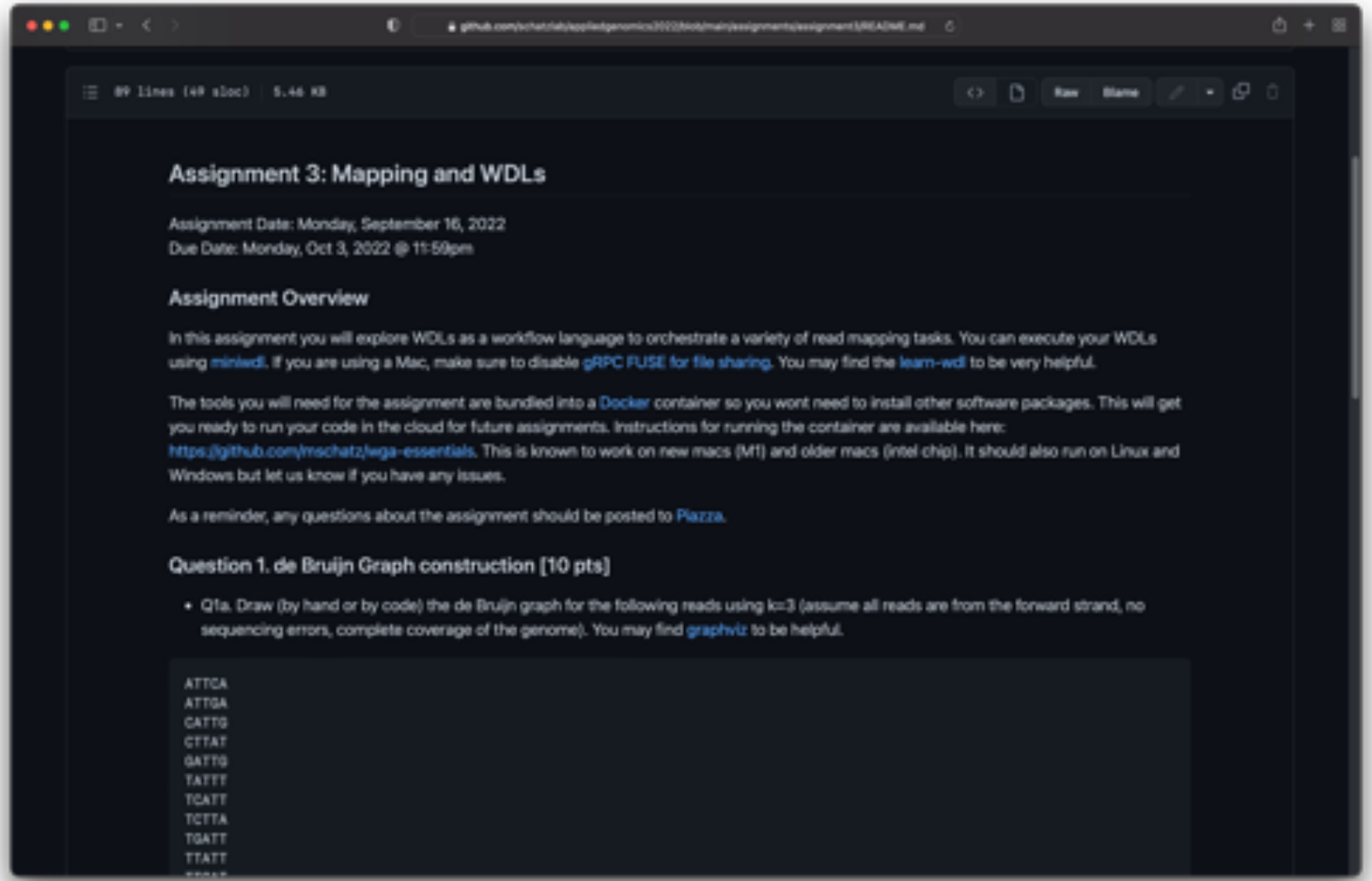
Structural Variant Calling

Michael Schatz

October 4, 2022

Lecture 11: Applied Comparative Genomics

Assignment 3: Mapping and WDL
Due Monday Oct 3 by 11:59pm



Assignment 3: Mapping and WDLs

Assignment Date: Monday, September 16, 2022
Due Date: Monday, Oct 3, 2022 @ 11:59pm

Assignment Overview

In this assignment you will explore WDLs as a workflow language to orchestrate a variety of read mapping tasks. You can execute your WDLs using miniwdl. If you are using a Mac, make sure to disable gRPC FUSE for file sharing. You may find the learn-wdl to be very helpful.

The tools you will need for the assignment are bundled into a Docker container so you wont need to install other software packages. This will get you ready to run your code in the cloud for future assignments. Instructions for running the container are available here: https://github.com/mschatz/wga-essentials. This is known to work on new macs (M1) and older macs (intel chip). It should also run on Linux and Windows but let us know if you have any issues.

As a reminder, any questions about the assignment should be posted to Piazza.

Question 1. de Bruijn Graph construction [10 pts]

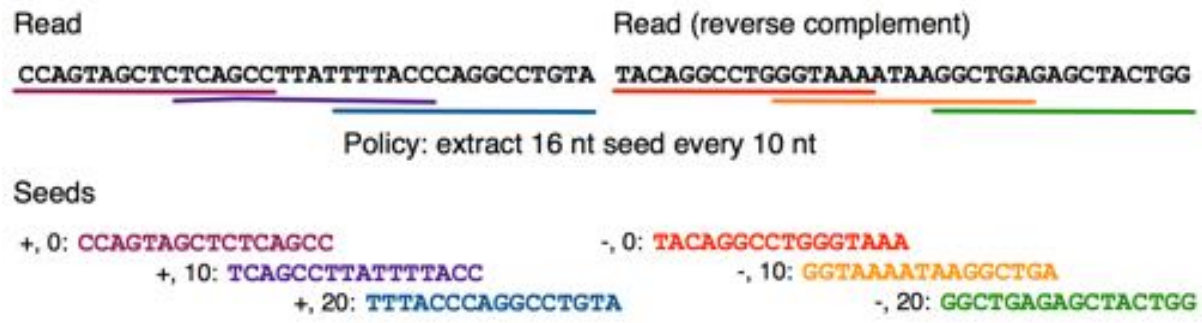
- Q1a. Draw (by hand or by code) the de Bruijn graph for the following reads using k=3 (assume all reads are from the forward strand, no sequencing errors, complete coverage of the genome). You may find graphviz to be helpful.

```
ATTCA
ATTGA
CATTG
CTTAT
GATTG
TATTT
TCATT
TCTTA
TGATT
TTATT
```

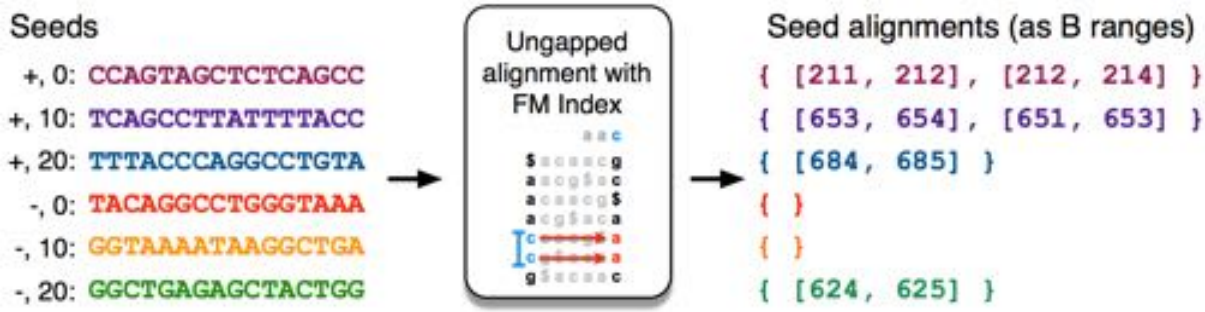
https://github.com/schatzlab/appliedgenomics2022/tree/main/assignments/assignment3
Check Piazza for questions!

