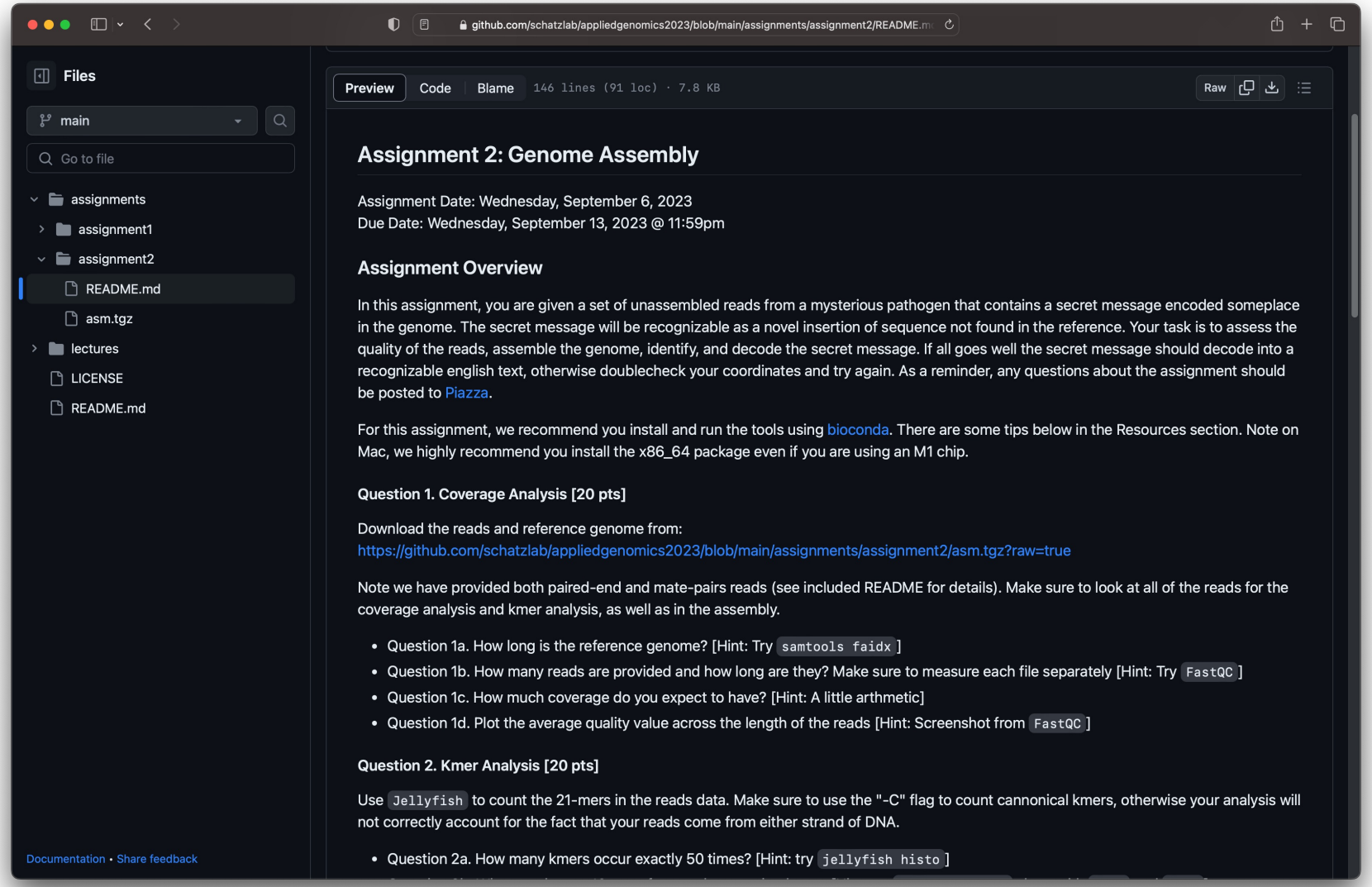
Practical Assembly pt2

Michael Schatz

Sept 13, 2023

Lecture 5: Applied Comparative Genomics

Assignment 2: Genome Assembly
Due Wednesday Sept 13 by 11:59pm



https://github.com/schatzlab/appliedgenomics2023/tree/main/assignments/assignment2

Check Piazza for questions!

Pop Quiz!

I want to sequence a 10Mbp genome to 24x coverage.
How many 120bp reads do I need?

I need 10Mbp x 24x = 240Mbp of data
240Mbp / 120bp / read = 2M reads

I want to sequence a 10Mbp genome so that
>97.5% of the genome has at least 24x coverage.
How many 120bp reads do I need?

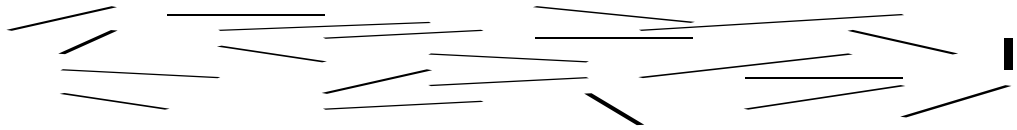
Find X such that X-2*sqrt(X) = 24

36-2*sqrt(36) = 24

I need 10Mbp x 36x = 360Mbp of data
360Mbp / 120bp / read = 3M reads

Assembling a Genome

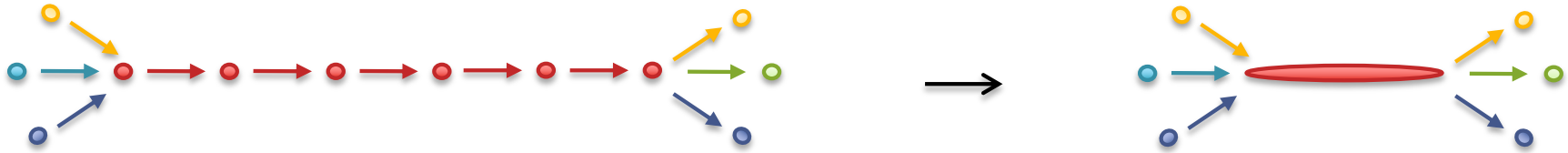
1. Shear & Sequence DNA



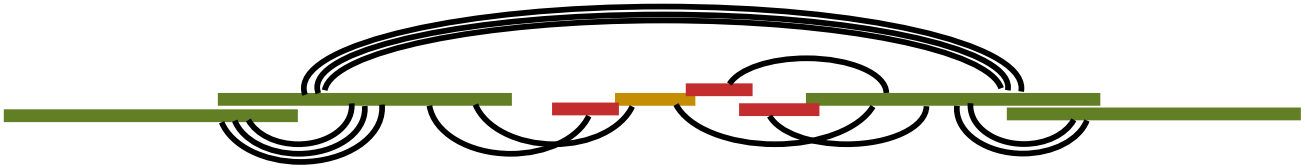
2. Construct assembly graph from reads (de Bruijn / overlap graph)

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

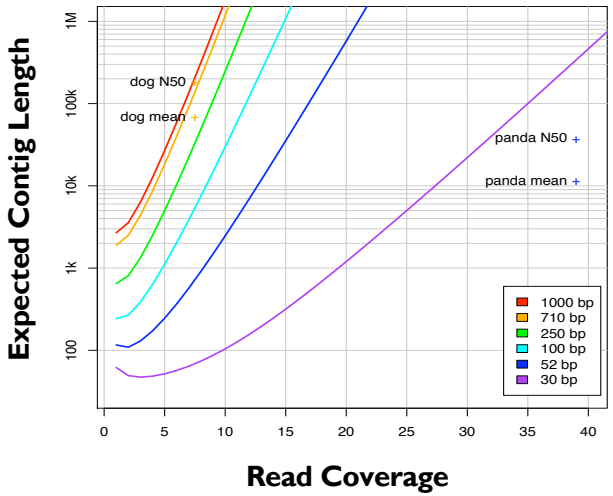


4. Detangle graph with long reads, mates, and other links



Ingredients for a good assembly

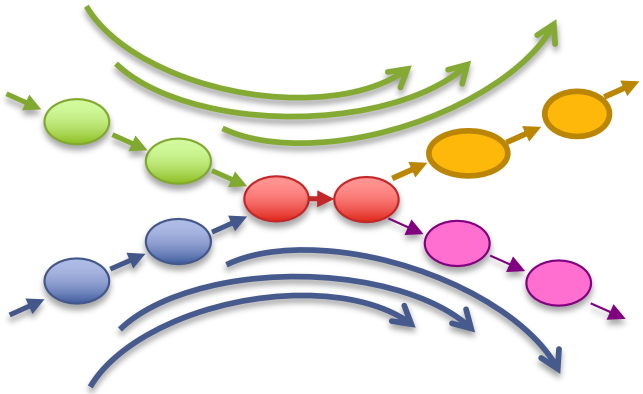
Coverage



High coverage is required

- Oversample the genome to ensure every base is sequenced with long overlaps between reads
- Biased coverage will also fragment assembly

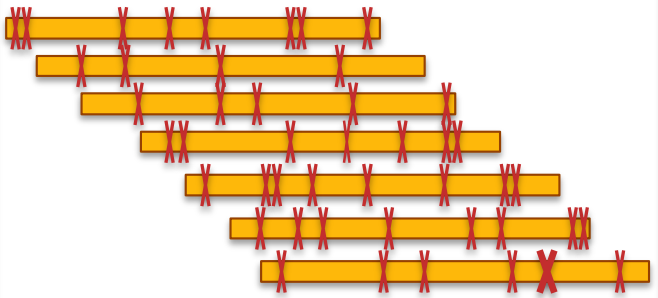
Read Length



Reads & mates must be longer than the repeats

- Short reads will have ***false overlaps*** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

Quality



Errors obscure overlaps

- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

Coverage Statistics

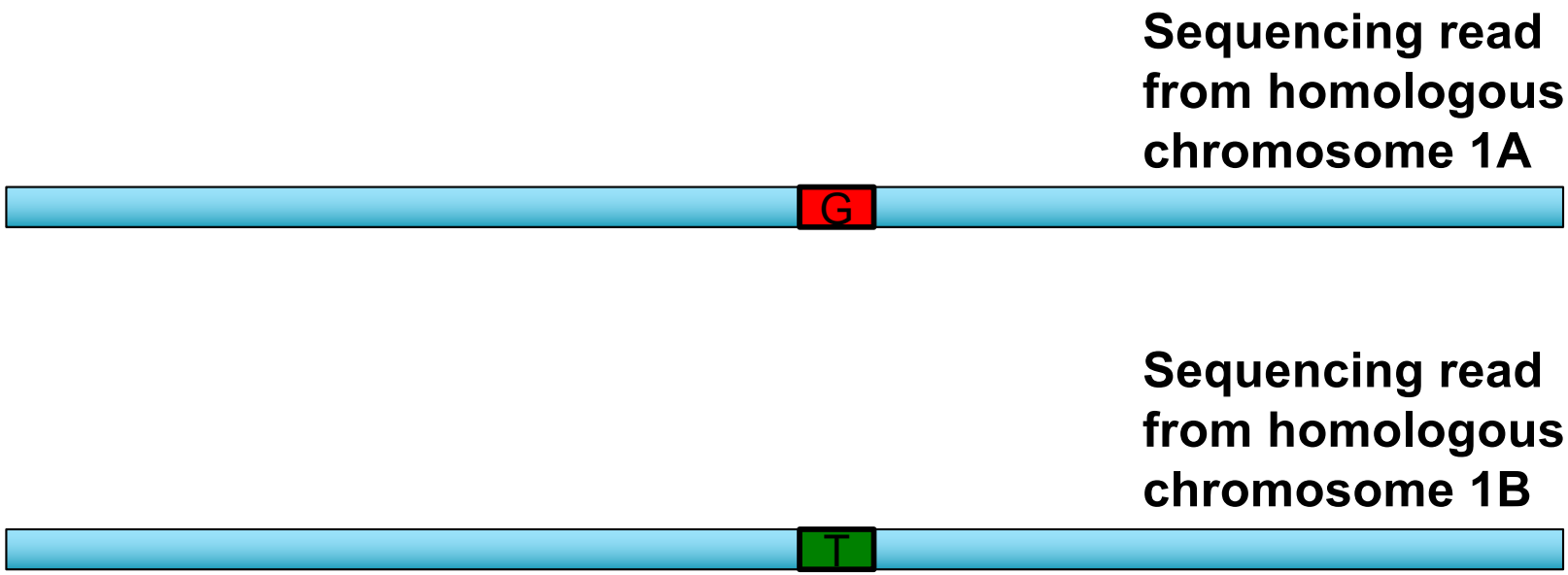
$$\text{sequencing\_coverage} = \frac{\text{total\_bases\_sequenced}}{\text{genome\_size}}$$

$$\text{genome\_size} = \frac{\text{total\_bases\_sequenced}}{\text{sequencing\_coverage}}$$

