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)

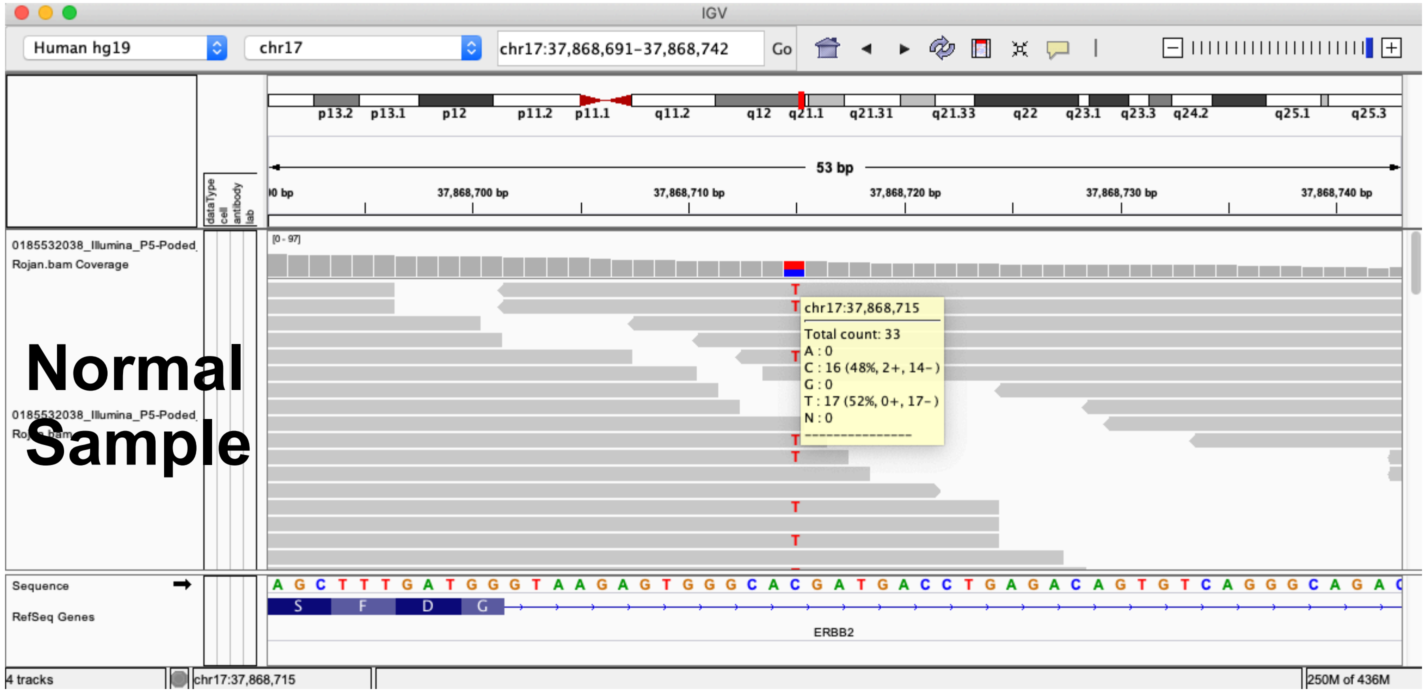
b. **Small insertions or deletions**
   - Germline or somatic insertion or deletion (INDEL)

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)