Algorithm Overview

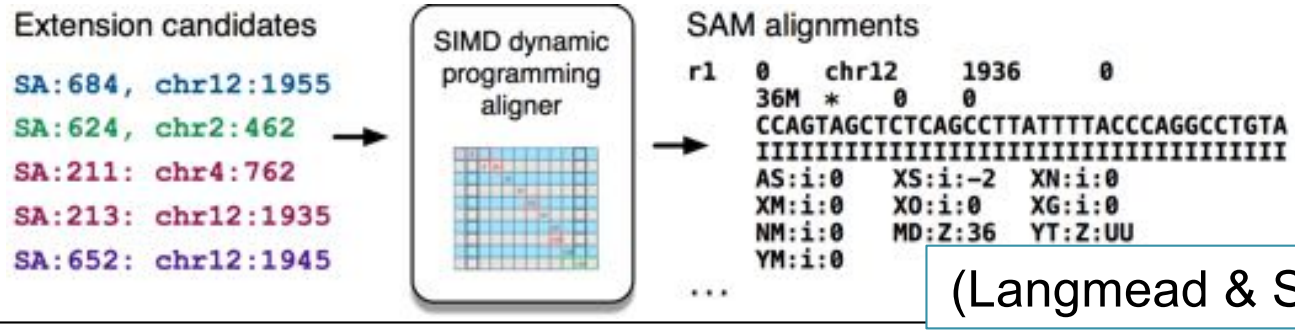
1. Split read into segments



2. Lookup each segment and prioritize



3. Evaluate end-to-end match



(Langmead & Salzberg, 2012)

Similarity metrics

- Hamming distance
  - Count the number of substitutions to transform one string into another

```
MIKESCHATZ
||X||XXXX|
MICESHATZZ
     5
```

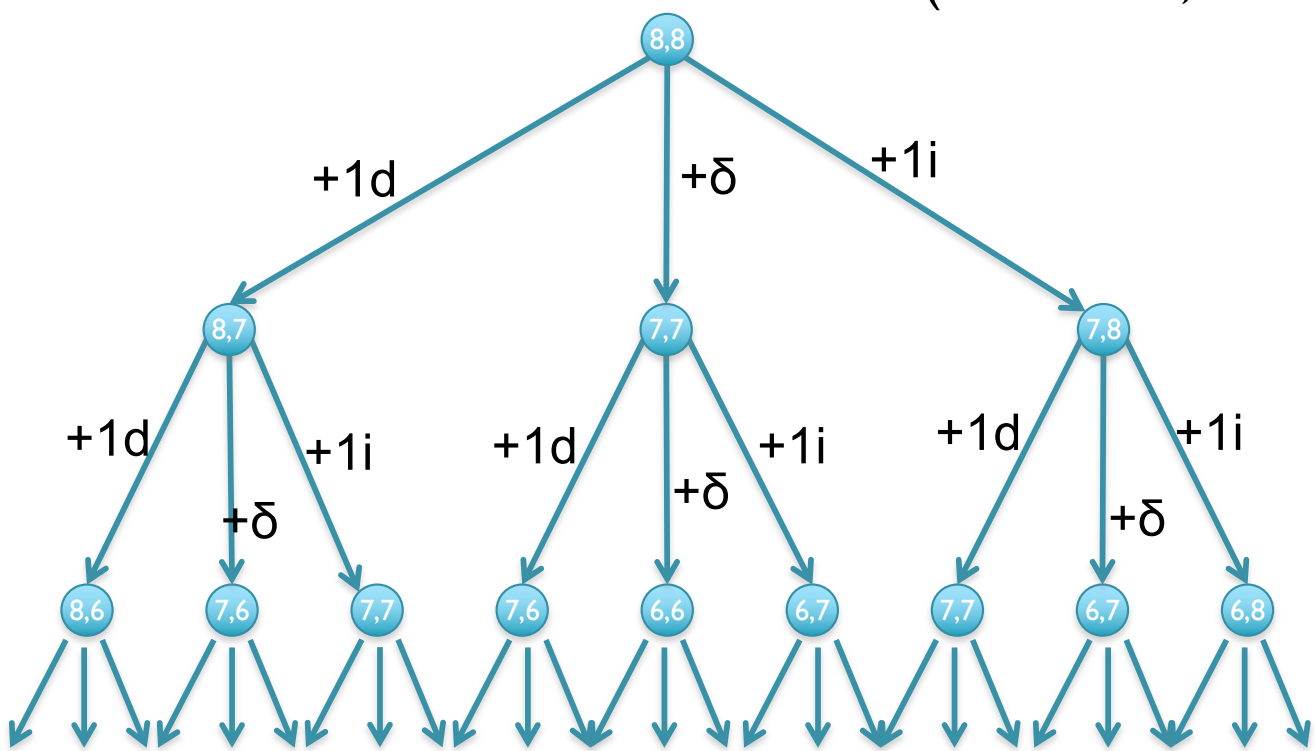
- Edit distance
  - The minimum number of substitutions, insertions, or deletions to transform one string into another

```
MIKESCHAT-Z
||X||X|||X|
MICES-HATZZ
     3
```

Recursive solution

- Computation of D is a recursive process.
  - At each step, we only allow matches, substitutions, and indels
  - D(i,j) in terms of D(i',j') for i' ≤ i and j' ≤ j.

D(AGCACACA, ACACACTA) = min{D(AGCACACA, ACACACT) + 1,
D(AGCACAC, ACACACTA) + 1,
D(AGCACAC, ACACACT) +δ(A, A)}



[What is the running time?]

Dynamic Programming Matrix

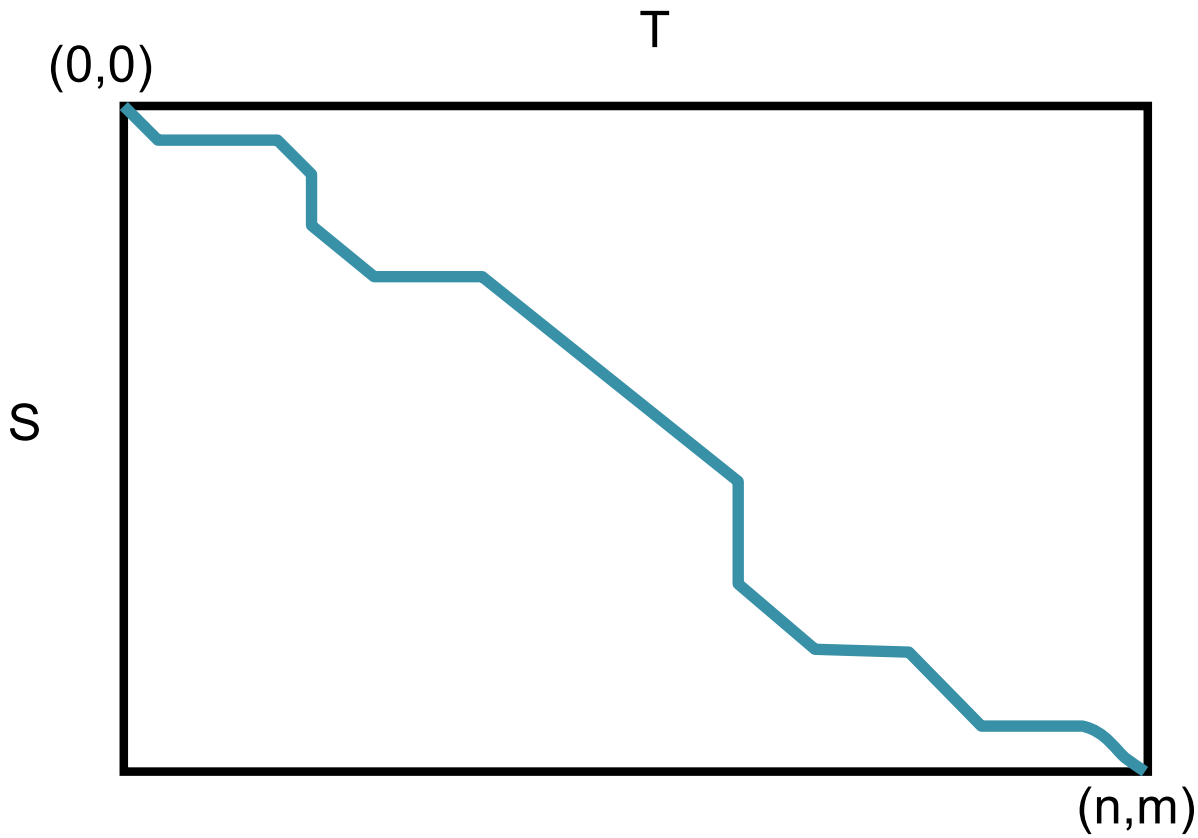
		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1	2	3	4	5	6	7
G	2	1	1	2	3	4	5	6	7
C	3	2	1	2	2	3	4	5	6
A	4	3	2	1	2	2	3	4	5
C	5	4	3	2	1	2	2	3	4
A	6	5	4	3	2	1	2	3	3
C	7	6	5	4	3	2	1	2	3
A	8	7	6	5	4	3	2	2	2

D[AGCACACA,ACACACTA] = 2

```
AGCACAC-A
|*|||||*|
A-CACACTA
```

[Can we do it any better?]

