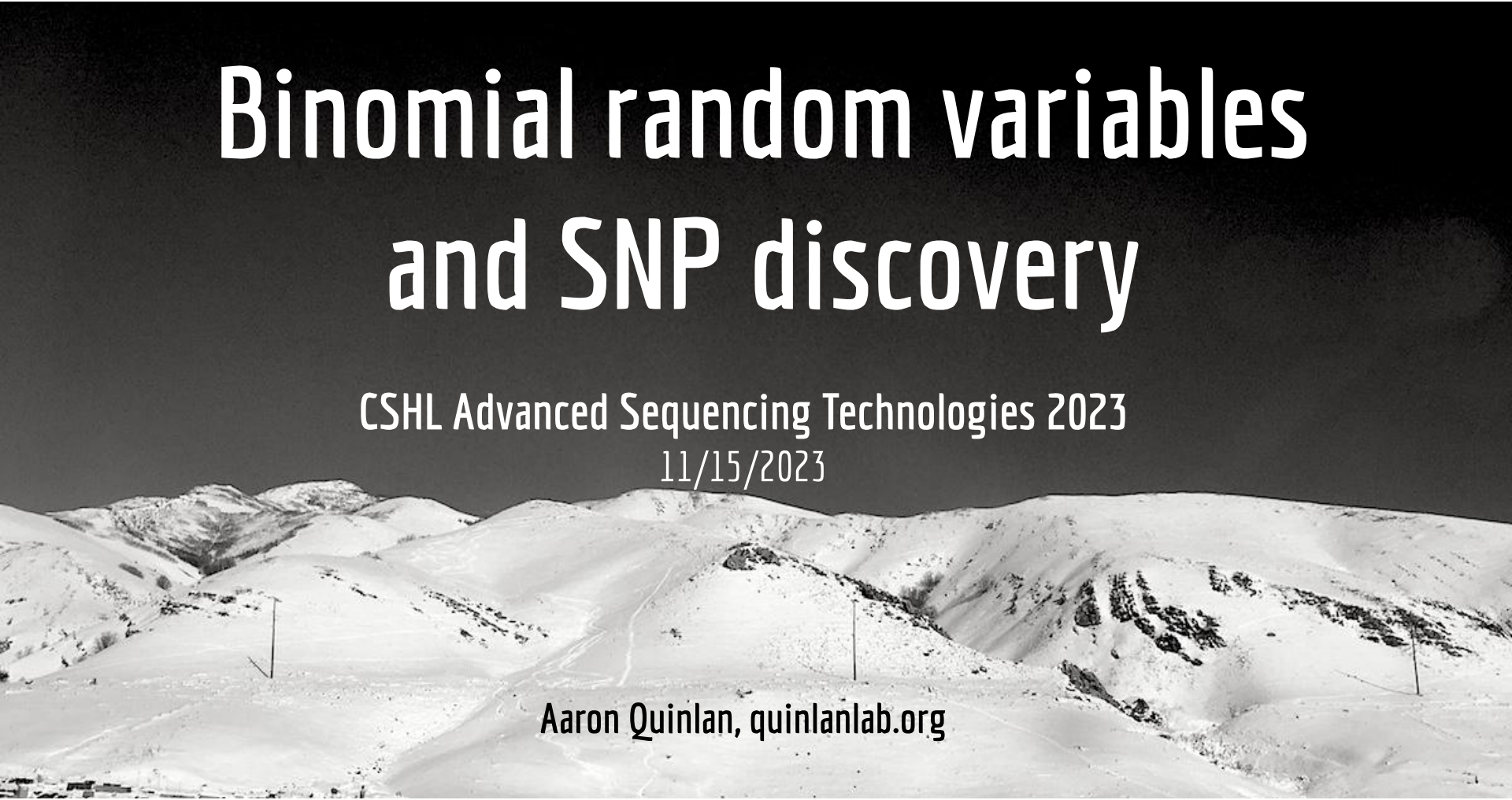
Binomial random variables and SNP discovery

CSHL Advanced Sequencing Technologies 2023

11/15/2023



Aaron Quinlan, quinlanlab.org

Thought experiment

Mutations arise on sperm and egg prior to fertilization.

Such mutations are observed in the offspring's DNA, but not in the parent: "**germline mutations**"

How many should we expect in a typical child?

