Fred Hutch
Cancer Center

Genomic Data Analysis using R

Lecture 15

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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 (Background; this Lecture)
   - 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

Genome Variant Analysis Background: Overview

1. Types of genomic variation

2. Visualization using IGV

3. File Formats for Variation Data

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

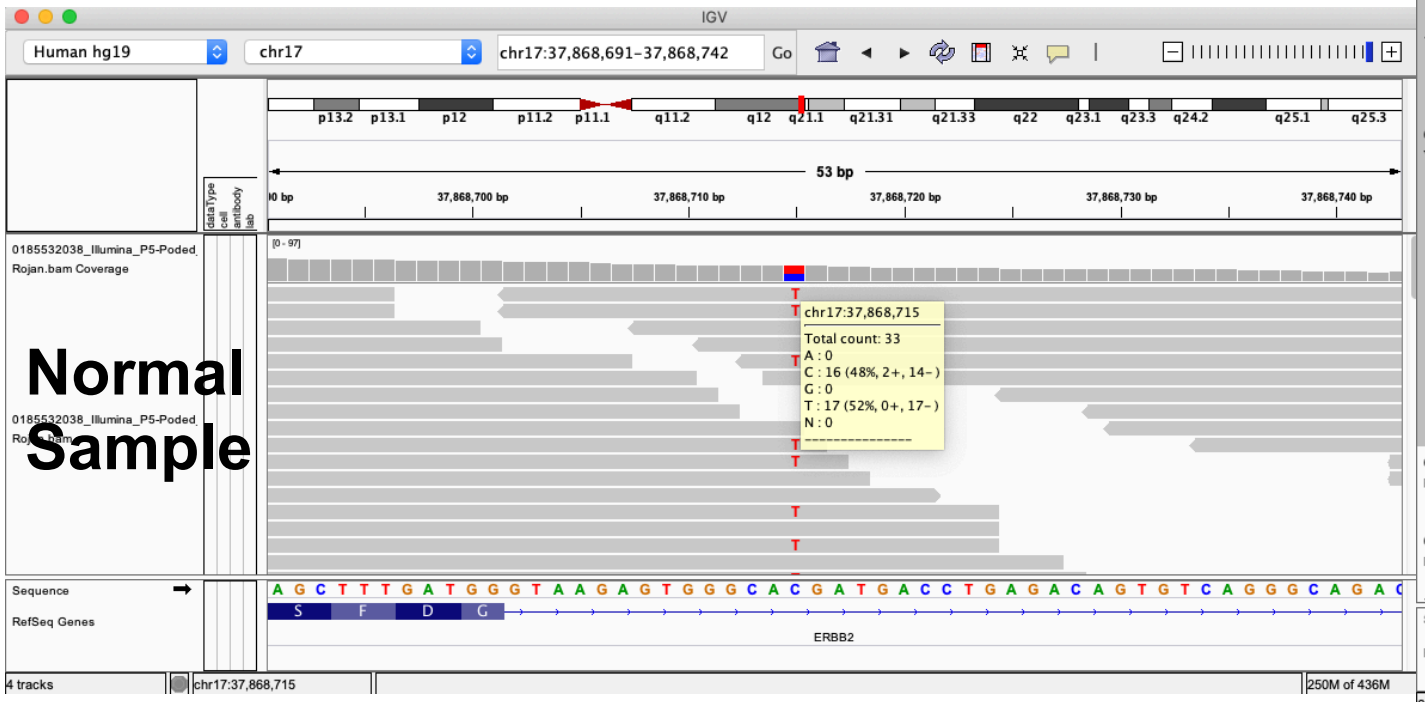
- ~1.5 to 2 million **SNPs** per individual
- Identify SNPs from normal peripheral blood mononuclear cells (PBMC)