Global Alignment Schematic



- A high quality alignment will stay close to the diagonal
  - If we are only interested in high quality alignments, we can skip filling in cells that can't possibly lead to a high quality alignment
  - Find the global alignment with at most edit distance d: O(2dn)

Variant Calling

Genotyping Theory

Heterozygous variant (3/7)

Homozygous variant (6/6)

```
                                                    GGTATAC…
…CCATAG     TGTGCGCCC    CGGAAATTT CGGTATAC
…CCAT    CTATGTGCG      TCGGAAATT  CGGTATAC
…CCAT GGCTATGTG     CTATCGGAAA    GCGGCATA
…CCA AGGCTATAT     CCTATCGGA    TTGCGGTA   C…
…CCA AGGCTATAT  GCCCTATCG     TTTGCGGT    C…
…CC  AGGCTATAT  GCCCTATCG  AAATTTGC    ATAC…
…CC TAGGCTATA GCGCCCTA     AAATTTGC GTATAC…
```

Subject

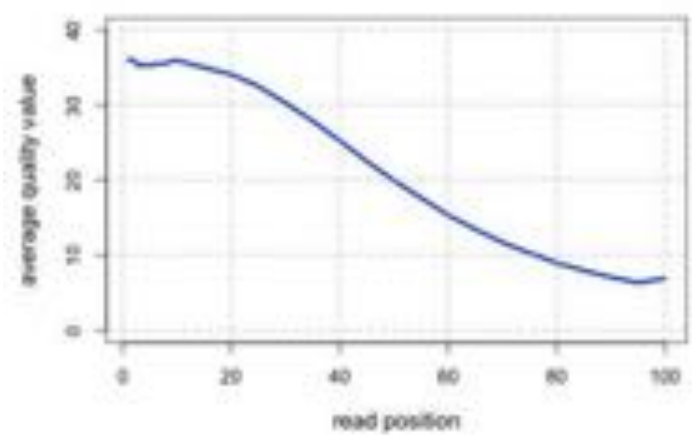
Reference

```
…CCATAGGCTATATGCGCCCTATCGGCAATTTGCGGTATAC…
```

Error or Het (1/7)?

- If there were no sequencing errors, identifying SNPs would be very easy: any time a read disagrees with the reference, it must be a variant!

- Sequencing instruments make mistakes
  - Quality of read decreases over the read length



- A single read differing from the reference is probably just an error, but it becomes more likely to be real as we see it multiple times

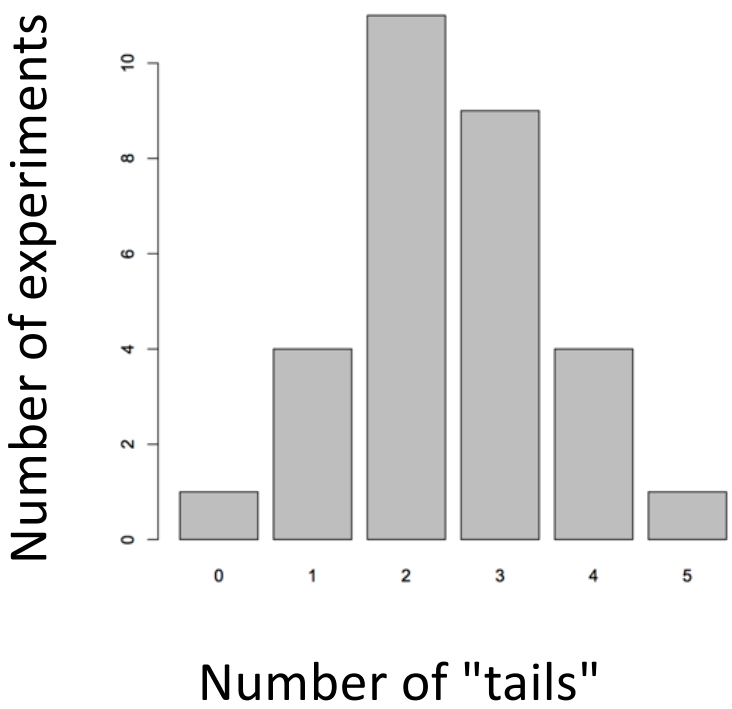
The Binomial Distribution: Adventures in Coin Flipping

P(heads) = 0.5

P(tails) = 0.5

Aaron Quinlan

What is the distribution of tails (alternate alleles) do we expect to see after 5 tosses (sequence reads)?



R code:

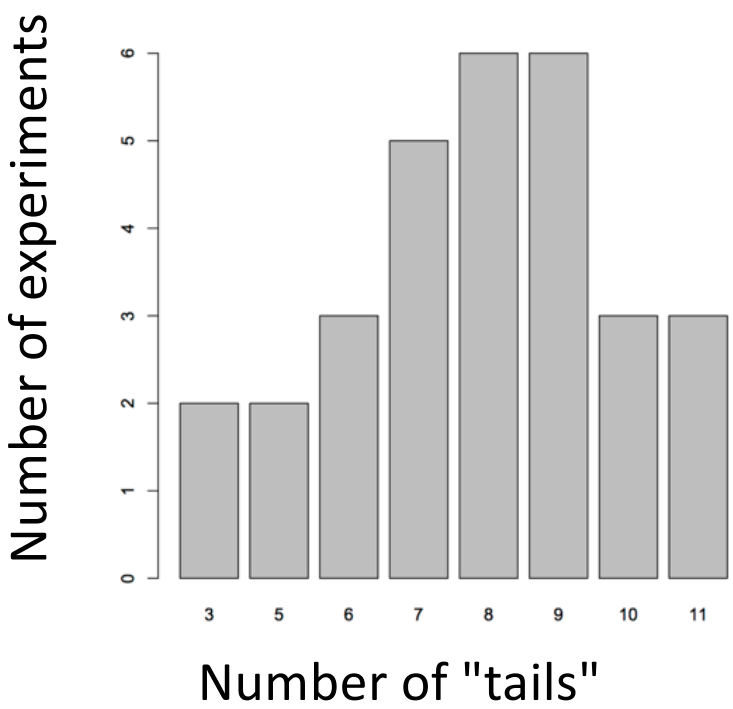
```
barplot(table(rbinom(30, 5, 0.5)))
```

30 experiments (students tossing coins)

5 tosses each

Probability of Tails

What is the distribution of tails (alternate alleles) do we expect to see after 15 tosses (sequence reads)?



R code:

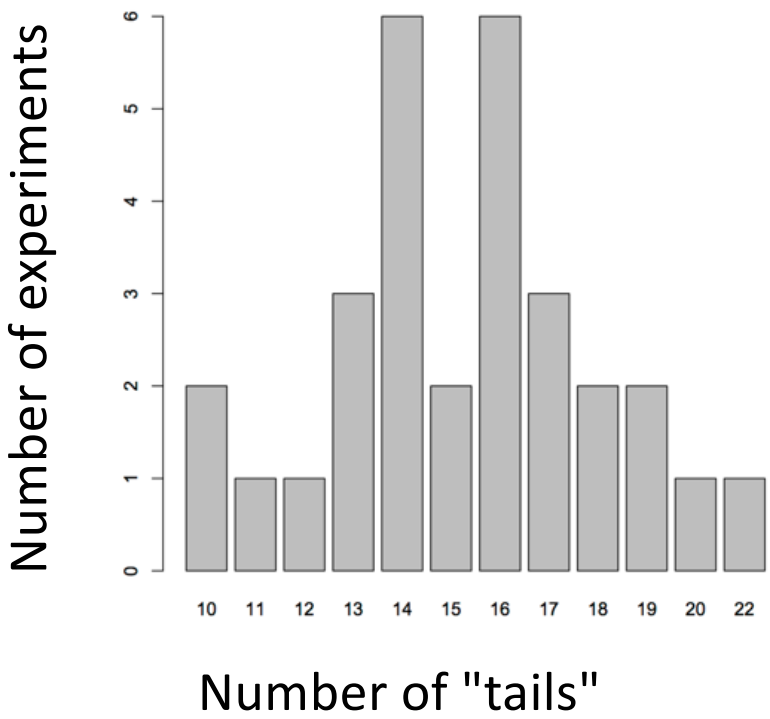
```
barplot(table(rbinom(30, 15, 0.5)))
```

30 experiments (students tossing coins)

15 tosses each

Probability of Tails

What is the distribution of tails (alternate alleles) do we expect to see after 30 tosses (sequence reads)?