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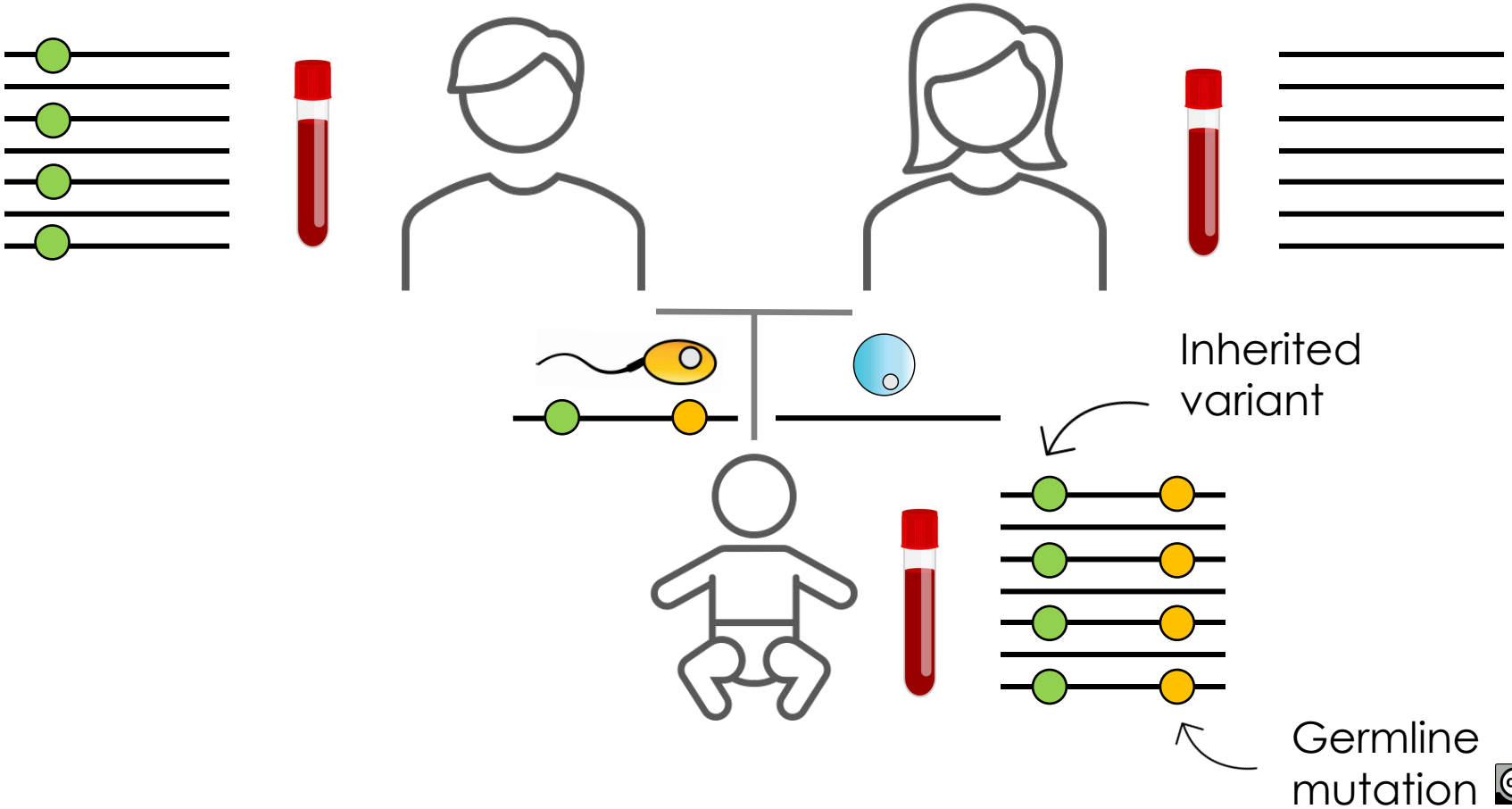
Finding mutations with family genome sequencing



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Germline mutation rate

~1 DNM per 100 million per generation
(1 x 10-8 / bp / gen)

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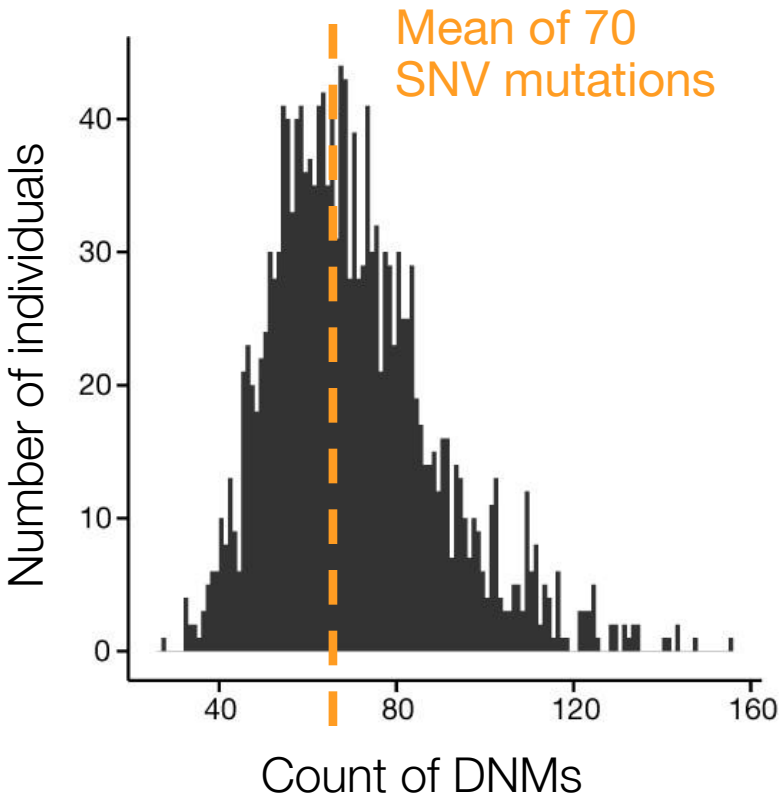
A typical human has 70 de novo SNV mutations. However, the mutation burden is variable.