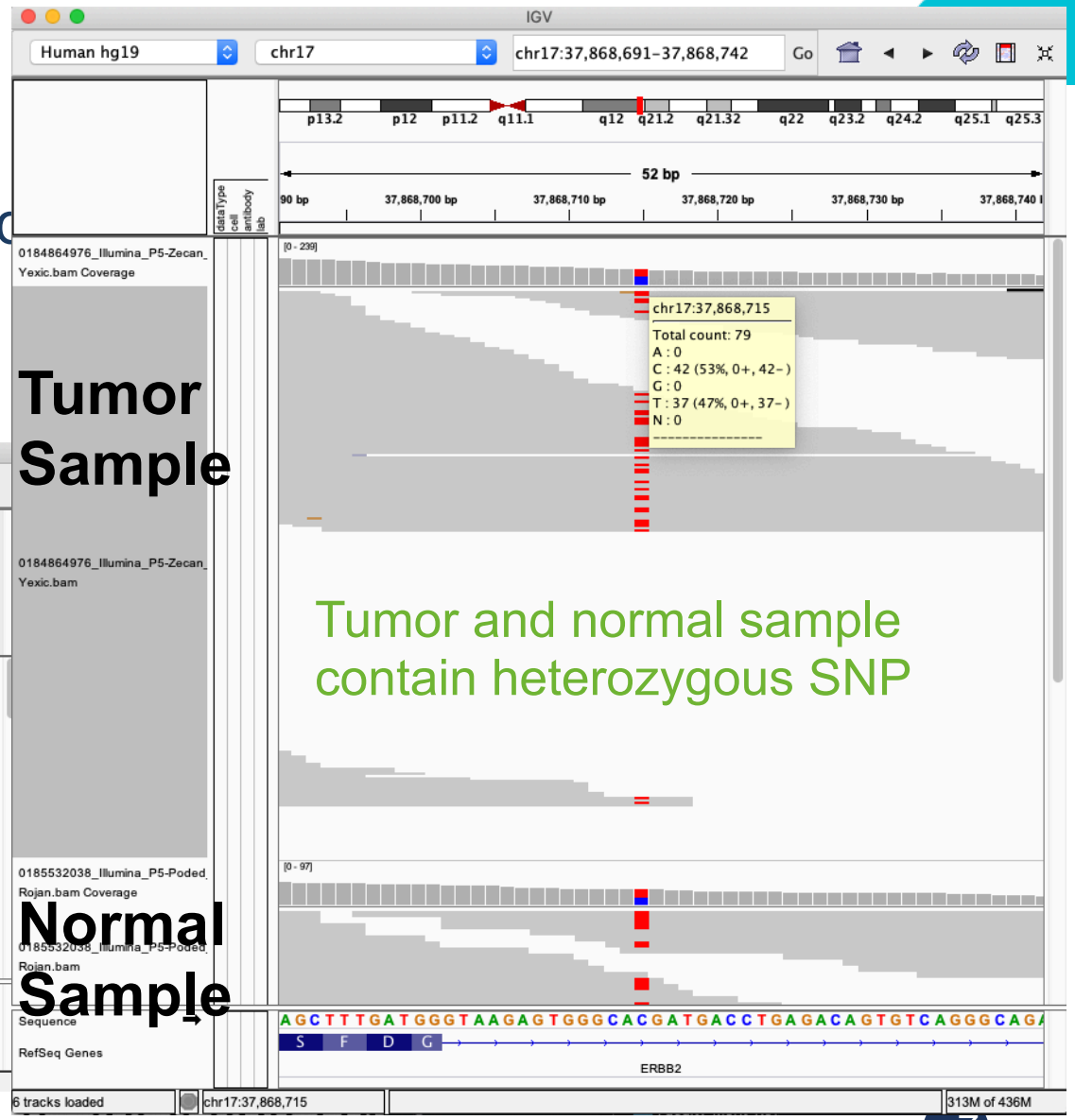
Heterozygous SNP with 37 reads containing the variant and having depth 79 reads

