De novo Assembly

Titus Brown

6/13/13

Assembly vs mapping

- No reference needed, for assembly!
  - De novo genomes, transcriptomes…

- But:
  - Scales poorly; need a much bigger computer.
  - Biology gets in the way (repeats!)
  - Need higher coverage

- But but:
  - Often your reference isn't that great, so assembly may actually be the best way to go.

Assembly

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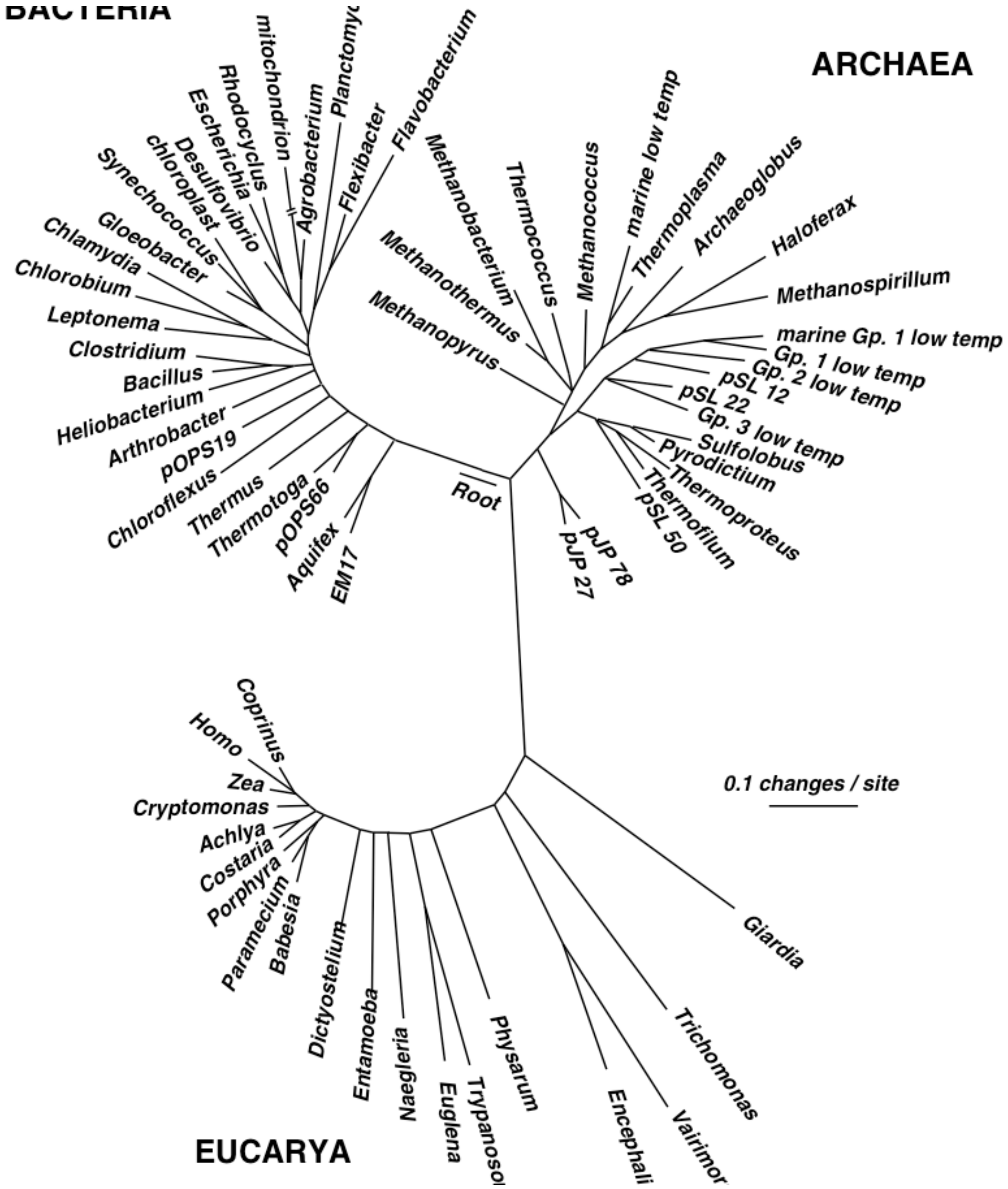
It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness

…but for lots and lots of fragments!


BACTERIA
ARCHAEA
EUCARYA
mitochondrion
Planctomyc
Flavobacterium
Rhodocyclus
Escherichia
Agrobacterium
Flexibacter
Desulfovibrio
chloroplast
Synechococcus
Gloeobacter
Chlamydia
Chlorobium
Leptonema
Clostridium
Bacillus
Heliobacterium
Arthrobacter
pOPS19
Chloroflexus
Thermus
Thermotoga
pOPS66
Aquifex
EM17
Root
Methanobacterium
Thermococcus
Methanococcus
marine low temp
Thermoplasma
Archaeoglobus
Haloferax
Methanospirillum
Methanothermus
Methanopyrus
marine Gp. 1 low temp
Gp. 1 low temp
Gp. 2 low temp
pSL 12
pSL 22
Gp. 3 low temp
Sulfolobus
Pyrodictium
Thermoproteus
Thermofilum
pSL 50
pJP 78
pJP 27
0.1 changes / site
Coprinus
Homo
Zea
Cryptomonas
Achlya
Costaria
Porphyra
Paramecium
Babesia
Dictyostelium
Entamoeba
Naegleria
Euglena
Trypanoso
Physarum
Encephali
Vairimor
Trichomonas
Giardia

Assemble based on word overlaps:

the quick brown fox jumped

jumped over the lazy dog

the quick brown fox jumped over the lazy dog

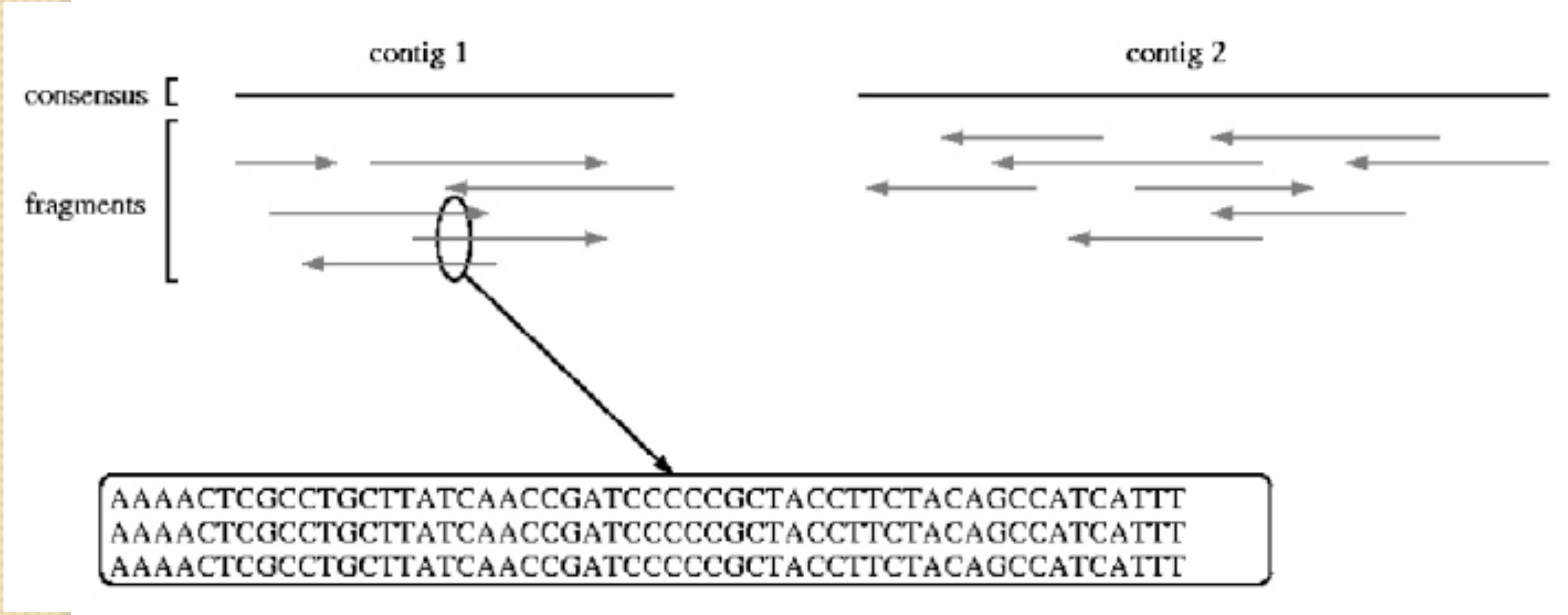
Repeats do cause problems:

my chemical romance: na na na

na na na, batman!

Shotgun sequencing & assembly

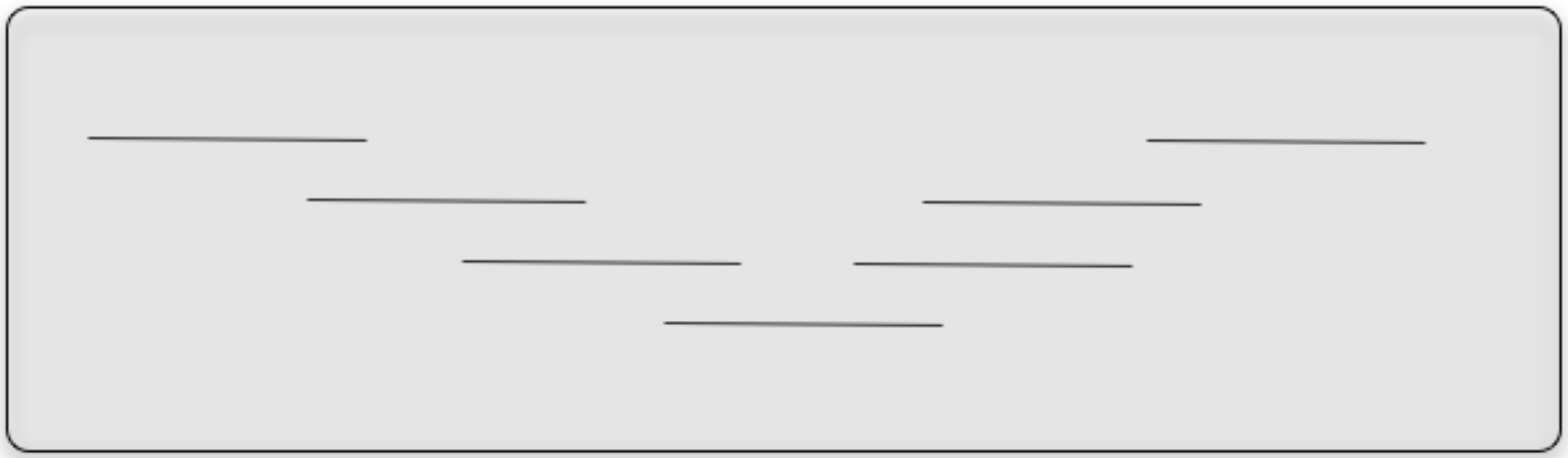
Randomly fragment & sequence from DNA; reassemble computationally.


contig 1
contig 2
consensus
fragments
AAAACTCGCCTGCTTATCAACCGATCCCCCGCTACCTTCTACAGCCATCATTT
AAAACTCGCCTGCTTATCAACCGATCCCCCGCTACCTTCTACAGCCATCATTT
AAAACTCGCCTGCTTATCAACCGATCCCCCGCTACCTTCTACAGCCATCATTT

UMD assembly primer (cbcb.umd.edu)

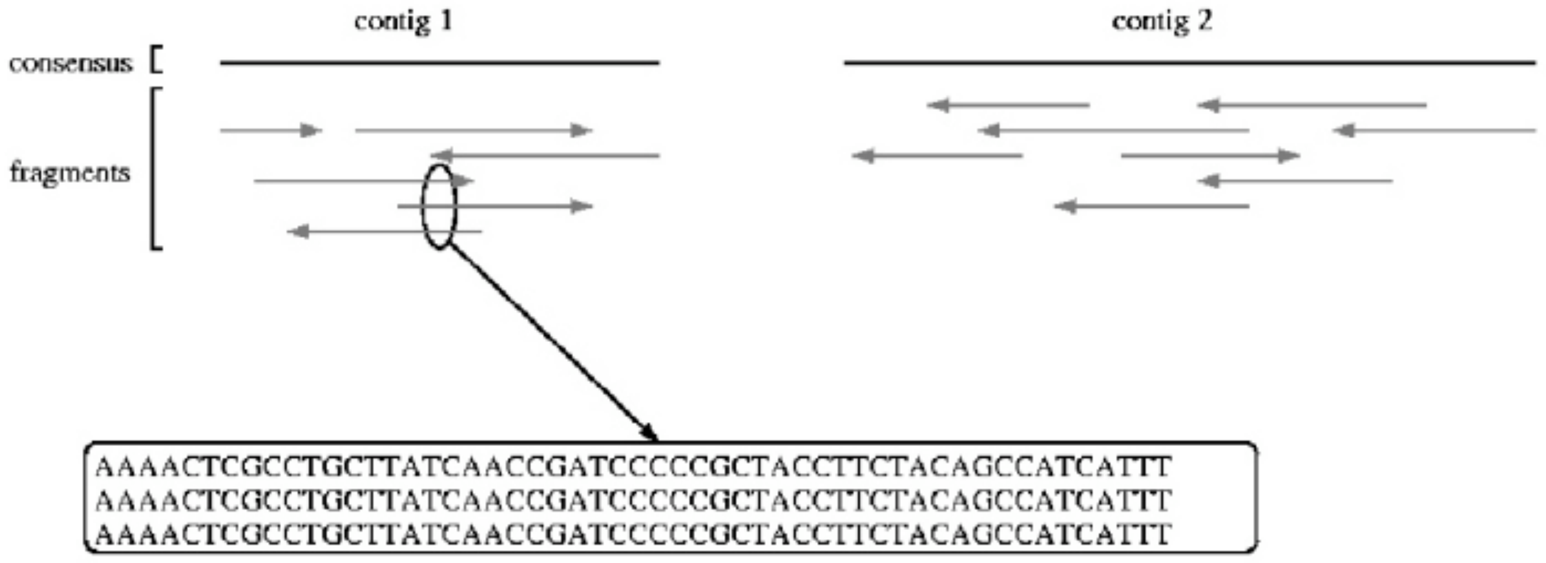
Assembly – no subdivision!