Jonnson et al, Nature, 2017

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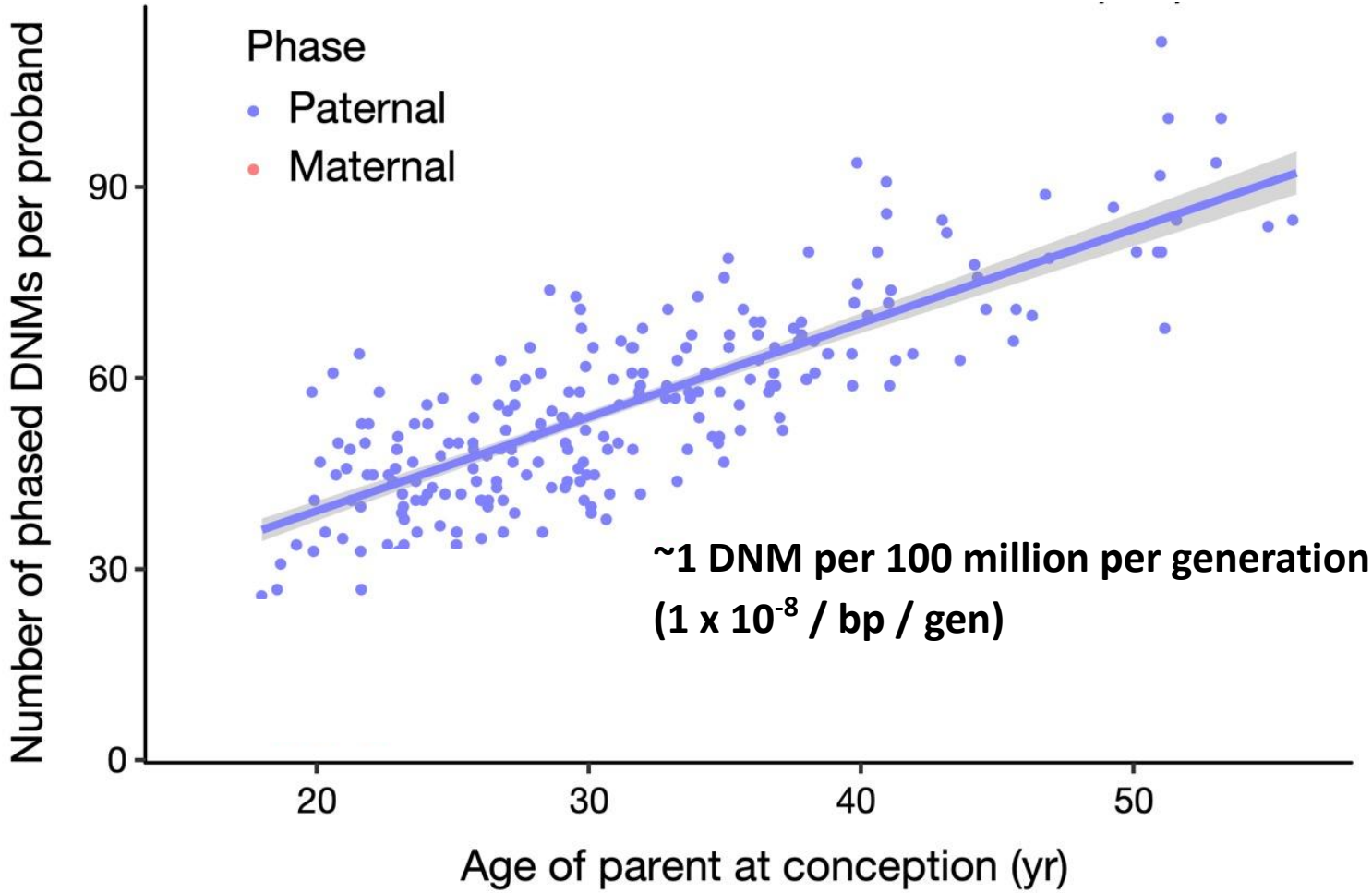

Sasani et al, eLife, 2019

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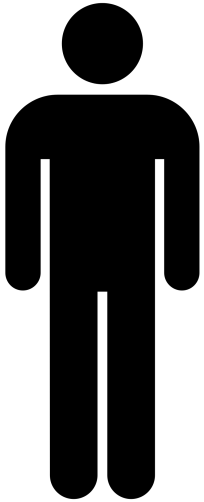
Goal: find all germline mutations in an individual's diploid genome.



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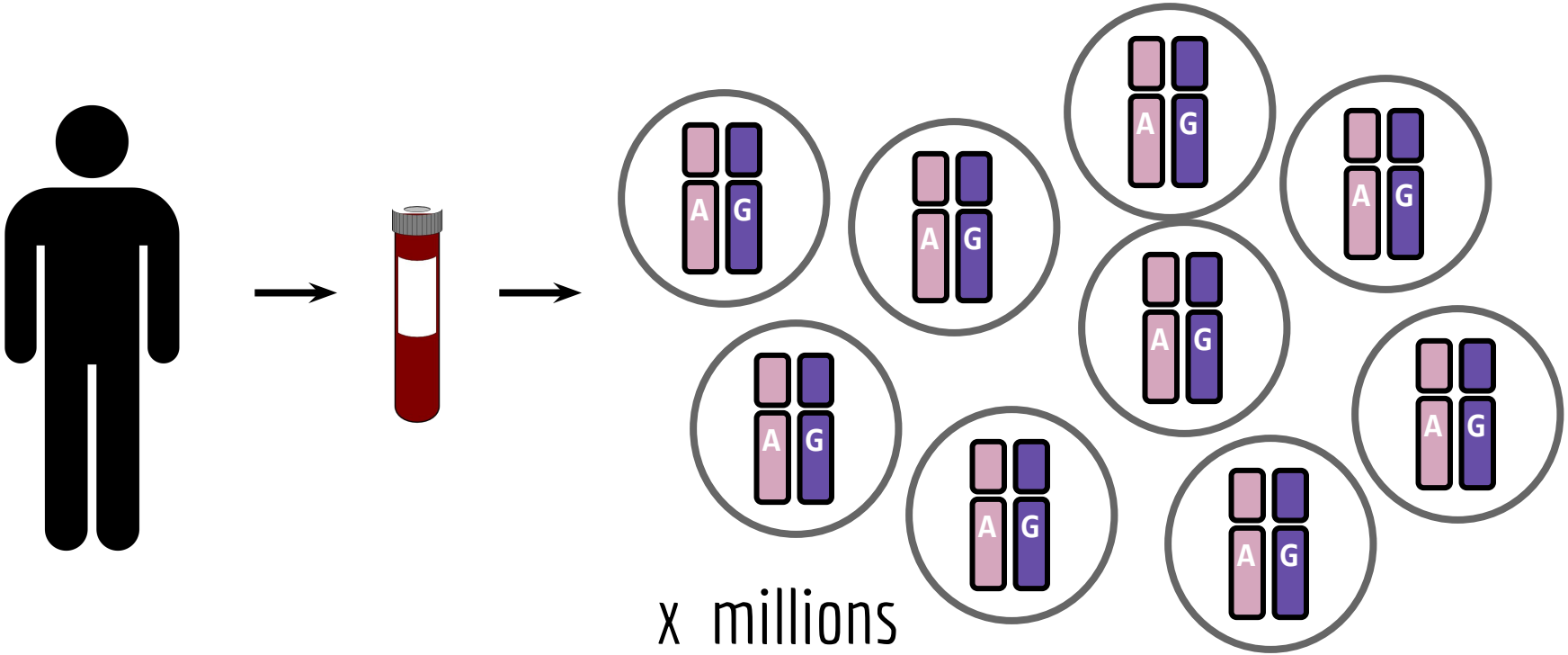
Find all germline mutations by sequencing DNA from millions of cells