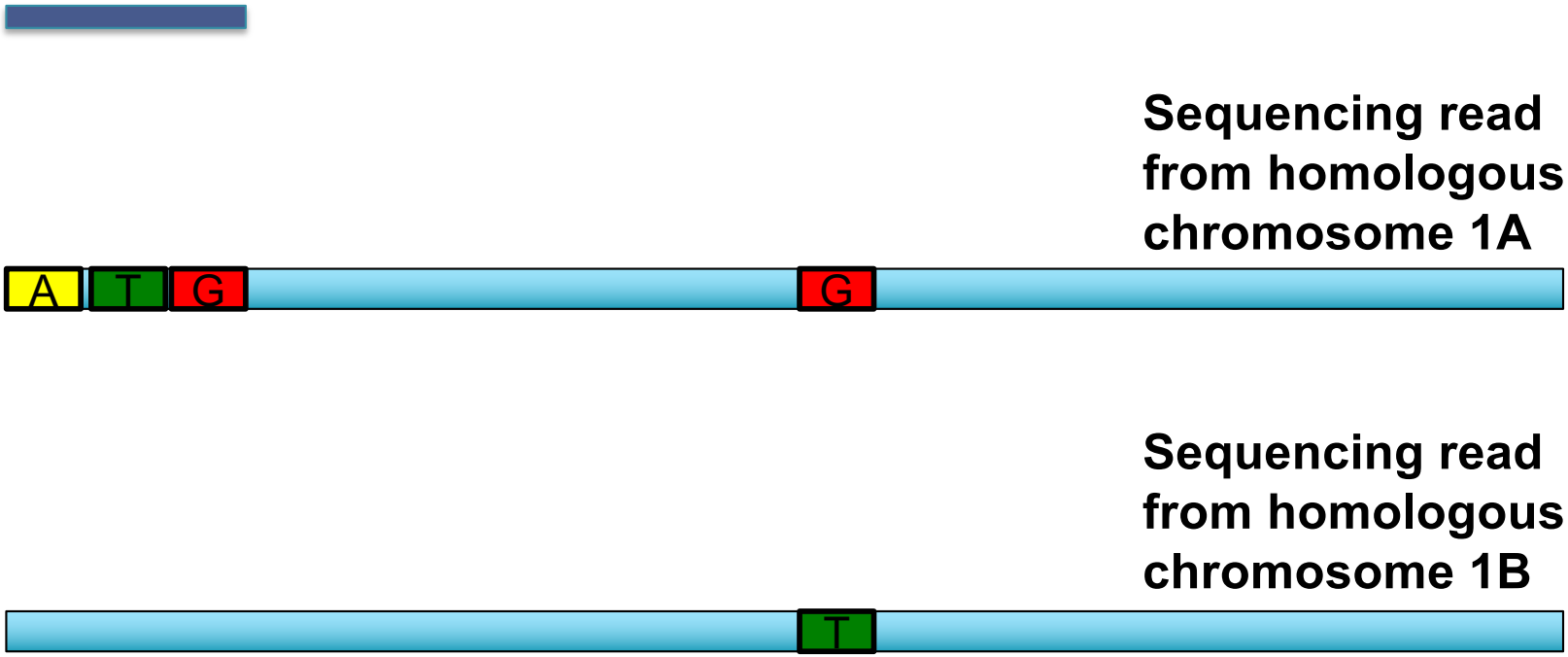
$$\text{genome\_size} = \frac{100\text{Gb}}{50\text{x}} = 2\text{Gb}$$

But how can you figure out
the coverage without a genome?