Assembly is inherently an *all by all* process. There is no good way to subdivide the reads without potentially missing a key connection



Short-read assembly

- Short-read assembly is problematic
- Relies on very deep coverage, ruthless read trimming, paired ends.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Short read lengths are hard.

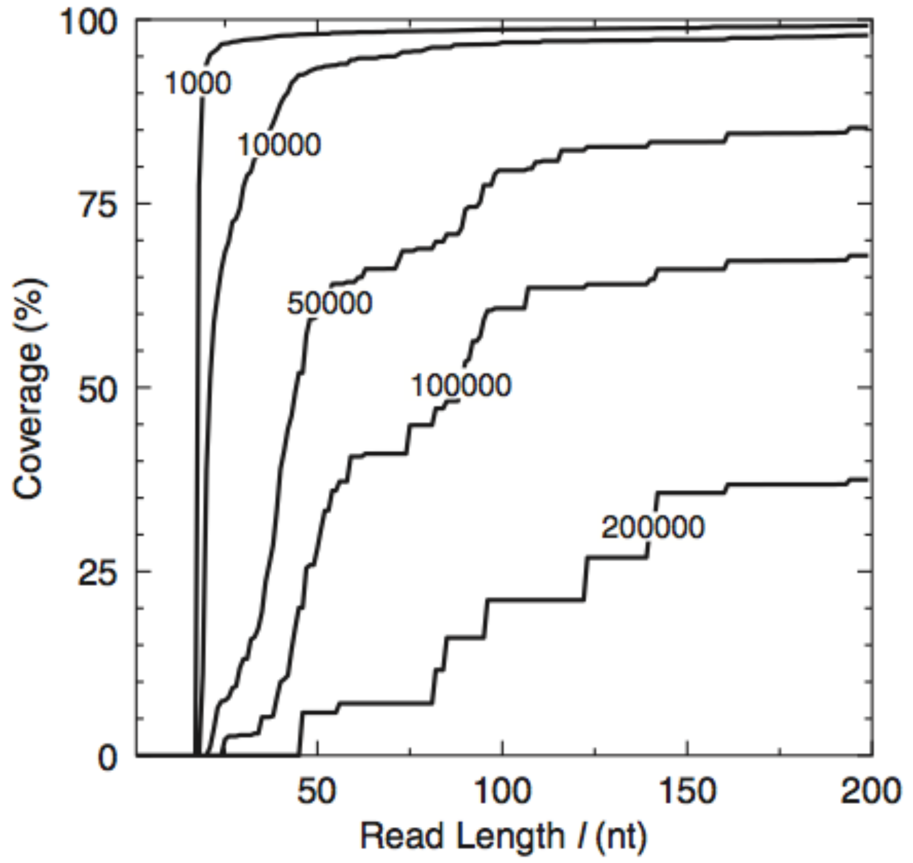


Figure 3. Percentage of the *E.coli* genome covered by contigs greater than a threshold length as a function of read length.

Whiteford et al., Nuc. Acid Res, 2005

Short read lengths are hard.

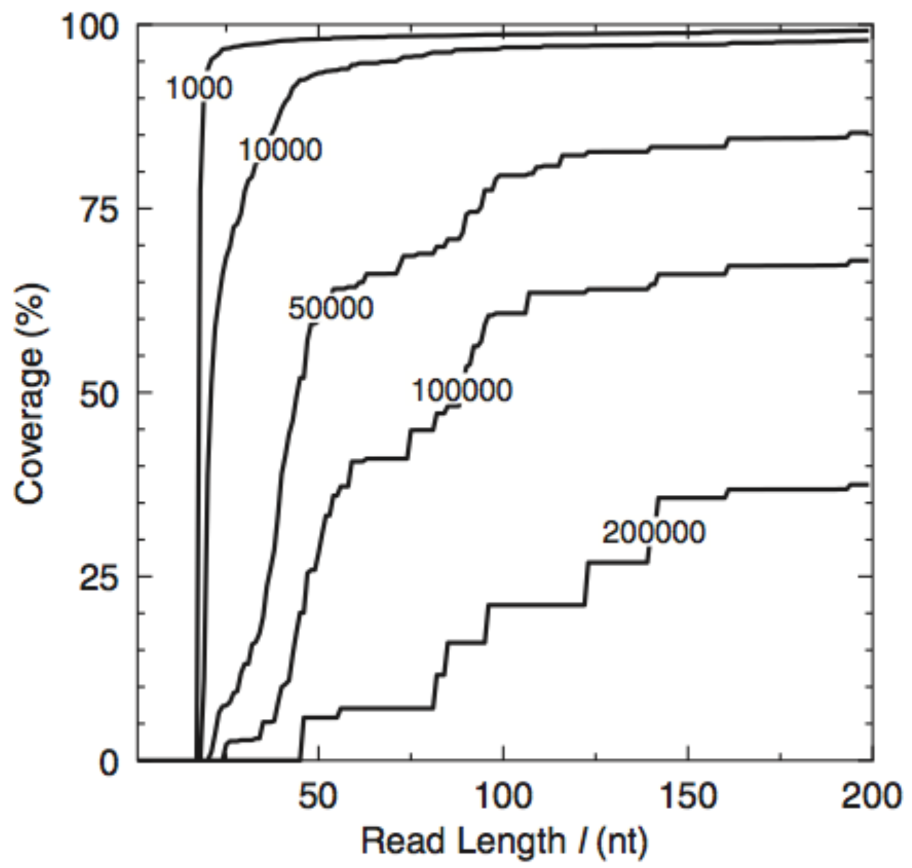


Figure 3. Percentage of the *E.coli* genome covered by contigs greater than a threshold length as a function of read length.

Conclusion: even with a read length of 200, the E. coli genome cannot be assembled completely.

Why?

Whiteford et al., Nuc. Acid Res, 2005

Short read lengths are hard.

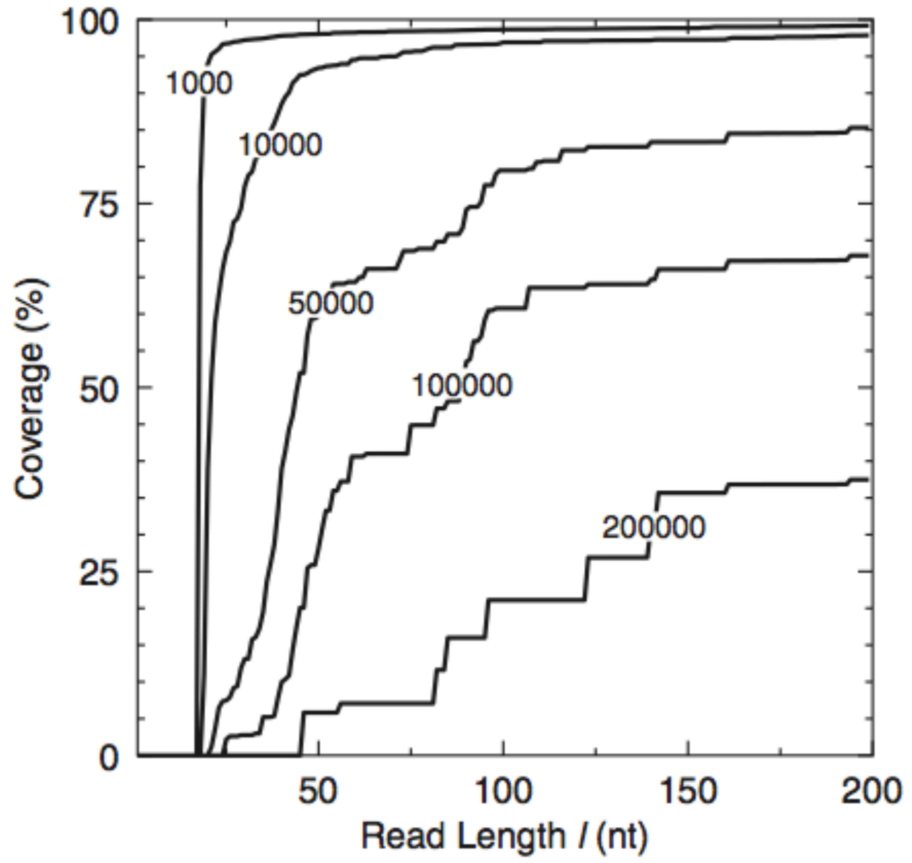


Figure 3. Percentage of the *E.coli* genome covered by contigs greater than a threshold length as a function of read length.

Conclusion: even with a read length of 200, the E. coli genome cannot be assembled completely.

Why? **REPEATS.**

This is why paired-end sequencing is so important for assembly.

Whiteford et al., Nuc. Acid Res, 2005

Four main challenges for *de novo* sequencing.

- Repeats.
- Low coverage.
- Errors

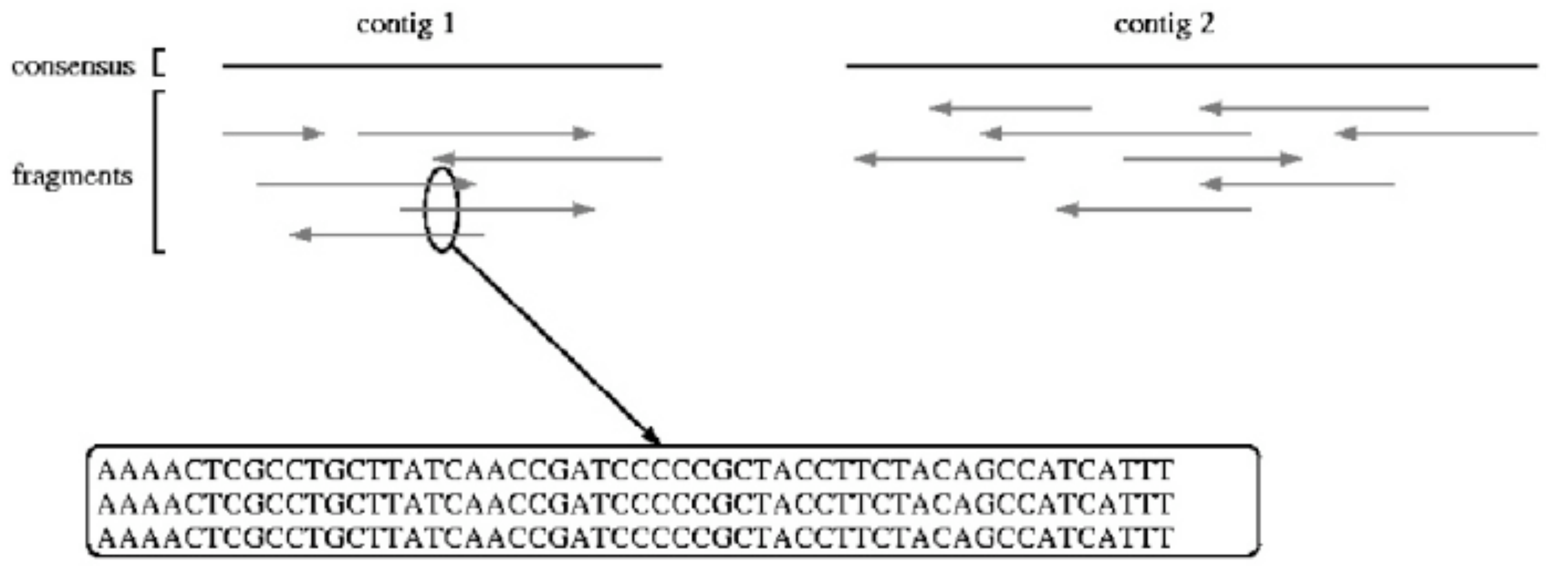
These introduce breaks in the construction of contigs.

- *Variation* in coverage – transcriptomes and metagenomes, as well as amplified genomic.

This challenges the assembler to distinguish between erroneous connections (e.g. repeats) and real connections.

