CS4330: Combinatorial Methods in Bioinformatics

Practical genome assembly based on de Bruijn graphs

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Practical issues in genome assembly based on de Bruijn graph

Read errors

Heterozygosity

Repeats

} De Bruijn graph becomes big, complicated, & contains many erroneous edges

Incomplete coverage } De Bruijn graph fragments into many connected components

Choice of K for constructing the de Bruijn graph

How successful practical genome assemblers deal with these issues

I will describe Velvet. You read up on the rest

Velvet

Efficient in assembling short-read sequencing data

Zerbino & Birney, "Velvet: Algorithms for de novo short read assembly using de Bruijn graphs", Genome Research, 18(5):821-829, 2008

SOAPdenovo

Suitable for large-scale genome assembly

Li et al., "De novo assembly of human genomes with massively parallel short read sequencing", Genome research, 20(2):265-272, 2010

SPAdes (aka St. Petersburg genome assembler)

Can handle diverse sequencing data types, including short reads, long reads, and mate-pair reads

Bakevich et al. "SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing", Journal of Computational Biology, 19(5):455-477, 2012

Velvet

https://en.wikipedia.org/wiki/Velvet_assembler

Simplification

Remove tips

Merge bubbles

Remove low-coverage edges

Step 1: Simplify the graph $DB_K(\mathcal{R})$

Whenever node x has only one outgoing edge, and it goes to a node y which has only one incoming edge, these two nodes are merged

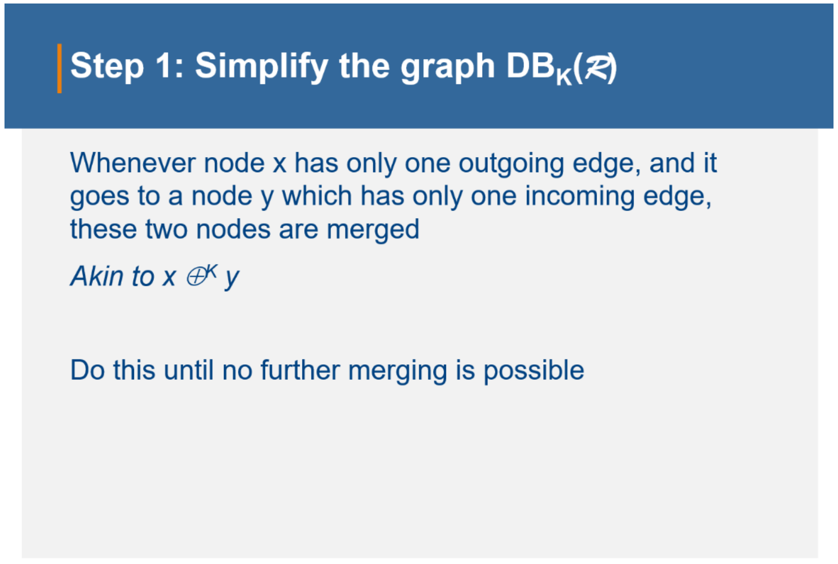
Akin to $x \oplus^K y$

Do this until no further merging is possible

Exercise

Is this step “safe”?

If not, how to make it safer?



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Whenever node x has only one outgoing edge, and it goes to a node y which has only one incoming edge, these two nodes are merged

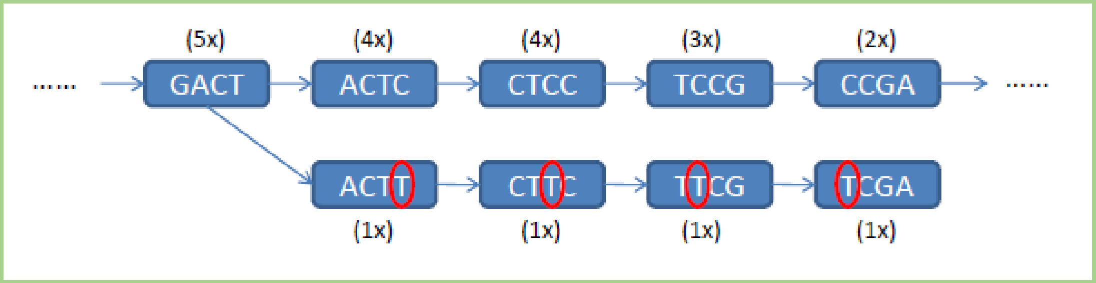
Akin to $x \oplus^K y$

Do this until no further merging is possible

Step 2: Remove "tips"