chr17:37,868,715

Total count: 33
A : 0
C : 16 (48%, 2+, 14-)
G : 0
T : 17 (52%, 0+, 17-)
N : 0

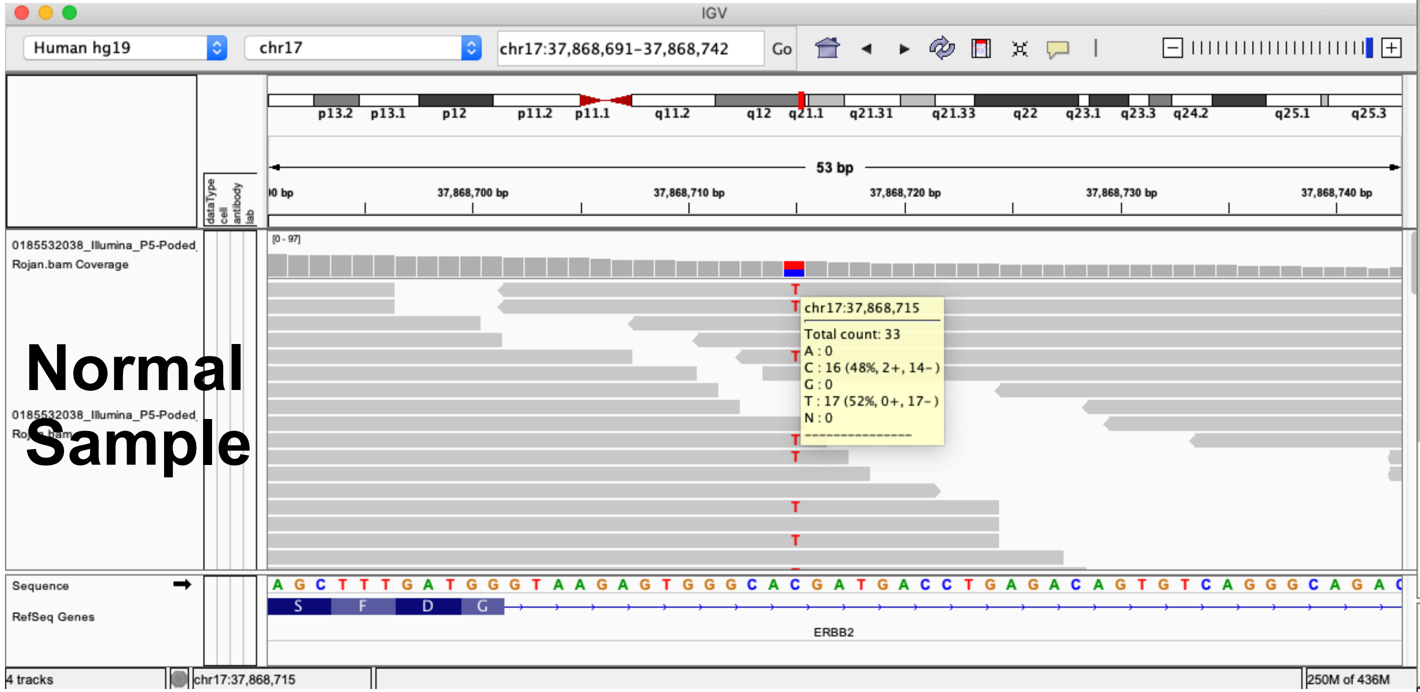
Normal Sample

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

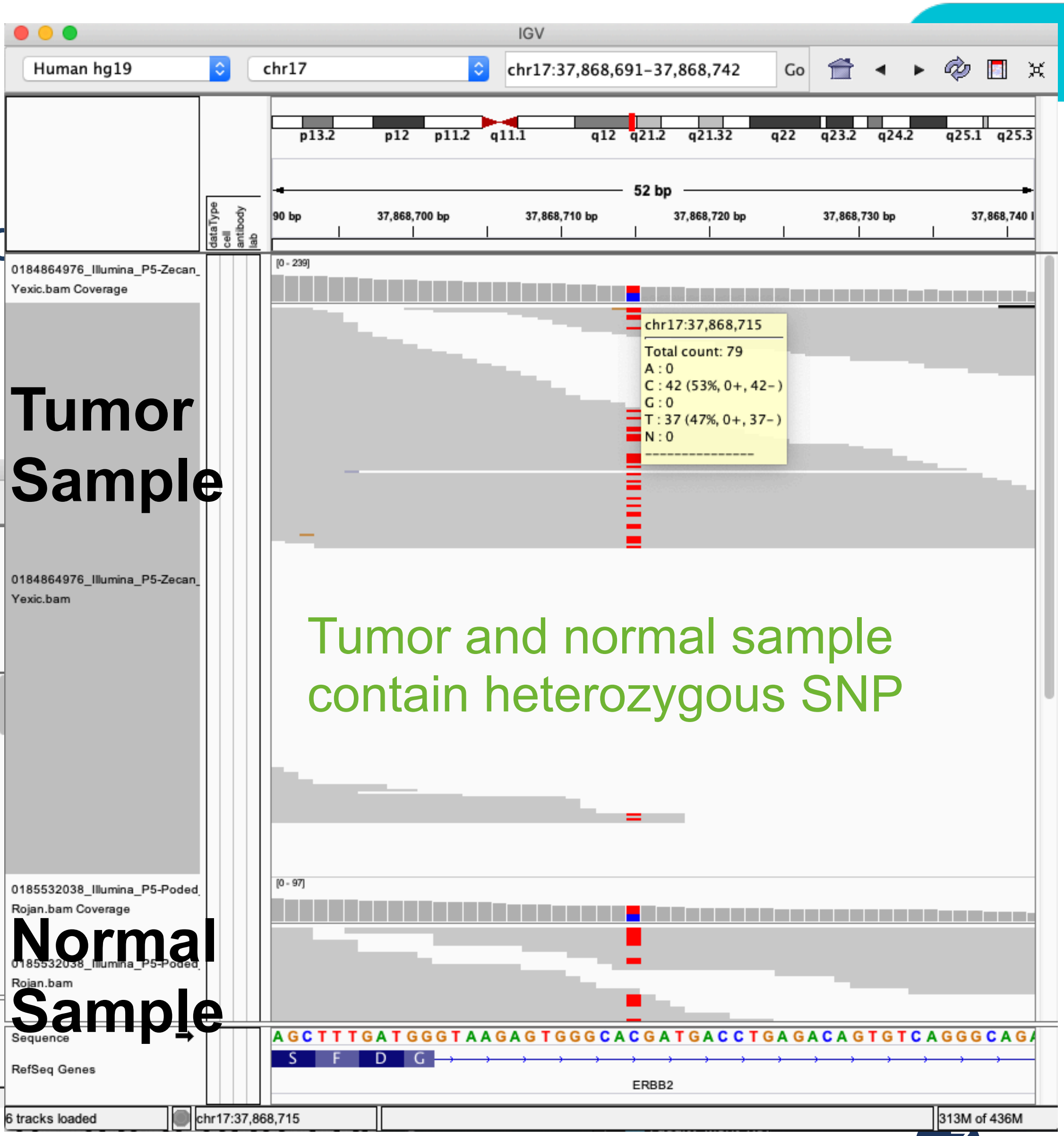


IGV

Human hg19 | chr17 | chr17:37,868,691-37,868,742 | Go

```
chr17:37,868,715
Total count: 33
A : 0
C : 16 (48%, 2+, 14- )
G : 0
T : 17 (52%, 0+, 17- )
N : 0
```

Normal Sample



IGV

Human hg19 | chr17 | chr17:37,868,691-37,868,742 | Go

```
chr17:37,868,715
Total count: 79
A : 0
C : 42 (53%, 0+, 42- )
G : 0
T : 37 (47%, 0+, 37- )
N : 0
```

p13.2
p12
p11.2
q11.1
q12
q21.2
q21.32
q22
q23.2
q24.2
q25.1
q25.3
52 bp
37,868,700 bp
37,868,710 bp
37,868,720 bp
37,868,730 bp
37,868,740 bp
dataType
cell
antibody
lab
0184864976_Illumina_P5-Zecan_Yexic.bam Coverage
[0 - 239]
0184864976_Illumina_P5-Zecan_Yexic.bam
0185532038_Illumina_P5-Poded_Rojan.bam Coverage
[0 - 97]
0185532038_Illumina_P5-Poded_Rojan.bam
Sequence
RefSeq Genes
A G C T T T G A T G G G T A A G A G T G G G C A C G A T G A C C T G A G A C A G T G T C A G G G C A G A
S F D G
ERBB2
6 tracks loaded
chr17:37,868,715
313M of 436M