R code:

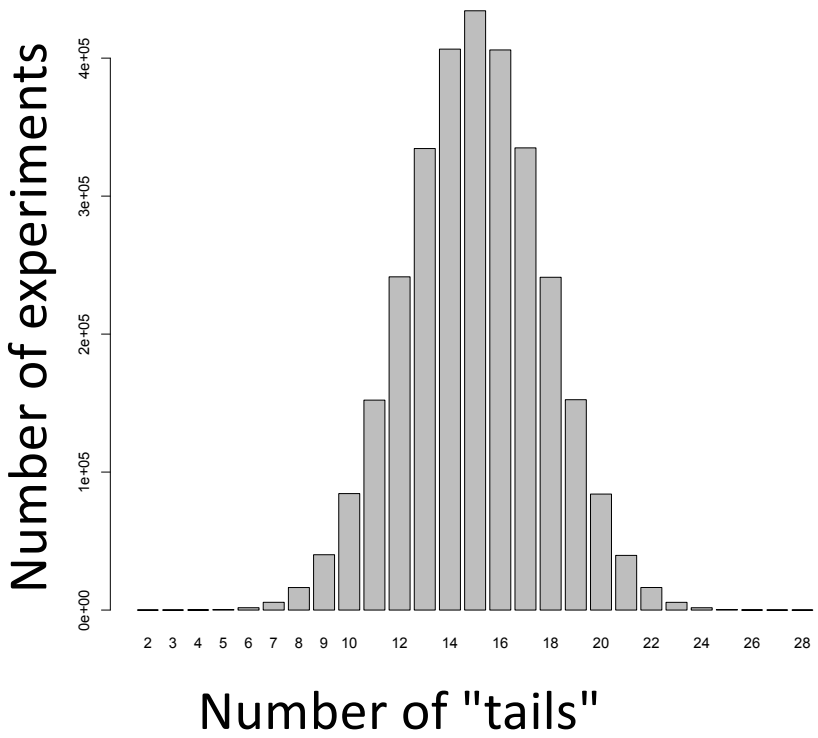
```
barplot(table(rbinom(30, 30, 0.5)))
```

30 experiments (students tossing coins)

30 tosses each

Probability of Tails

What is the distribution of tails (alternate alleles) do we expect to see after 30 tosses (sequence reads)?



R code:

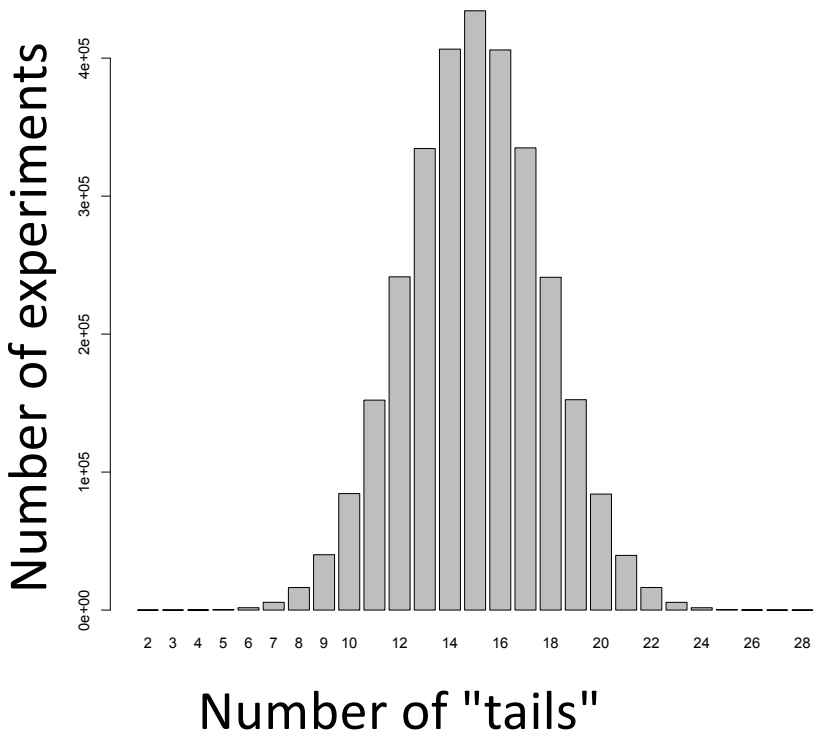
```
barplot(table(rbinom(3e6, 30, 0.5)))
```

3M experiments (students tossing coins)

30 tosses each

Probability of Tails

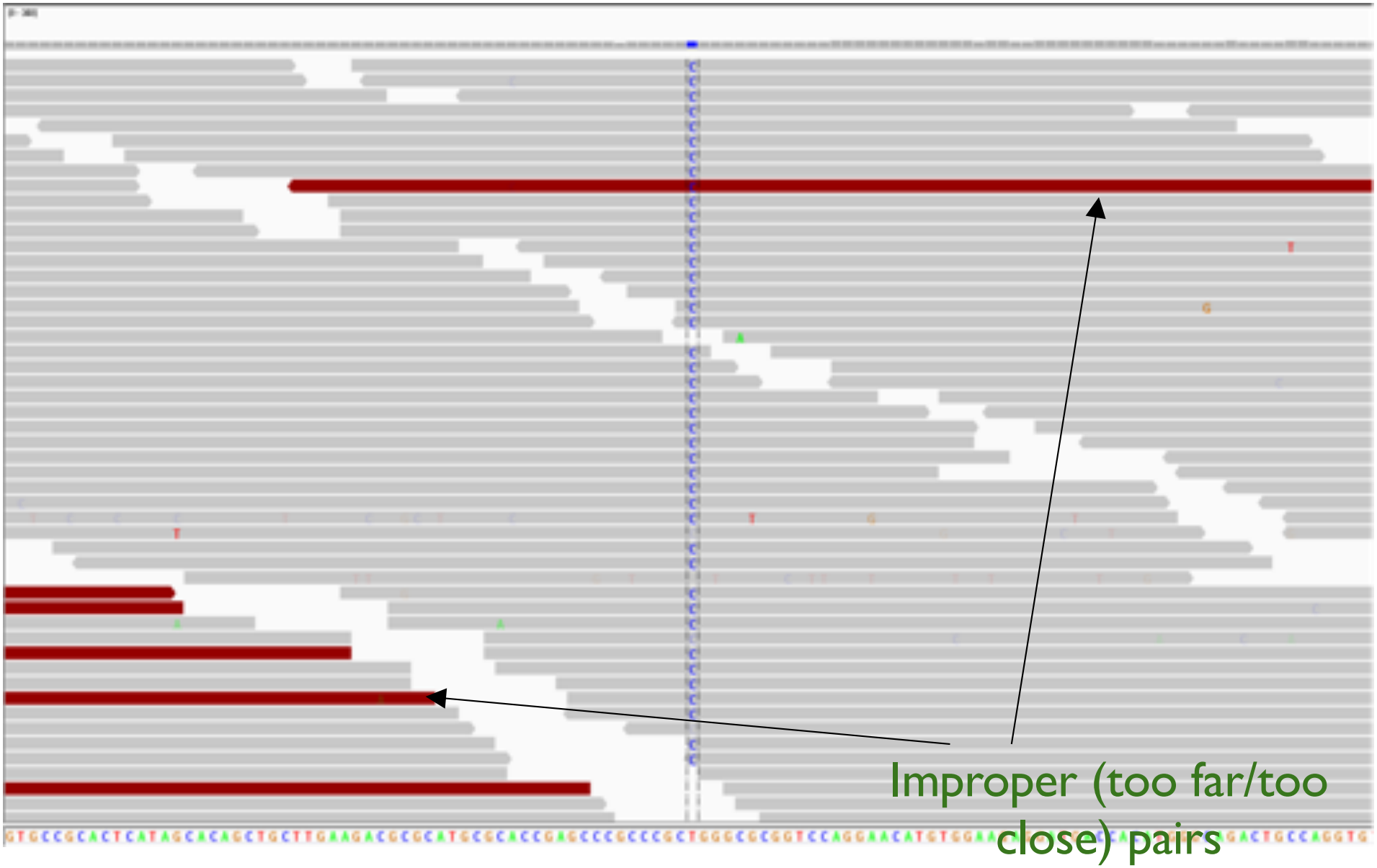
So, with 30 tosses (reads), we are much more likely to see an even mix of alternate and reference alleles at a heterozygous locus in a genome