"Tips" correspond to edges due to erroneous reads

Do tip removal until no further removal is possible



A "tip" is a chain of nodes such that

Length is at most 2K

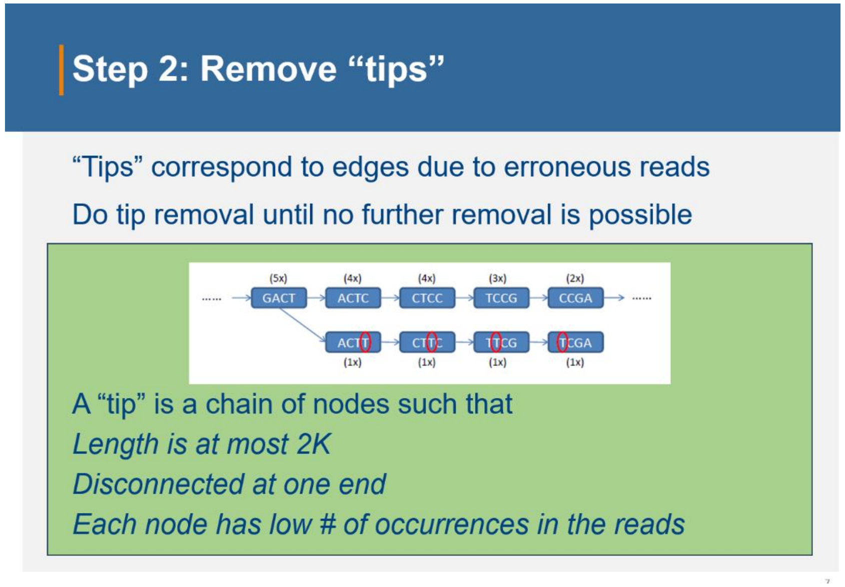
Disconnected at one end

Each node has low # of occurrences in the reads

Exercise

Why is the length of a tip defined to be at most 2K?

What might be other good choices?



(5x)
GACT
(4x)
ACTC
(4x)
CTCC
(3x)
TCCG
(2x)
CCGA
ACTT
(1x)
CTTC
(1x)
TTCG
(1x)
TCGA
(1x)

Step 2: Remove "tips"

"Tips" correspond to edges due to erroneous reads

Do tip removal until no further removal is possible

A "tip" is a chain of nodes such that

Length is at most 2K

Disconnected at one end

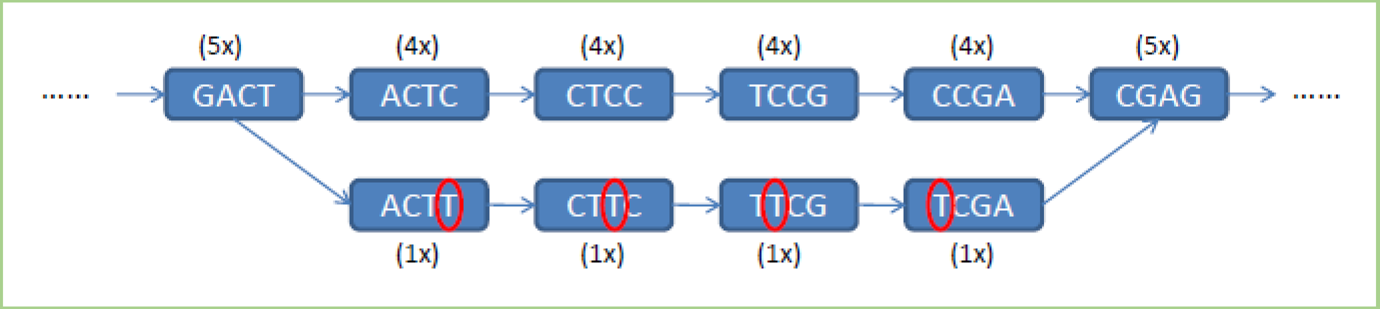
Each node has low # of occurrences in the reads

Step 3: Merge "bubbles"