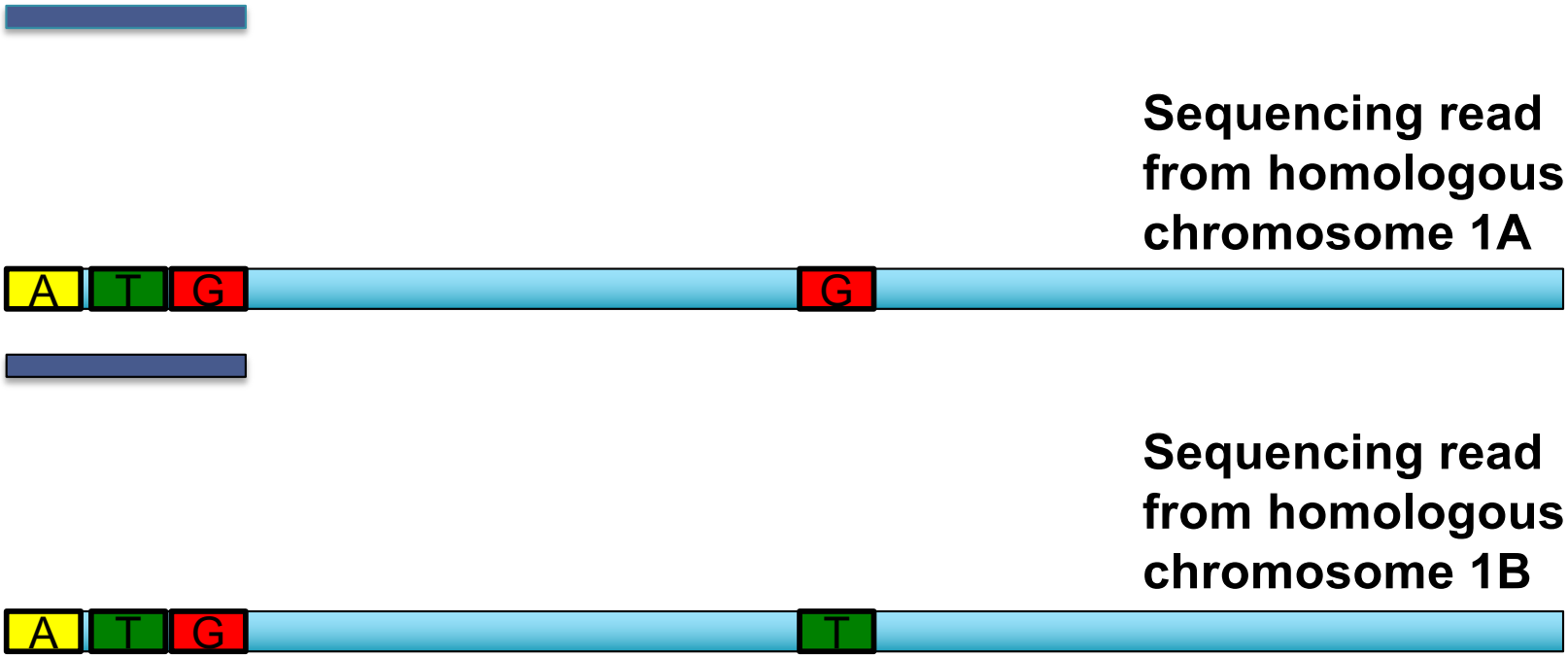
K-mer counting in heterozygous genomes



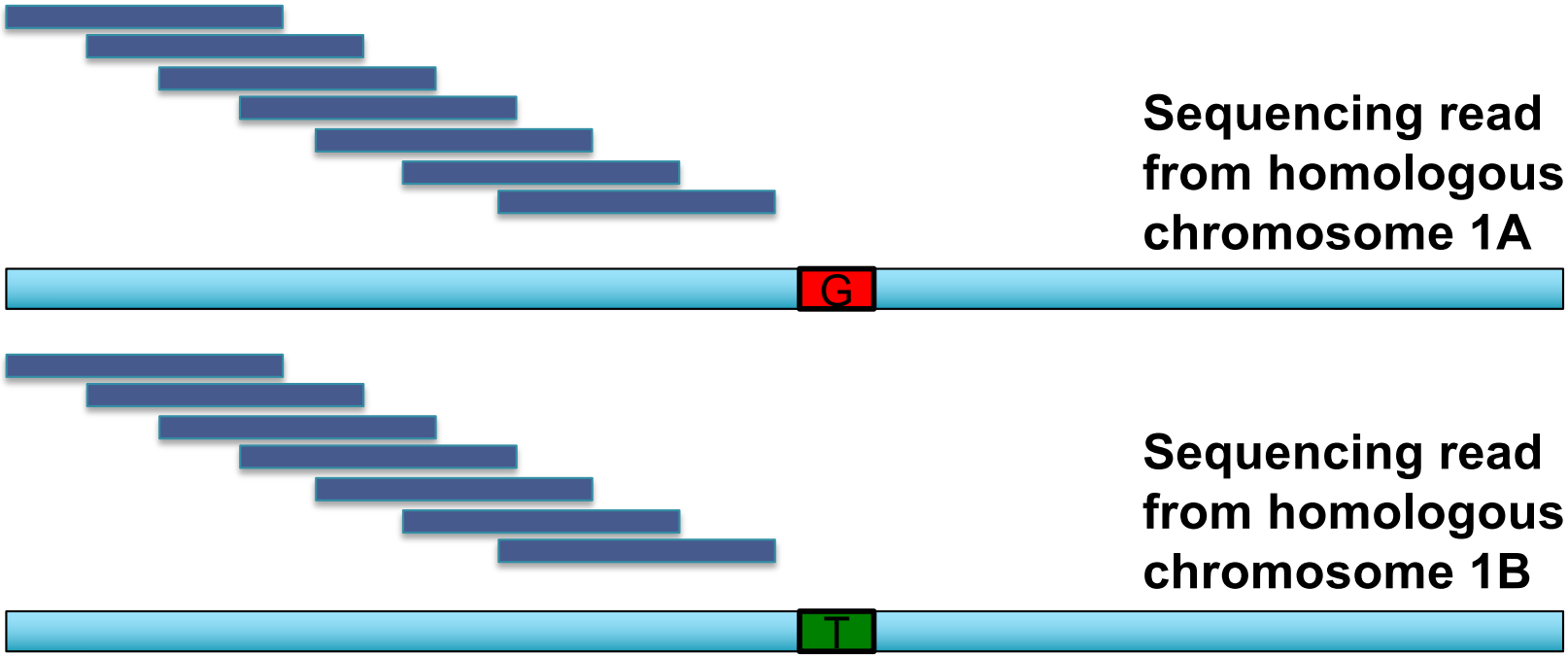
K-mer counting in heterozygous genomes



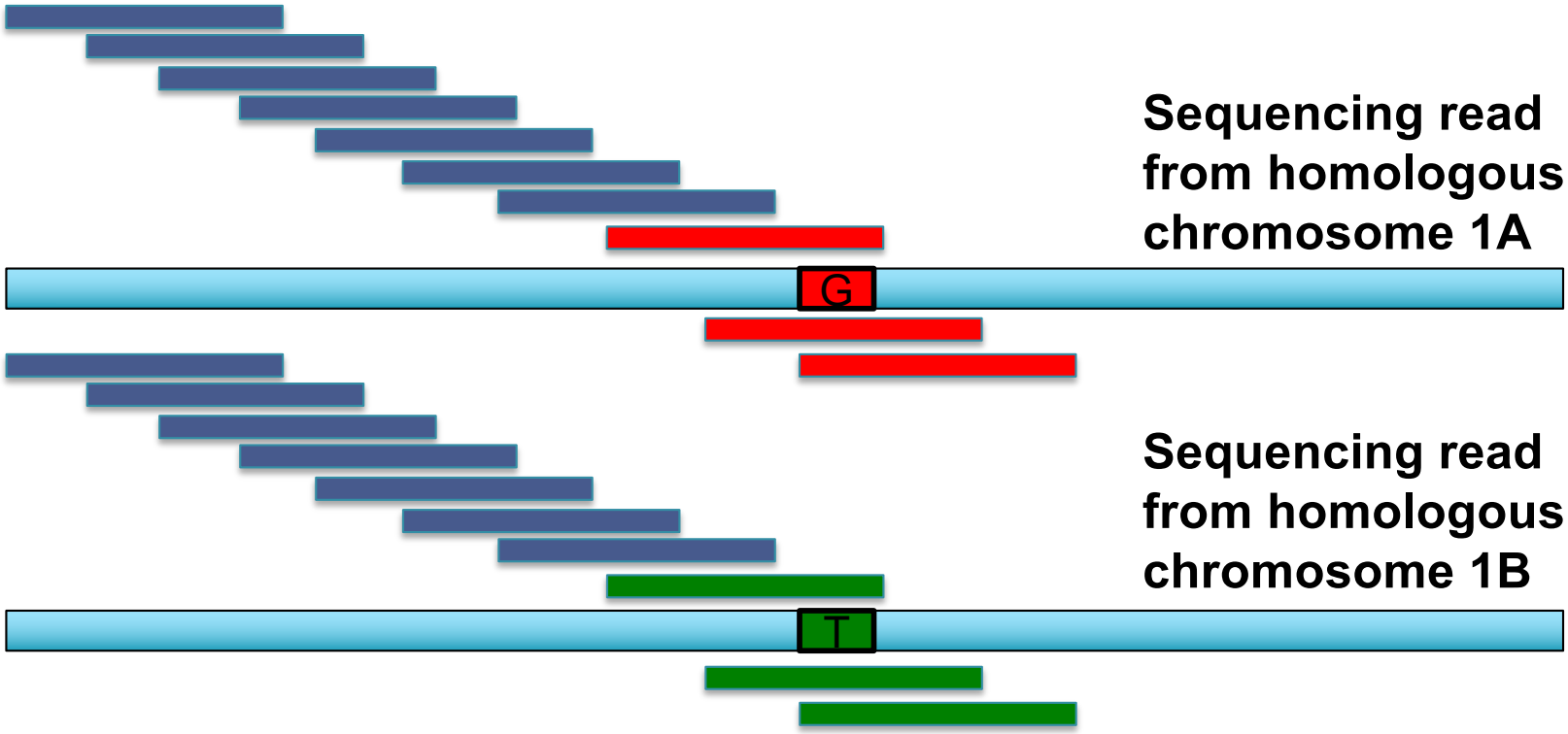
K-mer counting in heterozygous genomes



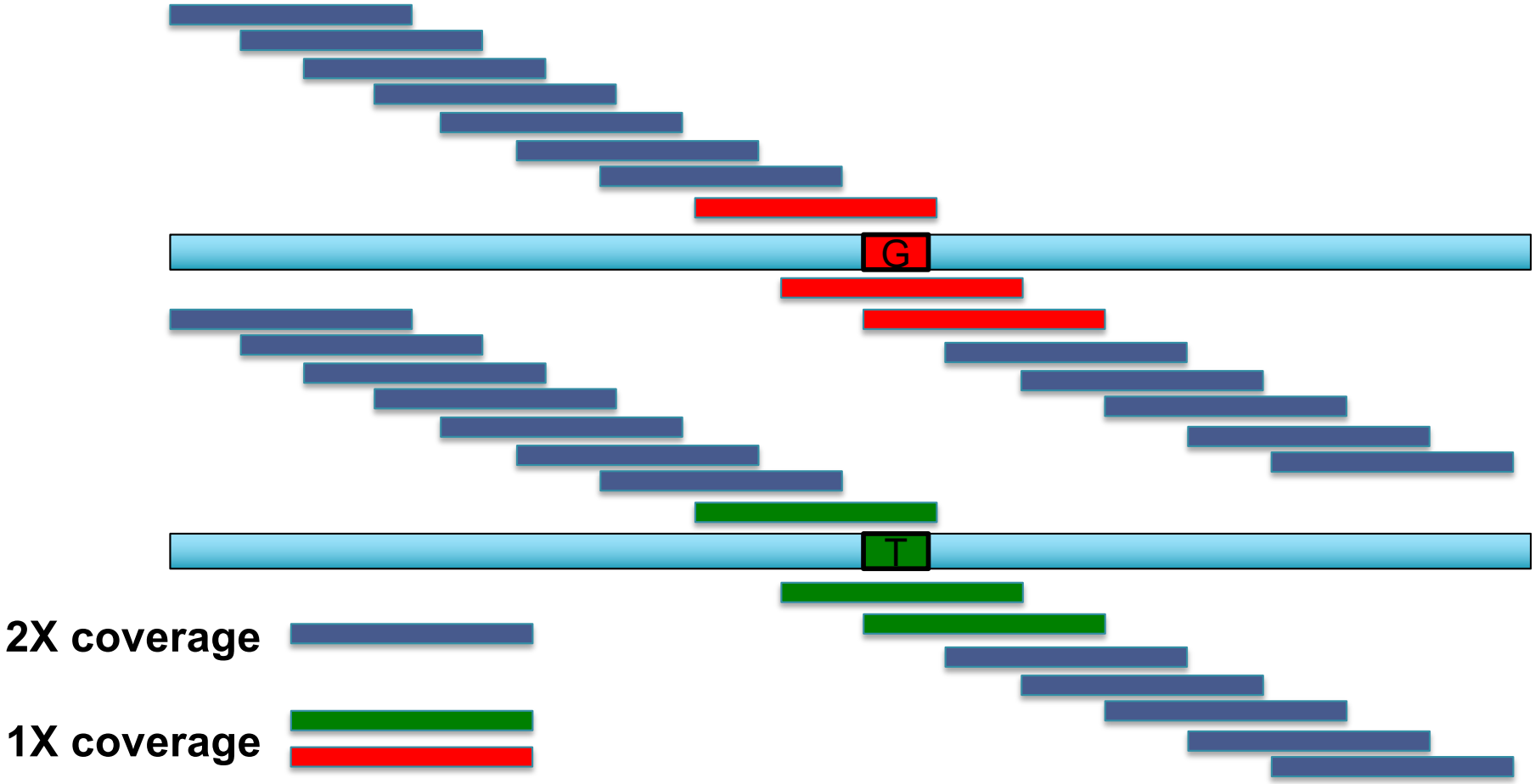
K-mer counting in heterozygous genomes



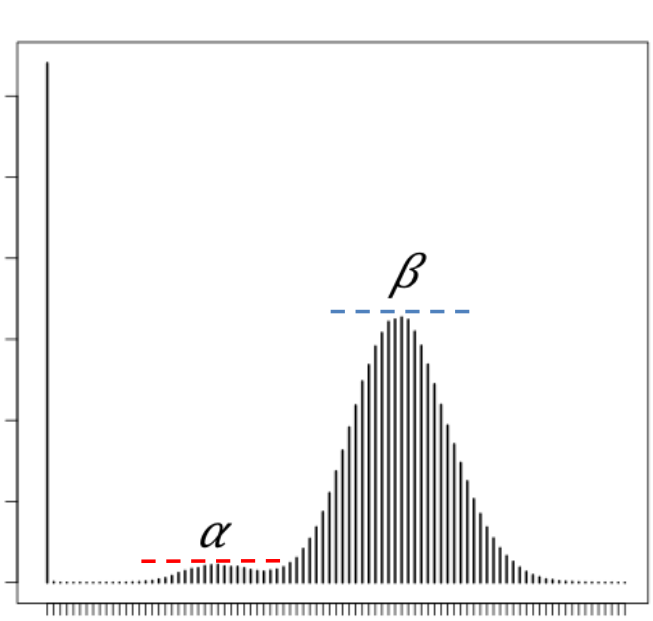
K-mer counting in heterozygous genomes



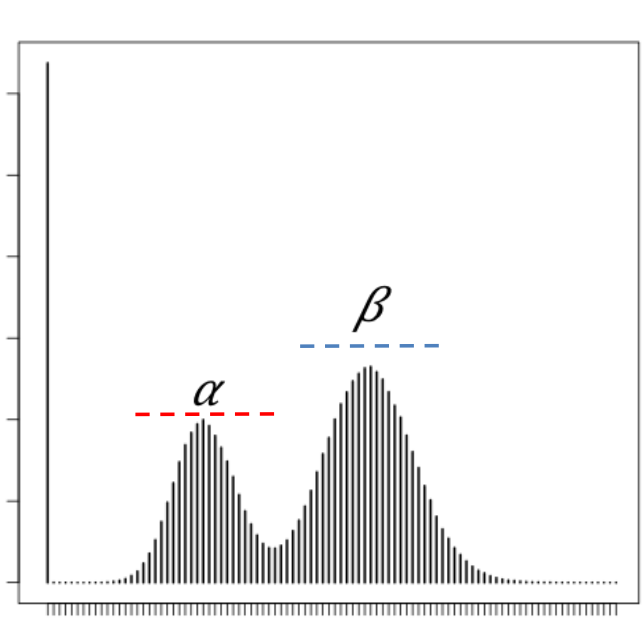
K-mer counting in heterozygous genomes



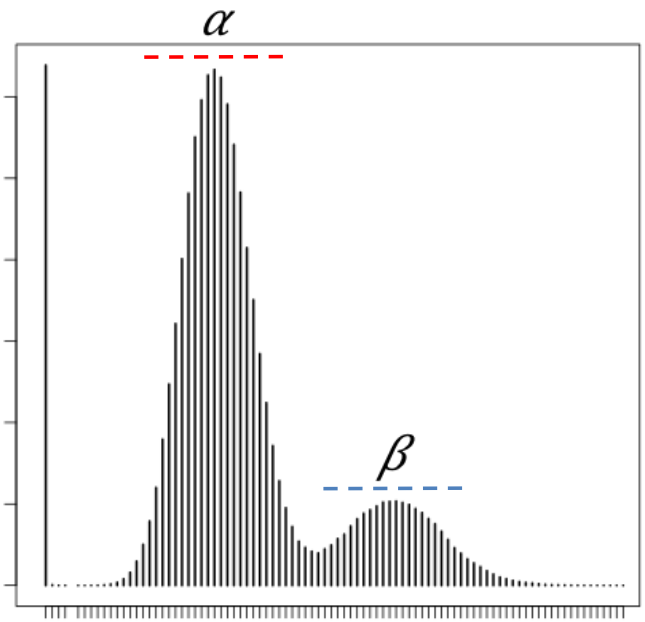
Heterozygous Kmer Profiles



0.1% heterozygosity



1% heterozygosity



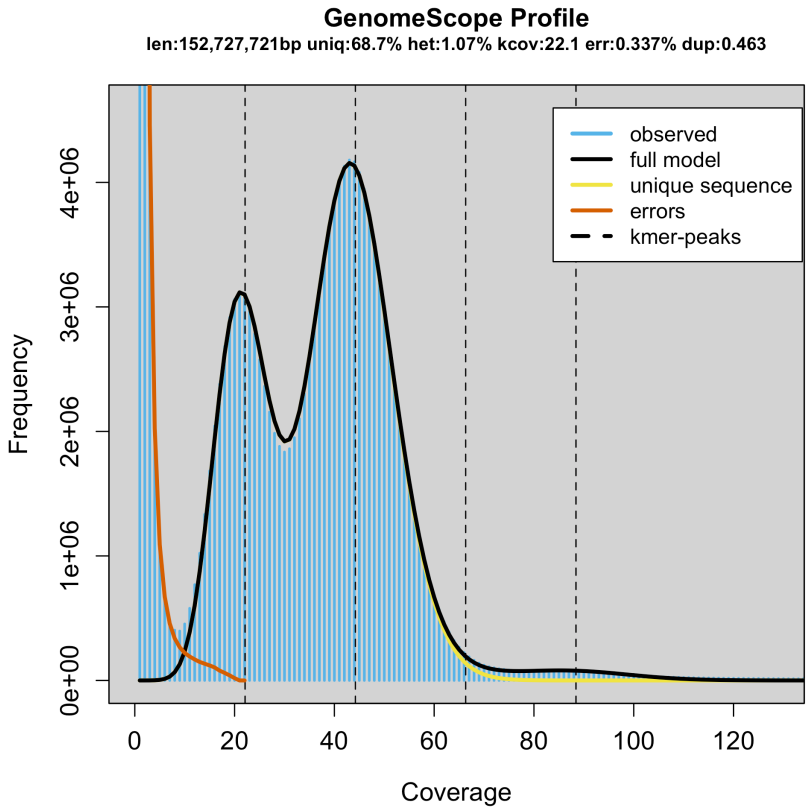
5% heterozygosity

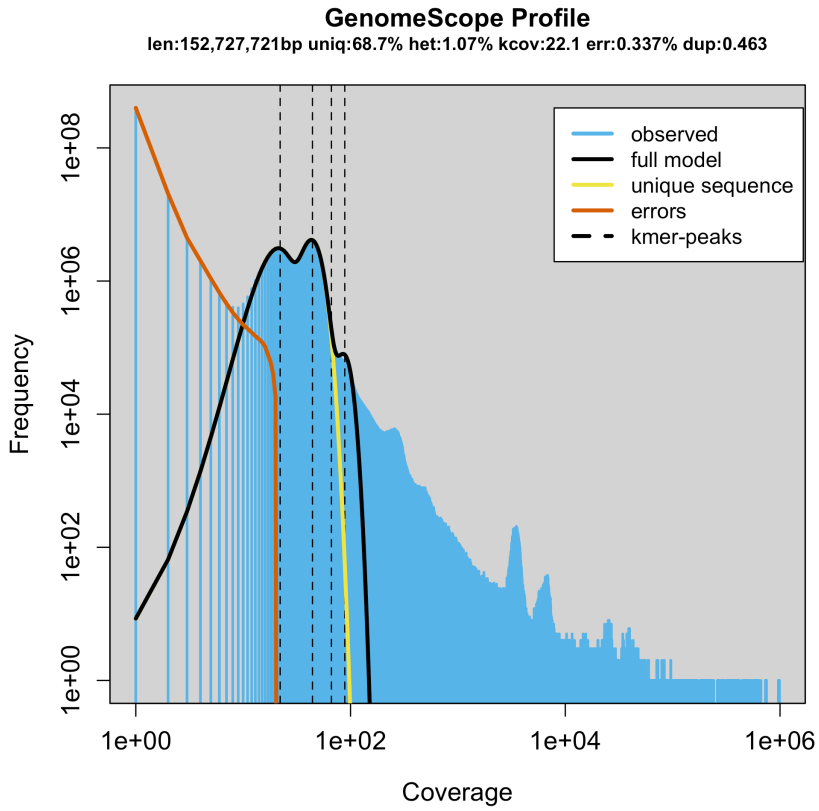
- ***Heterozygosity creates a characteristic "double-peak" in the Kmer profile***
  - Second peak at twice k-mer coverage as the first: heterozygous kmers average 50x coverage, homozygous kmers average 100x coverage

- ***Relative heights of the peaks is directly proportional to the heterozygosity rate***
  - The peaks are balanced at around 1.25% because each heterozygous SNP creates 2*k heterozygous kmers (typically k = 21)

GenomeScope: Fast genome analysis from short reads

http://genomescope.org