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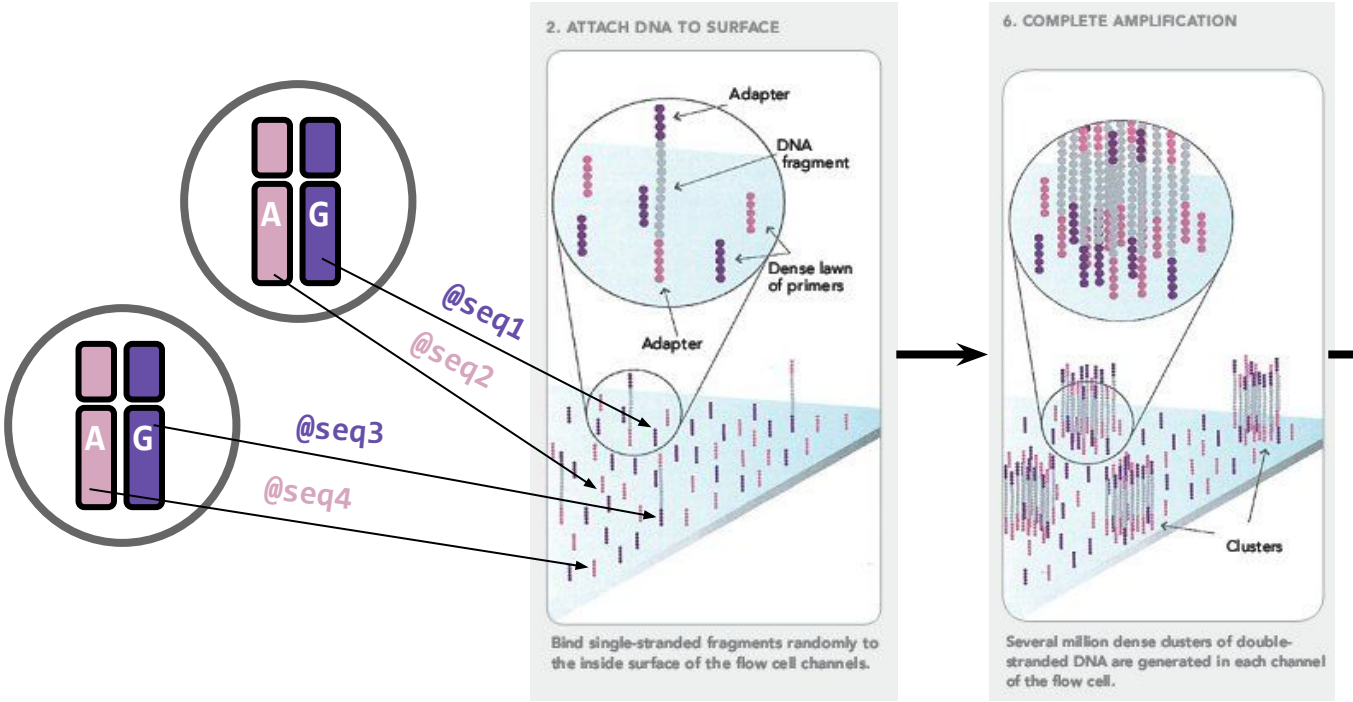
Each DNA cluster is amplified from a single strand from a single haploid chromosome from a single cell.




```
@seq1
ACCTTCGAACGGCGGGGGGTTACAA
+
!''*((((***+))%%%++).1***
@seq2
TGGAACCGAACGGCCCCGGTTACAT
+
!''*!!!!***+))+++++).1***
@seq3
ACCTTCGAACGGCGGGGGGTTACAA
+
!''*((((***+))%%%++).1***
@seq4
TGGAACCGAACGGCCCCGGTTACAT
+
!''*!!!!***+))+++++).1***
```

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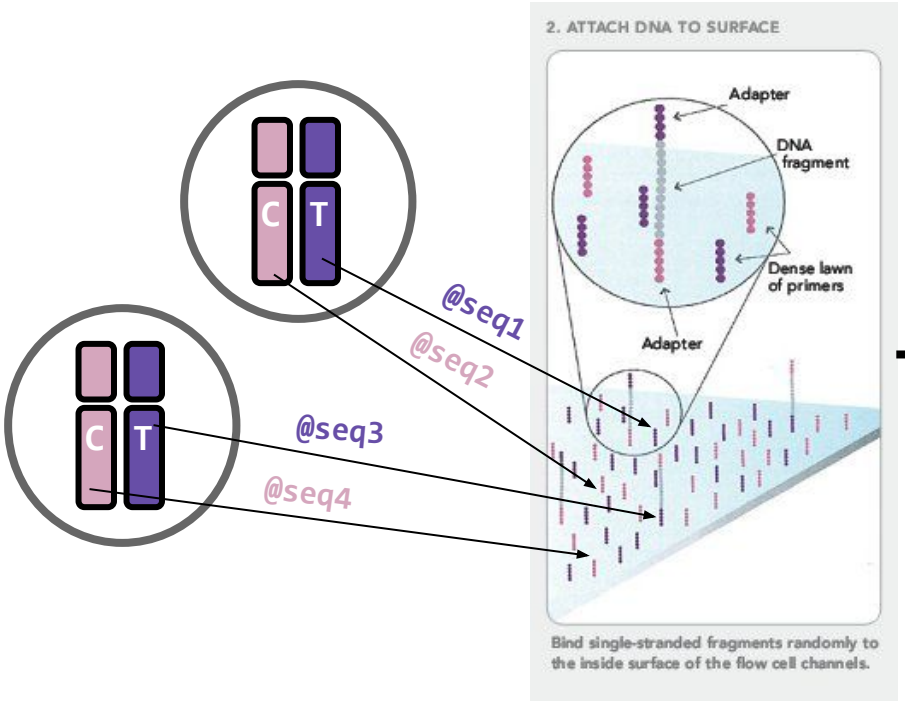
Scenario: An individual is heterozygous for an "alternate" allele.




```
@seq1
ACCTTCGAACGGCGGGGGGTTACAA
+
!''*((((***+))%%%++).1***
@seq2
TGGAACCGAACGGCCCCGGTTACAT
+
!''*!!!!***+))+++++).1***
@seq3
ACCTTCGAACGGCGGGGGGTTACAA
+
!''*((((***+))%%%++).1***
@seq4
TGGAACCGAACGGCCCCGGTTACAT
+
!''*!!!!***+))+++++).1***
```