Repeats

- Overlaps don't place sequences uniquely when there are repeats present.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AAAACTCGCCTGCTTATCAACCGATCCCCCGCTACCTTCTACAGCCATCATTT

UMD assembly primer (cbcb.umd.edu)

Coverage

Easy calculation:

(# reads x avg read length) / genome size

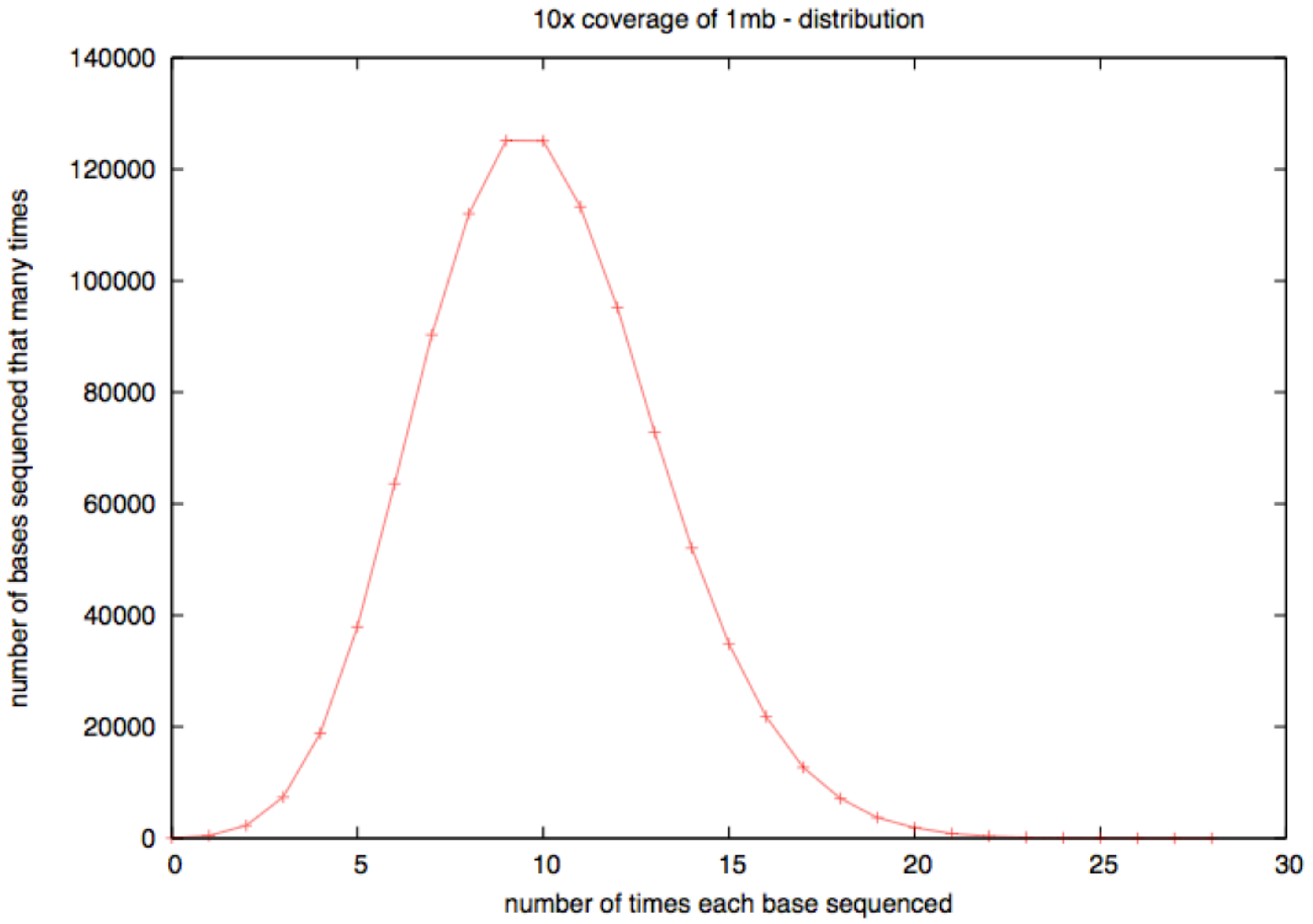
So, for haploid human genome:

30m reads x 100 bp = 3 bn

Coverage

- "1x" doesn't mean every DNA sequence is read once.
- It means that, if sampling were *systematic*, it would be.
- Sampling isn't systematic, it's random!

Actual coverage varies widely from the average, for low avg coverage



Two basic assembly approaches

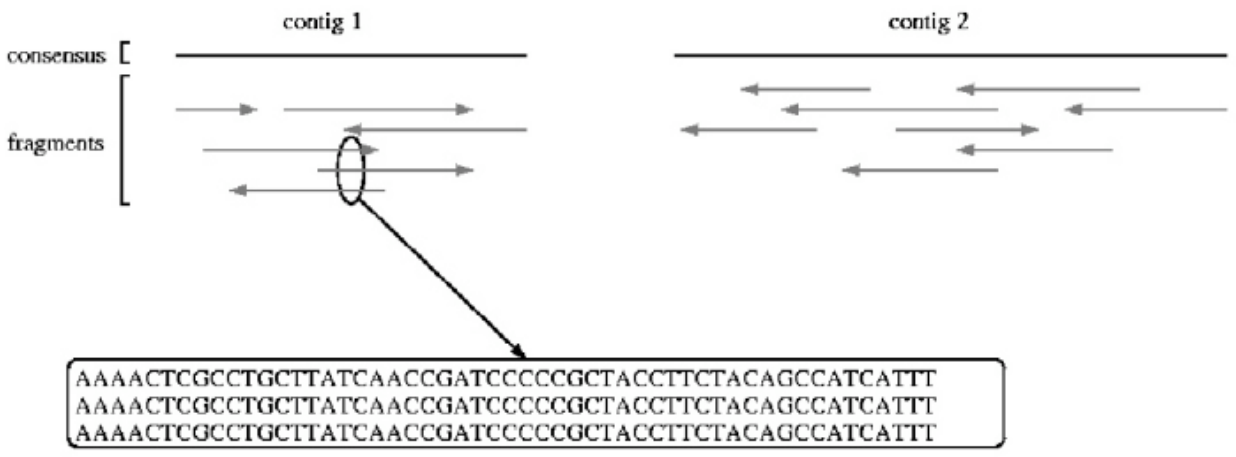
- Overlap/layout/consensus
- De Bruijn k-mer graphs

The former is used for long reads, esp all Sanger-based assemblies. The latter is used because of memory efficiency.

Overlap/layout/consensus

Essentially,

1. Calculate all overlaps
2. Cluster based on overlap.
3. Do a multiple sequence alignment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UMD assembly primer (cbcb.umd.edu)

K-mers

Break reads (of any length) down into multiple overlapping words of fixed length *k*.

```
ATGGACCAGATGACAC (k=12) =>

ATGGACCAGATG
 TGGACCAGATGA
  GGACCAGATGAC
   GACCAGATGACA
    ACCAGATGACAC
```

K-mers – what k to use?

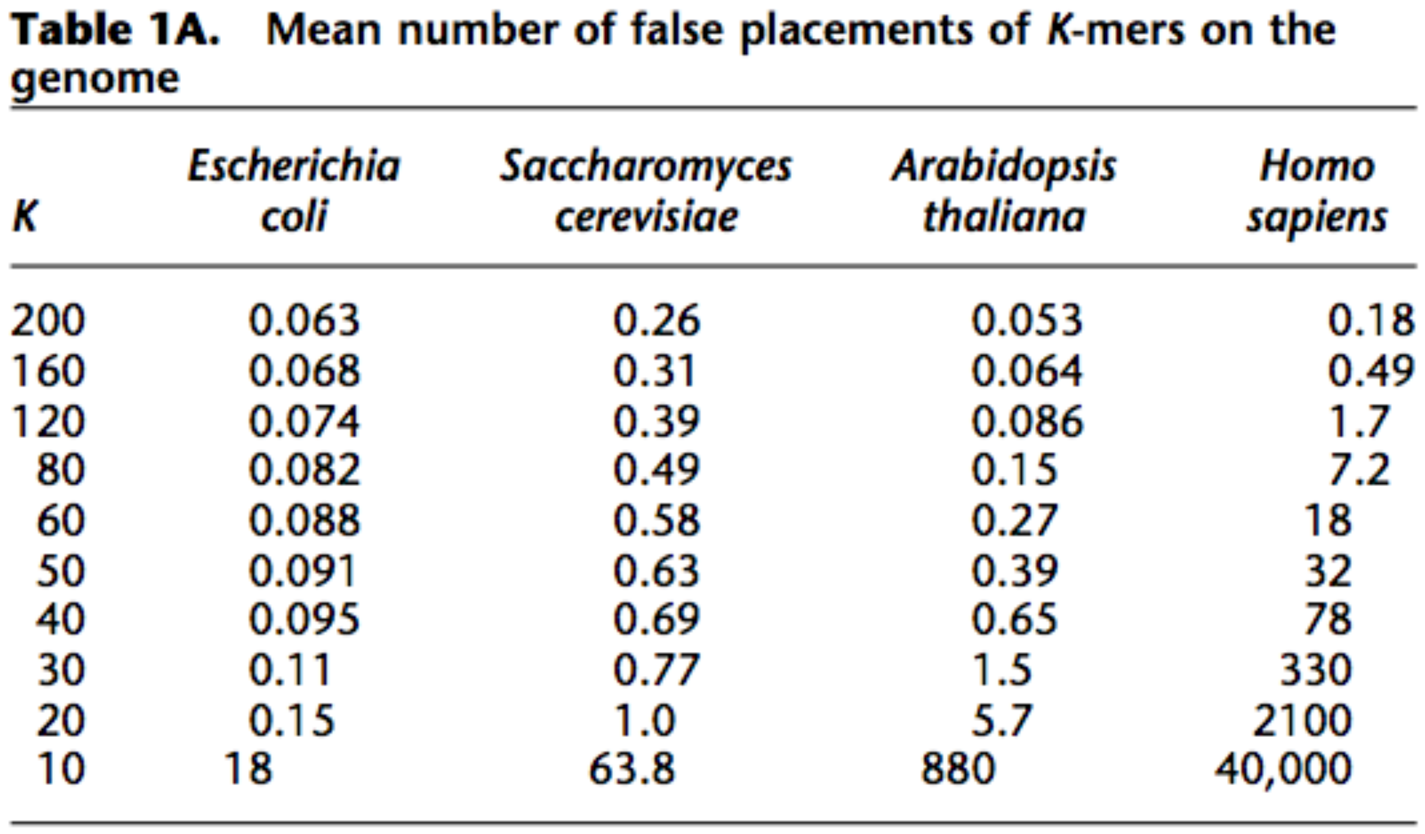


Table 1A. Mean number of false placements of *K*-mers on the genome

K	*Escherichia coli*	*Saccharomyces cerevisiae*	*Arabidopsis thaliana*	*Homo sapiens*
200	0.063	0.26	0.053	0.18
160	0.068	0.31	0.064	0.49
120	0.074	0.39	0.086	1.7
80	0.082	0.49	0.15	7.2
60	0.088	0.58	0.27	18
50	0.091	0.63	0.39	32
40	0.095	0.69	0.65	78
30	0.11	0.77	1.5	330
20	0.15	1.0	5.7	2100
10	18	63.8	880	40,000

Butler et al., Genome Res, 2009

K-mers – what k to use?

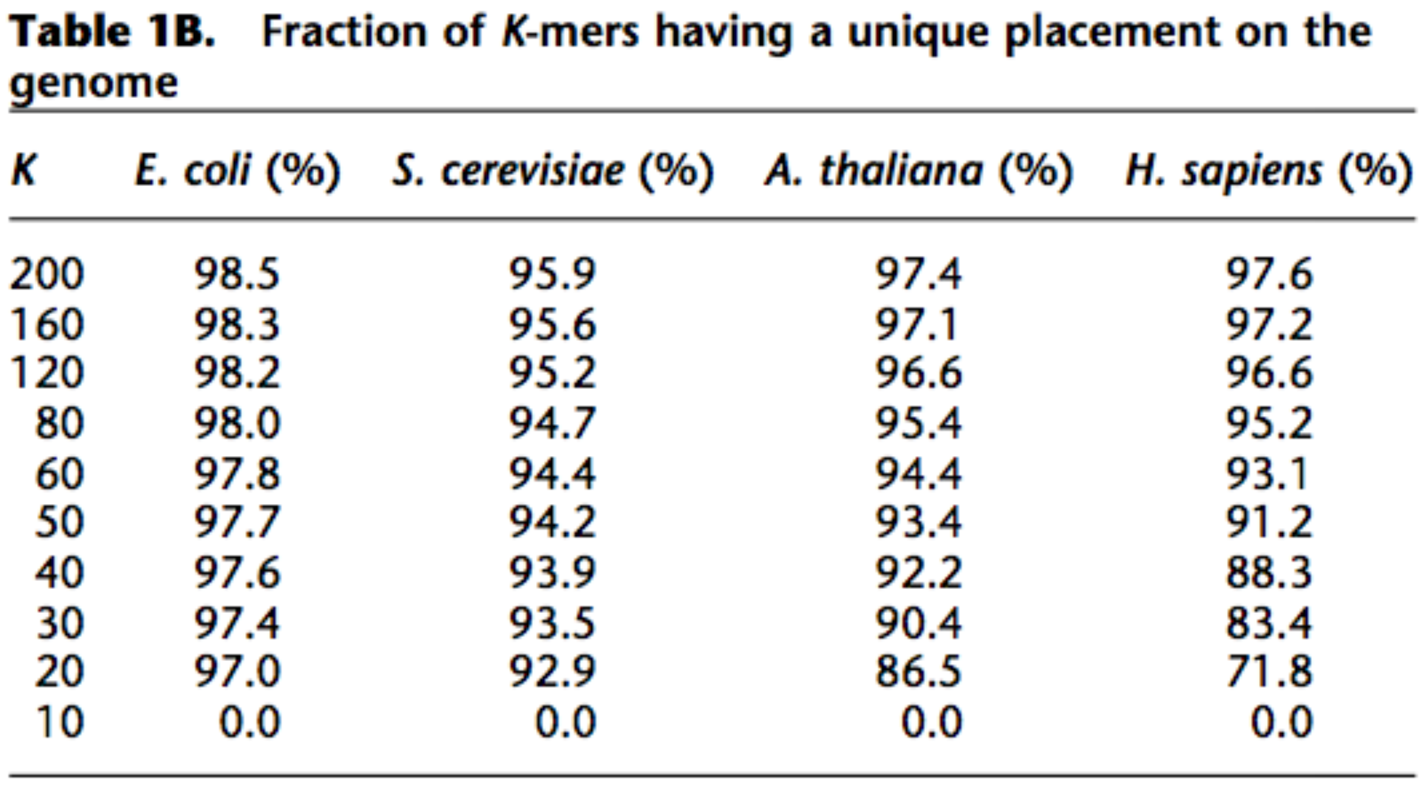
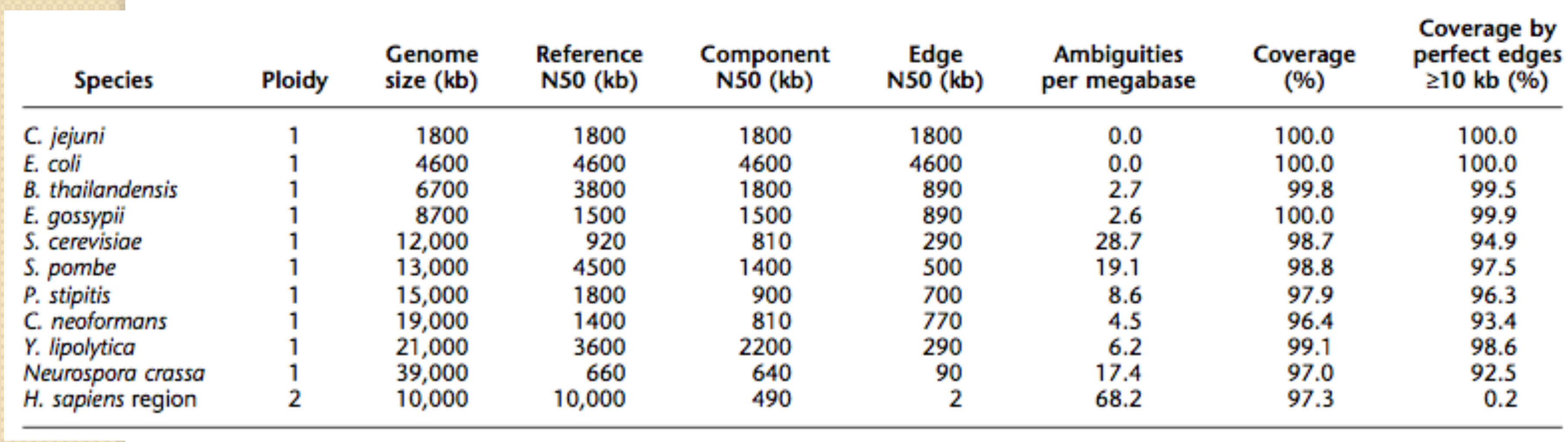