- Theoretical model agrees well with published results:
  - Rate of heterozygosity is higher than reported by other approaches but likely correct.
  - Genome size of plants inflated by organelle sequences (exclude very high freq. kmers)

Vurture, GW*, Sedlazeck FJ*, et al. (2017) *Bioinformatics*
Ranallo-Benavidez, TR. *et al.* (2020) *Nature Communication*

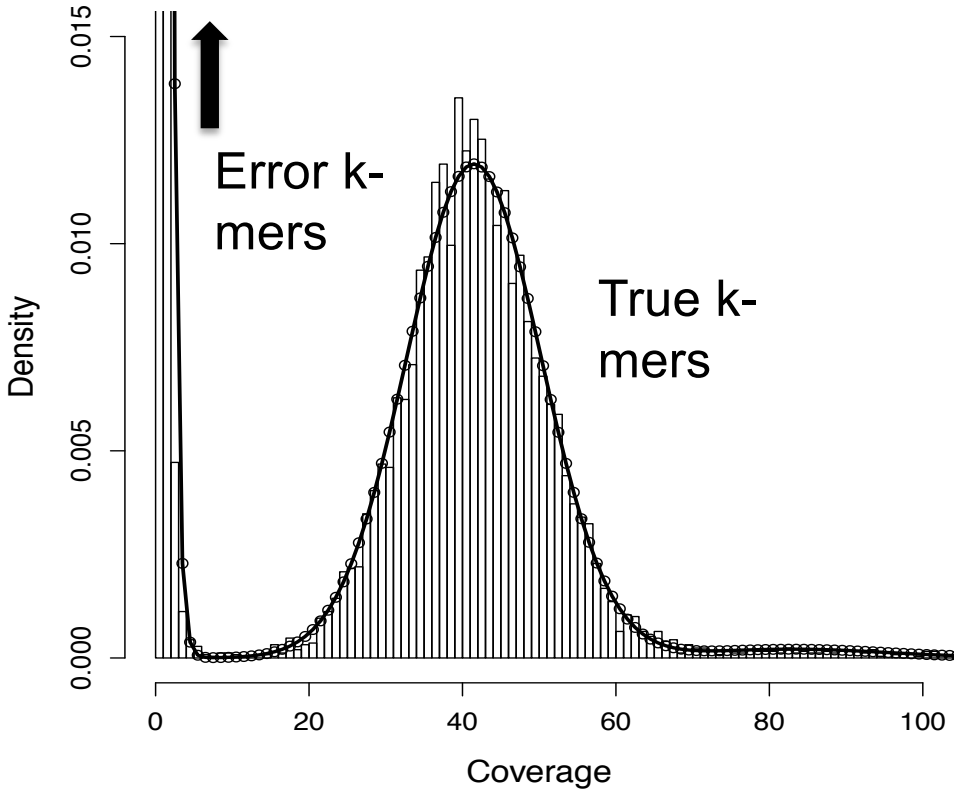
Error Correction with Quake

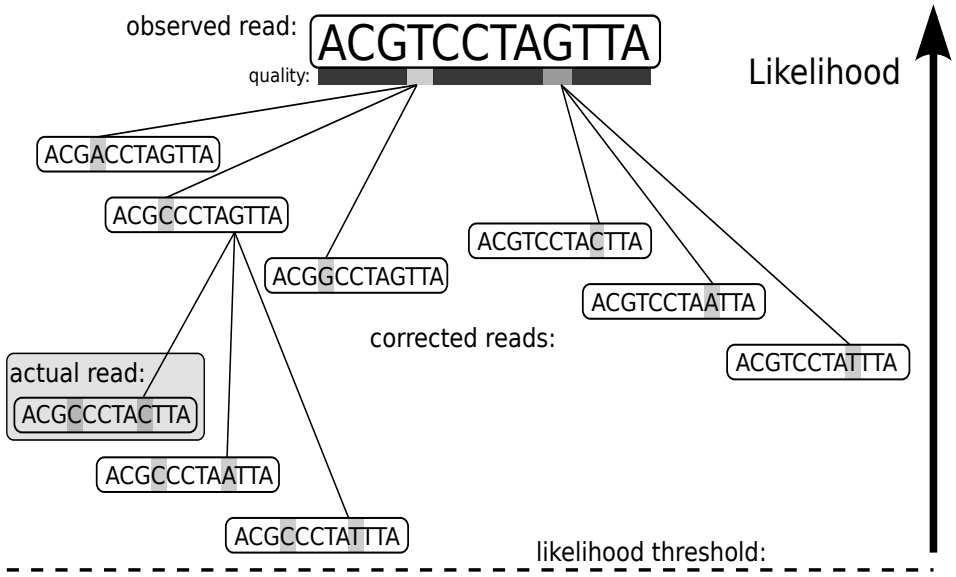
1. Count all "Q-mers" in reads

- Fit coverage distribution to mixture model of errors and regular coverage
- Automatically determines threshold for trusted k-mers

2. Correction Algorithm

- Considers editing erroneous kmers into trusted kmers in decreasing likelihood
- Includes quality values, nucleotide/nucleotide substitution rate



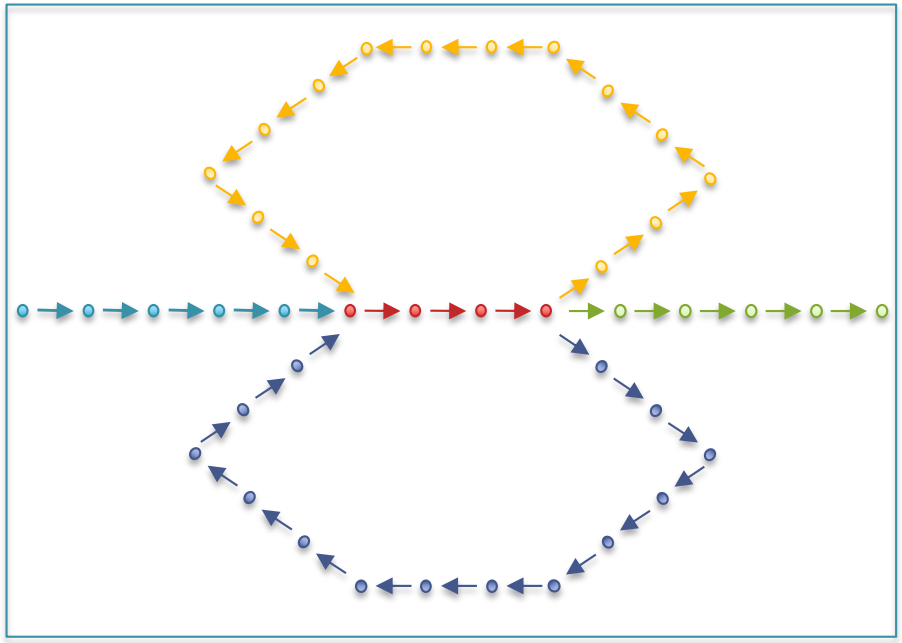


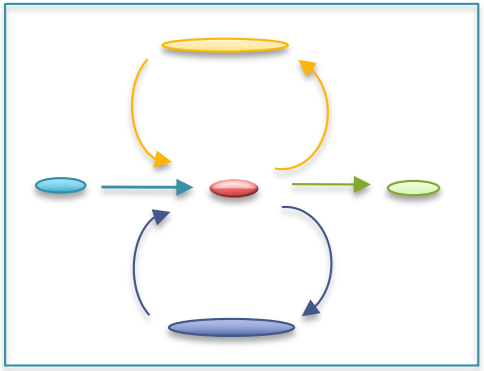
Quake: quality-aware detection and correction of sequencing reads.
Kelley, DR, Schatz, MC, Salzberg SL (2010) *Genome Biology*. 11:R116

Errors

Unitigging / Unipathing

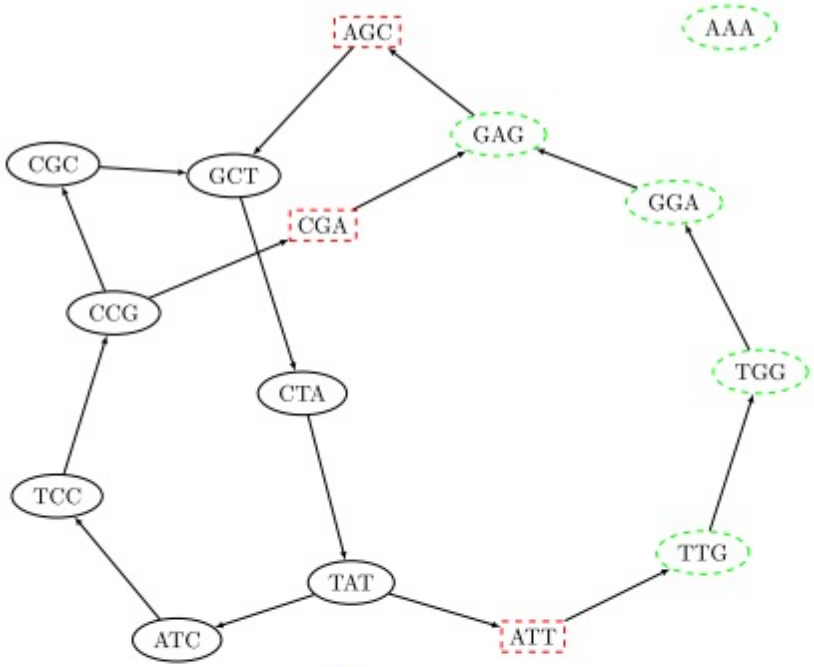
- After simplification and correction, compress graph down to its non-branching initial contigs
  - Aka "unitigs", "unipaths"





Why do contigs end?