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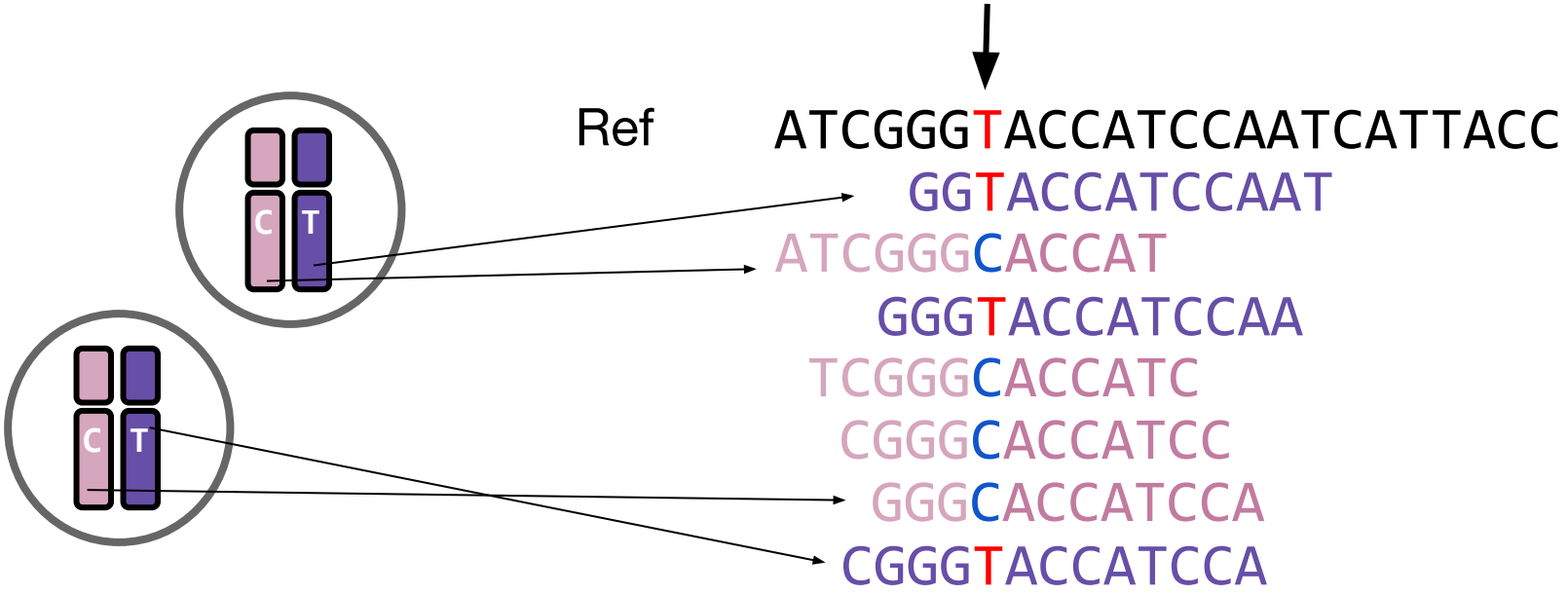
Scenario 3: An individual is heterozygous for an "alternate" allele.



Why might finding heterozygous variants be harder?

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Binomial random variables: adventures in coin flipping

P(heads) = 0.5

P(tails) = 0.5

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Thinking about allele sampling with the binomial distribution

The **binomial distribution** with parameters *n* and *p* is the discrete probability distribution of the number of successes in a sequence of *n* independent yes (e.g., "heads" or "reference allele") or no (e.g., "tails", or "alternate allele") experiments, each of which yields success with probability *p*.

The probability of getting exactly k successes in n trials is given by the probability mass function:

$$\Pr(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

What is the probability of seeing k=1 tails in n=3 flips of a fair coin with the probability of a tail (p) = 0.5?

3 choose 1 = 3; 0.5^1 = 0.5; $(1-0.5)^{(3-1)}$ = 0.25. So.... 3*0.5*0.25 = **0.375**

In R, the function would be: `dbinom(1, size=3, prob=0.5)`

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

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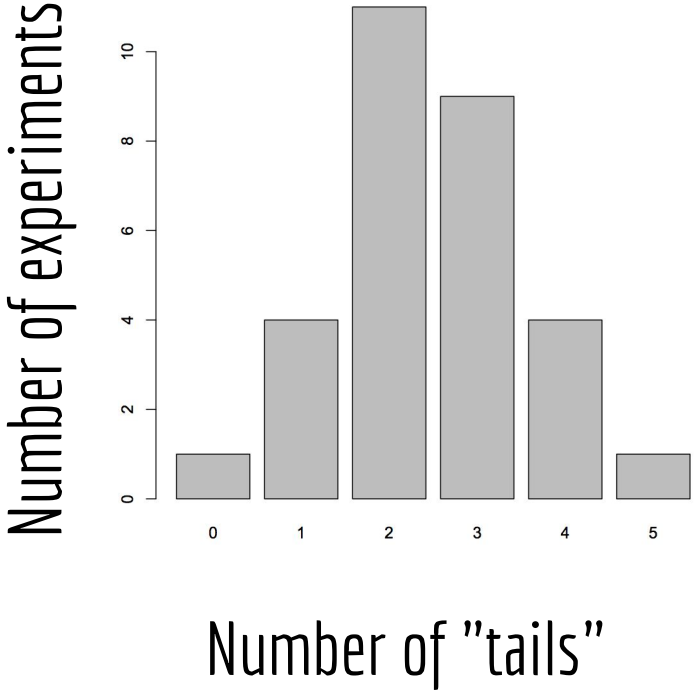