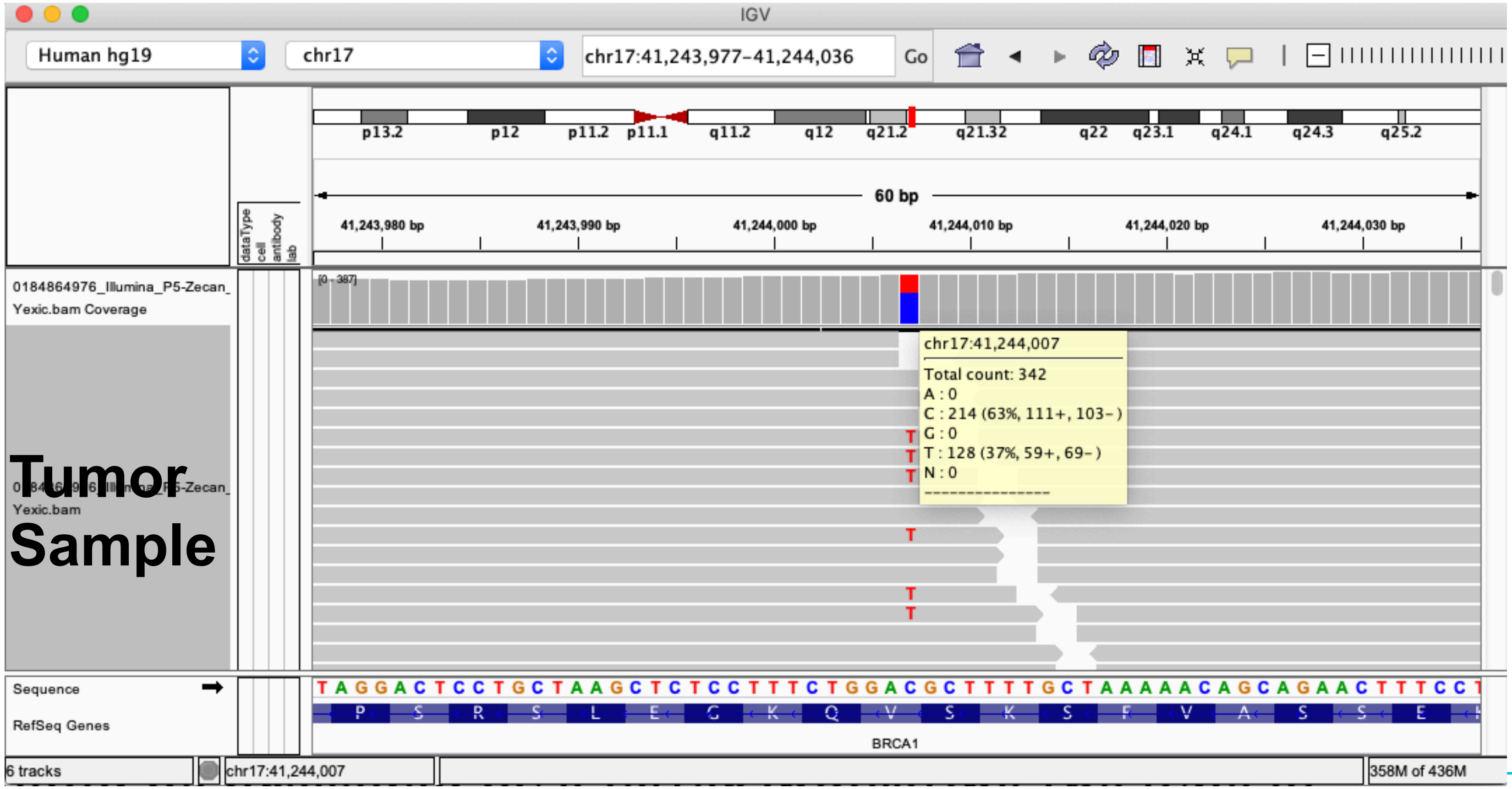
Tumor Sample

Tumor and normal sample contain heterozygous SNP

Normal Sample

Genome Variant Analysis: Single Nucleotide Variant (SNV)

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



IGV
Human hg19
chr17
chr17:41,243,977-41,244,036
Go
p13.2
p12
p11.2
p11.1
q11.2
q12
q21.2
q21.32
q22
q23.1
q24.1
q24.3
q25.2
60 bp
41,243,980 bp
41,243,990 bp
41,244,000 bp
41,244,010 bp
41,244,020 bp
41,244,030 bp
dataType
cell
antibody
lab
0184864976_Illumina_P5-Zecan_Yexic.bam Coverage
[0 - 387]
0184864976_Illumina_P5-Zecan_Yexic.bam
T
T
T
T
T
T
Sequence
RefSeq Genes
T A G G A C T C C T G C T A A G C T C T C C T T T C T G G A C G C T T T T G C T A A A A A C A G C A G A A C T T T C C T
P S R S L E G K Q V S K S F V A S S E
BRCA1
6 tracks
chr17:41,244,007
358M of 436M

```
chr17:41,244,007
Total count: 342
A : 0
C : 214 (63%, 111+, 103- )
G : 0
T : 128 (37%, 59+, 69- )
N : 0
---------------
```