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

Genome Variant Analysis: Single Nucleotide Polymorphism

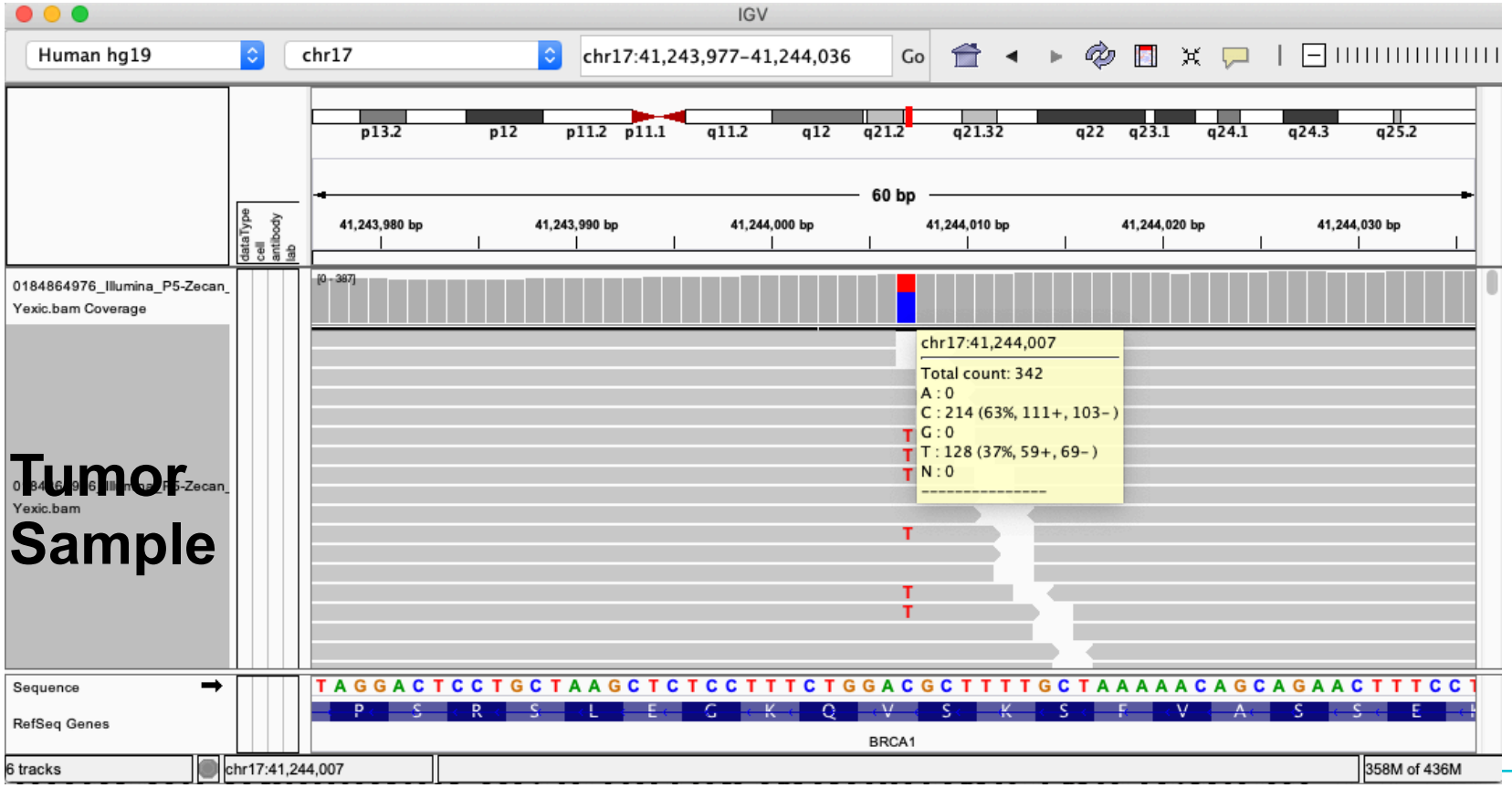
- ~1.5 to 2 million **SNPs** per individual
- Identify SNPs from normal peripheral bloo





Genome Variant Analysis: Single Nucleotide Variant (SNV)