Table 1B. Fraction of *K*-mers having a unique placement on the genome

K	*E. coli* (%)	*S. cerevisiae* (%)	*A. thaliana* (%)	*H. sapiens* (%)
200	98.5	95.9	97.4	97.6
160	98.3	95.6	97.1	97.2
120	98.2	95.2	96.6	96.6
80	98.0	94.7	95.4	95.2
60	97.8	94.4	94.4	93.1
50	97.7	94.2	93.4	91.2
40	97.6	93.9	92.2	88.3
30	97.4	93.5	90.4	83.4
20	97.0	92.9	86.5	71.8
10	0.0	0.0	0.0	0.0

Butler et al., Genome Res, 2009

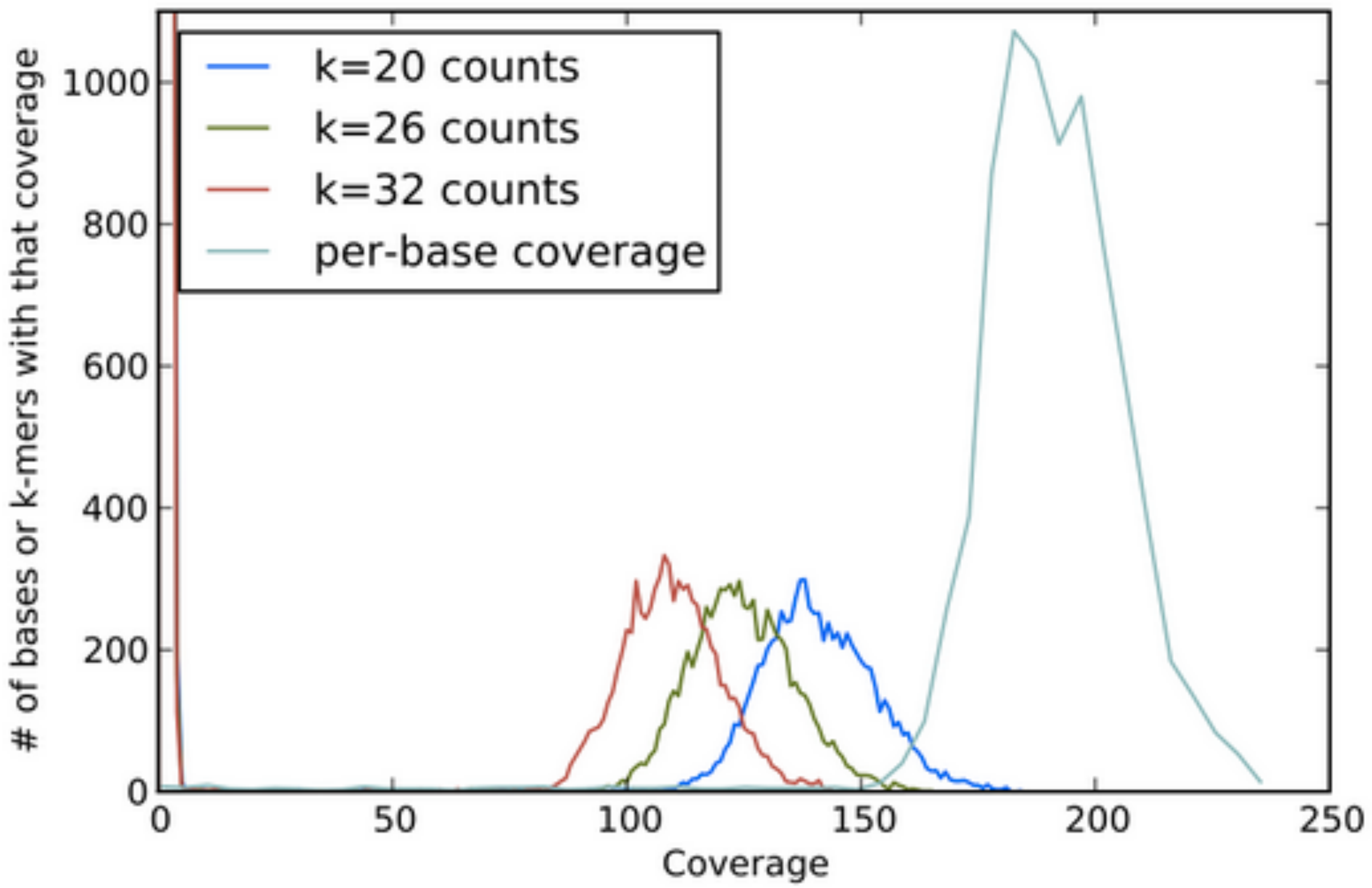
Big genomes are problematic



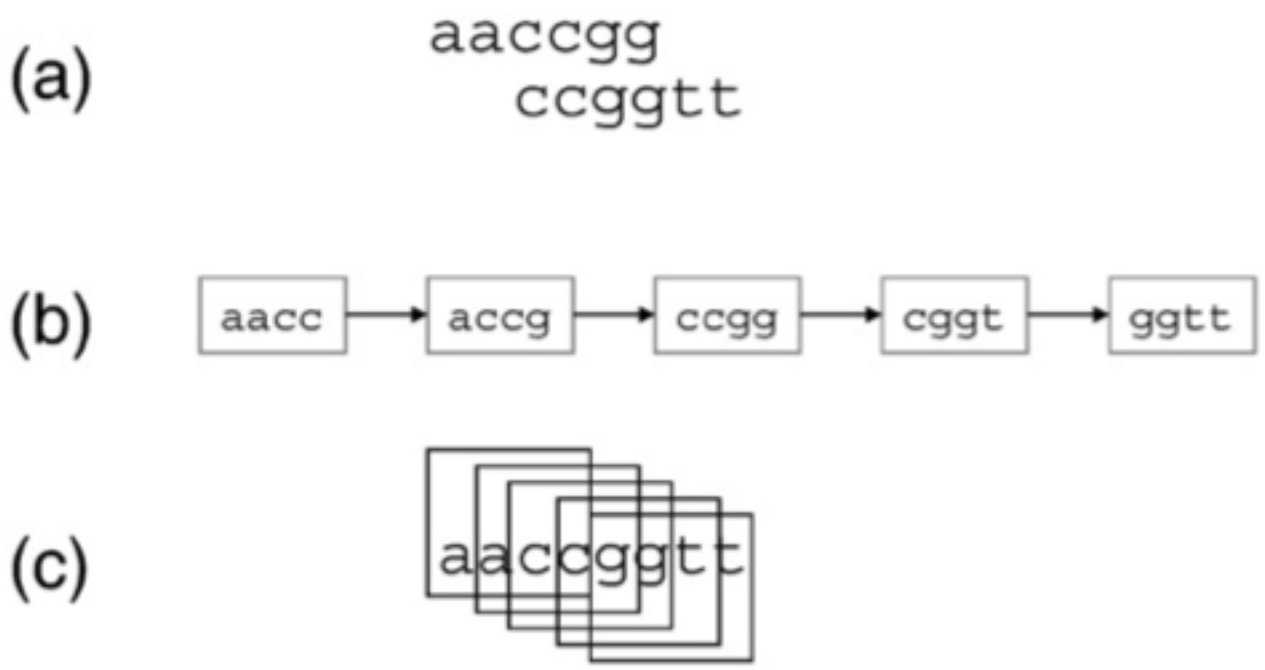
Species	Ploidy	Genome size (kb)	Reference N50 (kb)	Component N50 (kb)	Edge N50 (kb)	Ambiguities per megabase	Coverage (%)	Coverage by perfect edges ≥10 kb (%)
C. jejuni	1	1800	1800	1800	1800	0.0	100.0	100.0
E. coli	1	4600	4600	4600	4600	0.0	100.0	100.0
B. thailandensis	1	6700	3800	1800	890	2.7	99.8	99.5
E. gossypii	1	8700	1500	1500	890	2.6	100.0	99.9
S. cerevisiae	1	12,000	920	810	290	28.7	98.7	94.9
S. pombe	1	13,000	4500	1400	500	19.1	98.8	97.5
P. stipitis	1	15,000	1800	900	700	8.6	97.9	96.3
C. neoformans	1	19,000	1400	810	770	4.5	96.4	93.4
Y. lipolytica	1	21,000	3600	2200	290	6.2	99.1	98.6
Neurospora crassa	1	39,000	660	640	90	17.4	97.0	92.5
H. sapiens region	2	10,000	10,000	490	2	68.2	97.3	0.2

Butler et al., Genome Res, 2009

Choice of k affects apparent coverage

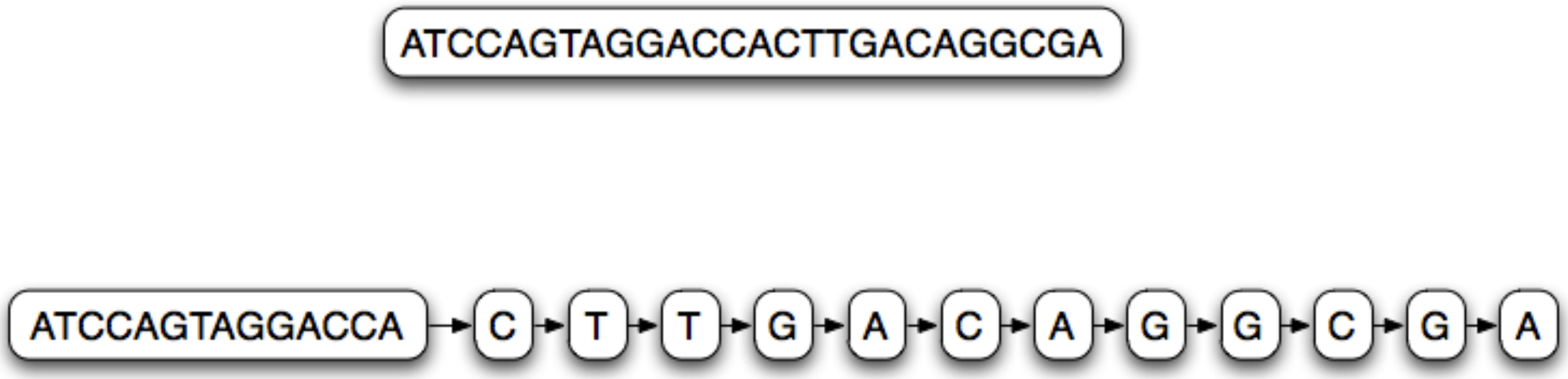


K-mer graphs - overlaps



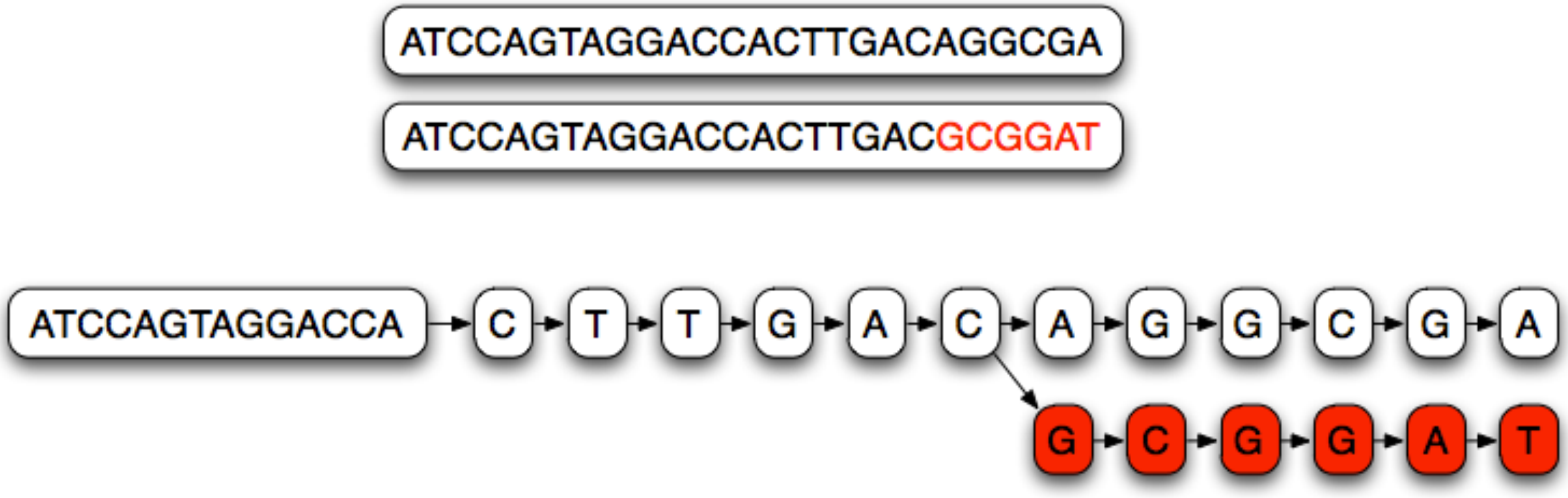
J.R. Miller et al. / Genomics (2010)

