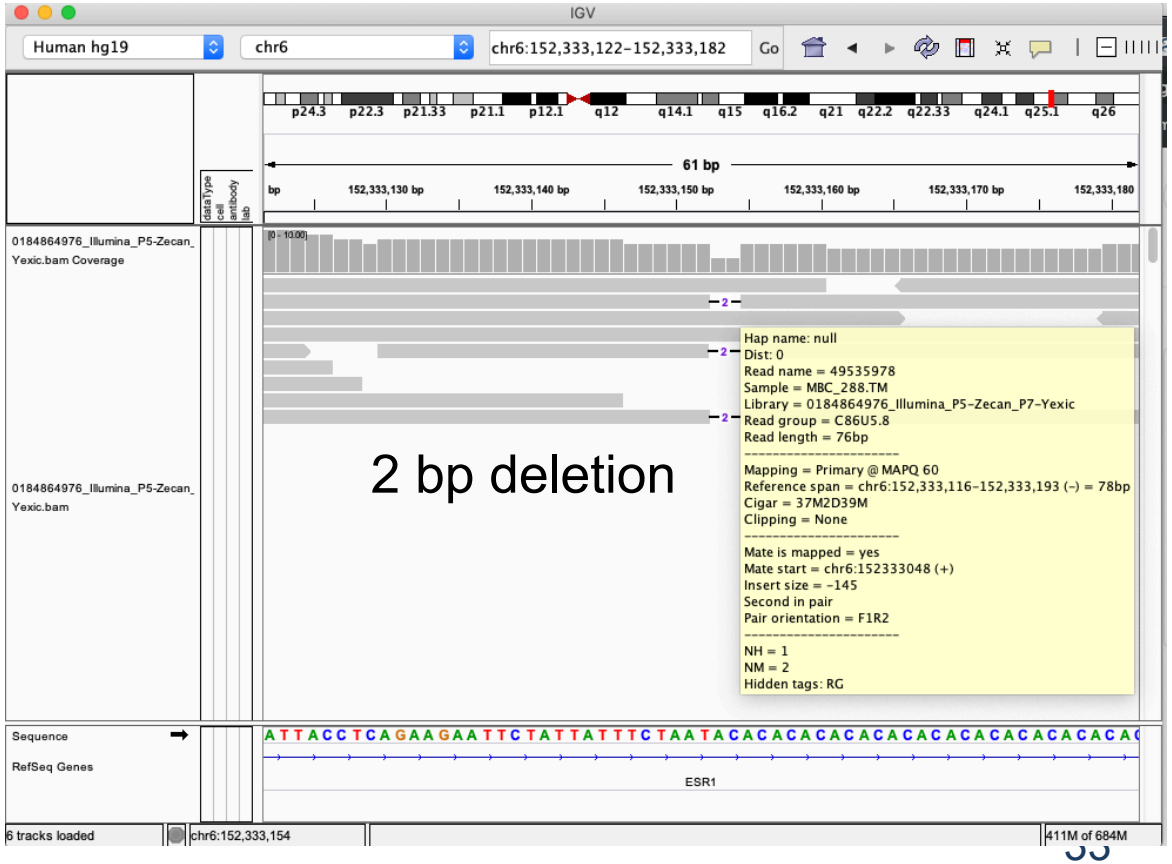




Genome Variant Analysis: Insertion & Deletion (INDEL)

- 1 to 10,000 bps size range
- Can lead to in-frame or frame-shift mutations
- Recall: CIGAR strings