(1) End of chromosome! ☺, (2) lack of coverage, (3) errors,
(4) heterozygosity and (5) repeats



(a)

$a_1 \ldots a_k$	$\sum_{i=1}^{k} a_i^i \bmod 10$
ATC	0
CCG	0
TCC	5
CGC	6
...	...

(b)

Bloom filter
1
0
0
0
0
1
1
0
0
0

(c)

Nodes self-information:

$$\lceil log_2 \binom{4^3}{7} \rceil = 30 \text{ bits}$$

Structure size:

$$\underbrace{10}_{\text{Bloom}} + \underbrace{3 \cdot 6}_{\text{False positives}} = 28 \text{ bits}$$

(d)

Table 2 de novo human genome (NA18507) assemblies

Method	Minia	C. & B.	ABySS	SOAPdenovo
Value of *k* chosen	27	27	27	25
Number of contigs (M)	3.49	7.69	4.35	-
Longest contig (kbp)	18.6	22.0	15.9	-
Contig N50 (bp)	1156	250	870	886
Sum (Gbp)	2.09	1.72	2.10	2.08
Nb of nodes/cores	1/1	1/8	21/168	1/16
Time (wall-clock, h)	23	50	15	33
Memory (sum of nodes, GB)	**5.7**	**32**	**336**	**140**

de novo human genome (NA18507) assemblies reported by our assembler (Minia), Conway and Bromage assembler [9], ABySS [8], and SOAPdenovo [7]. Contigs shorter than 100 bp were discarded. Assemblies were made without any pairing information.

Space-efficient and exact de Bruijn graph representation based on a Bloom filter

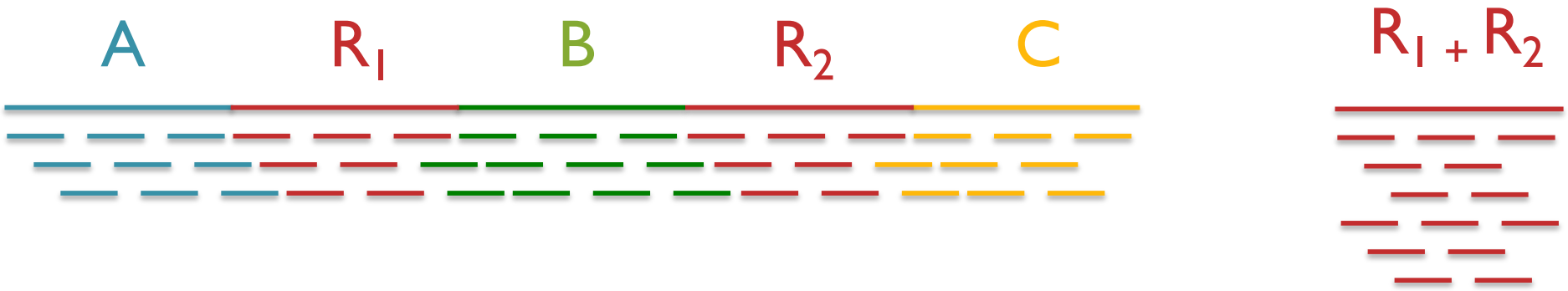
Chikhi and Rizk (2013) *Algorithms for Molecular Biology*. 8:22

Repetitive regions

Repeat Type	Definition / Example	Prevalence
Low-complexity DNA / Microsatellites	$(b_1 b_2 \ldots b_k)^N$ where $1 \leq k \leq 6$ CACACACACACACACACACA	2%
SINEs (Short Interspersed Nuclear Elements)	*Alu* sequence (~280 bp) Mariner elements (~80 bp)	13%
LINEs (Long Interspersed Nuclear Elements)	~500 – 5,000 bp	21%
LTR (long terminal repeat) retrotransposons	Ty1-copia, Ty3-gypsy, Pao-BEL (~100 – 5,000 bp)	8%
Other DNA transposons		3%
Gene families & segmental duplications		4%

- Over 50% of mammalian genomes are repetitive
  - Large plant genomes tend to be even worse
  - Wheat: 16 Gbp; Pine: 24 Gbp

Repeats and Coverage Statistics



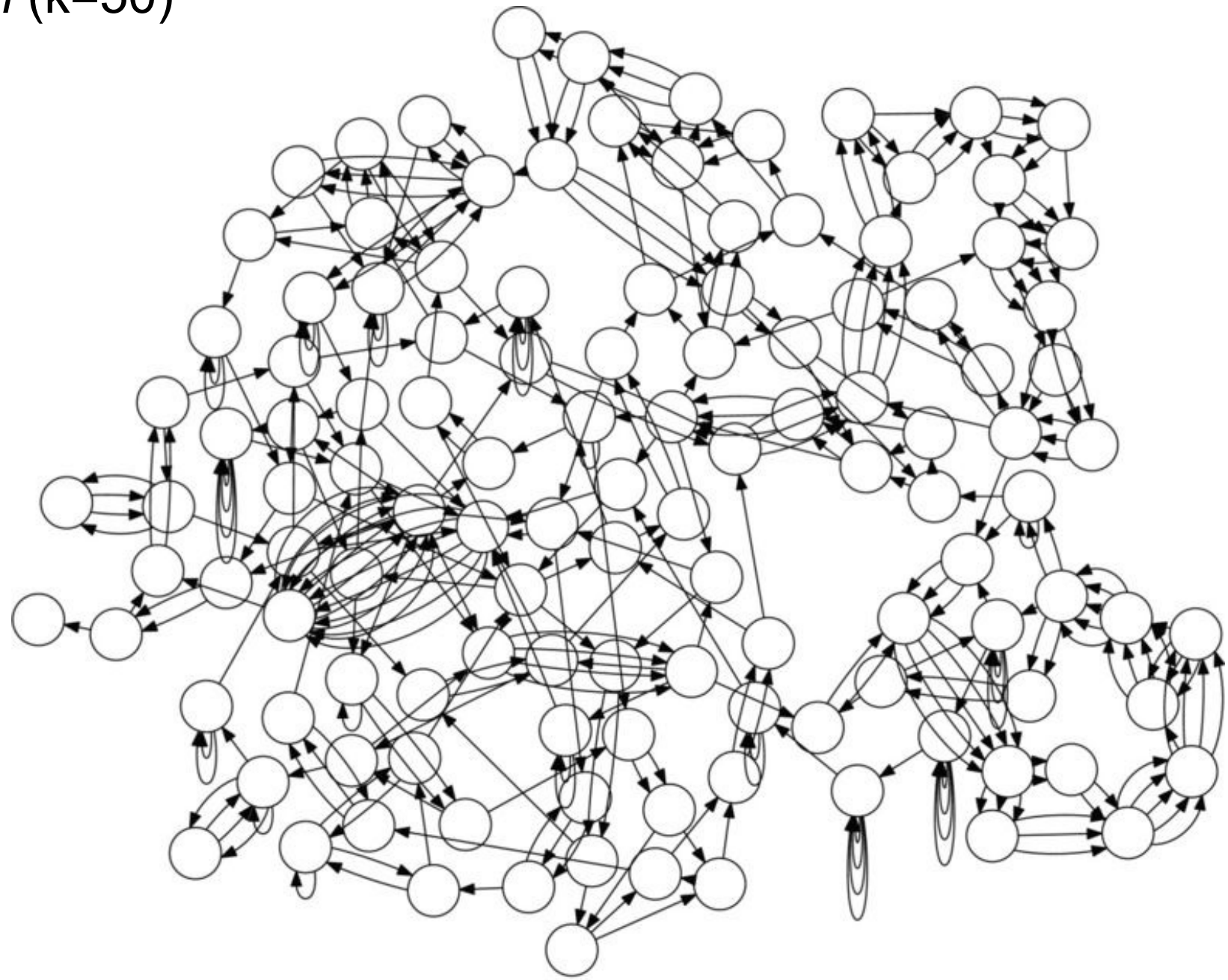
- If *n* reads are a uniform random sample of the genome of length *G*, we expect $k=n\Delta/G$ reads to start in a region of length Δ.
  - If we see many more reads than k (if the arrival rate is > A) , it is likely to be a collapsed repeat

$$\Pr(X-copy)=\binom{n}{k}\left(\frac{X\Delta}{G}\right)^{k}\left(\frac{G-X\Delta}{G}\right)^{n-k}$$

$$A(\Delta,k)=\ln\left(\frac{\Pr(1-copy)}{\Pr(2-copy)}\right)=\ln\left(\frac{\frac{(\Delta n/G)^{k}}{k!}e^{\frac{-\Delta n}{G}}}{\frac{(2\Delta n/G)^{k}}{k!}e^{\frac{-2\Delta n}{G}}}\right)=\frac{n\Delta}{G}-k\ln 2$$

The fragment assembly string graph
Myers, EW (2005) Bioinformatics. 21(suppl 2): ii79-85.

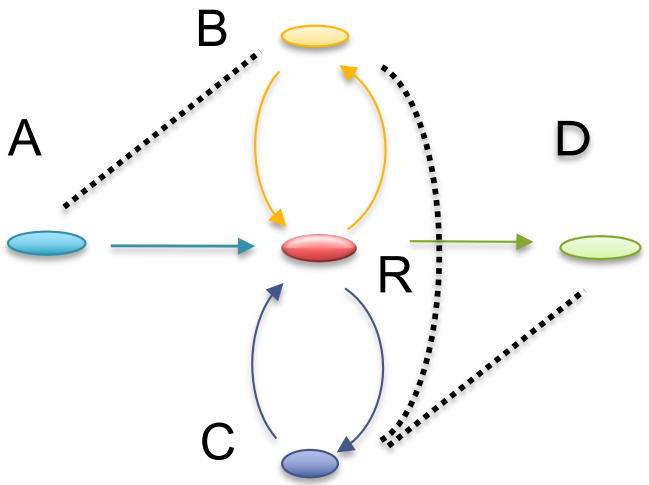
E. coli (k=50)



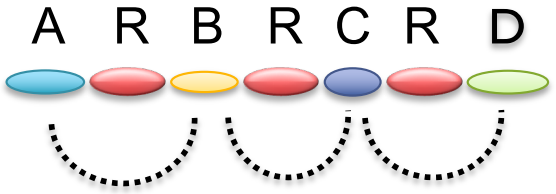
Reducing assembly complexity of microbial genomes with single-molecule sequencing
Koren et al (2013) Genome Biology. **14**:R101 https://doi.org/10.1186/gb-2013-14-9-r101

Scaffolding

- Initial contigs (*aka* unipaths, unitigs) terminate at
  - *Coverage gaps*: especially extreme GC
  - *Conflicts*: errors, repeat boundaries



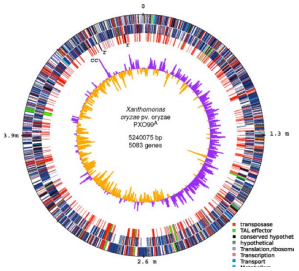
- Use mate-pairs to resolve correct order through assembly graph
  - Place sequence to satisfy the mate constraints
  - Mates through repeat nodes are tangled



- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

Why do scaffolds end?