"Bubbles" correspond to errors or SNPs

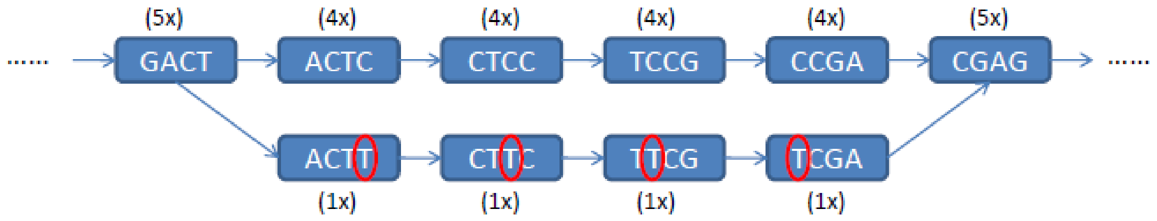
Use "Tour bus" algo to merge bubbles

"Bubbles" are two paths having the same starting node and the same ending node where these two paths represent two strings differing by very few nucleotides (e.g., 1 nucleotide)

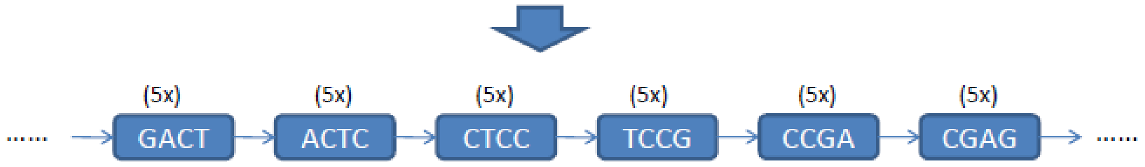


Merge a bubble

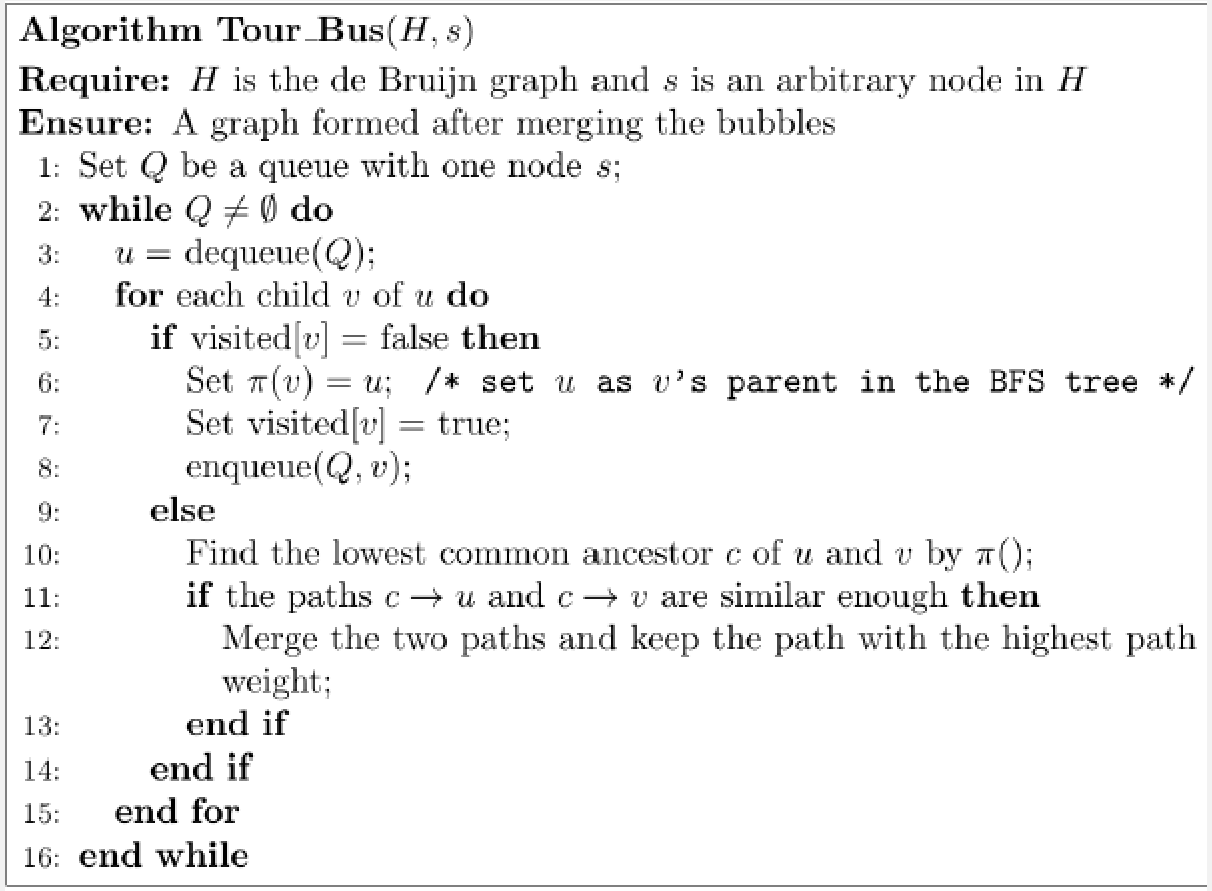
- The top path represents GACTCCGAG.
- The bottom path represents GACTTCGAG.