Genome Variant Analysis: Tools to Predict SNP/SNV/INDEL

1. GATK4 (https://software.broadinstitute.org/gatk/)

   a. `HaplotypeCaller`

   - Call germline SNPs and INDELs using local reassembly of haplotypes
   - Variant Quality Score Recalibration (VQSR)
     - VariantRecalibrator + ApplyVQSR

   b. `Mutect2`

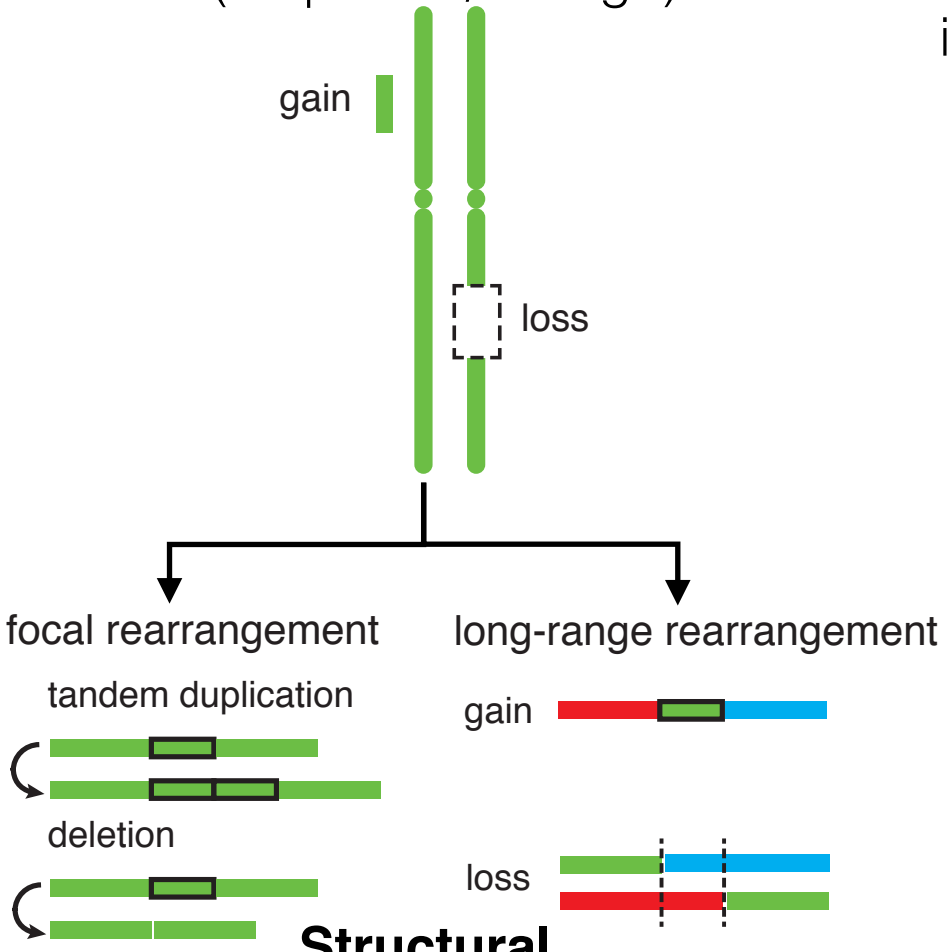
   - Call somatic SNVs using with tumor and normal pairing
   - https://software.broadinstitute.org/gatk/documentation/tooldocs/4.beta.5/org_broadinstitute_hellbender_tools_walkers_mutect_Mutect2.php

2. Strelka (https://github.com/Illumina/strelka, Kim et al. Nature Methods, 2018)

3. Others: VarScan2, SomaticSniper, MuSE, LoLoPicker, deepSNV, FreeBayes, Platypus, CaVEMan, DeepVariant, JointSNVMix2, ShearWater,

Genome Variant Analysis: Copy Number and Structural Variation

Copy number alterations
(amplitude/dosage)



Structural rearrangements
(location/configuration)