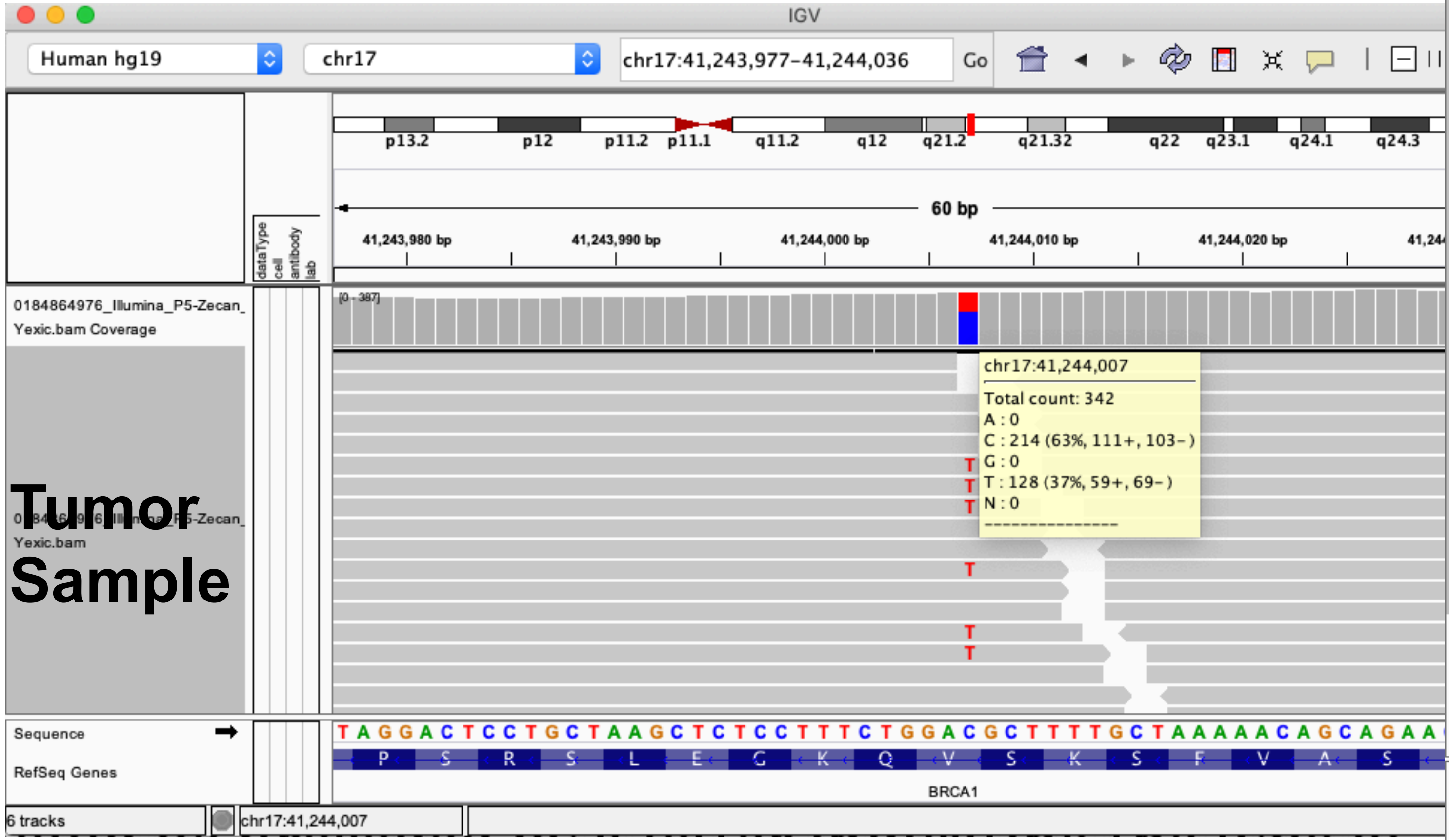
Tumor Sample

Potential SNV with 128/342 (37%) VAF

p.V1181I

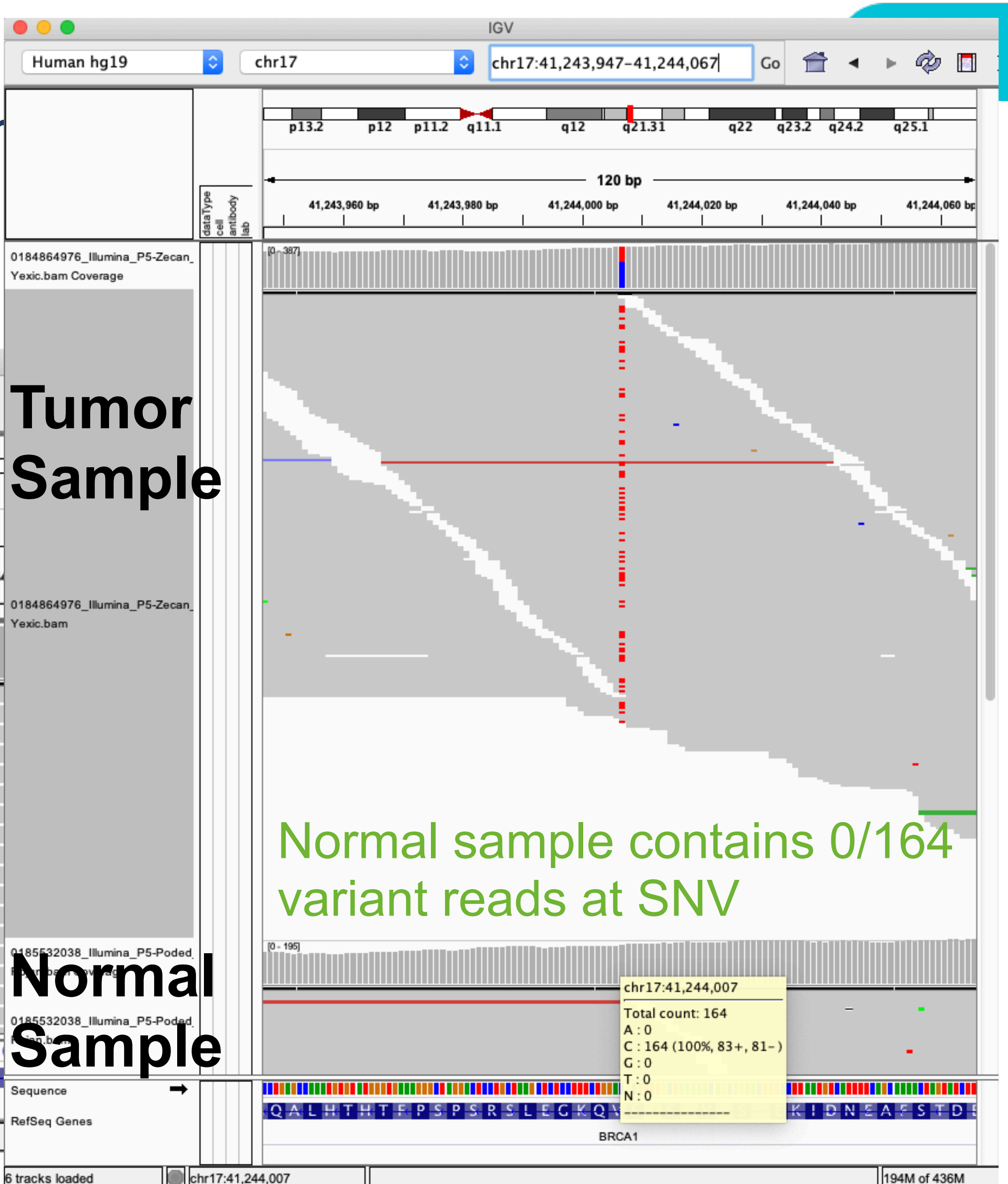
Genome Variant Analysis: Single Nucleotide Variant (SNV)

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


IGV
Human hg19
chr17
chr17:41,243,977-41,244,036
Go
p13.2
p12
p11.2
p11.1
q11.2
q12
q21.2
q21.32
q22
q23.1
q24.1
q24.3
60 bp
41,243,980 bp
41,243,990 bp
41,244,000 bp
41,244,010 bp
41,244,020 bp
dataType
cell
antibody
lab
0184864976_Illumina_P5-Zecan_Yexic.bam Coverage
[0 - 387]
0184864976_Illumina_P5-Zecan_Yexic.bam
Sequence
RefSeq Genes
T A G G A C T C C T G C T A A G C T C T C C T T T T C T G G A C G C T T T T G C T A A A A A C A G C A G A A
P S R S L E G K Q V S K S F V A S
BRCA1
6 tracks
chr17:41,244,007

Tumor Sample

```
chr17:41,244,007
Total count: 342
A : 0
C : 214 (63%, 111+, 103- )
G : 0
T : 128 (37%, 59+, 69- )
N : 0
---------------
```