K-mer graph (k=14)



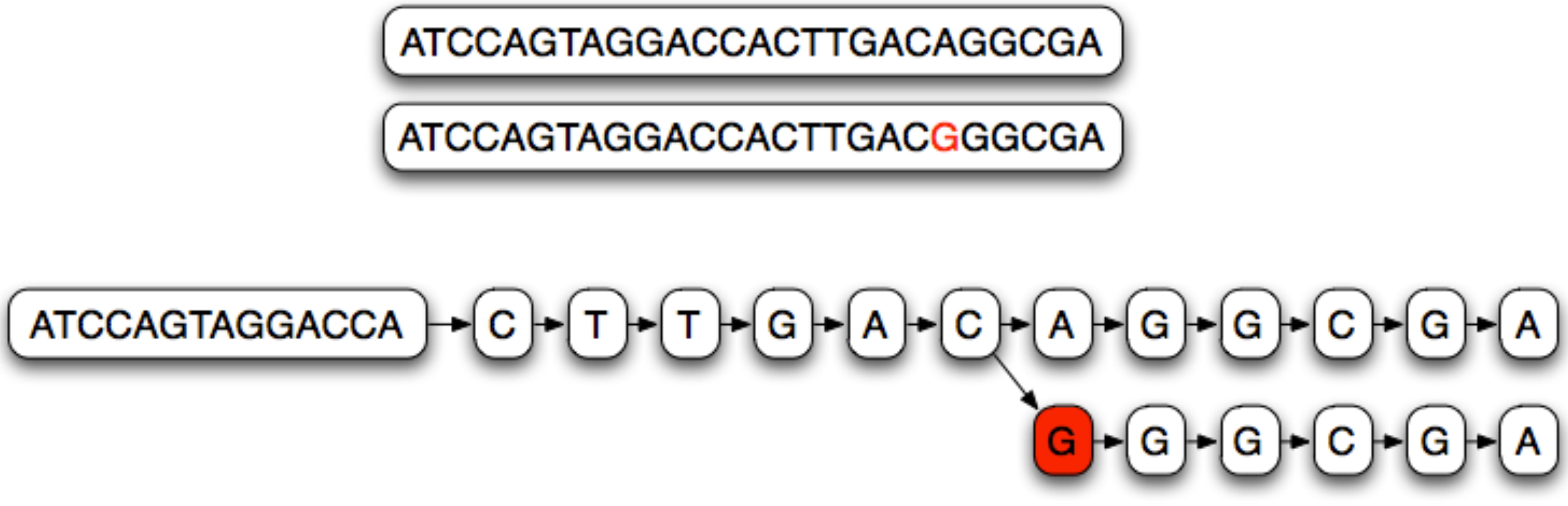
Each node represents a 14-mer;
Links between each node are 13-mer overlaps

K-mer graph (k=14)



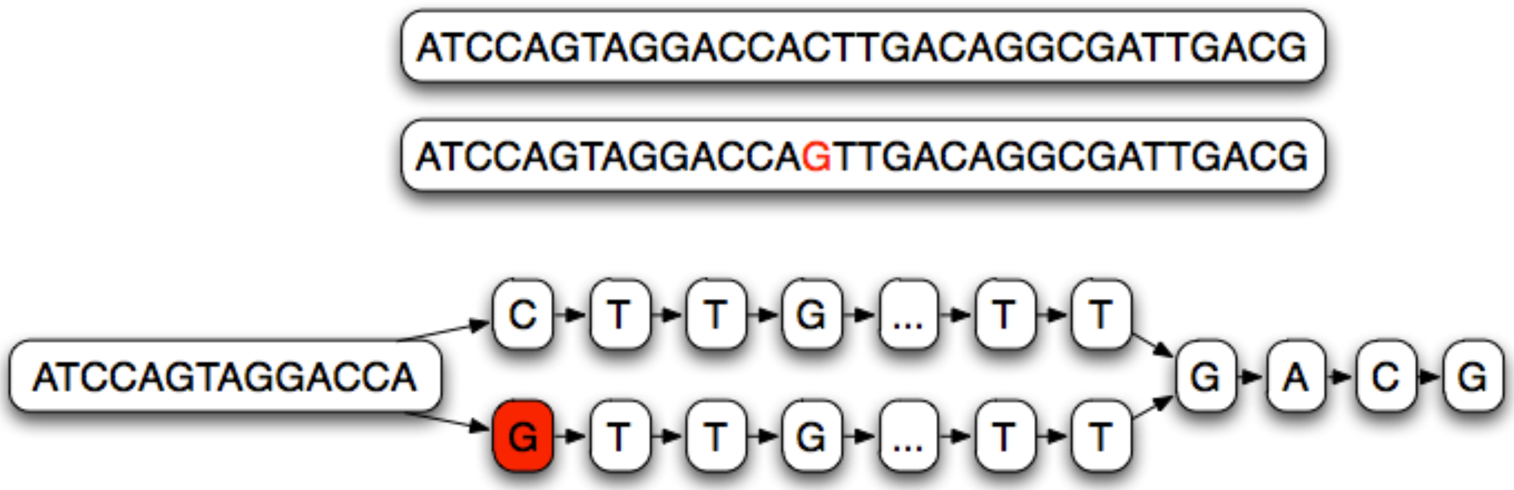
Branches in the graph represent partially overlapping sequences.

K-mer graph (k=14)



Single nucleotide variations cause long branches

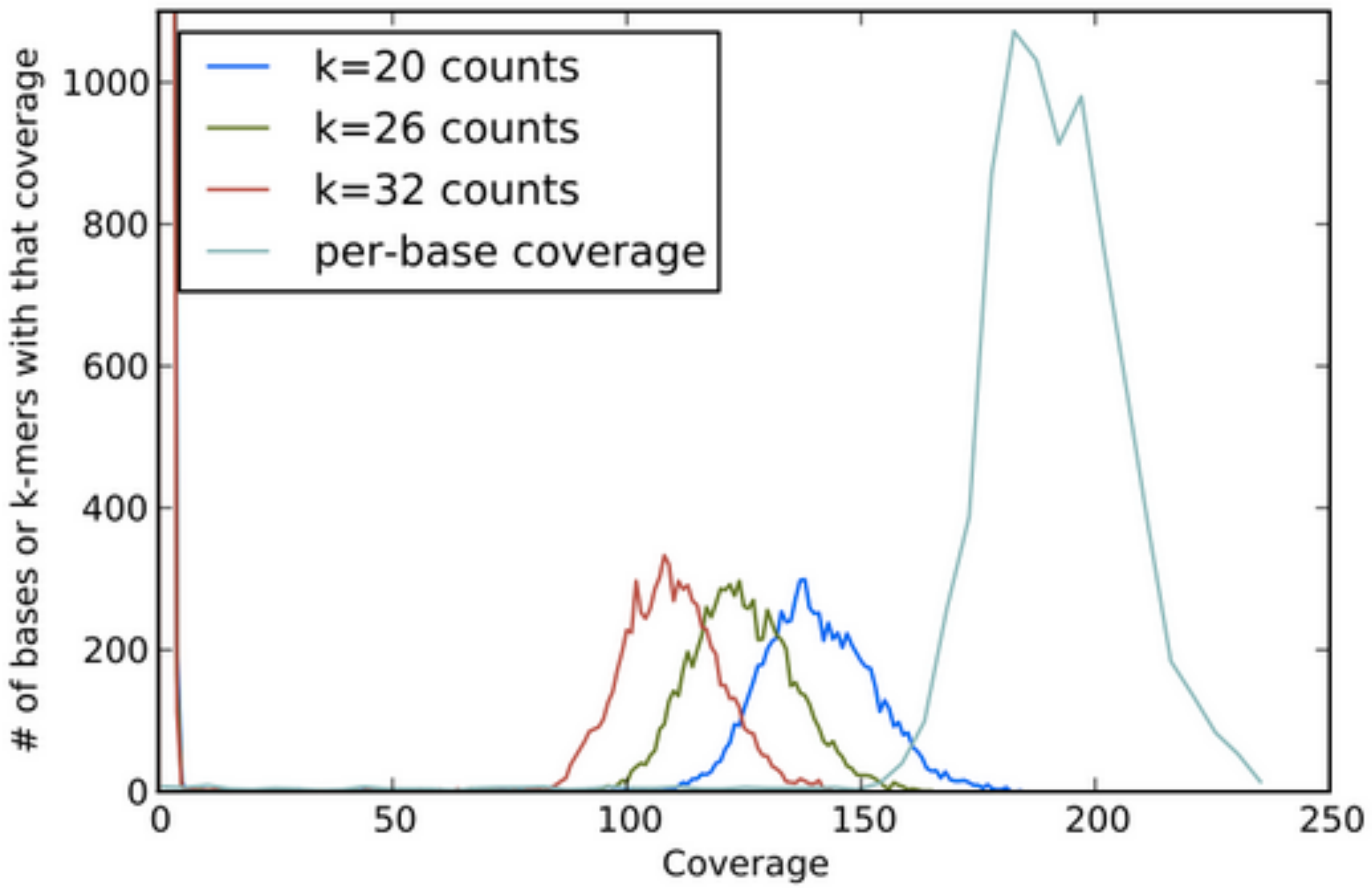
K-mer graph (k=14)



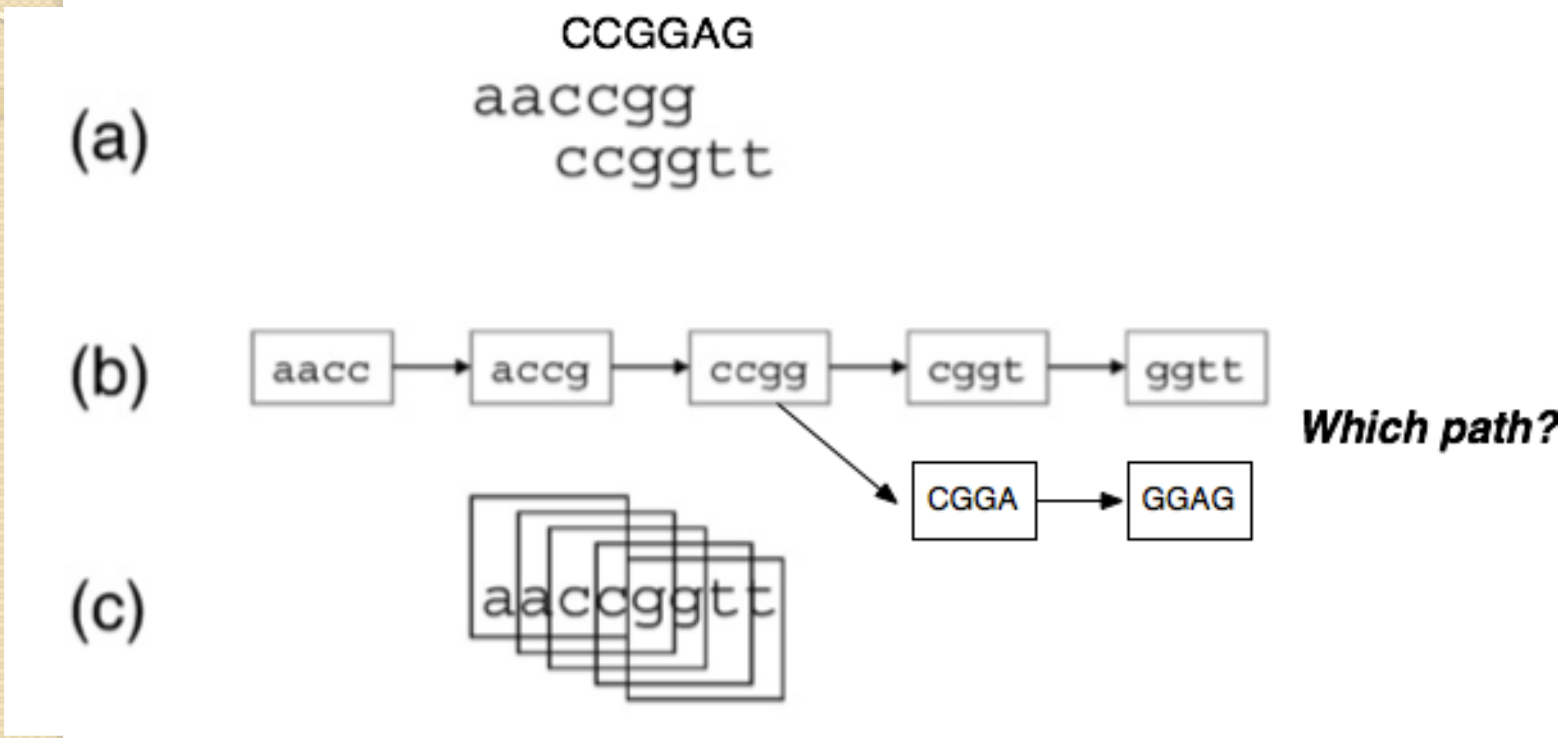
ATCCAGTAGGACCACTTGACAGGCGATTGACG
ATCCAGTAGGACCAGTTGACAGGCGATTGACG
ATCCAGTAGGACCA
C T T G ... T T
G T T G ... T T
G A C G

Single nucleotide variations cause long branches;
They don’t rejoin quickly.

Choice of k affects apparent coverage

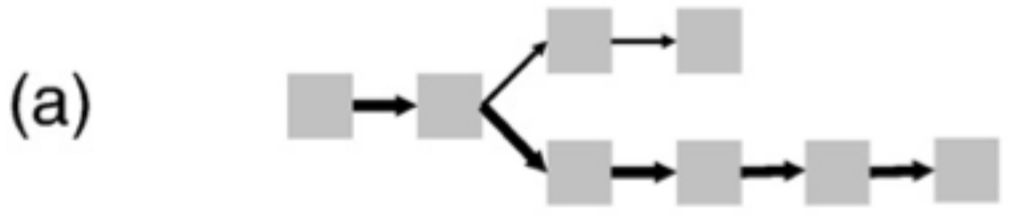


K-mer graphs - branching



For decisions about which paths etc, biology-based heuristics come into play as well.