"discordant read pair"
read pairs with aberrant inferred fragment length

"copy number change"
abrupt change in read coverage

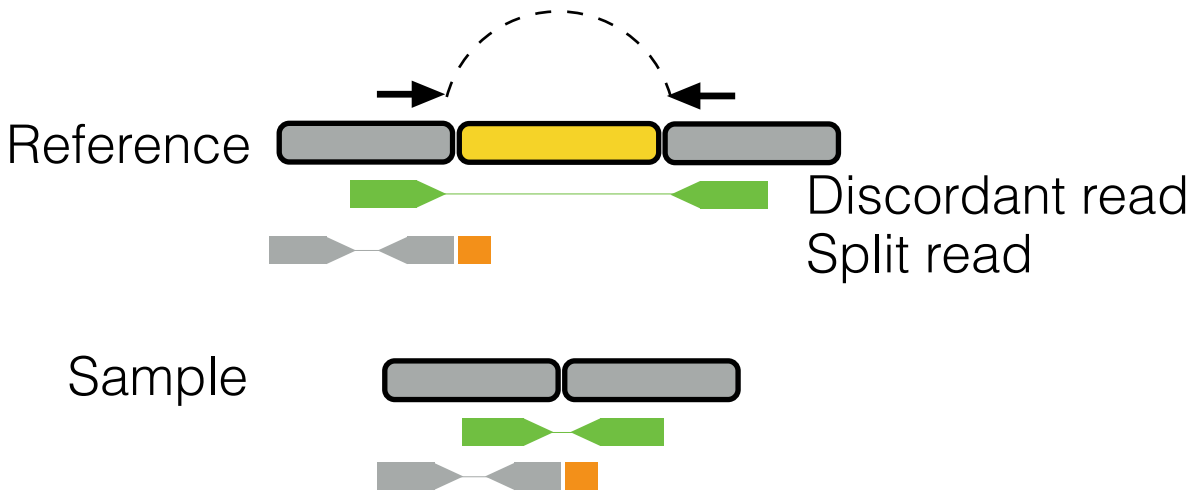
pair-mates unmapped

"split read"
split alignments

Zhang and Pellman. *CSH Symp Quant Biol.* **80**:117-37 (2016)

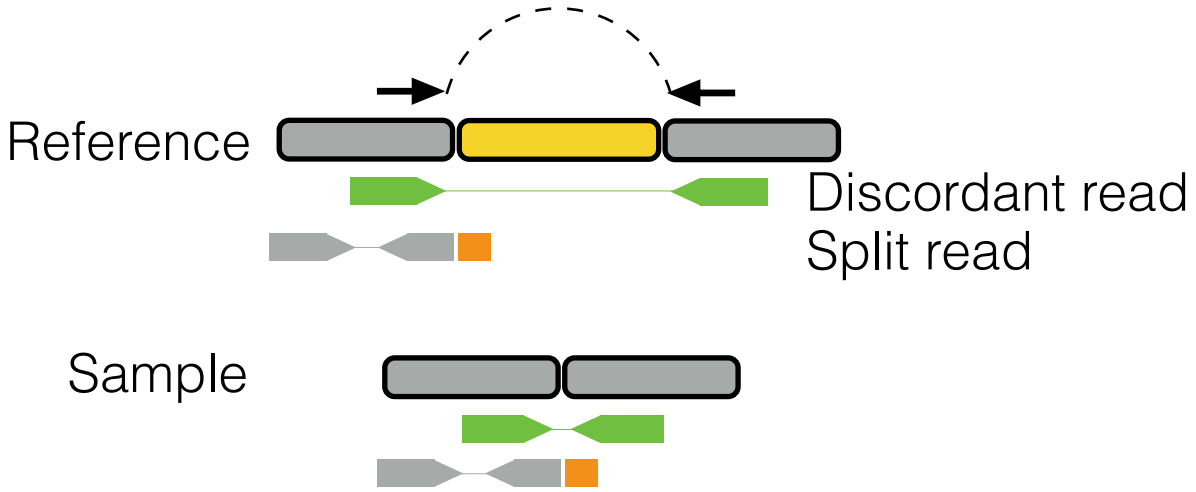
Genome Variant Analysis: Structural Variation