Assembly Summary



Assembly quality depends on

1. ***Coverage***: low coverage is mathematically hopeless
2. ***Repeat composition***: high repeat content is challenging
3. ***Read length***: longer reads help resolve repeats
4. ***Error rate***: errors reduce coverage, obscure true overlaps

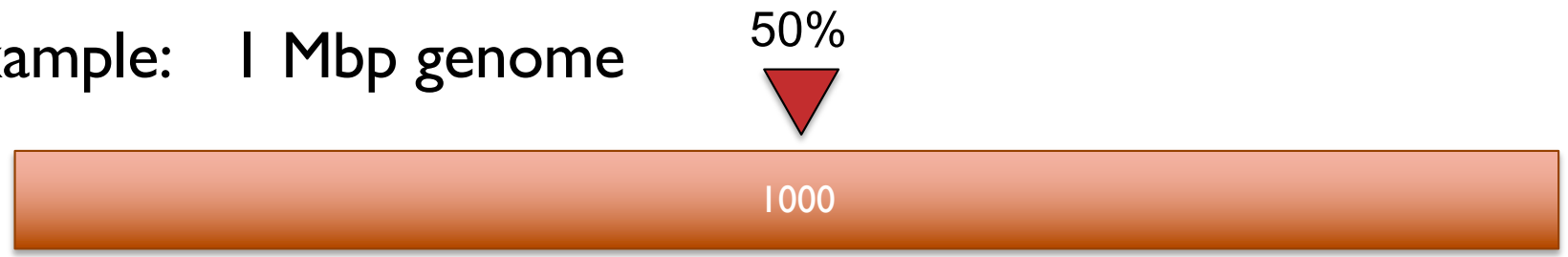
- Assembly is a hierarchical, starting from individual reads, build high confidence contigs/unitigs, incorporate the mates to build scaffolds
  - Extensive error correction is the key to getting the best assembly possible from a given data set

- Watch out for collapsed repeats & other misassemblies
  - Globally/Locally reassemble data from scratch with better parameters & stitch the 2 assemblies together

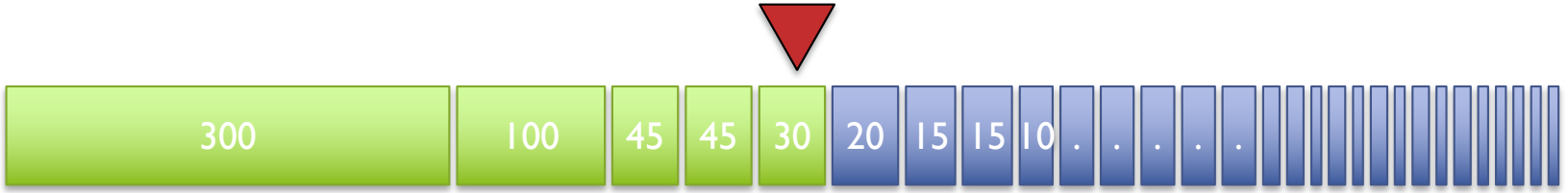
Contig N50

Def: 50% of the genome is in contigs as large as the N50 value

Example: 1 Mbp genome

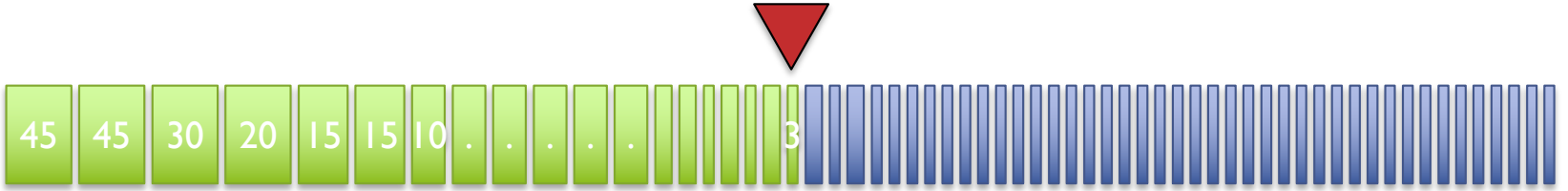


A



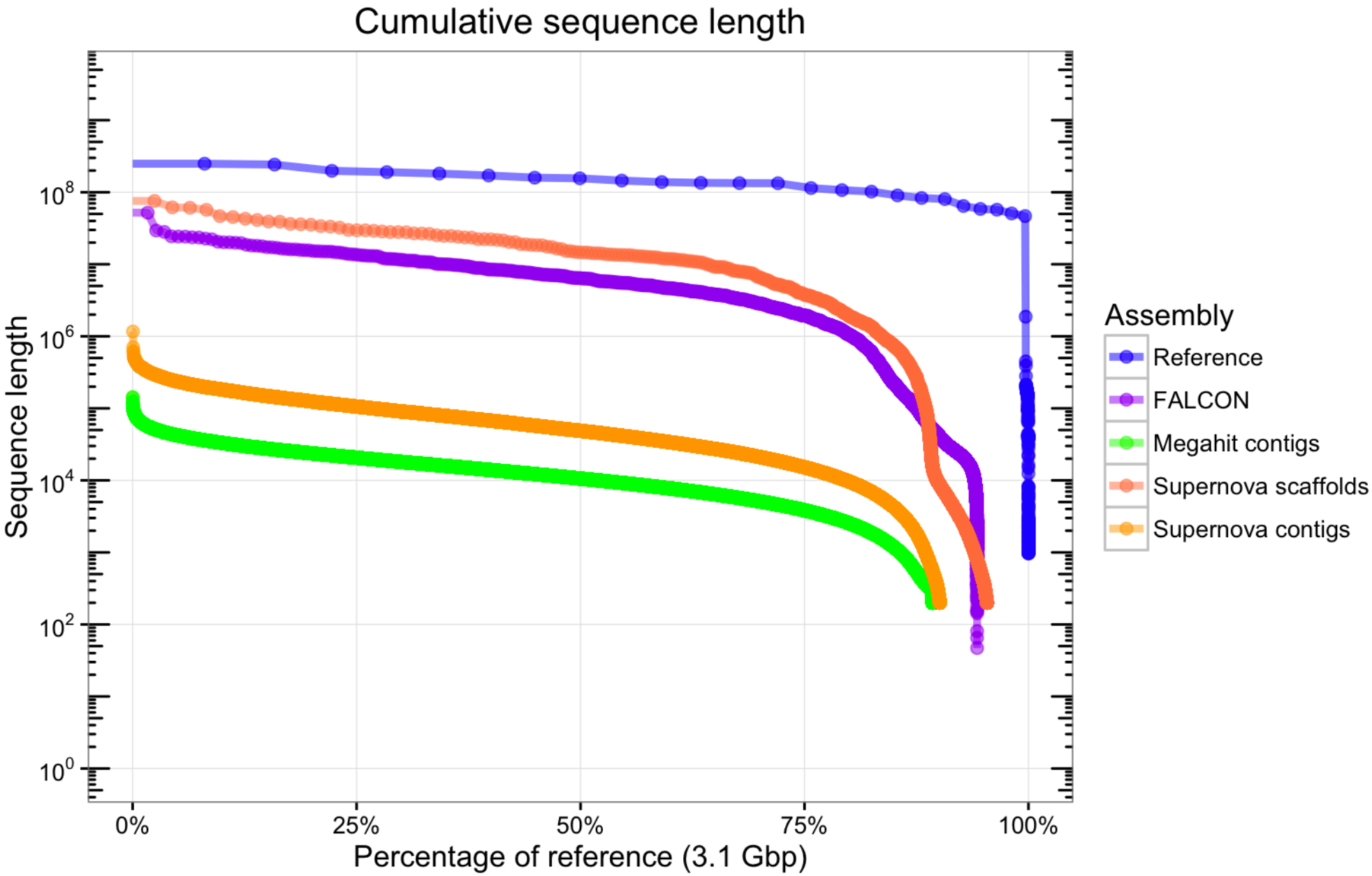
N50 size = 30 kbp

B

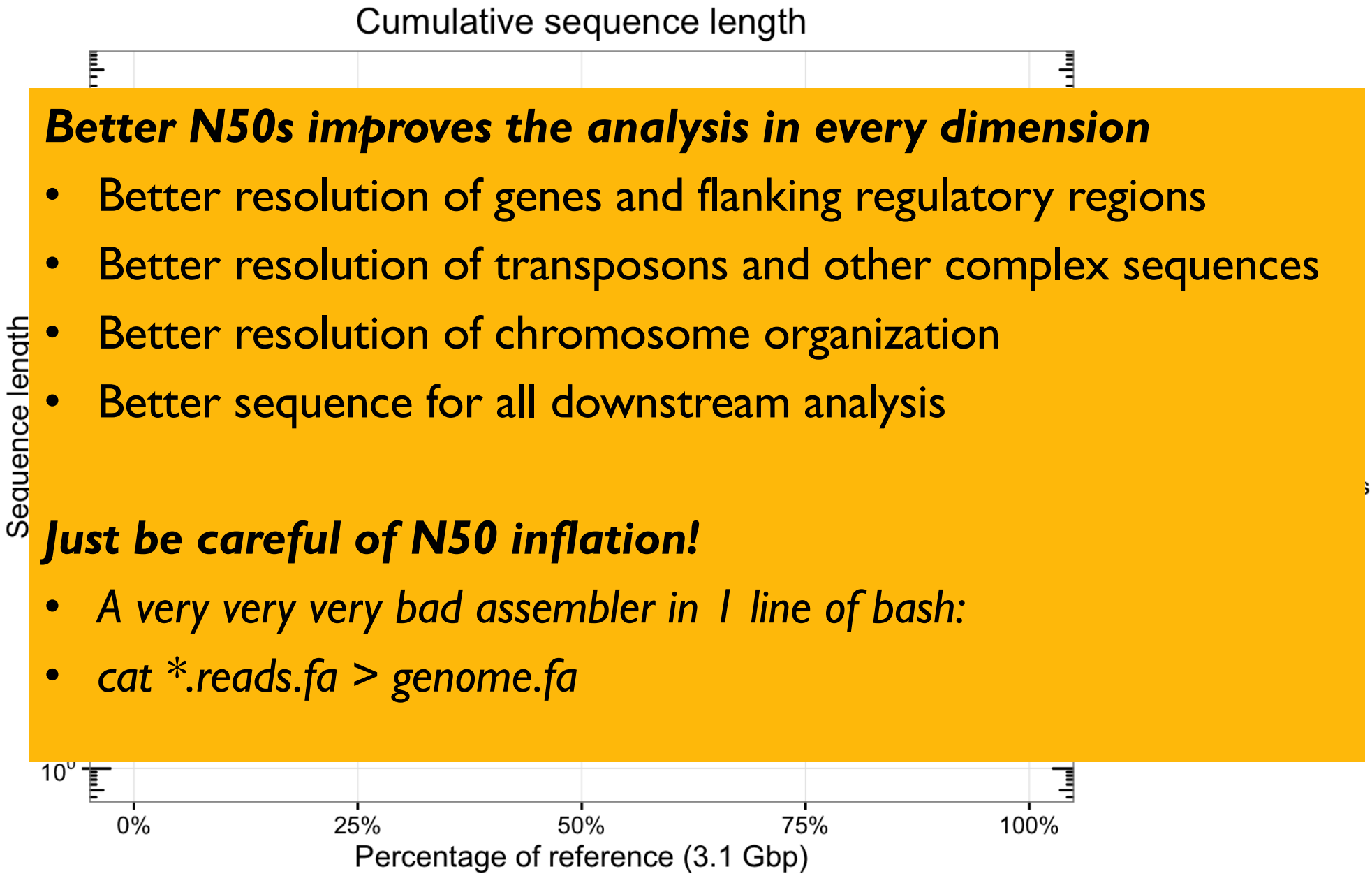


N50 size = 3 kbp

Contig Nchart



Contig Nchart



Better N50s improves the analysis in every dimension

- Better resolution of genes and flanking regulatory regions
- Better resolution of transposons and other complex sequences
- Better resolution of chromosome organization
- Better sequence for all downstream analysis

Just be careful of N50 inflation!