R code:

```
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)?

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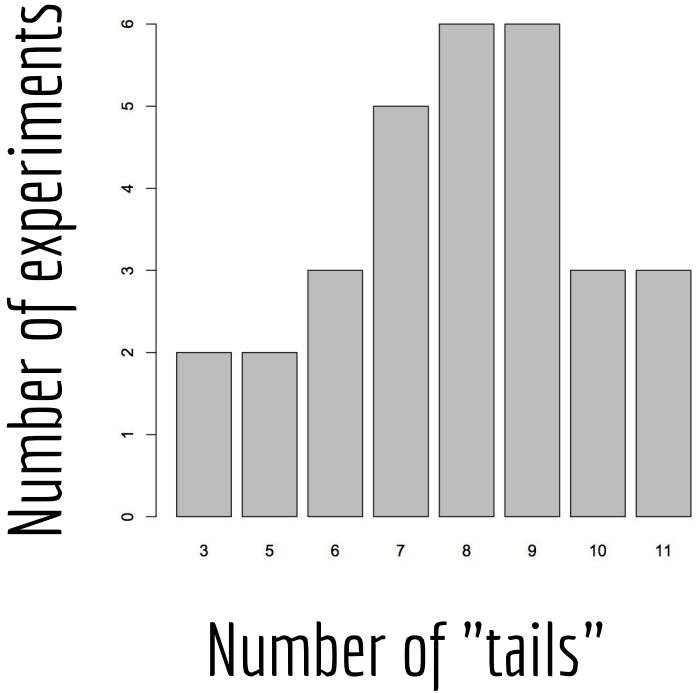

R code:

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

30 experiments (students tossing coins)
15 tosses each
Probability of Tails

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

Record your result in the following spreadsheet:

https://docs.google.com/spreadsheets/d/1i8sA1KMeYc9UhWTnCg0tLFjCy8x5LIsBITcXrz5La94/edit?usp=sharing

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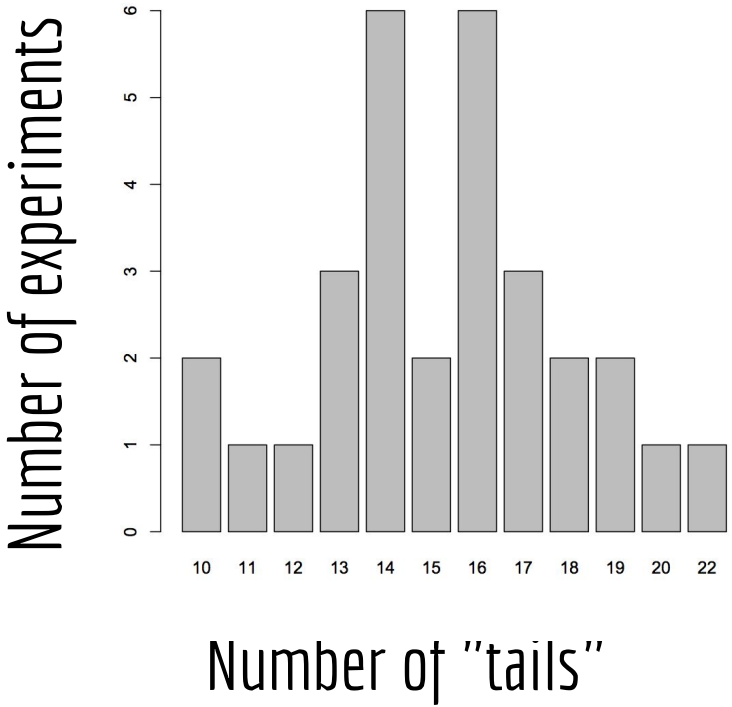
What is the distribution of tails (alternate alleles) do we expect to see after 30 tosses (sequence reads)?