K-mer graph complexity - spur

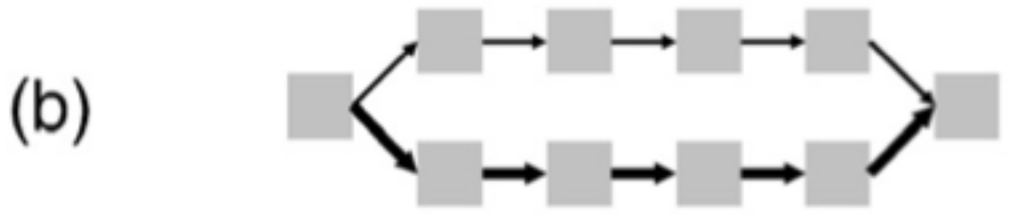


(Short) dead-end in graph.

Can be caused by error at the end of some overlapping reads, or low coverage

J.R. Miller et al. / Genomics (2010)

K-mer graph complexity - bubble



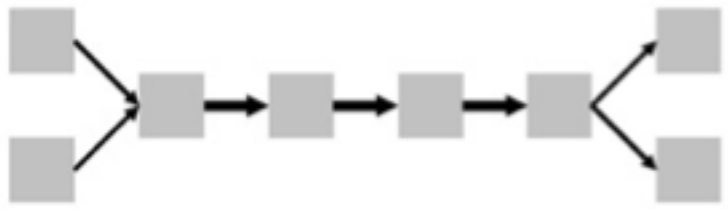
Multiple parallel paths that diverge and join.

Caused by sequencing error and true polymorphism / polyploidy in sample.

J.R. Miller et al. / Genomics (2010)

K-mer graph complexity – "frayed rope"

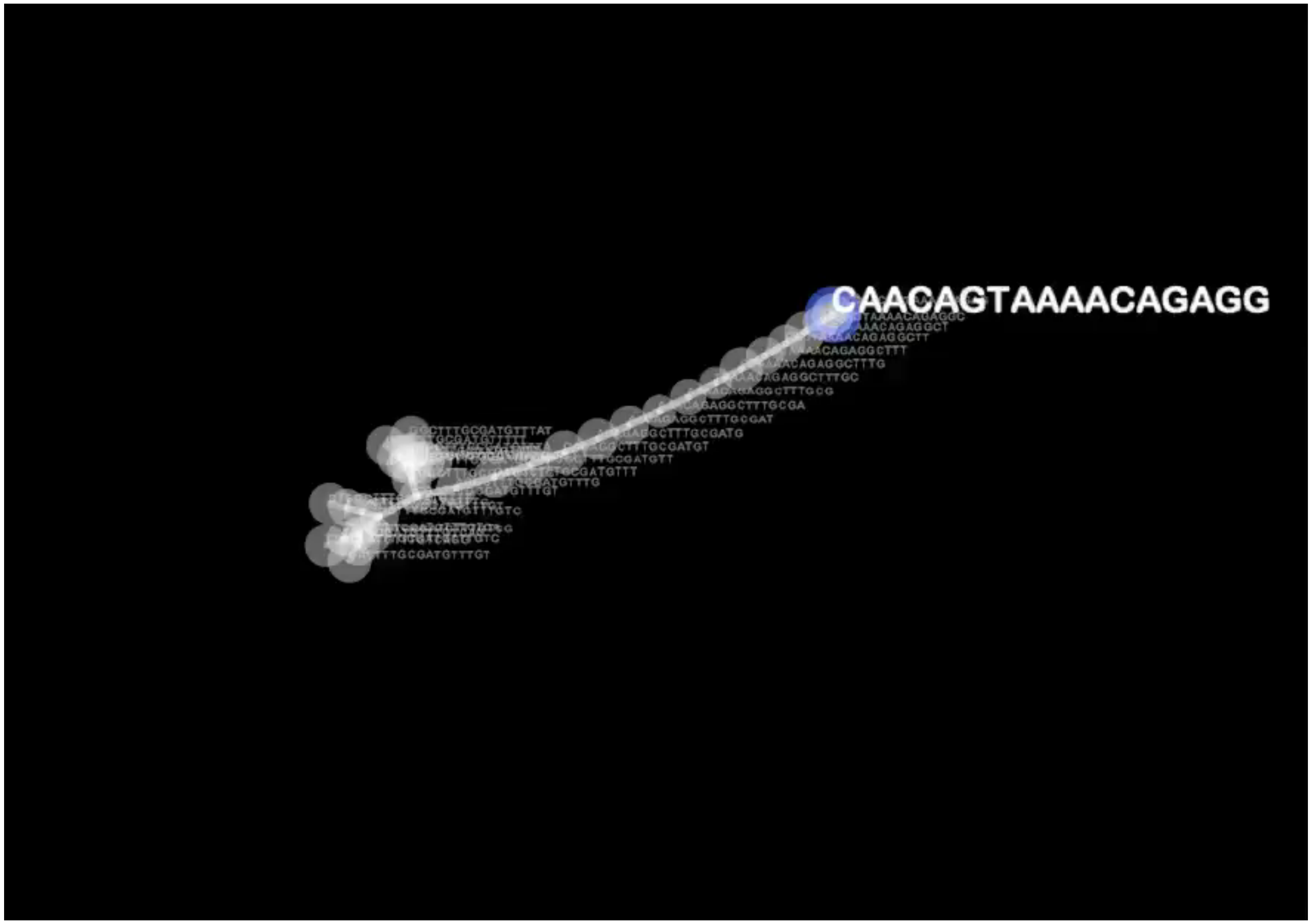
(c)



Converging, then diverging paths.

Caused by repetitive sequences.

J.R. Miller et al. / Genomics (2010)

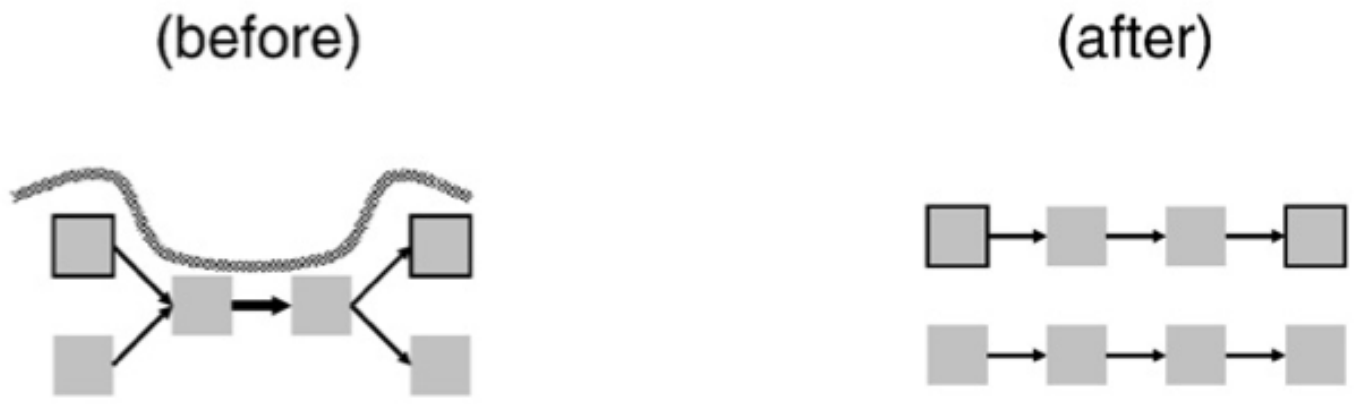


Groxel view of repeat region / Arend Hintze

Resolving graph complexity

- Primarily heuristic (approximate) approaches.
- Detecting complex graph structures can generally not be done efficiently.
- Much of the divergence in functionality of new assemblers comes from this.
- Three examples:

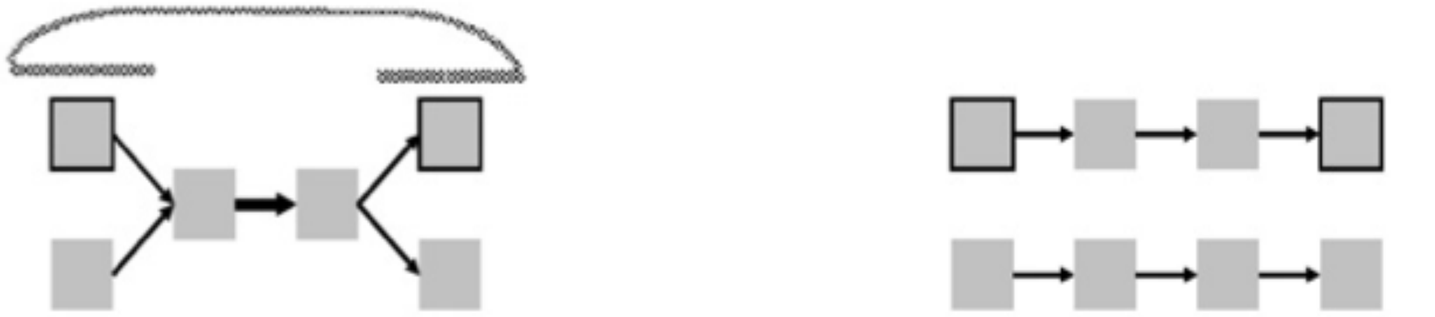
Read threading



Single read spans k-mer graph => extract the single-read path.

J.R. Miller et al. / Genomics (2010)

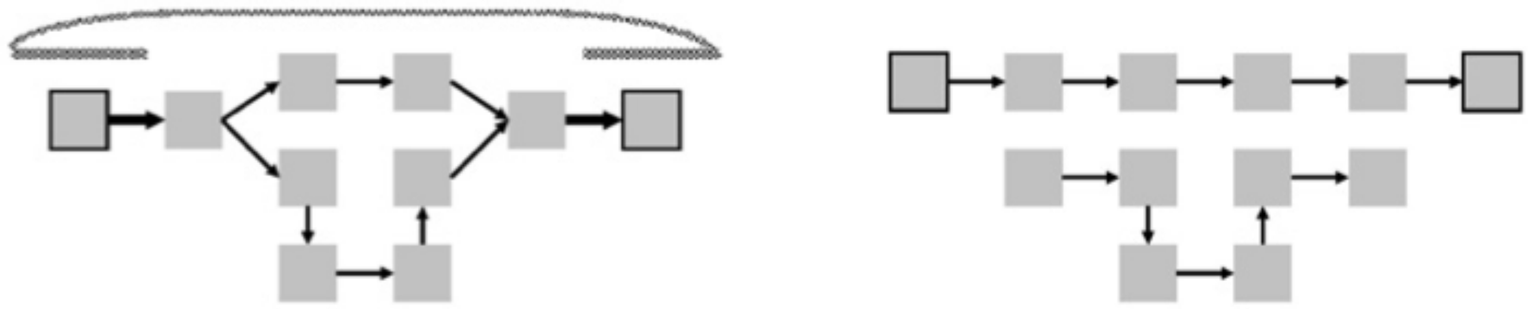
Mate threading



Resolve “frayed-rope” pattern caused by repeats, by separating paths based on mate-pair reads.

J.R. Miller et al. / Genomics (2010)

Path following



Reject inconsistent paths based on mate-pair reads and insert size.

J.R. Miller et al. / Genomics (2010)

More assembly issues

- Many parameters to optimize!
- RNAseq has variation in copy number; naïve assemblers can treat this as repetitive and eliminate it.
- Some assemblers require gobs of memory (4 lanes, 60m reads => ~ 150gb RAM)
- How do we evaluate assemblies?
  - What's the best assembler?

K-mer based assemblers scale poorly

Why do big data sets require big machines??