Deletion

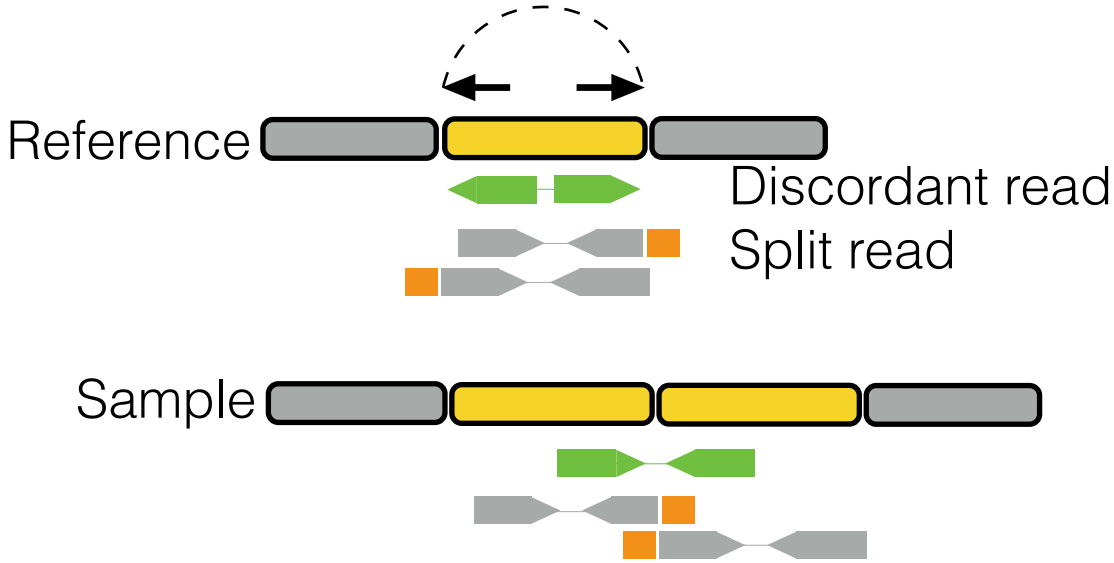


Genome Variant Analysis: Structural Variation

Deletion

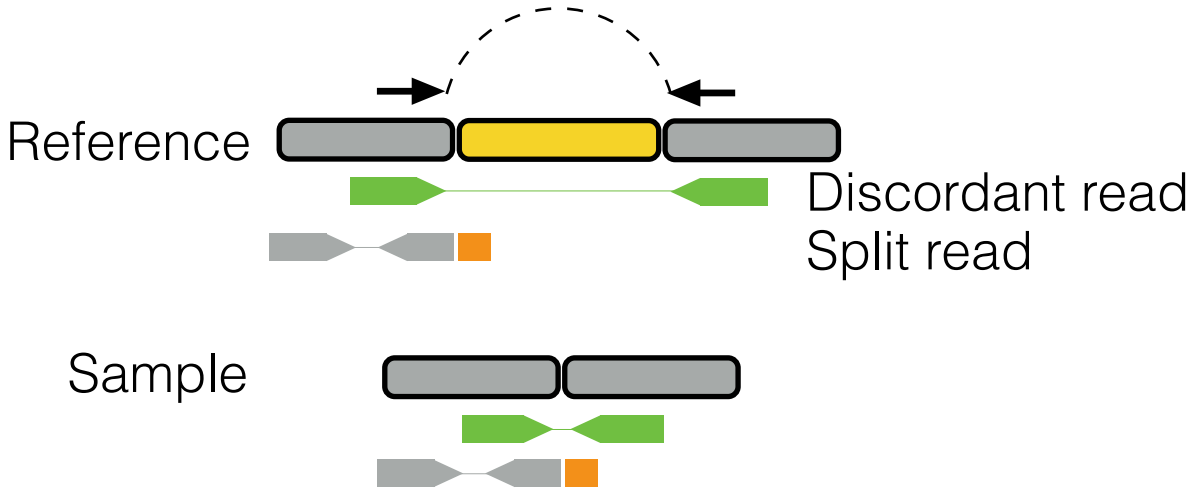


Tandem Duplication

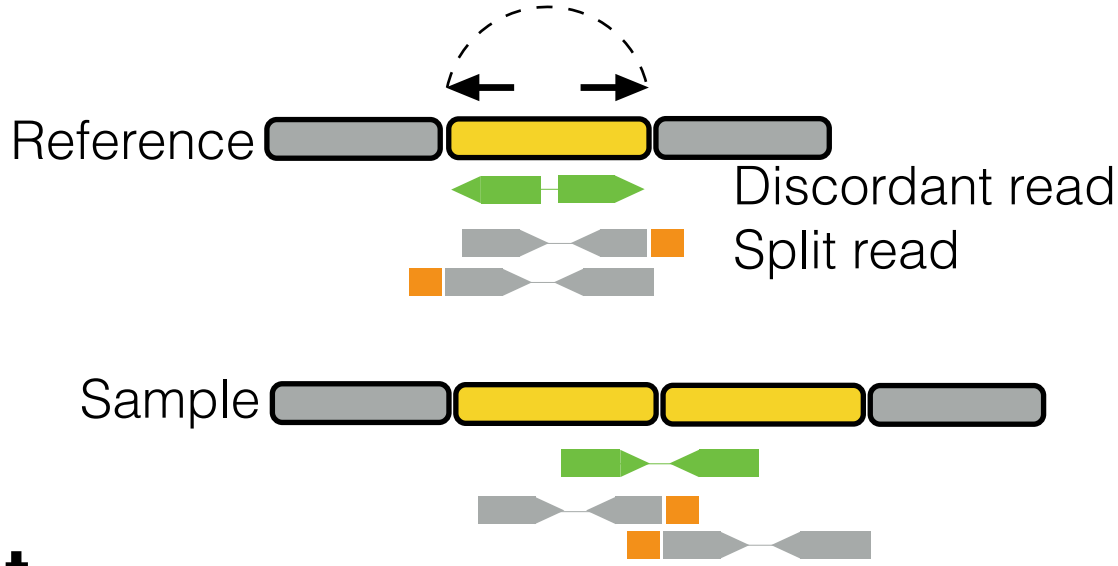


Genome Variant Analysis: Structural Variation

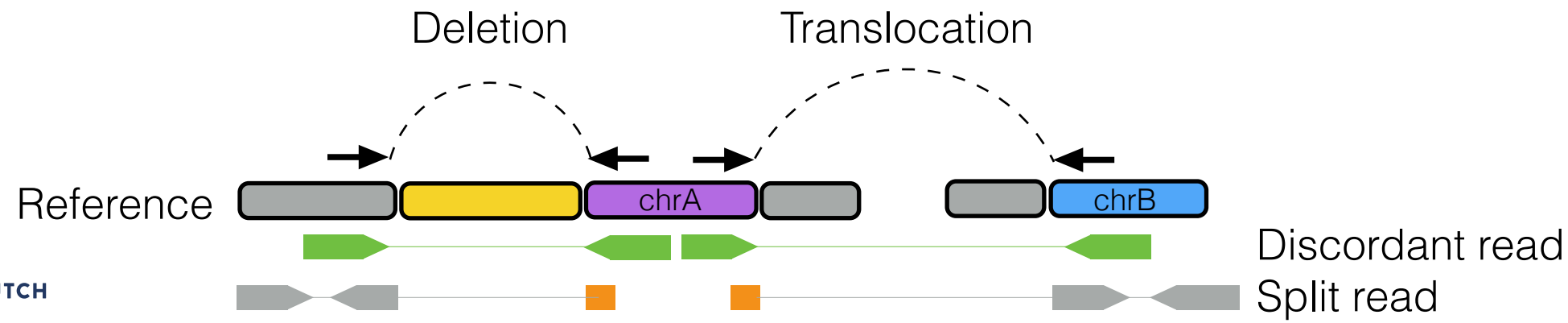
Deletion



Tandem Duplication



Complex Event



Genome Variant Analysis: Tools to Predict SVs

1. Germline SV
    - GATK4
    - LUMPY (https://github.com/arq5x/lumpy-sv)
    - DELLY (https://github.com/dellytools/delly)
    - Manta (https://github.com/Illumina/manta)
2. Somatic SV
    - BreakDancer (https://github.com/genome/breakdancer)
    - SvABA (https://github.com/walaj/svaba)
3. Others: Comparison of 69 SV tools (Kosugi et al. *Genome Biol*, 2019)

Genome Variant Analysis: Copy Number Variation

1098 Samples

TCGA BRCA

http://firebrowse.org/?cohort=BRCA
https://portal.gdc.cancer.gov/projects/TCGA-BRCA