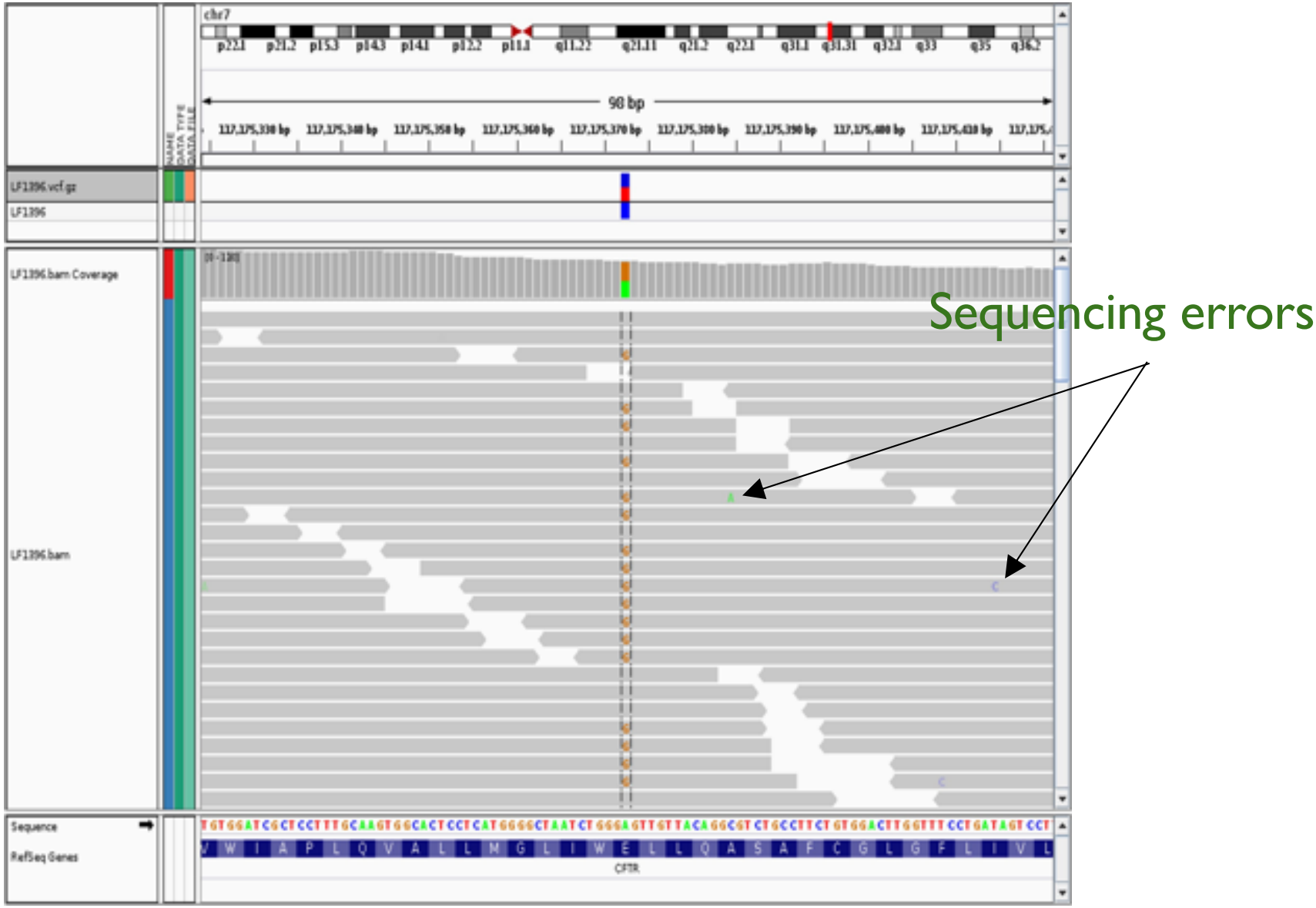
This is why <u>at least</u> a "30X" (30 fold sequence coverage) genome is recommended: it confers sufficient power to distinguish heterozygous alleles and from mere sequencing errors

P(3/30 het) <?> P(3/30 err)

Homozygous for the "C" allele

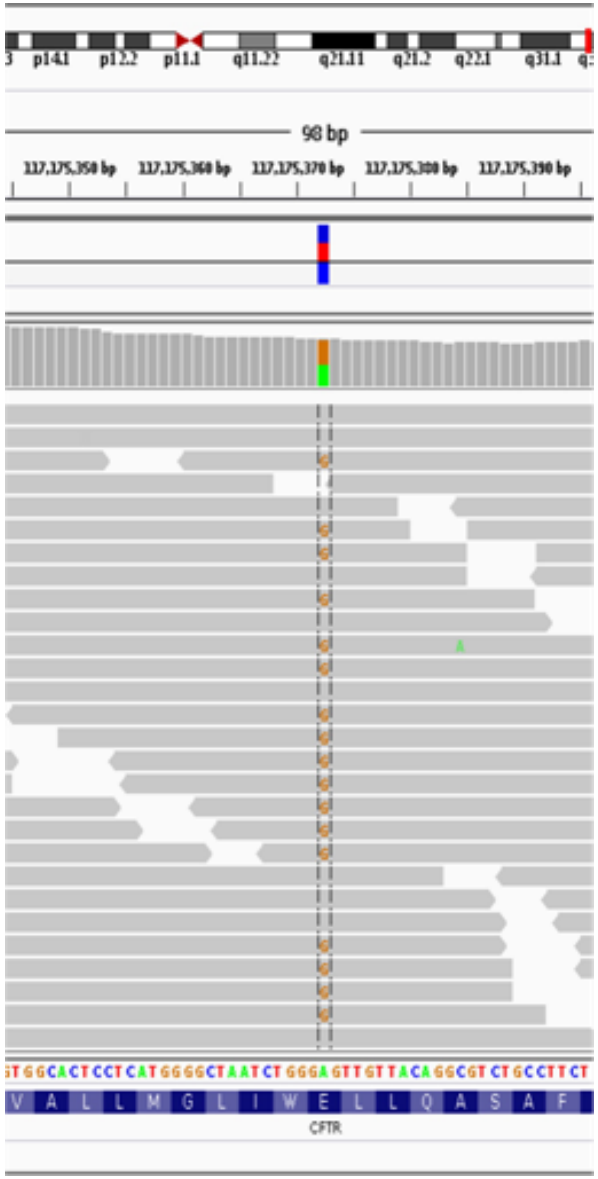


Sequencing errors fall out as noise (most of the time)



https://jchoigt.files.wordpress.com/2012/07/igv_e217g_snapshot.png

What information is needed to decide if a variant exists?



- Depth of coverage at the locus
- Bases observed at the locus
- The base qualities of each allele
- The strand composition
- Mapping qualities
- Proper pairs?
- Expected polymorphism rate

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-10-r99

PolyBayes: The first statistically rigorous variant detection tool.

letter © 1999 Nature America Inc. · http://genetics.nature.com

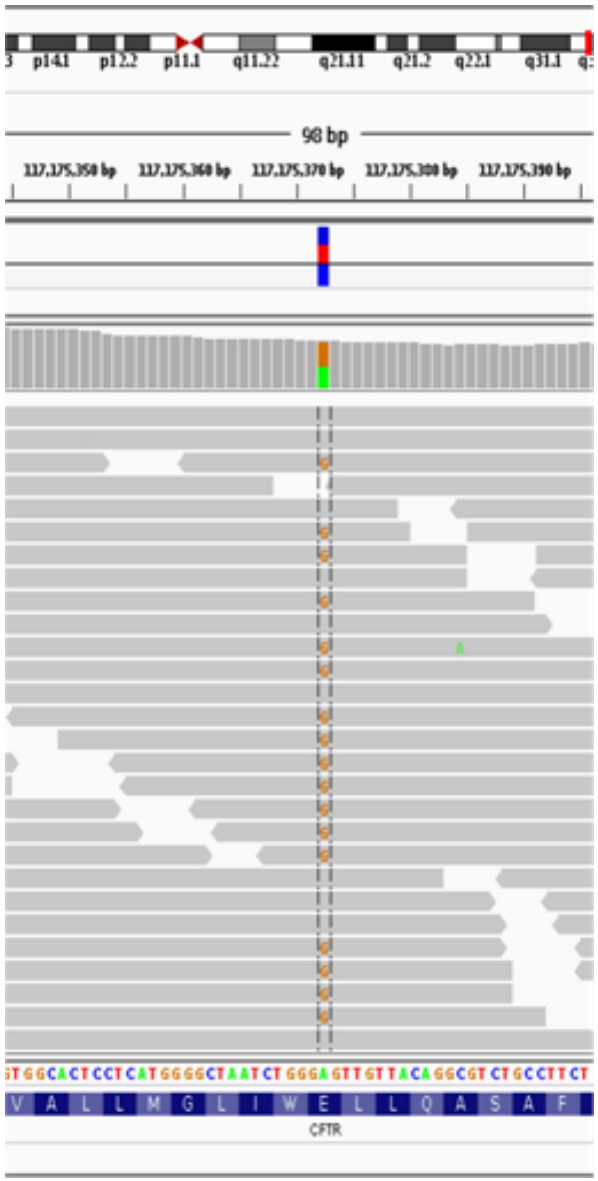
A general approach to single-nucleotide polymorphism discovery

Gabor T. Marth[1], Ian Korf[1], Mark D. Yandell[1], Raymond T. Yeh[1], Zhijie Gu[2], Hamideh Zakeri[2], Nathan O. Stitziel[1], LaDeana Hillier[1], Pui-Yan Kwok[2] & Warren R. Gish[1]

Its main innovation was the use of Bayes's theorem

http://www.nature.com/ng/journal/v23/n4/full/ng1299_452.html

Bayesian SNP calling



$$P(SNP|Data) = \frac{P(Data|SNP) * P(SNP)}{P(Data)}$$

http://www.nature.com/ng/journal/v23/n4/full/ng1299_452.html

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letter

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Bayesian posterior probability