- There is only one nucleotide different. We merge them.



Tour bus algorithm



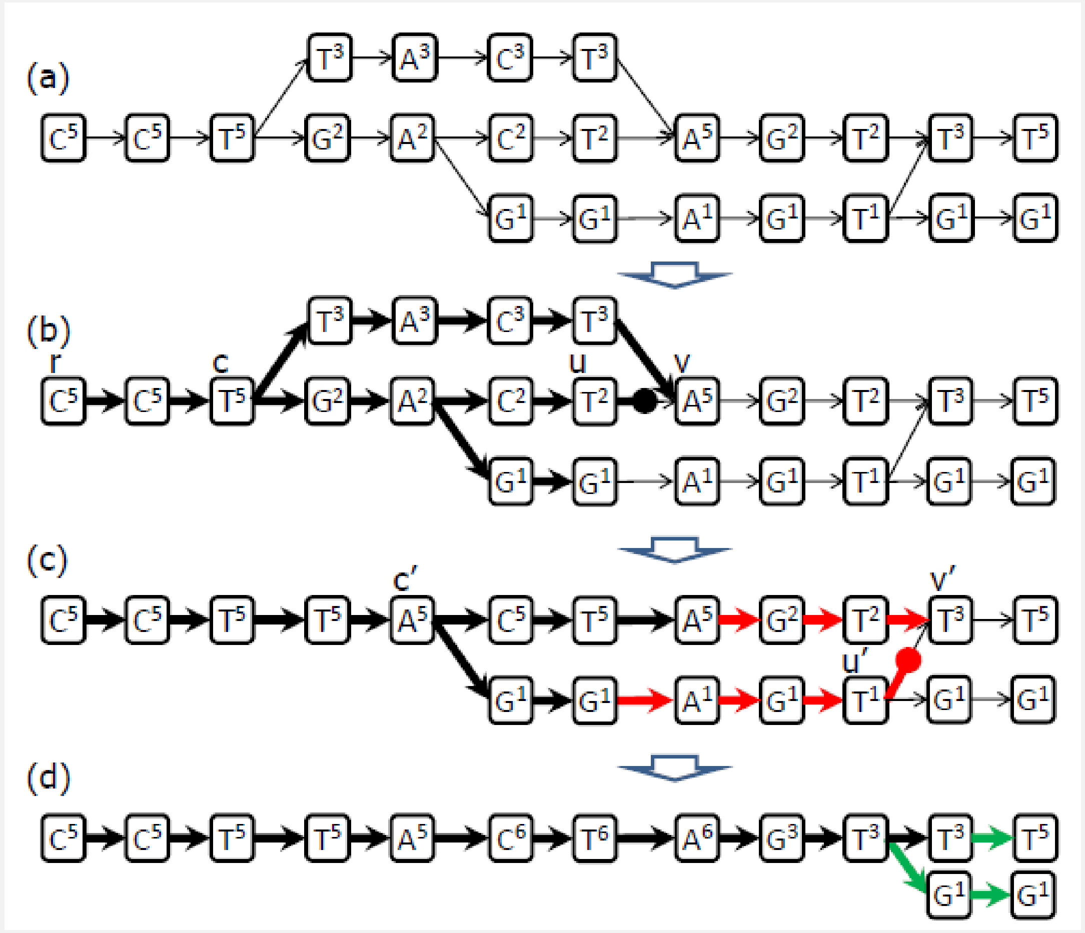
Algorithm Tour_Bus(H, s)

Require: H is the de Bruijn graph and s is an arbitrary node in H

Ensure: A graph formed after merging the bubbles

```
Set $Q$ be a queue with one node $s$;
while $Q \neq \emptyset$ do
    $u$ = dequeue($Q$);
    for each child $v$ of $u$ do
        if visited[$v$] = false then
            Set $\pi(v) = u$;  /* set $u$ as $v$'s parent in the BFS tree */
            Set visited[$v$] = true;
            enqueue($Q, v$);
        else
            Find the lowest common ancestor $c$ of $u$ and $v$ by $\pi()$;
            if the paths $c \to u$ and $c \to v$ are similar enough then
                Merge the two paths and keep the path with the highest path weight;
            end if
        end if
    end for
end while
```

Example



Step 4: Remove erroneous connections

Remove edges with low coverage (a cutoff set by users)

Exercise

How to derive a reasonable threshold for this step?