- *A very very very bad assembler in 1 line of bash:*
- *cat *.reads.fa > genome.fa*

Pop Quiz 1

Assemble these reads using a de Bruijn graph approach (k=3):

ATTA

GATT

TACA

TTAC

Pop Quiz 1

Assemble these reads using a de Bruijn graph approach (k=3):

```
ATTA: ATT -> TTA
GATT: GAT -> ATT
TACA: TAC -> ACA
TTAC: TTA -> TAC
```

Pop Quiz 1

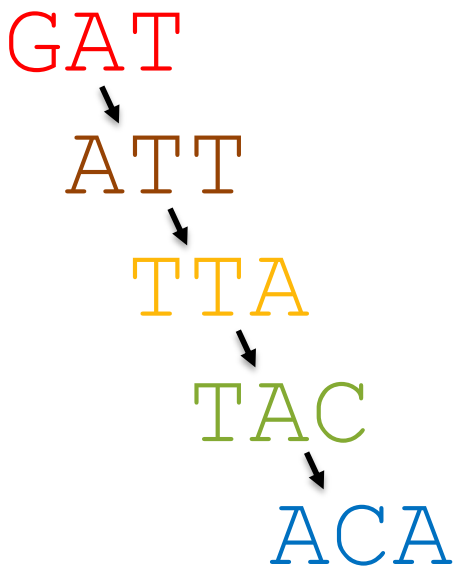
Assemble these reads using a de Bruijn graph approach (k=3):

ATTA: ATT -> TTA

GATT: GAT -> ATT

TACA: TAC -> ACA

TTAC: TTA -> TAC



GATTACA

Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

ACGA

ACGT

ATAC

CGAC

CGTA

GACG

GTAT

TACG

Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

~~ACGA~~

ACGT

ATAC

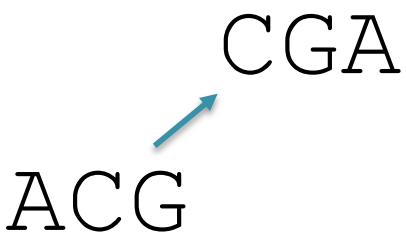
CGAC

CGTA

GACG

GTAT

TACG



Pop Quiz 2

Assemble these reads using a de Bruijn graph approach (k=3):

~~ACGA~~

~~ACGT~~

ATAC

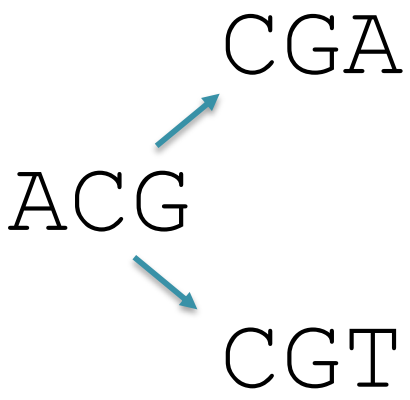
CGAC

CGTA

GACG

GTAT

TACG



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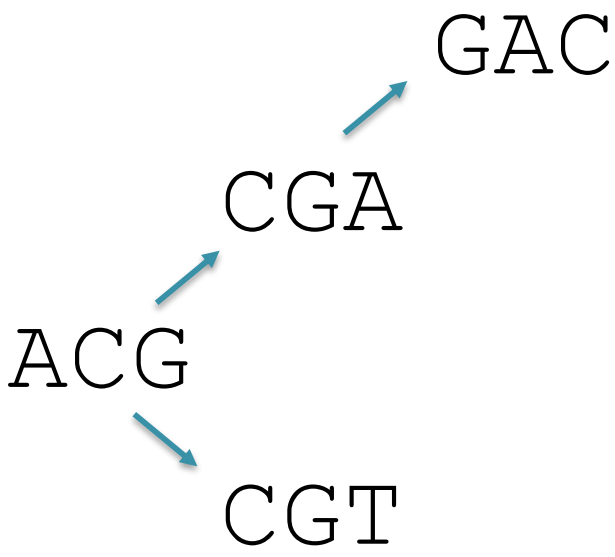

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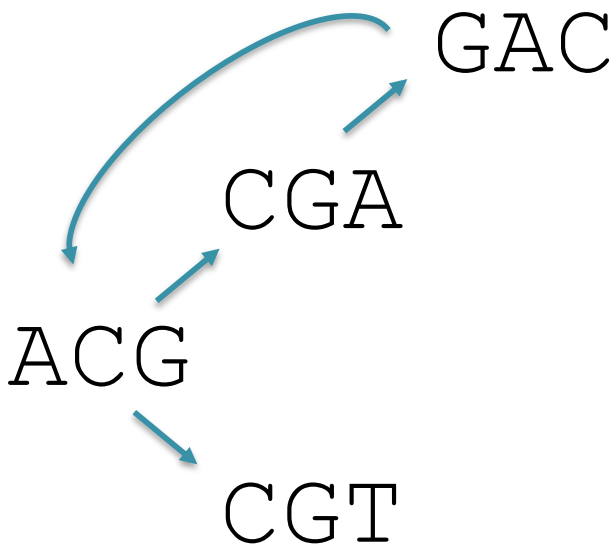

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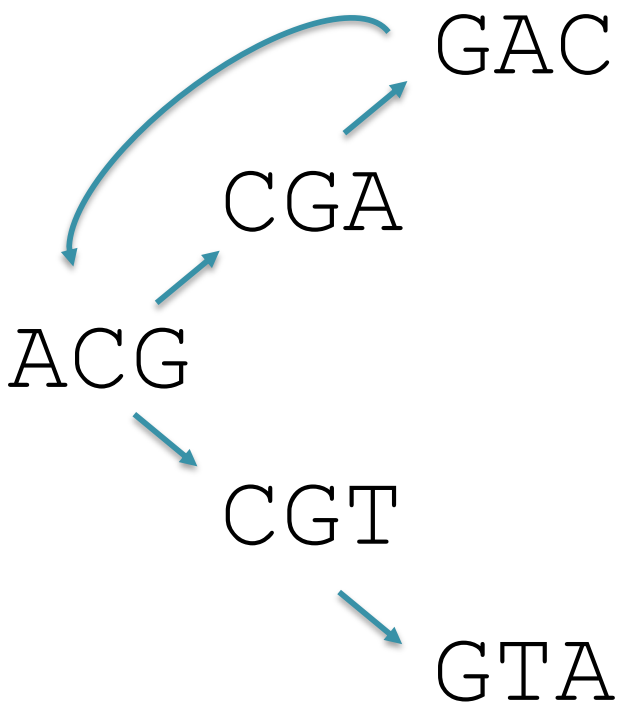

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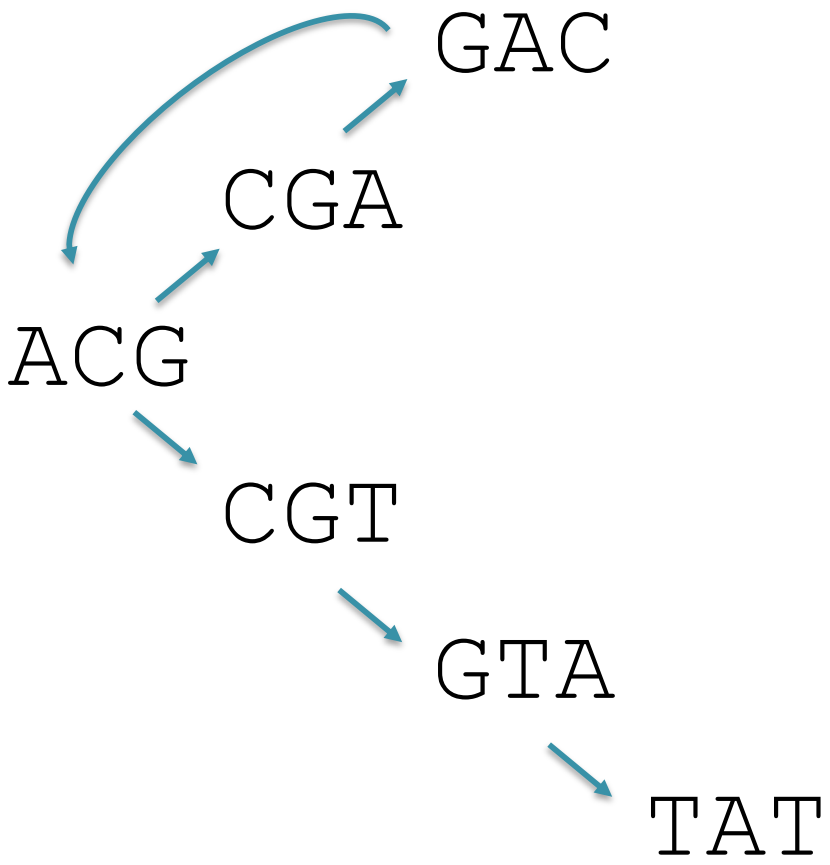

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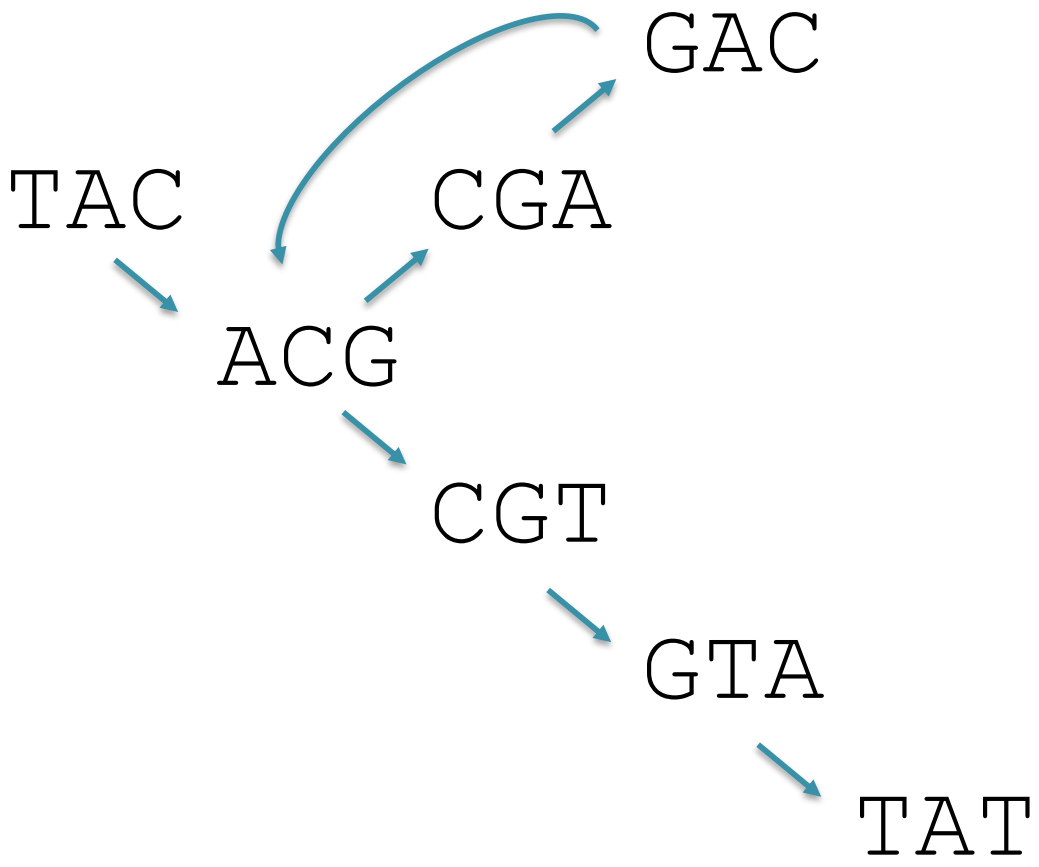

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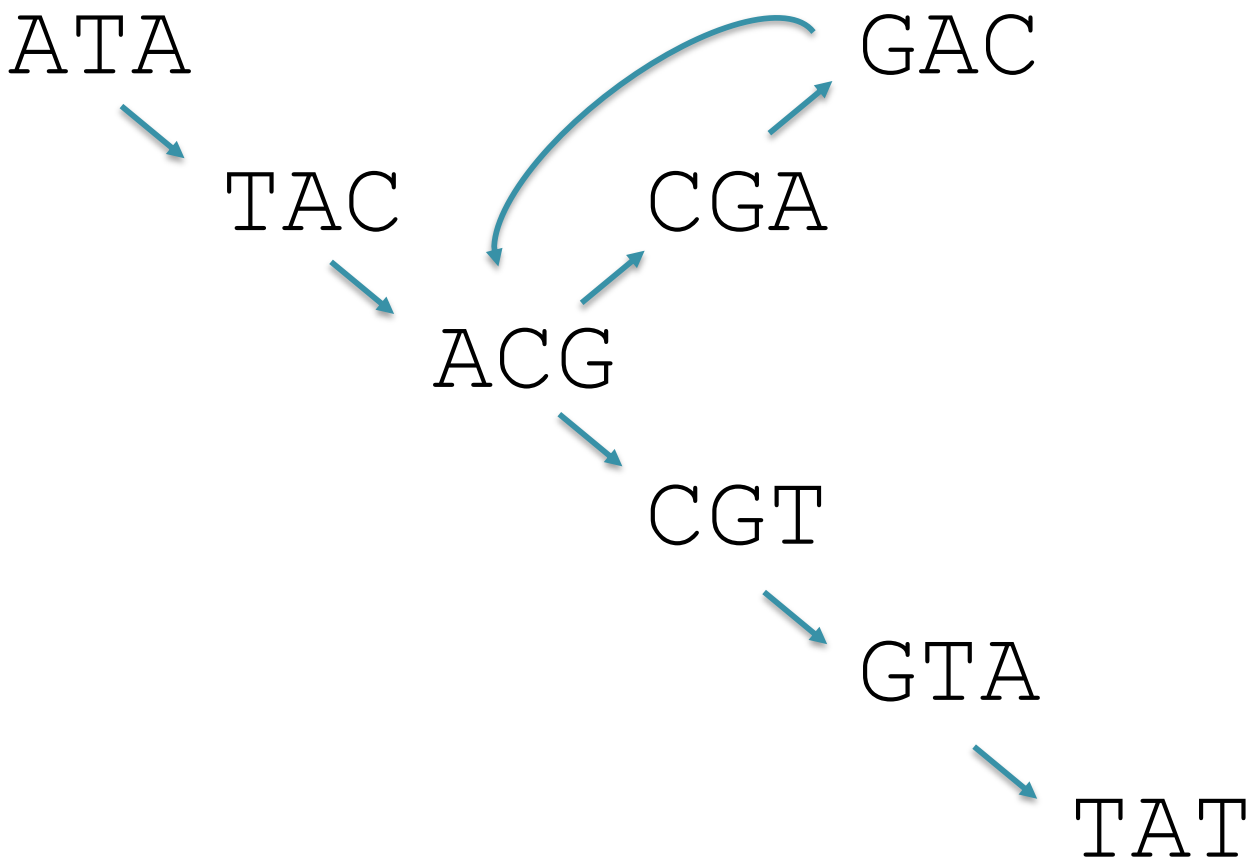