Memory usage ~ "real" variation + number of errors

Number of errors ~ size of data set

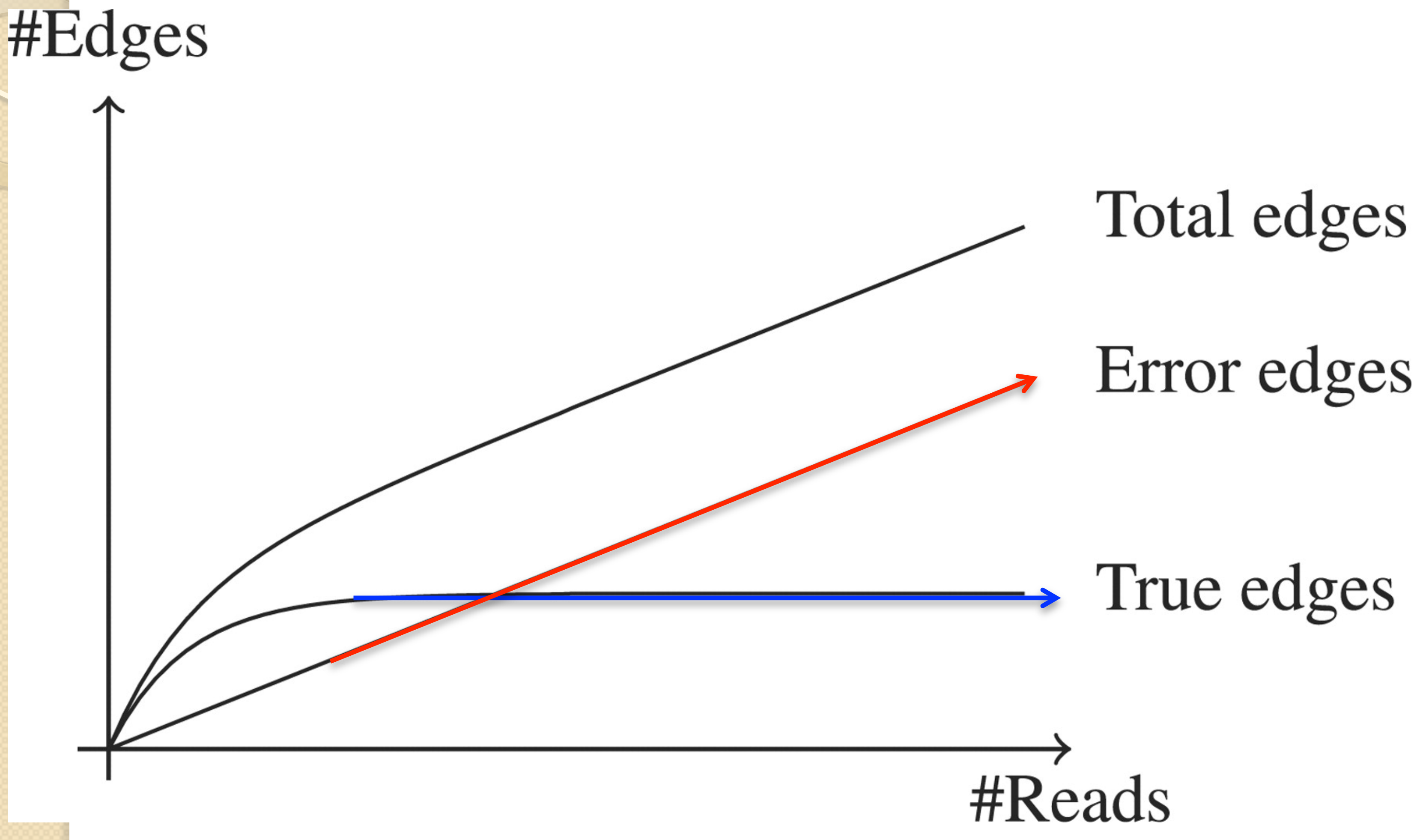
GCGTCAGGTAG**C**AGACCACCGCCATGGCGACGATG

GCGTCAGGTAGGAGACCACCG**T**CATGGCGACGATG

GCGT**T**AGGTAGGAGACCACCGCCATGGCGACGATG

GCGTCAGGTAGGAGACC**G**CCGCCATGGCGACGATG

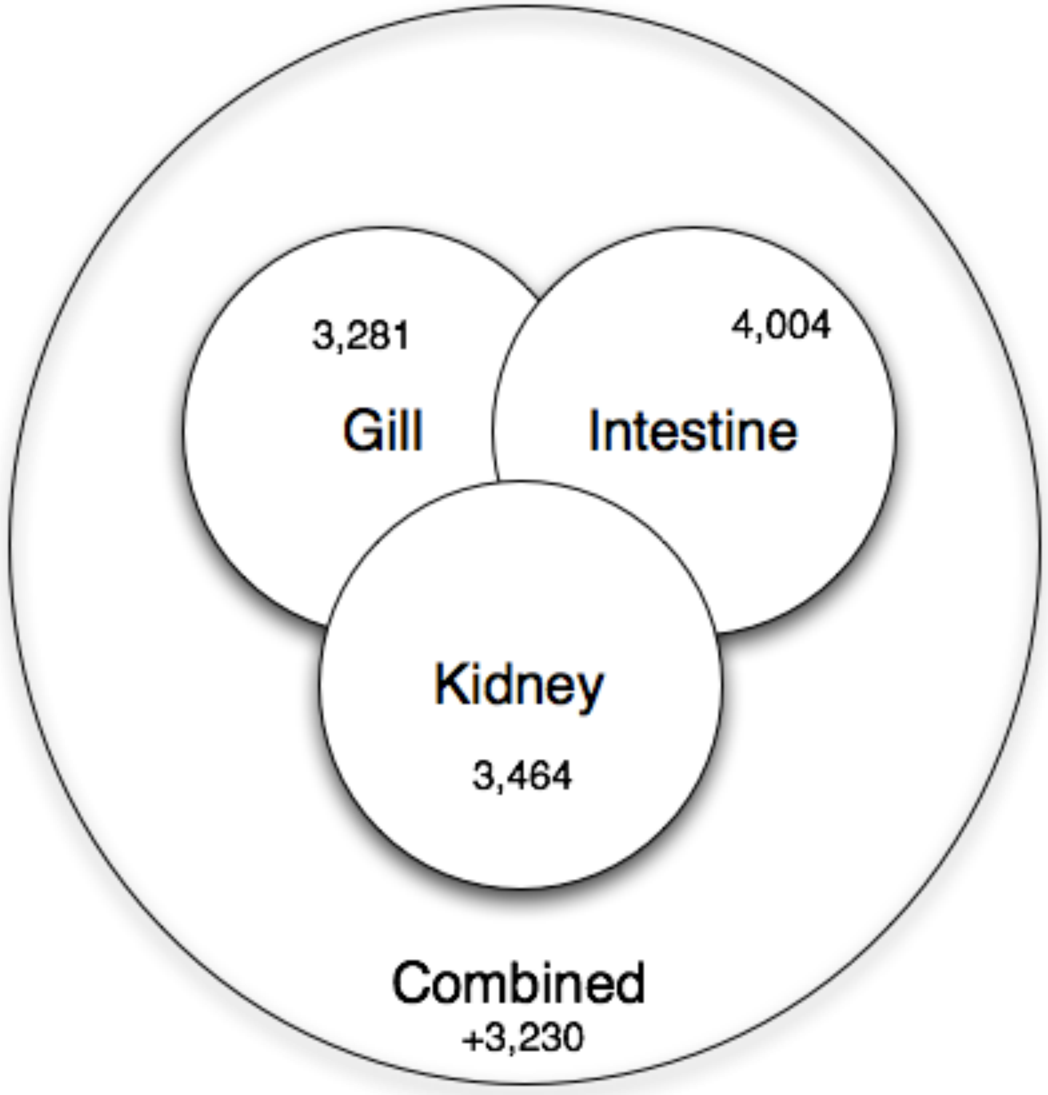
De Bruijn graphs scale poorly with erroneous data



Conway T C , Bromage A J Bioinformatics 2011;27:479-486

Co-assembly is important for sensitivity

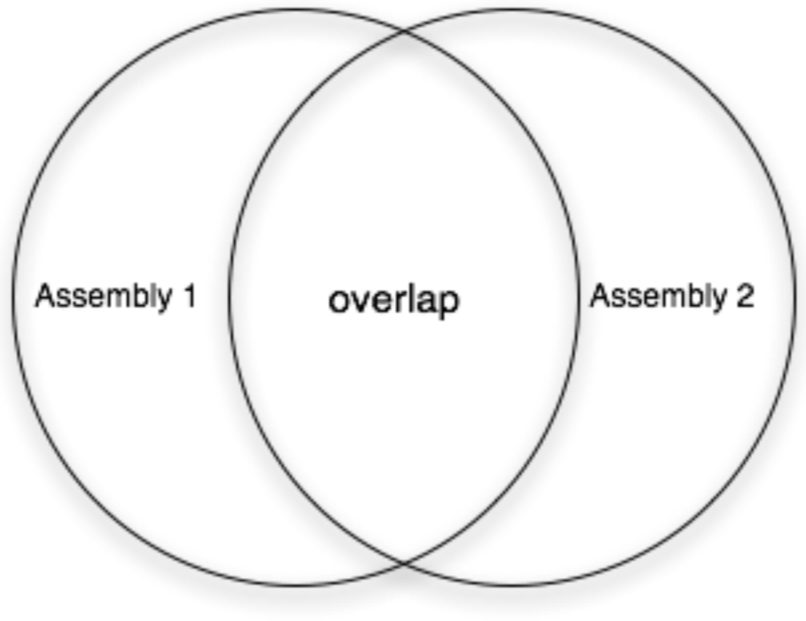
Shared low-level transcripts may not reach the threshold for assembly.



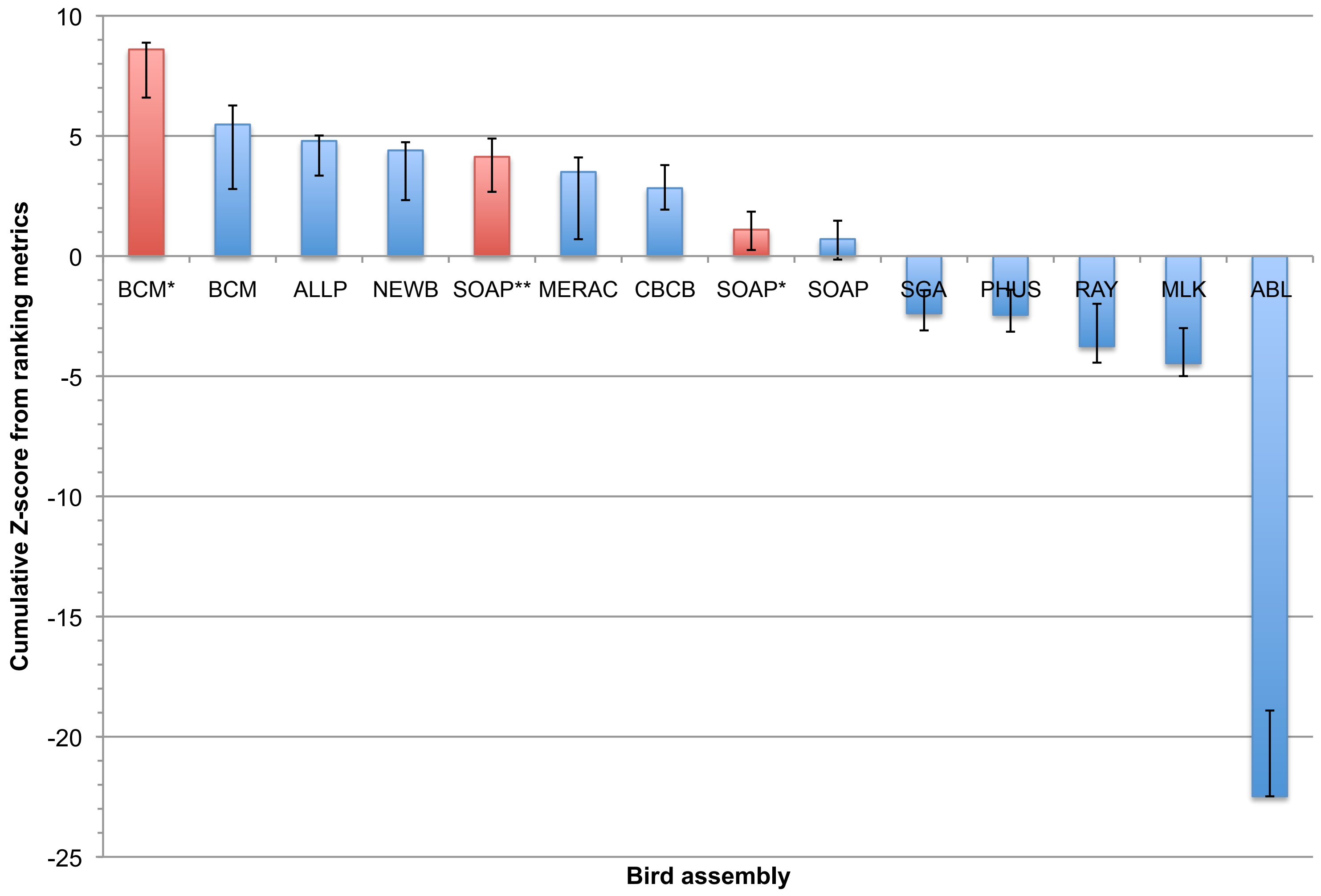
Is your assembly good?

- For genomes, N50 is an OK measure:
  - "50% or more of the genome is in contigs > this number"
- That assumes your contigs are correct…!
- What about mRNA and metagenomes??
- **Truly reference-free assembly is hard to evaluate.**

How do you compare assemblies?



What's the best assembler?



Bradnam et al., Assemblathon 2:
http://arxiv.org/pdf/1301.5406v1.pdf

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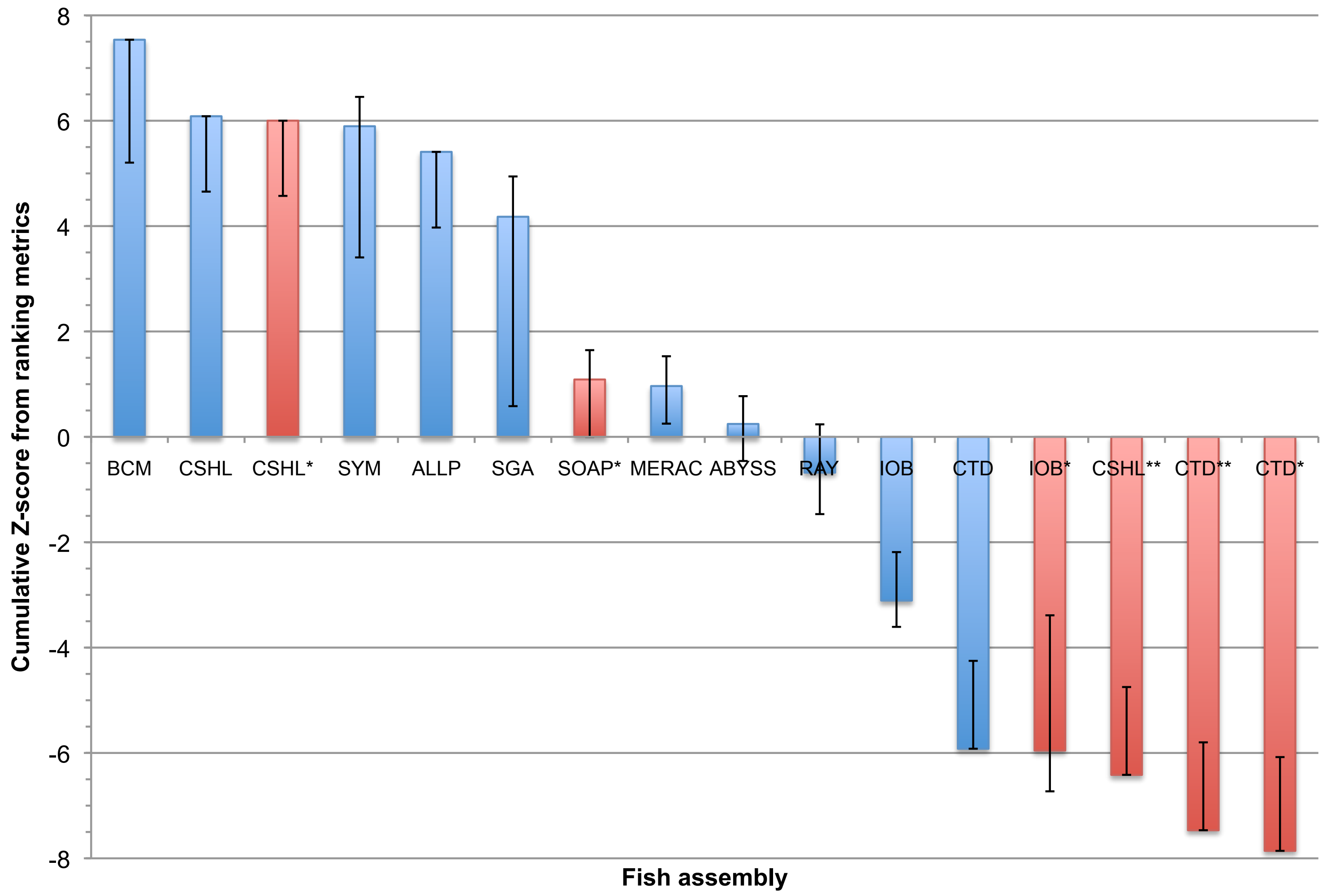