Base call + Base quality

Expected (prior) polymorphism rate

$$P(SNP) = \sum_{all\ variable\ S} \frac{\frac{P(S_1 \mid R_1)}{P_{Prior}(S_1)} \cdot \ldots \cdot \frac{P(S_N \mid R_N)}{P_{Prior}(S_N)} \cdot P_{Prior}(S_1, \ldots, S_N)}{\sum_{S_{i_1} \in [A,C,G,T]} \ldots \sum_{S_{i_N} \in [A,C,G,T]} \frac{P(S_{i_1} \mid R_1)}{P_{Prior}(S_{i_1})} \cdot \ldots \cdot \frac{P(S_{i_N} \mid R_1)}{P_{Prior}(S_{i_N})} \cdot P_{Prior}(S_{i_1}, \ldots, S_{i_N})}$$

Probability of observed base composition (should model sequencing error rate)

http://www.nature.com/ng/journal/v23/n4/full/ng1299_452.html

PolyBayes: The first statistically rigorous variant detection tool.

letter

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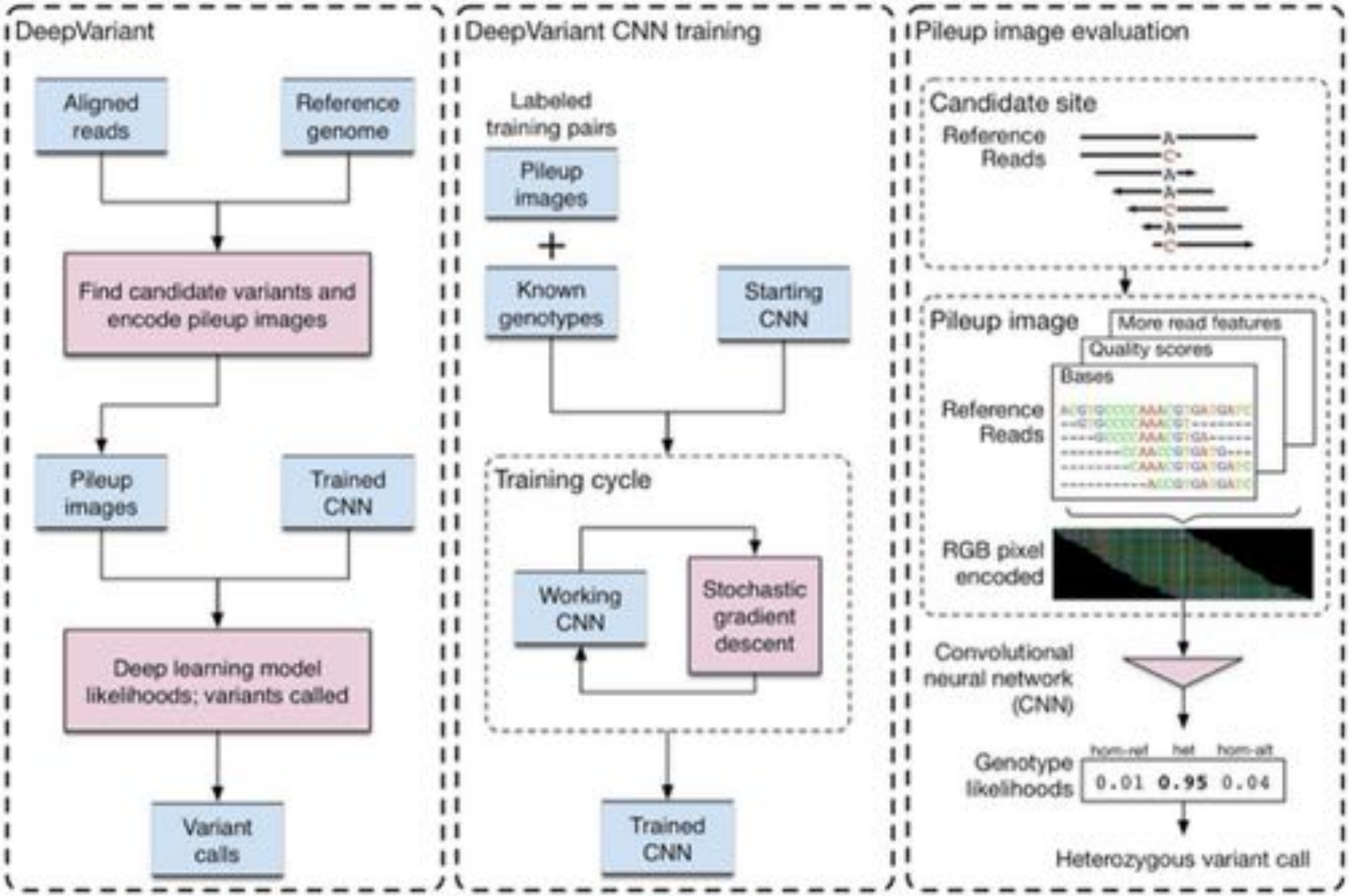
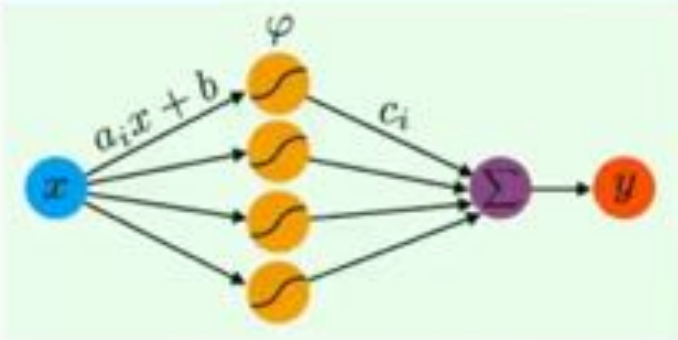
This Bayesian statistical framework has been adopted by many modern statistical SNP/INDEL callers such as FreeBayes, GATK, and samtools

http://www.nature.com/ng/journal/v23/n4/full/ng1299_452.html

DeepVariant



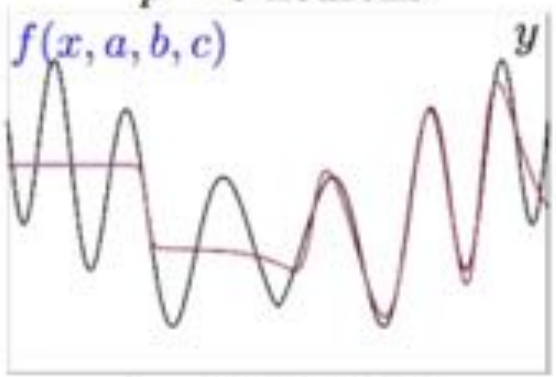
Creating a universal SNP and small indel variant caller with deep neural networks
Poplin et al. (2018) Nature Biotechnology. https://www.nature.com/articles/nbt.4235



1 hidden layer perceptron:

$$y \approx f(x, a, b, c) \stackrel{\text{def.}}{=} \sum_{i=1}^{p} c_i \varphi(a_i x + b_i)$$