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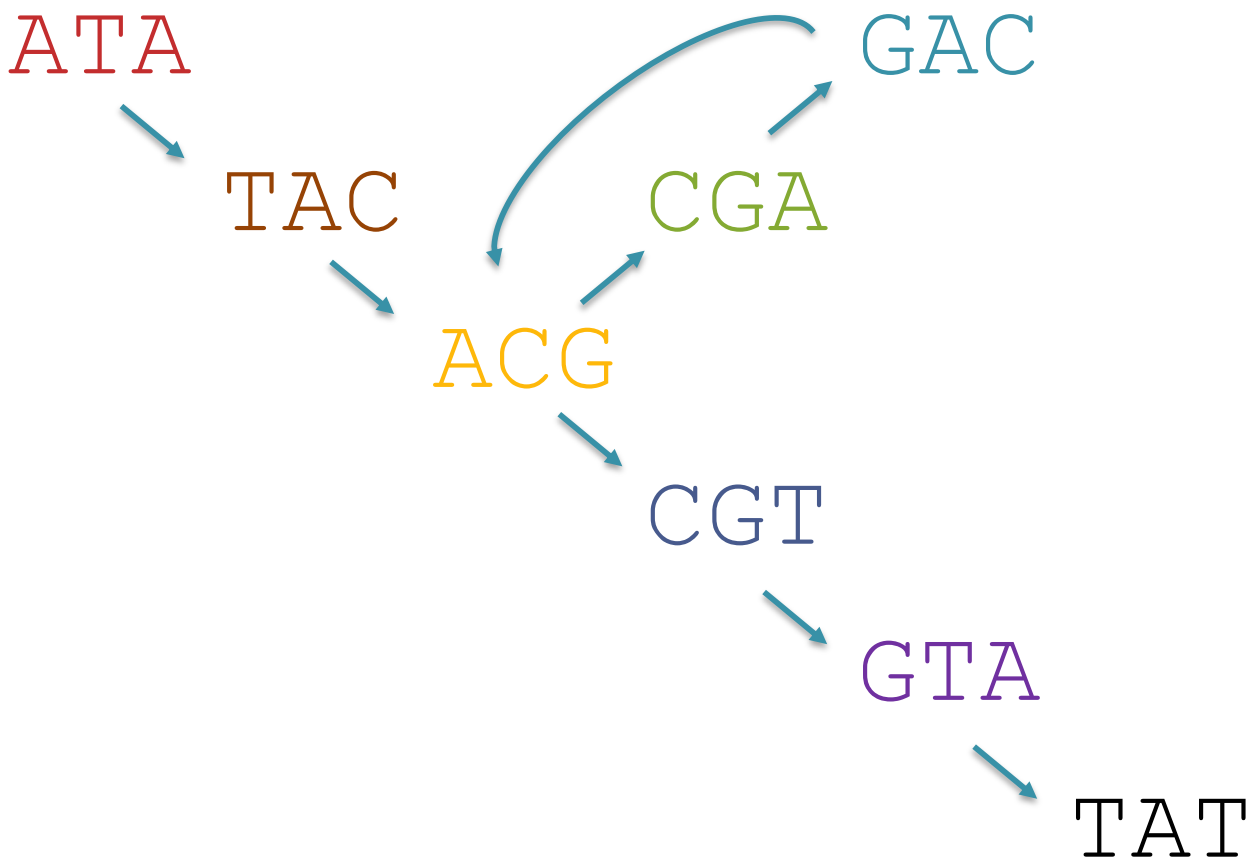

ATACGACGTAT

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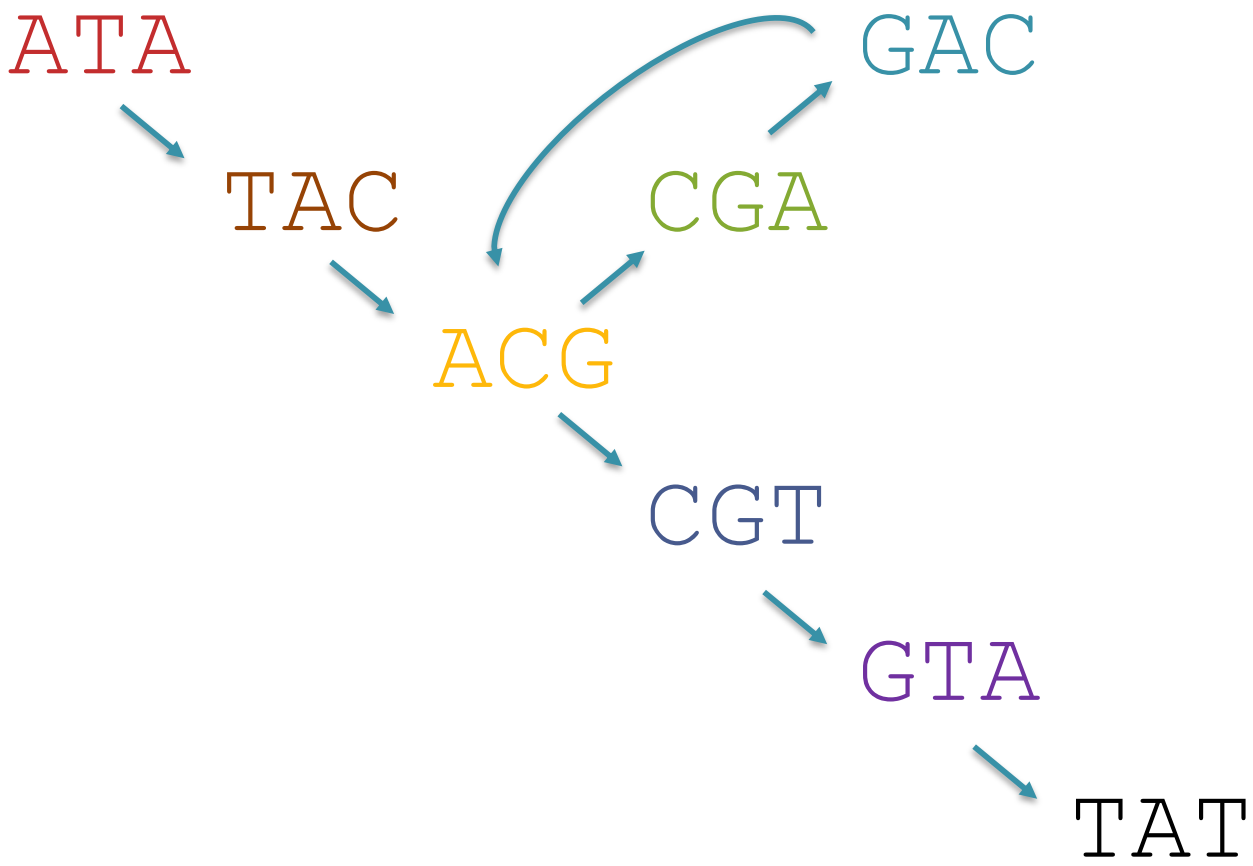

Whats another possible genome?

ATACGACGTAT

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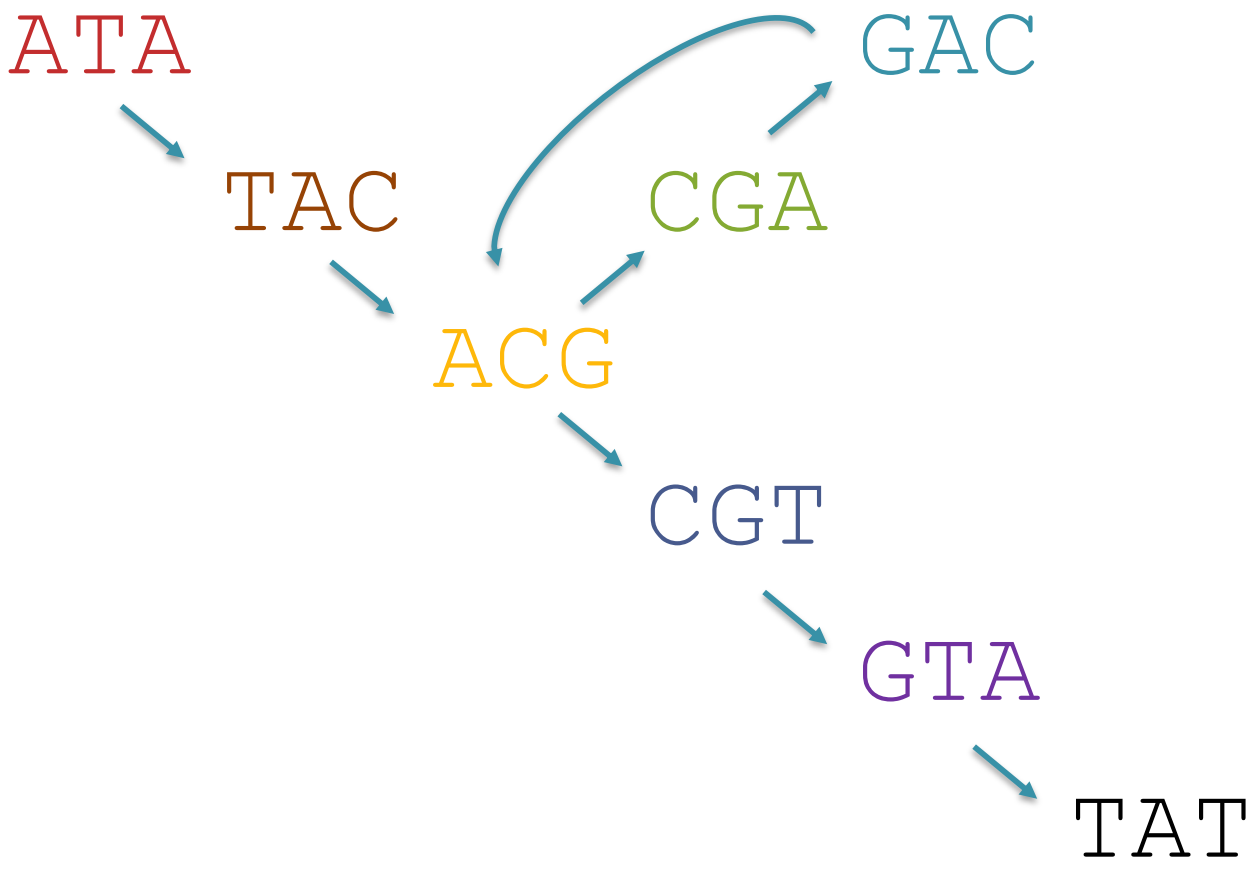

Should we add the edge TAT -> ATA?

ATACGACGTAT