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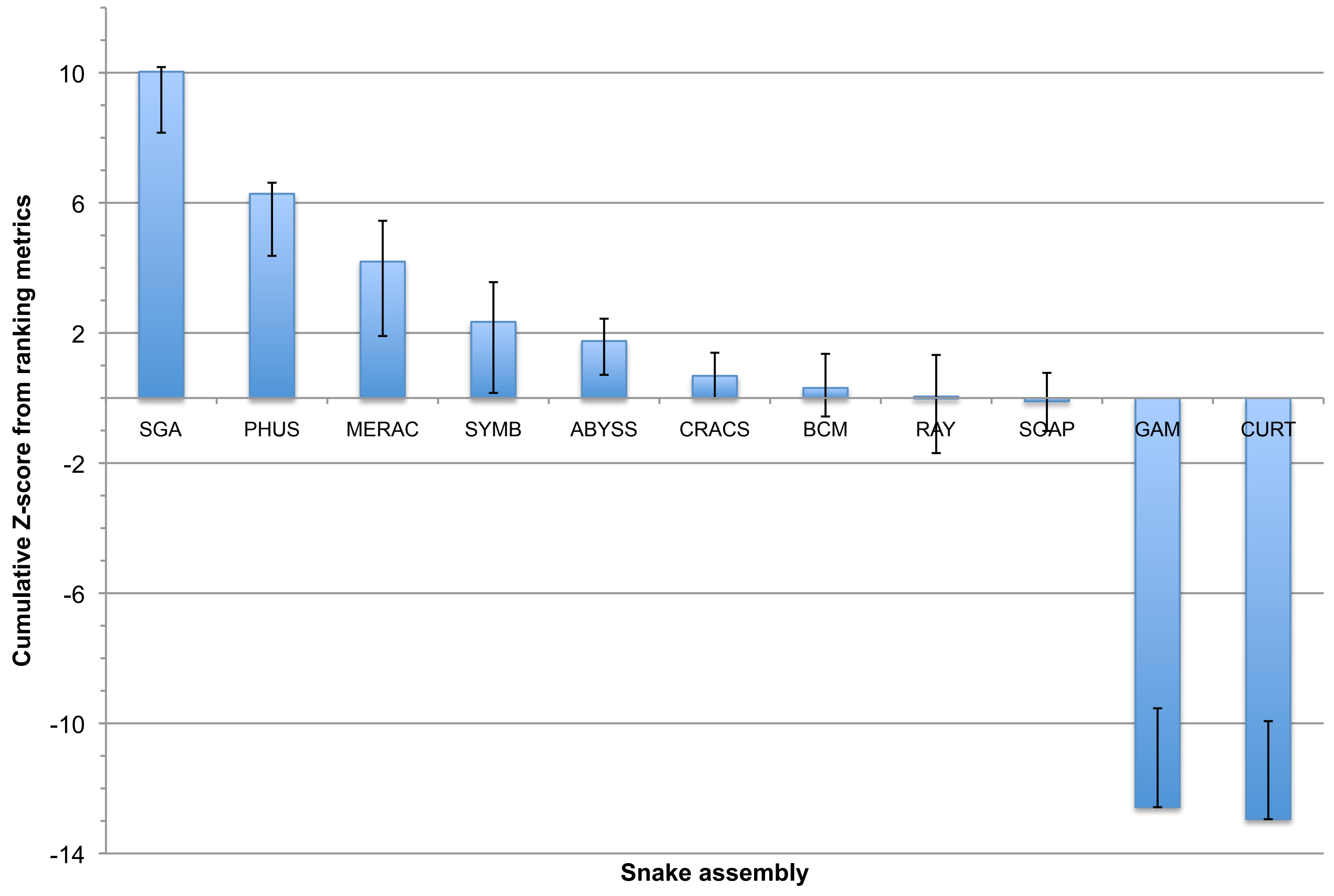


Bradnam et al., Assemblathon 2:
http://arxiv.org/pdf/1301.5406v1.pdf

Note: the teams mostly used *multiple* software packages

BCM-HGSC	BCM	2	1	1	4 + I + P[1]	Baylor College of Medicine Human Genome Sequencing Center	SeqPrep, KmerFreq, Quake, BWA, Newbler, ALLPATHS-LG, Atlas-Link, Atlas-GapFill, Phrap, CrossMatch, Velvet, BLAST, and BLASR

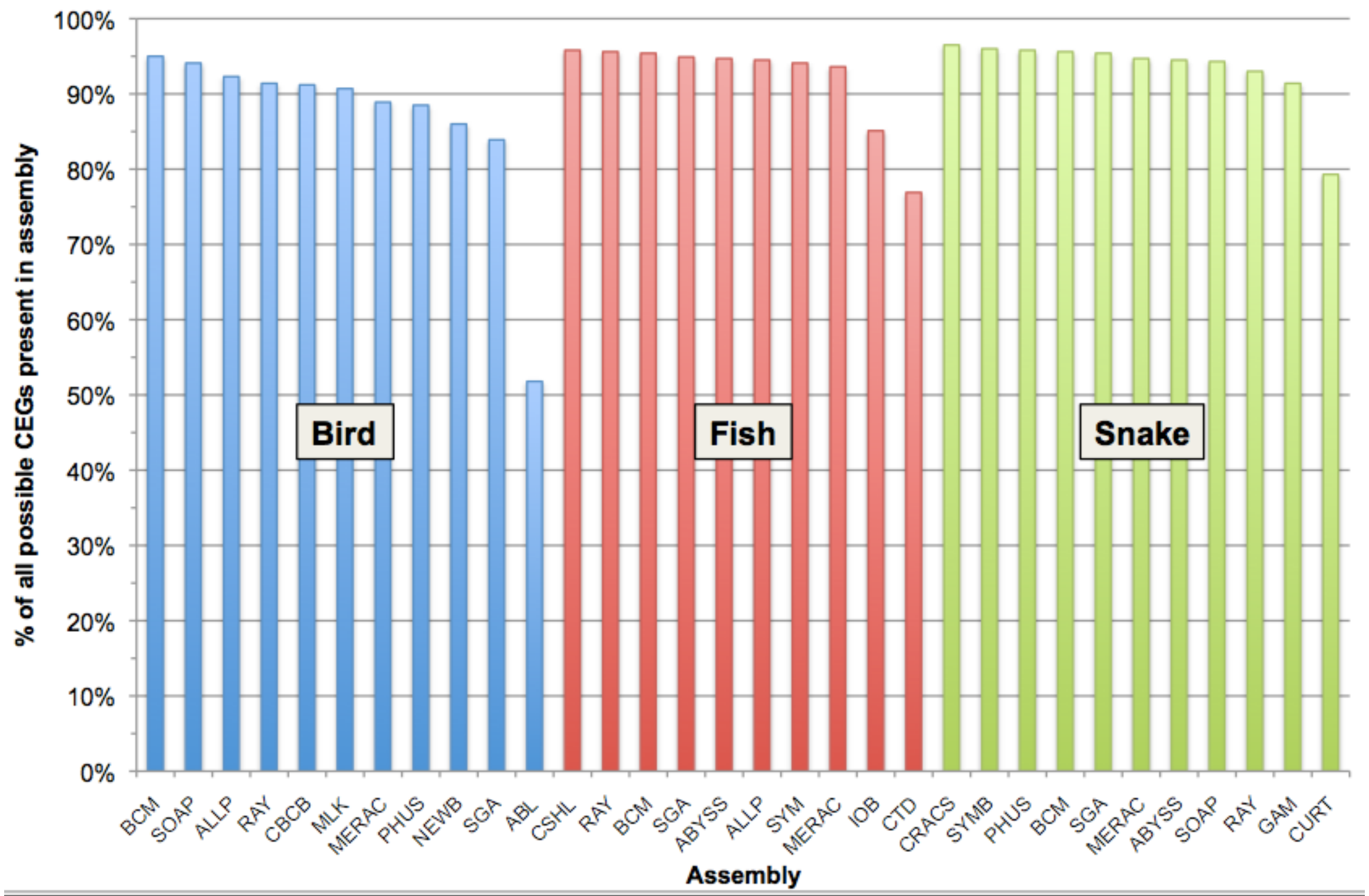
Answer: it depends

- Different assemblers perform differently, depending on
  - Repeat content
  - Heterozygosity

- Generally the results are very good (est completeness, etc.) but *different* between different assemblers (!)

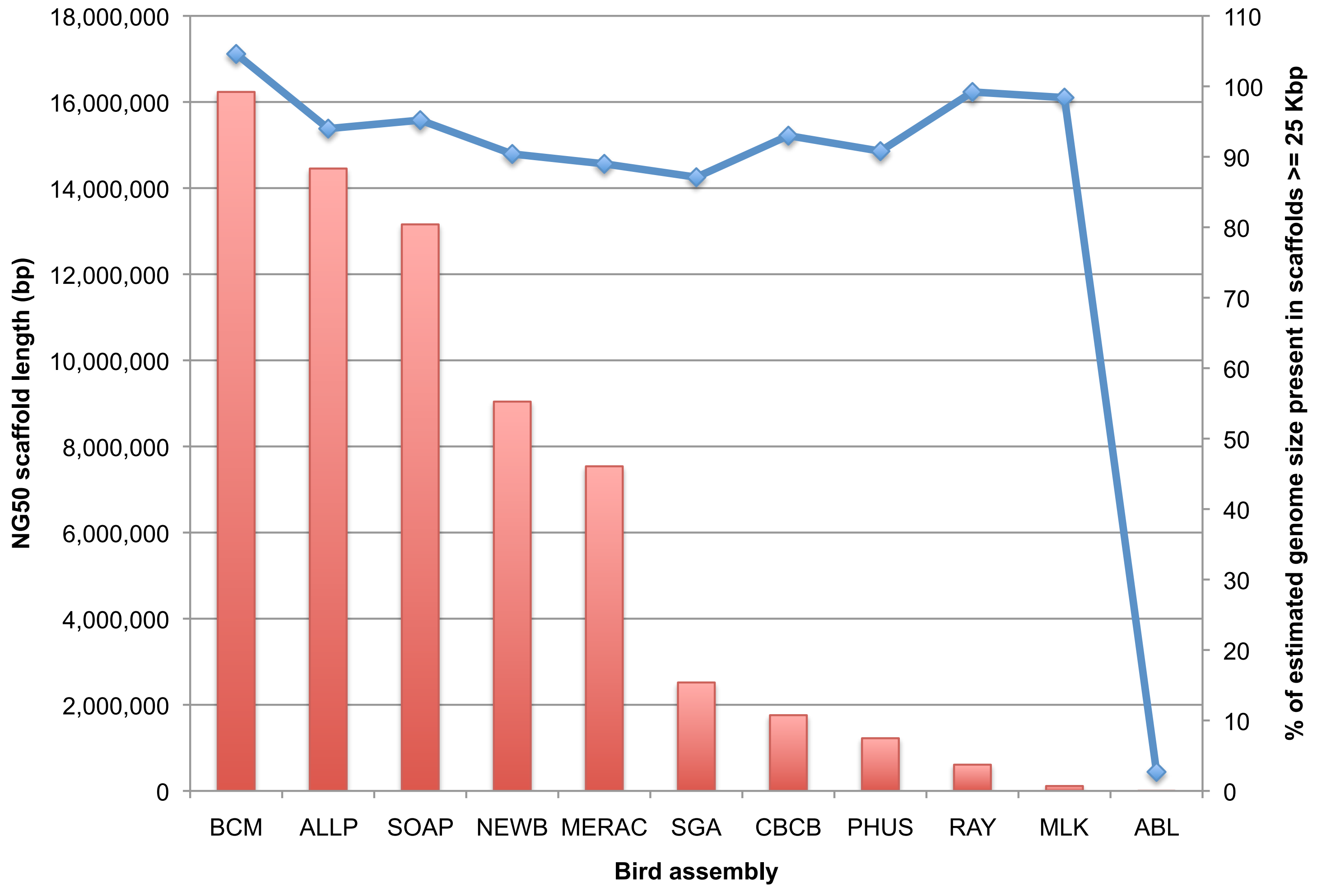
- There Is No One Answer.

Estimated completeness: CEGMA



Each assembler lost *different* ~5% CEGs

Tradeoffs in N 50 and % incl.



Practical issues

- Do you have enough memory?
- Trim vs use quality scores?
- When is your assembly as good as it gets?
- Paired-end vs longer reads?

- More data is not *necessarily* better, if it introduces more errors.

Practical issues

- Many bacterial genomes can be completely assembled with a combination of PacBio and Illumina.

- As soon as repeats, heterozygosity, and GC variation enter the picture, all bets are off (eukaryotes are trouble!)

Mapping & assembly

- Assembly and mapping (and variations thereof) are the two basic approaches used to deal with next-gen sequencing data.
- Go forth! Map! Assemble!