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

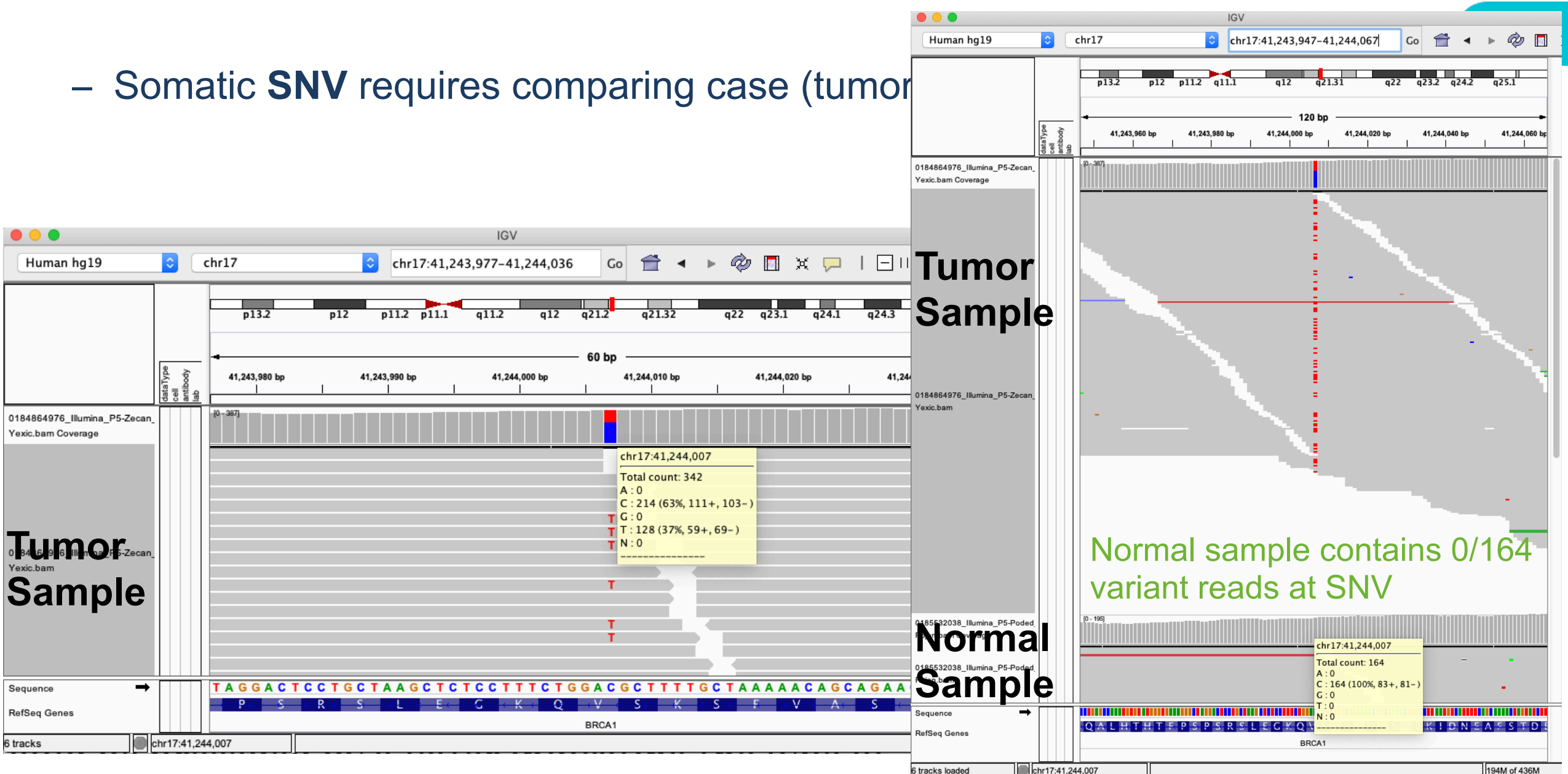


Potential SNV with 128/342 (37%) VAF

p.V1181I

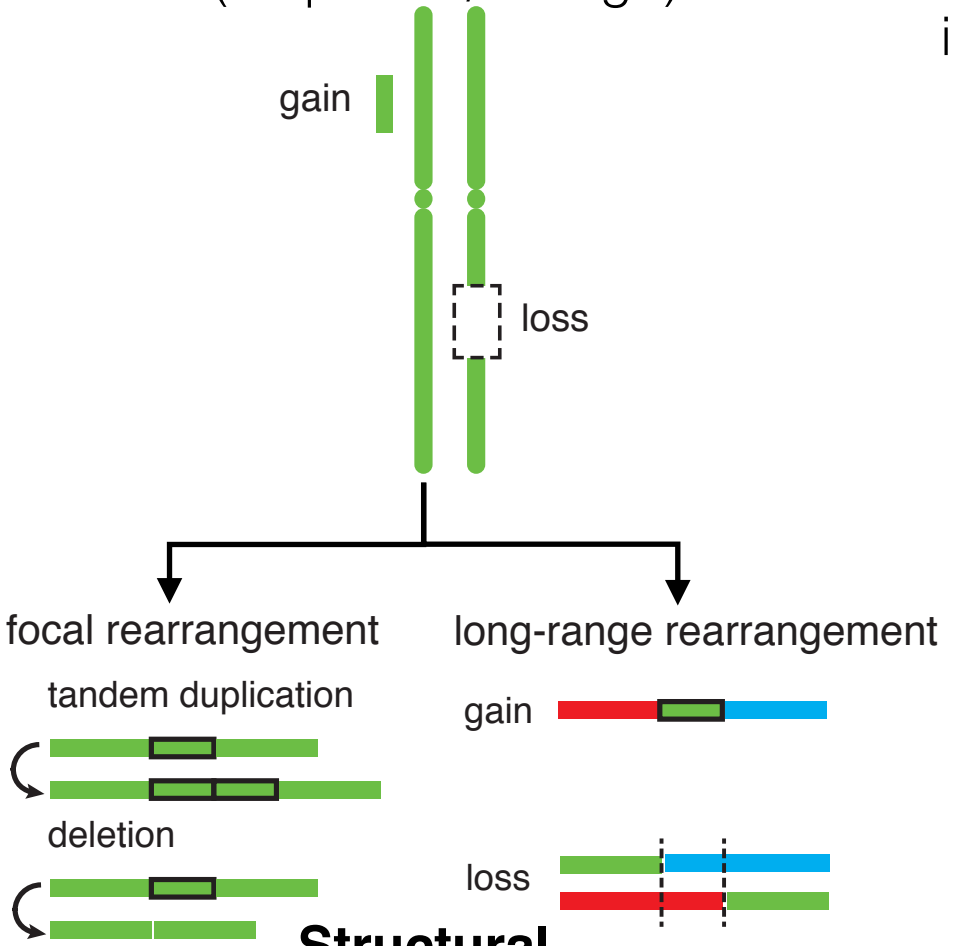
Genome Variant Analysis: Single Nucleotide Variant (SNV)

– Somatic **SNV** requires comparing case (tumor



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: Copy Number Variation

1098 Samples

TCGA BRCA

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

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 (UCSC, https://genome.ucsc.edu/cgi-bin/hgVai)

BioMart (http://www.biomart.org/)

For your reference.

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/)

For your reference.

R/Bioconductor Packages for Genomic Data

Tutorials

1. Analyzing Genomic Data
2. Analyzing and Annotating Variants

Overview: Learning Objectives

R Bioconductor Packages for Genomic Data

- `GenomicRanges, plyranges, VariantAnnotation`

Tutorials

1. Genomic Data Analysis (`GenomicRanges, plyranges`)
    i. Load, inspect, query a BED/SEG file
    ii. Genomic regions overlap
2. Genomic Variants and Annotations (`VariantAnnotation`)
    i. Load, inspect, query a VCF file

Tutorial #1: Genomic Data Analysis