R code:

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

30 experiments (students tossing coins)
30 tosses each
Probability of Tails

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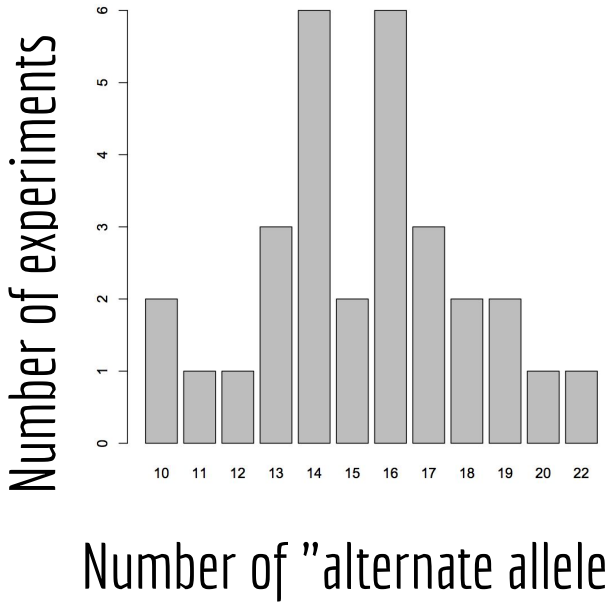
So, with 30 tosses (reads), we are much more likely to see an even mix of alternate and reference alleles at a heterozygous locus in a genome



This is why at least a "30X" (30 fold sequence coverage) genome is recommended: it confers sufficient power to find the majority of heterozygous alleles

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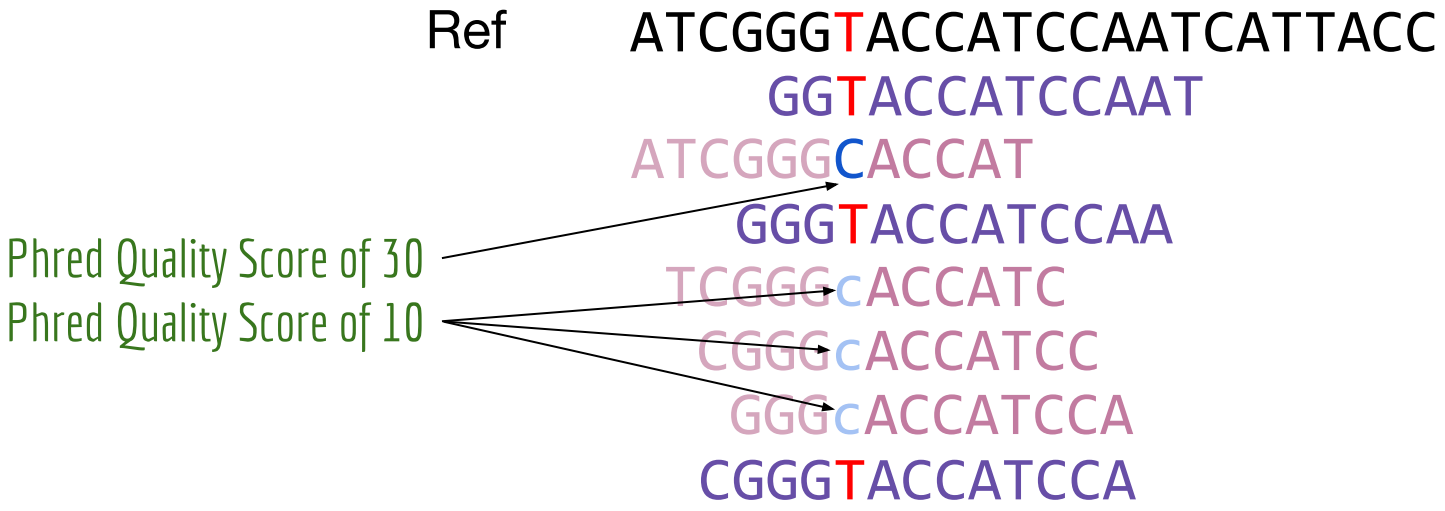