Tumor Sample

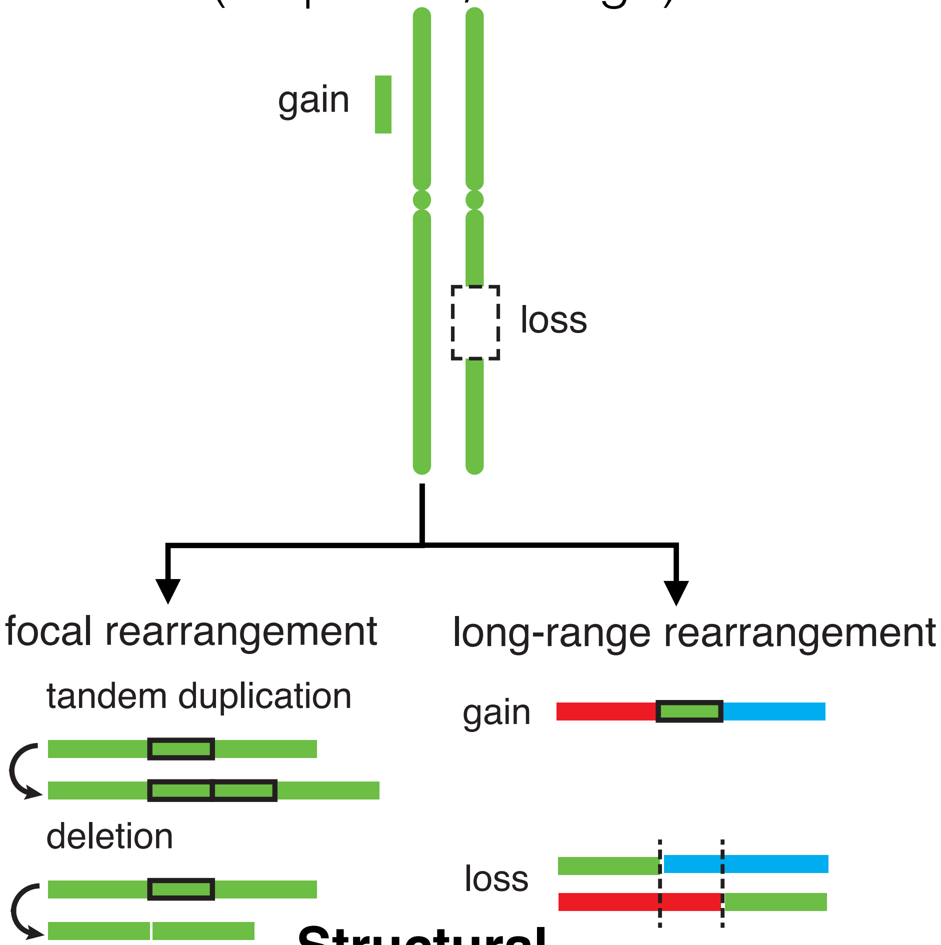
Normal sample contains 0/164 variant reads at SNV

Normal Sample

```
chr17:41,244,007
Total count: 164
A : 0
C : 164 (100%, 83+, 81- )
G : 0
T : 0
N : 0
---------------
```

Genome Variant Analysis: Copy Number and Structural Variation

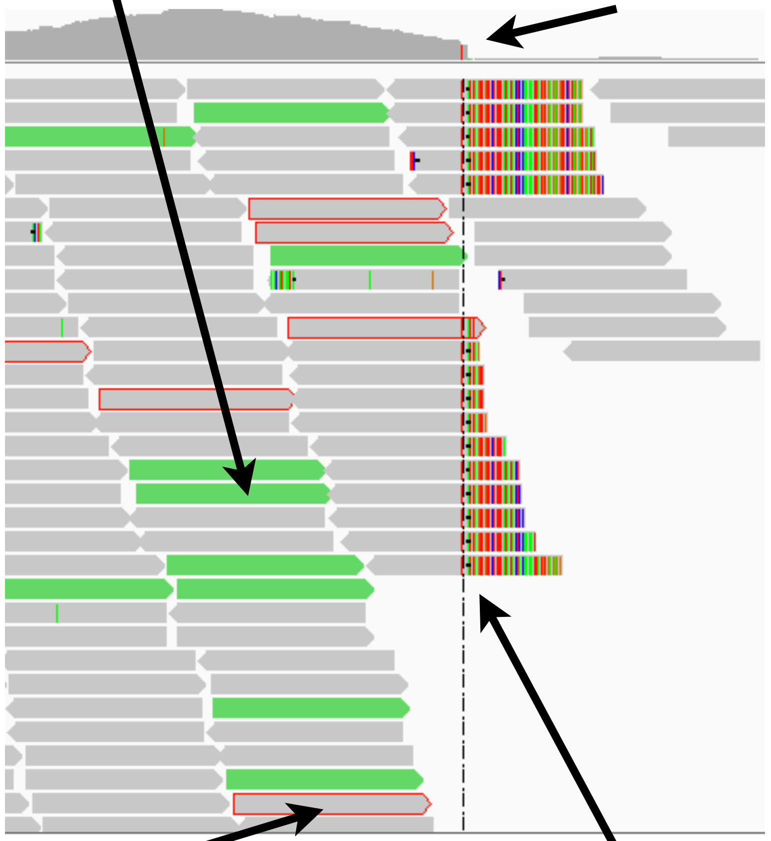
Copy number alterations
(amplitude/dosage)



Structural rearrangements
(location/configuration)