1. Loading and querying BED/SEG text files
    a. Use packages `GenomicRanges, plyranges`
2. Download the VCF and SEG files for this tutorial
    - https://www.dropbox.com/sh/zoitjnobgp7l7c2/AABBIpTQcNA4lWYOFnV5dlMKa?dl=0
    - BRCA.genome_wide_snp_6_broad_Level_3_scna.seg
3. R Markdown file for tutorial on GitHub: Lecture16_GenomicData.Rmd

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

Tutorial #2: Variant Call Format (VCF)

1. Loading and querying VCF files in R
    a. Use packages `VariantAnnotation`
    b. Download the VCF files for this tutorial
    - https://www.dropbox.com/sh/zoitjnobgp7l7c2/AABBIpTQcNA4lWYOFnV5dlMKa?dl=0
    - GIAB_highconf_v.3.3.2.vcf.gz
    - GIAB_highconf_v.3.3.2.vcf.gz.tbi
2. R Markdown file for tutorial on GitHub: Lecture16_VariantCalls.Rmd

Homework #7: Genomic Data Analysis in R

Problem Sets in R Markdown or Jupyter Notebook

- Contains 3 Problems with some code to prepare you for the questions.
- Please complete the assignment within the Markdown or Jupyter file
- You will be evaluated on
  i. the results and outputs
  ii. your code and documentation
  iii. Partial points awarded for code with correct logic/function even if the final answer may be incorrect