$p = 6$ neurons



$p = 20$ neurons

Approximation by Superpositions of a Sigmoidal Function*

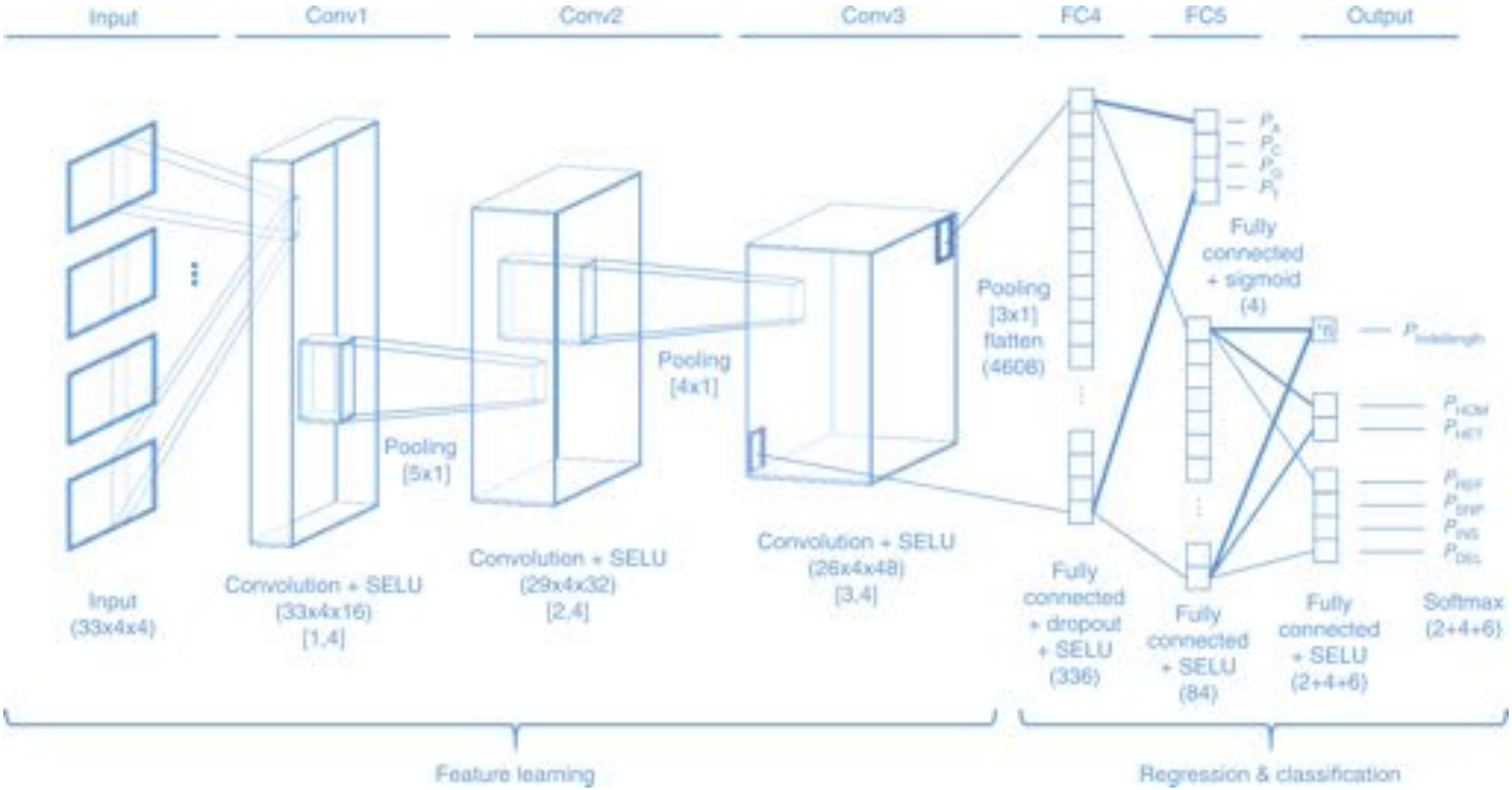
G. Cybenko†

Abstract. In this paper we demonstrate that finite linear combinations of compositions of a fixed, univariate function and a set of affine functionals can uniformly approximate any continuous function of n real variables with support in the unit hypercube; only mild conditions are imposed on the univariate function. Our results settle an open question about representability in the class of single hidden layer neural networks. In particular, we show that arbitrary decision regions can be arbitrarily well approximated by continuous feedforward neural networks with only a single internal, hidden layer and any continuous sigmoidal nonlinearity. The paper discusses approximation properties of other possible types of nonlinearities that might be implemented by artificial neural networks.

Key words. Neural networks, Approximation, Completeness.

Approximation by superpositions of a sigmoidal function

Cybenko, G. (1989) Mathematics of Control Signal Systems doi: 10.1007/BF02551274



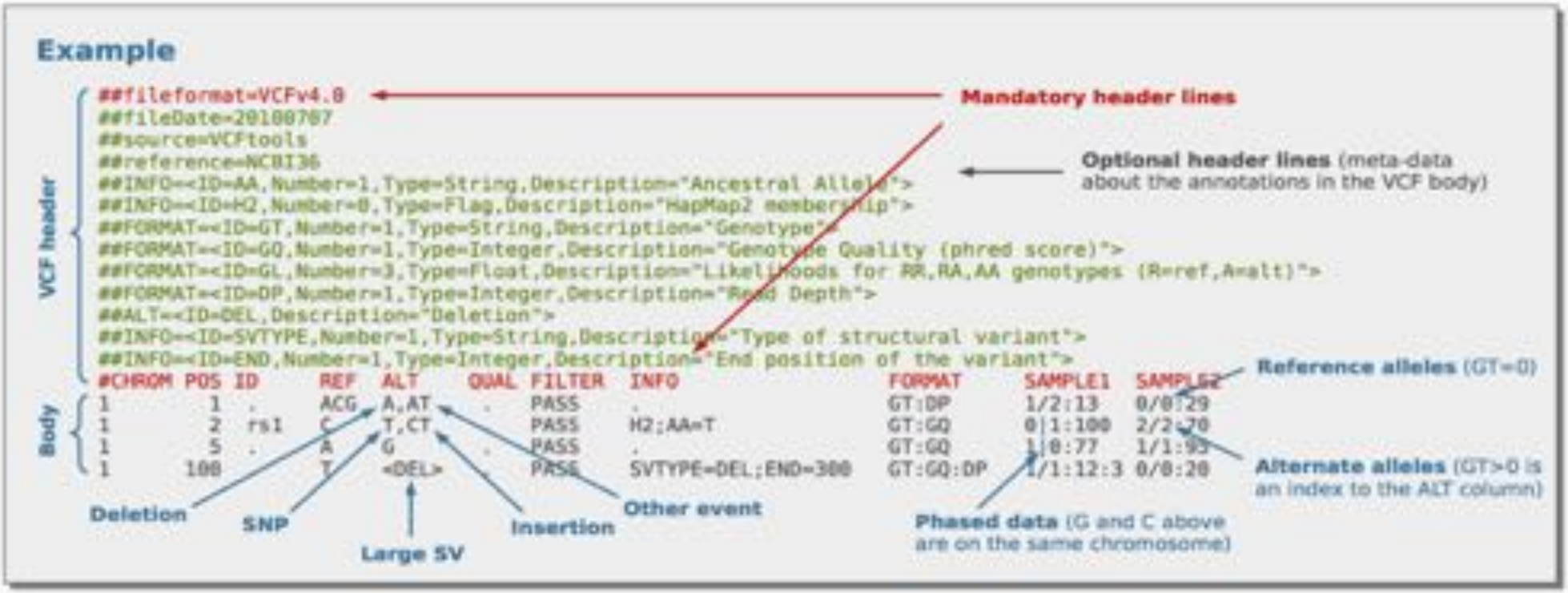
A multi-task convolutional deep neural network for variant calling in single molecule sequencing
Luo et al. (2019) Nature Communication. https://doi.org/10.1038/s41467-019-09025-z

Figure 2: Error counts over the years for HG003. For Ilumina WGS, we use a HiSeqX PCR-free dataset at 30x coverage. For PacBio HiFi, we use the same BAM as the one in our case study.