Step 4: Remove erroneous connections

Remove edges with low coverage (a cutoff set by users)

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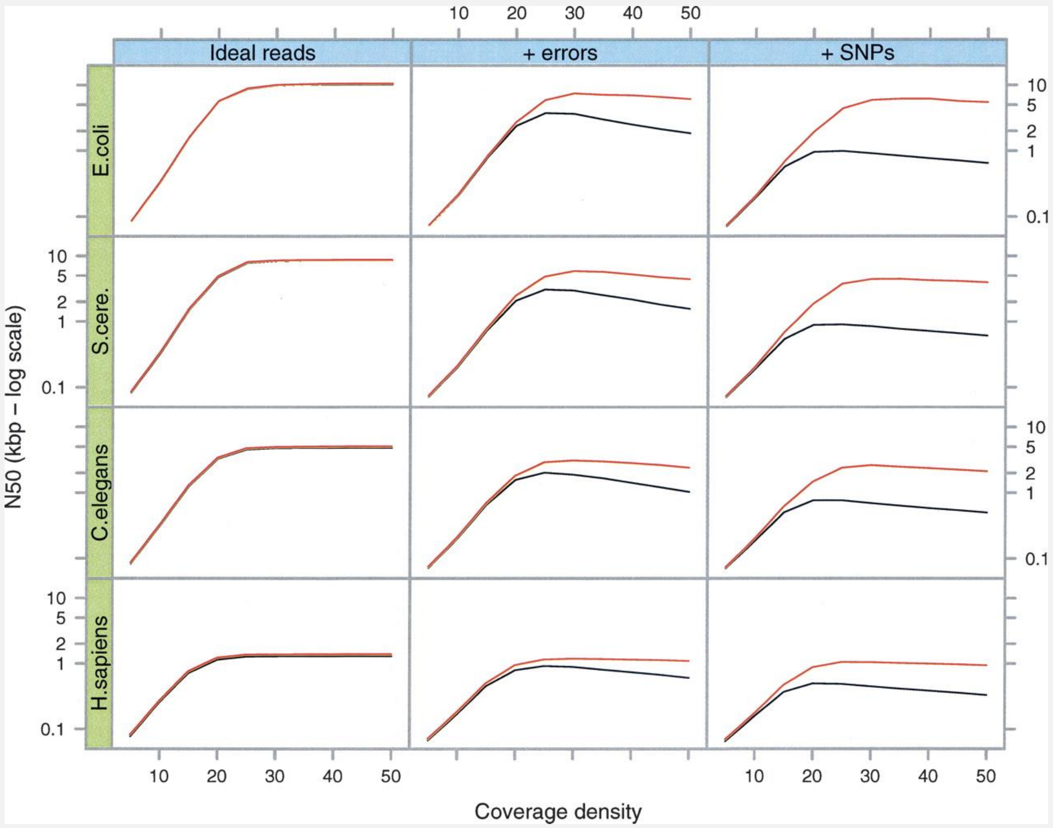
Assembly quality

Simulations of Tour Bus. The genome of *E. coli* and 5-Mb samples of DNA from three other species (*S. cerevisiae*, *C. elegans*, *and H. sapiens*, respectively) were used to generate 35-bp read sets of varying read depths (*X*-axis of each plot). We measured the contig length N50 (*Y*-axis, log scale) after tip-clipping (black curve) then after the subsequent bubble smoothing (red curve). In the first column are the results for perfect, error-free reads. In the second column, we inserted errors in the reads at a rate of 1%. In the third column, we generated a slightly variant genome from the original by inserting random SNPs at a rate of 1 in 500. The reads were then generated with errors from both variants, thus simulating a diploid assembly.