Genome Variant Analysis: Tools to Predict CNVs

1. Germline CNV
    - GATK4
    - DNAcopy (https://github.com/veseshan/DNAcopy)
    - Others: cn.MOPS, VarScan2
2. Somatic CNV for Cancer
    - ASCAT (https://github.com/Crick-CancerGenomics/ascat)
    - ABSOLUTE (https://software.broadinstitute.org/cancer/cga/absolute)
    - TITAN (https://github.com/gavinha/TitanCNA)
    - Battenberg (https://github.com/cancerit/cgpBattenberg)
    - Others: CNVkit, Sequenza, ichorCNA, HMMcopy

Genome Variant Analysis: Common Variant File Formats

a. Variant Call Format (VCF)
   - http://samtools.github.io/hts-specs/VCFv4.2.pdf
   - Used mostly for SNV/SNP, INDEL, and SV

b. Mutation Annotation Format (MAF)
   - https://docs.gdc.cancer.gov/Data/File_Formats/MAF_Format/
   - http://software.broadinstitute.org/software/igv/MutationData
   - Tab-delimited format containing columns for mutation information and annotations
   - Used primarily for SNV/SNP and INDEL data

c. Browser Embedded Data (BED)
   a. https://bedtools.readthedocs.io/
   b. Used for any genomic features/region and annotations, including CNV and SV (BEDPE)

d. Others
   a. http://genome.ucsc.edu/FAQ/FAQformat
   b. GFF, WIG/bigWIG, etc.

Genome Variant Analysis: Variant Call Format (VCF)

http://samtools.github.io/hts-specs/VCFv4.2.pdf

a. Header information

```
##fileformat=VCFv4.2
##GATKCommandLine=<ID=HaplotypeCaller,CommandLine="HaplotypeCaller">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the same order as listed">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for genotypes as defined in
the VCF specification">
##FORMAT=<ID=PS,Number=1,Type=Integer,Description="ID of Phase Set for Variant">
##FILTER=<ID=PASS,Description="All filters passed">
##FILTER=<ID=LowQual,Description="Low quality">
```

b. Variant record

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Sample_1
chr1	11542	.	A	T	49.77	PASS	AC=1;AF=0.5;AN=2;DP=4	GT:AD:DP:GQ:PL:PS	0|1:2,2:4:78:78,0,78

Genome Variant Analysis: Variant Call Format (VCF)

http://samtools.github.io/hts-specs/VCFv4.2.pdf

c. Genotype Field (GT)
    a. 0=Reference allele, 1=Alternate allele
    b. 0/1=heterozygous, 0/0 or 1/1=homozygous
    c. 0|1 or 1|0 = heterozygous (phased)

SNP	S1	S2	S3
Reference	A	T	G
Haplotype 1	A	C	G
Haplotype 2	C	T	A
GT (unphased)	0/1	0/1	0/1
GT (phased)	0|1	1|0	??

Haplotype 1 | Haplotype 2

A(0)|C(1)

C(1)|T(0)

?? | ??

Genome Variant Analysis: Variant Annotation Tools

ANNOVAR (http://annovar.openbioinformatics.org)

SnpEff (http://snpeff.sourceforge.net)

SIFT (https://sift.bii.a-star.edu.sg/) - predict amino acid substitution effects on protein function

GATK VariantAnnotator

VariantAnnotation R Package (https://bioconductor.org/packages/release/bioc/html/VariantAnnotation.html)

Variant Annotation Integrator (USCS, https://genome.ucsc.edu/cgi-bin/hgVai)

Genome Variant Analysis: Variant Databases

1000 Genomes Project (https://www.internationalgenome.org/)

dbSNP (https://www.ncbi.nlm.nih.gov/snp/)

dbVar (https://www.ncbi.nlm.nih.gov/dbvar/)

ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)

Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/)

- Lek et al. Nature, 536, 285-91 (2016)

Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/)

- Karczewski et al. bioRxiv (2019)

Genome Data Commons (https://portal.gdc.cancer.gov/)