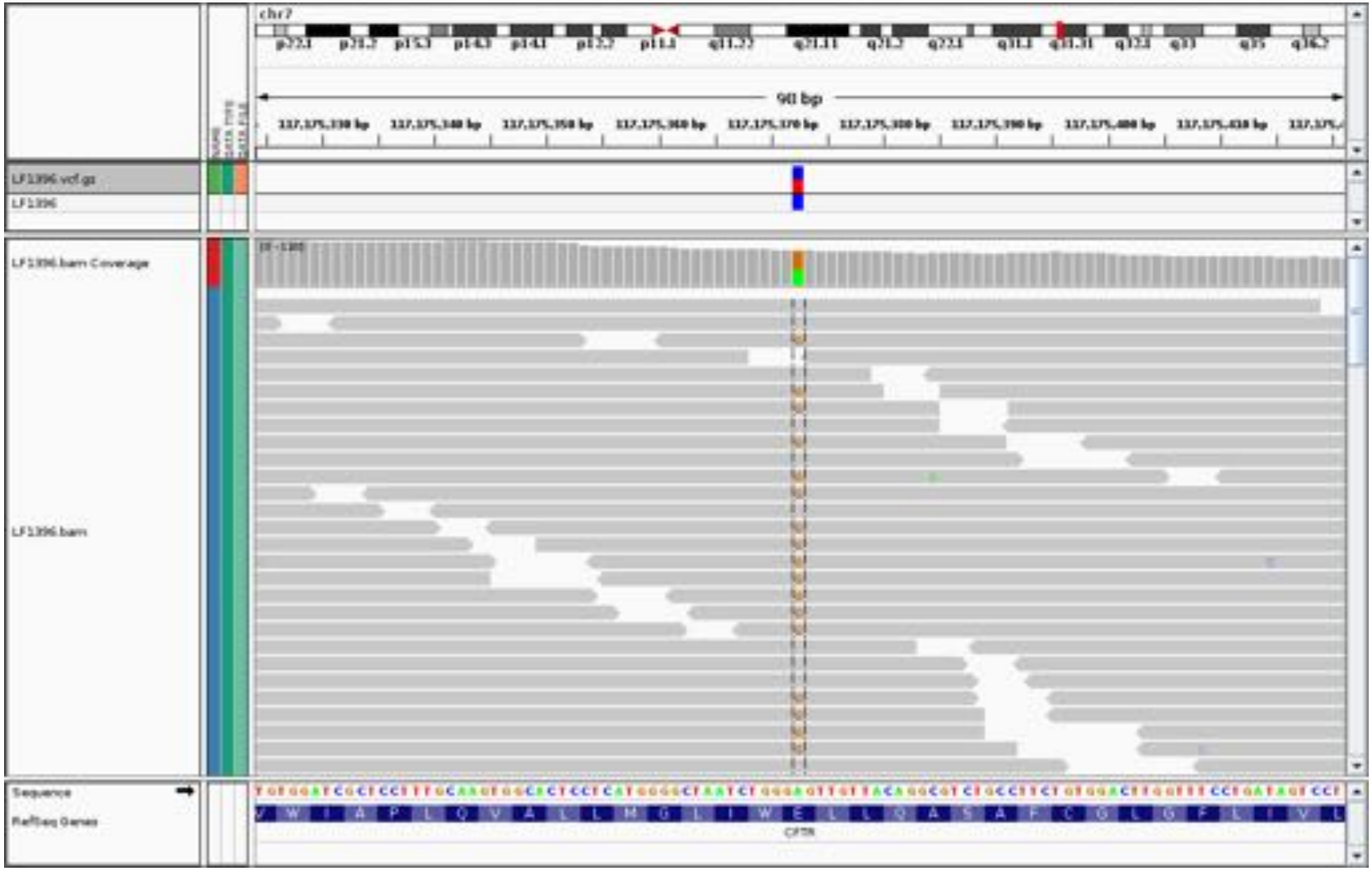
DeepVariant over the years

https://google.github.io/deepvariant/posts/2021-06-08-deepvariant-over-the-years/

VCF Format


Example
VCF header
Body
##fileformat=VCFv4.0
##fileDate=20100707
##source=VCFtools
##reference=NCBI36
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality (phred score)">
##FORMAT=<ID=GL,Number=3,Type=Float,Description="Likelihoods for RR,RA,AA genotypes (R=ref,A=alt)">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT SAMPLE1 SAMPLE2
1 1 . ACG A,AT . PASS . GT:DP 1/2:13 0/0:29
1 2 rs1 C T,CT PASS H2;AA=T GT:GQ 0|1:100 2/2:70
1 5 . A G . PASS . GT:GQ 1|0:77 1/1:95
1 100 T <DEL> . PASS SVTYPE=DEL;END=300 GT:GQ:DP 1/1:12:3 0/0:20
Mandatory header lines
Optional header lines (meta-data about the annotations in the VCF body)
Reference alleles (GT=0)
Alternate alleles (GT>0 is an index to the ALT column)
Phased data (G and C above are on the same chromosome)
Deletion
SNP
Large SV
Insertion
Other event

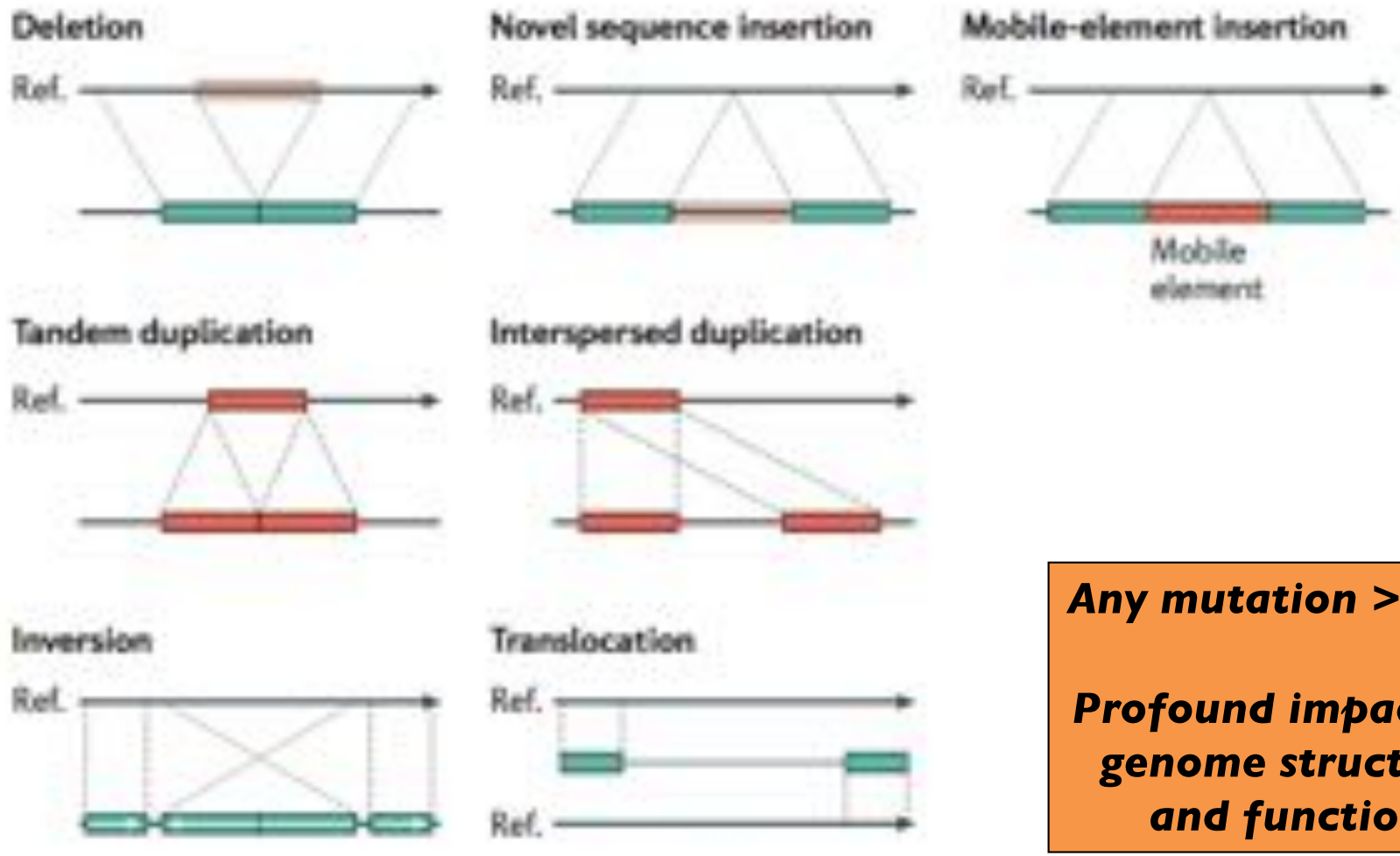
VCF Format



#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	LF1396
chr7	117175373	.	A	G	90	PASS	AF=0.5	GT	0/1

What about indels & structural variants

Structural Variations



Any mutation >50bp

Profound impact on genome structure and function

Genome structural variation discovery and genotyping

Alkan, C, Coe, BP, Eichler, EE (2011) *Nature Reviews Genetics*. May;12(5):363-76. doi: 10.1038/nrg2958.

Early 2000s dogma: SNPs account for most human genetic variation

https://hapmap.ncbi.nlm.nih.gov

Discovery of abundant copy-number variation

Science, **July 2004**

Large-Scale Copy Number Polymorphism in the Human Genome

Jonathan Sebat,[1] B. Lakshmi,[1] Jennifer Troge,[1] Joan Alexander,[1] Janet Young,[2] Pär Lundin,[3] Susanne Månér,[3] Hillary Massa,[2] Megan Walker,[2] Maoyen Chi,[1] Nicholas Navin,[1] Robert Lucito,[1] John Healy,[1] James Hicks,[1] Kenny Ye,[4] Andrew Reiner,[1] T. Conrad Gilliam,[5] Barbara Trask,[2] Nick Patterson,[6] Anders Zetterberg,[3] Michael Wigler[1*]

76 CNVs in 20 individuals
70 genes

Nature Genetics, **Aug. 2004**

Detection of large-scale variation in the human genome

A John Iafrate[1,2], Lars Feuk[3], Miguel N Rivera[1,2], Marc L Listewnik[1], Patricia K Donahoe[2,4], Ying Qi[3], Stephen W Scherer[3,5] & Charles Lee[1,2,5]

255 CNVs in 55 individuals
127 genes

- 331 CNVs, only 11 in common
- Half observed in only 1 individual
- Impact "plenty" of genes
- Correlated with segmental duplications in the reference genome

Why is structural variation relevant / important?

- They are common and affect a large fraction of the genome
  - In total, SVs impact more base pairs than all single-nucleotide differences.

- They are a major driver of genome evolution
  - Speciation can be driven by rapid changes in genome architecture
  - Genome instability and aneuploidy: hallmarks of solid tumor genomes