Depth tackles the allele sampling issue and lower quality scores



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Some real examples of SNPs in IGV: validating variants via manual review

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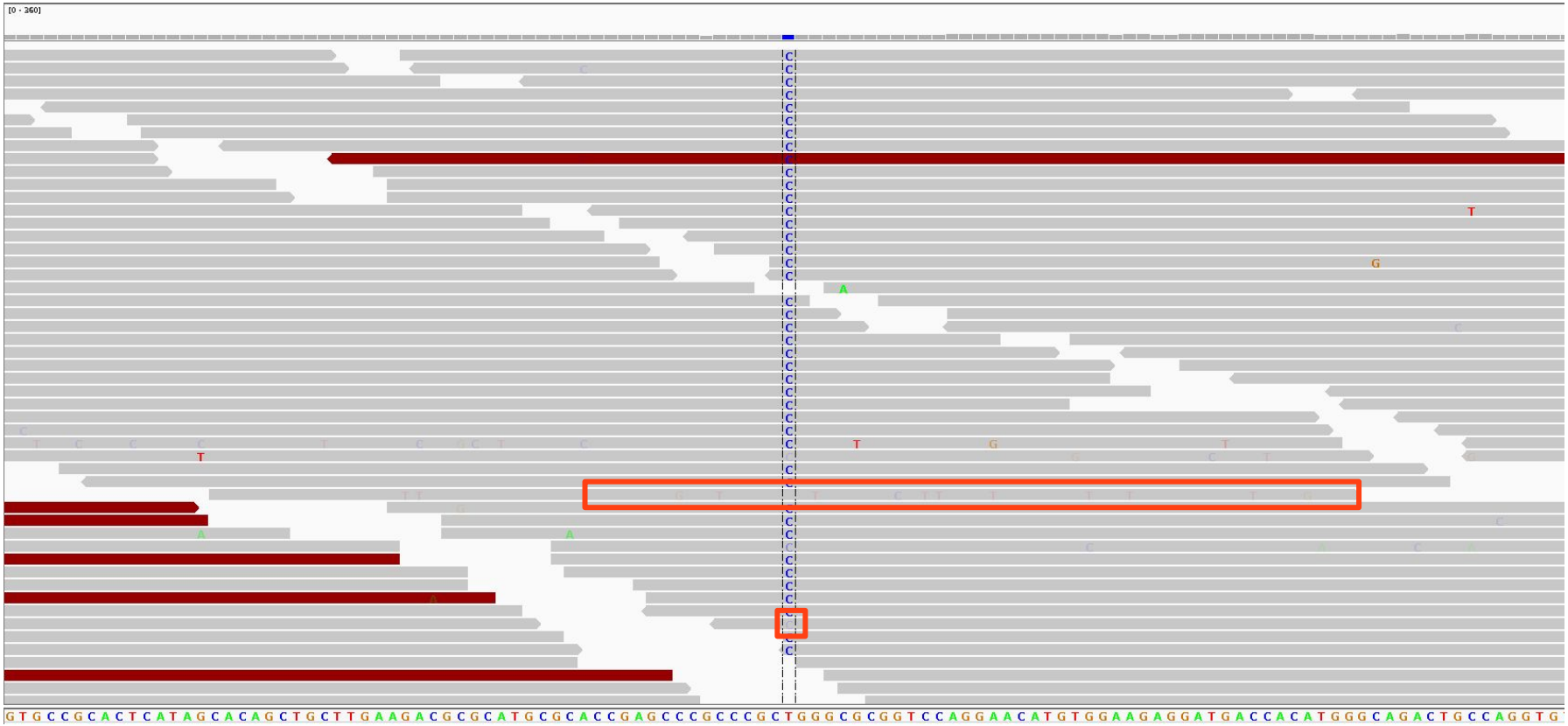
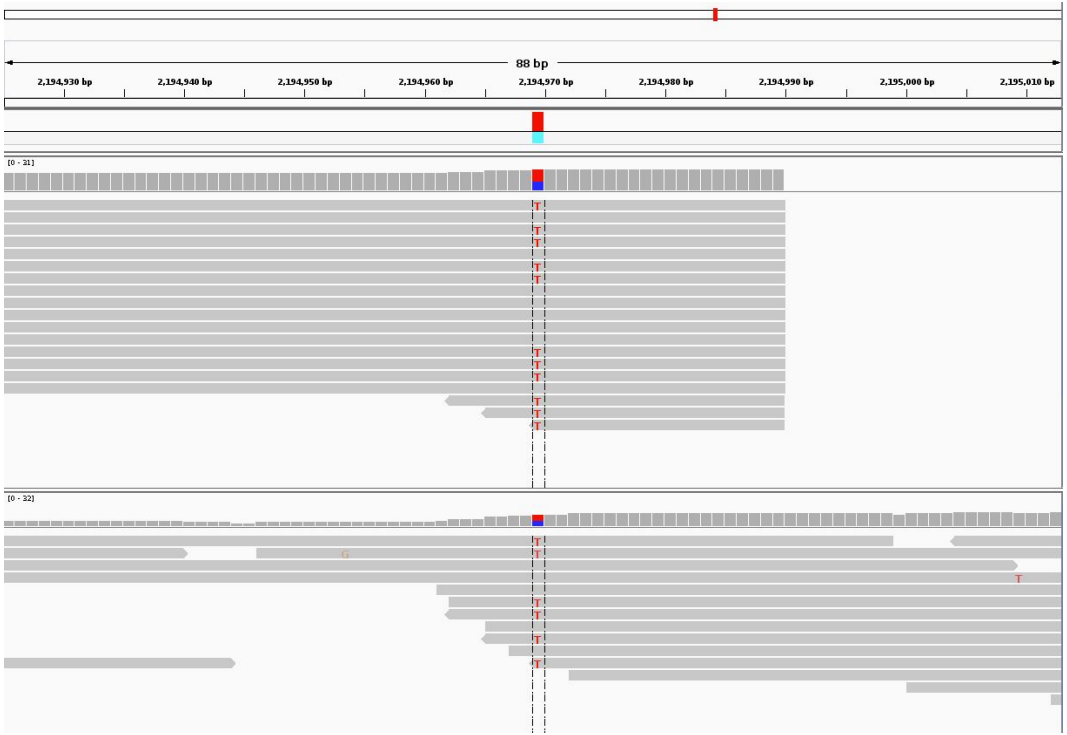
Homozygous for the "C" allele



Heterozygous for the alternate allele

Individual 1

Individual 2



Which genotype prediction would you have more confidence in?

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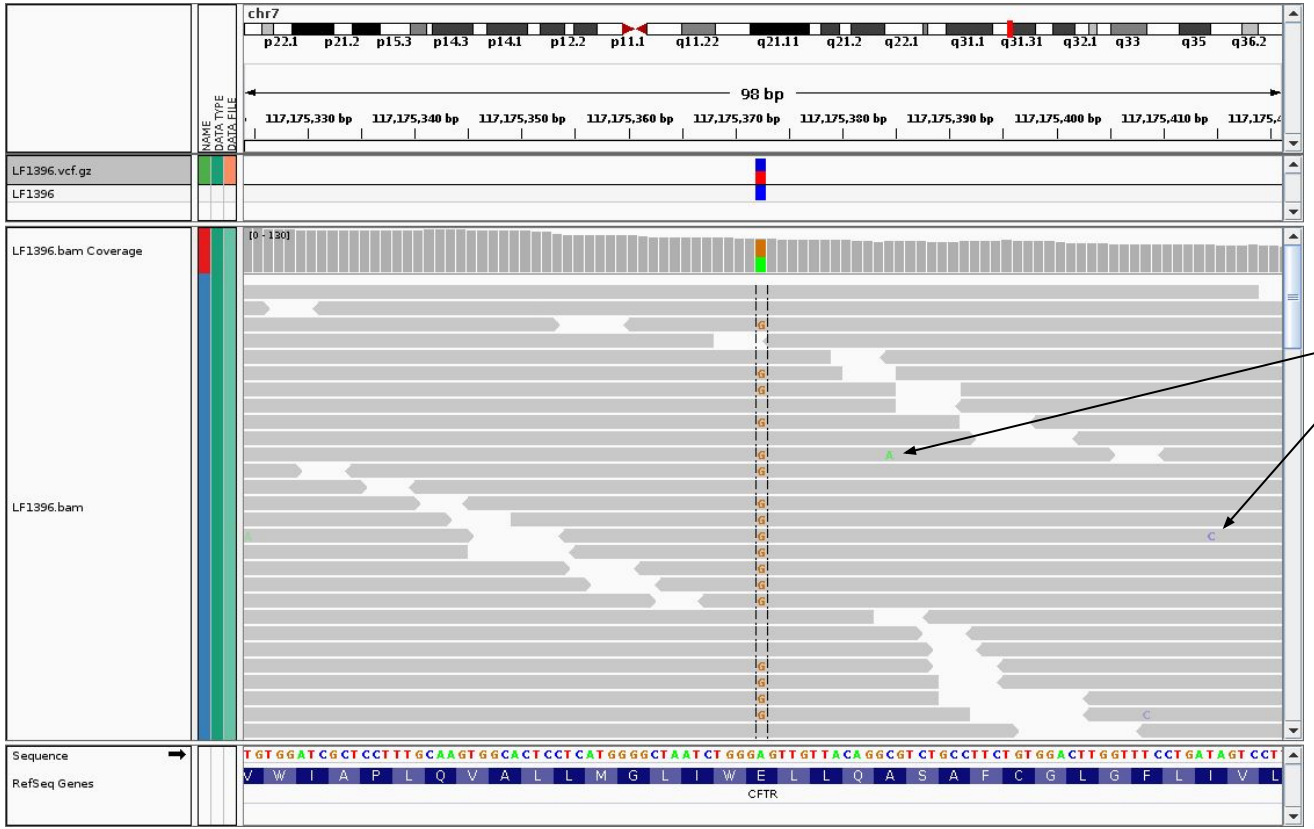
Sequencing errors fall out as noise (most of the time)



Sequencing errors

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