“discordant read pair”
read pairs with aberrant inferred fragment length

“copy number change”
abrupt change in read coverage

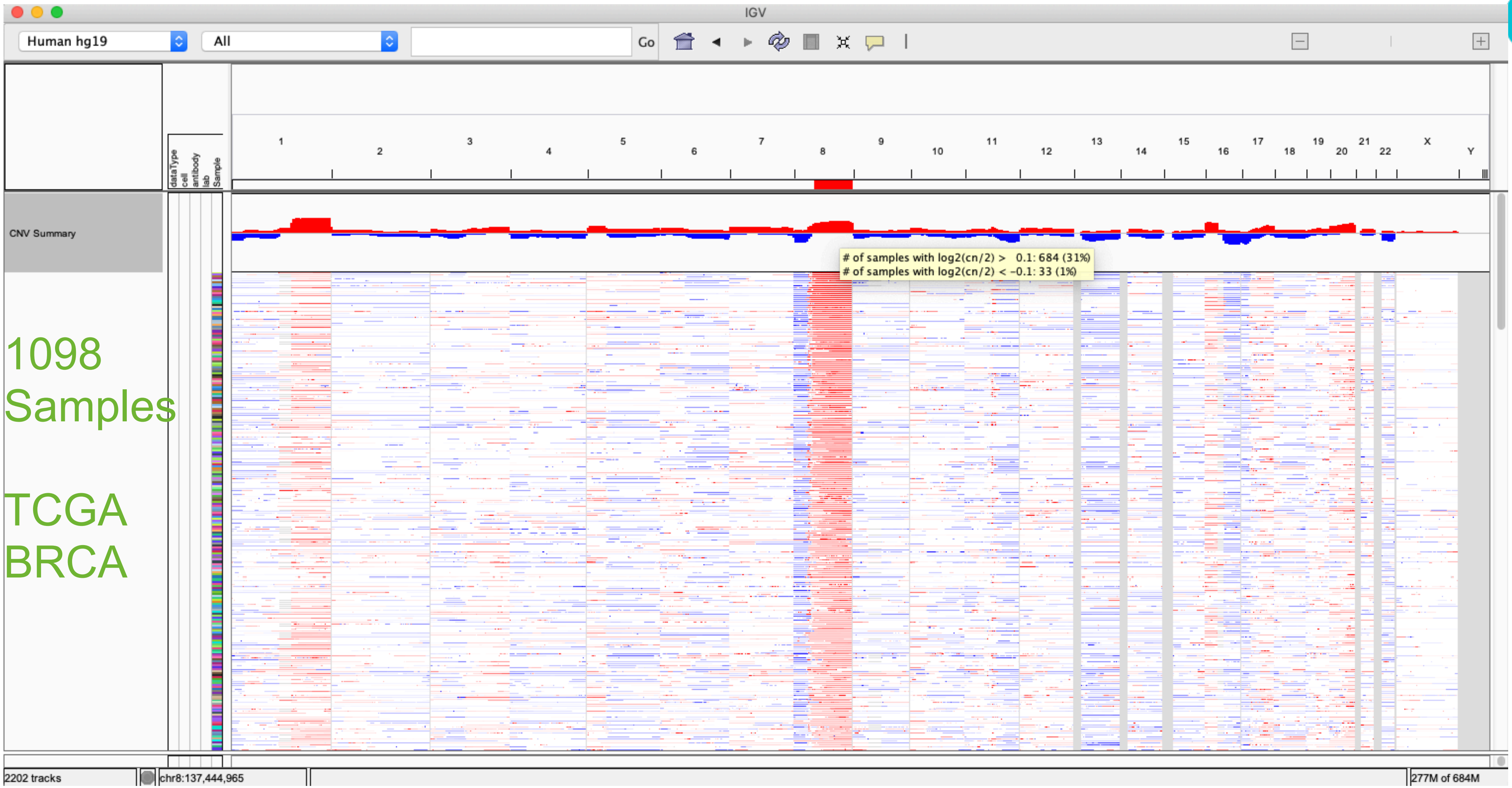


pair-mates unmapped

“split read”
split alignments

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

Genome Variant Analysis: Copy Number Variation



# of samples with log2(cn/2) > 0.1: 684 (31%)
# of samples with log2(cn/2) < -0.1: 33 (1%)

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`)
   1. Load, inspect, query a BED/SEG file
   2. Genomic regions overlap
2. Genomic Variants and Annotations (`VariantAnnotation`)
   1. 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. Files for tutorial on GitHub:
    - R Markdown: Lecture15_GenomicData.Rmd
    - Jupyter Notebook: Lecture15_GenomicData.ipynb

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. Files for tutorial on GitHub:
   - R Markdown: Lecture15_VariantCalls.Rmd
   - Jupyter Notebook: Lecture15_VariantCalls.ipynb

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
  1. the results and outputs
  2. your code and documentation
  3. Partial points awarded for code with correct logic/function even if the final answer may be incorrect