Zerbino & Birney, *Genome Research*, 18(5):821-829, 2008

Efficiency

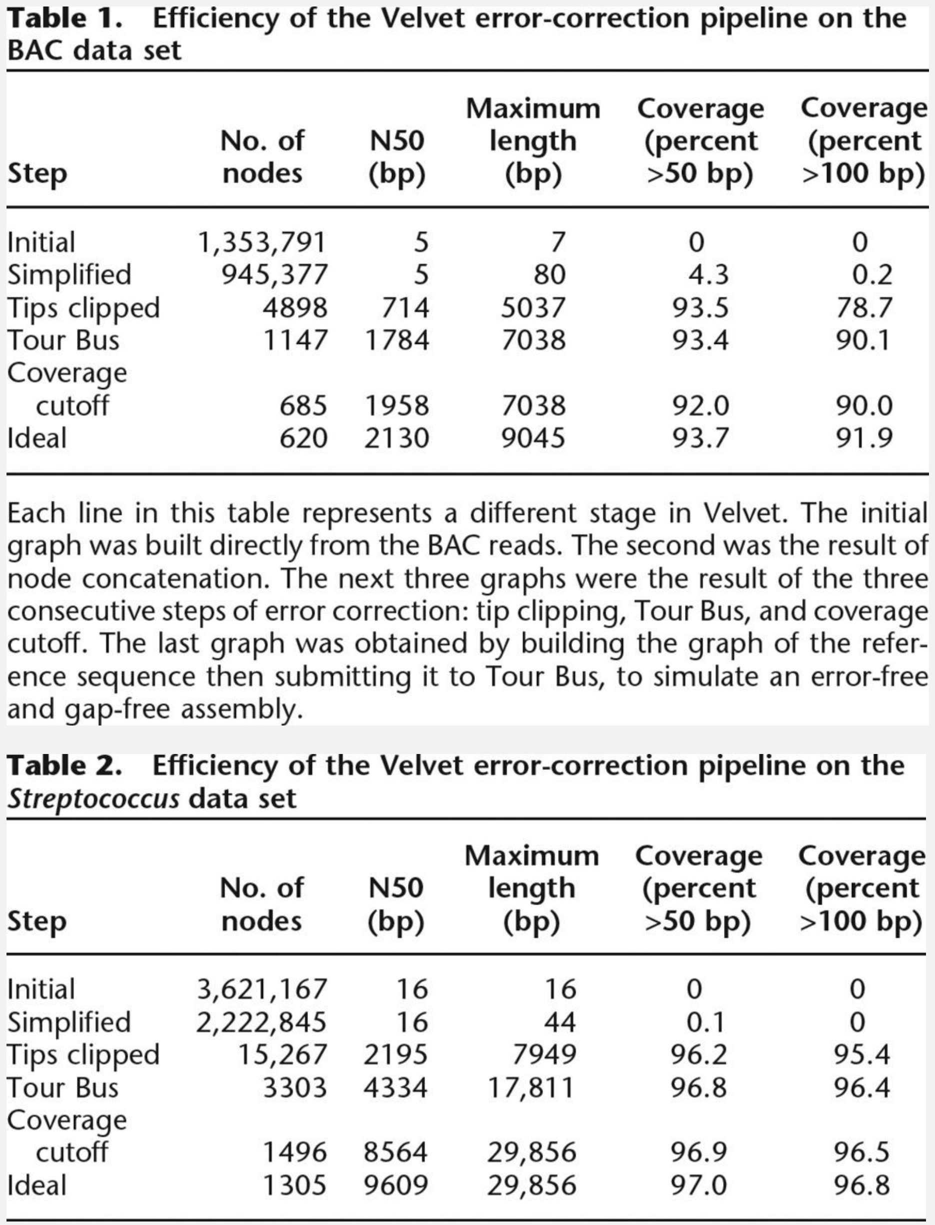


Table 1. **Efficiency of the Velvet error-correction pipeline on the BAC data set**

Step	No. of nodes	N50 (bp)	Maximum length (bp)	Coverage (percent >50 bp)	Coverage (percent >100 bp)
Initial	1,353,791	5	7	0	0
Simplified	945,377	5	80	4.3	0.2
Tips clipped	4898	714	5037	93.5	78.7
Tour Bus	1147	1784	7038	93.4	90.1
Coverage cutoff	685	1958	7038	92.0	90.0
Ideal	620	2130	9045	93.7	91.9

Each line in this table represents a different stage in Velvet. The initial graph was built directly from the BAC reads. The second was the result of node concatenation. The next three graphs were the result of the three consecutive steps of error correction: tip clipping, Tour Bus, and coverage cutoff. The last graph was obtained by building the graph of the reference sequence then submitting it to Tour Bus, to simulate an error-free and gap-free assembly.

Table 2. **Efficiency of the Velvet error-correction pipeline on the *Streptococcus* data set**

Step	No. of nodes	N50 (bp)	Maximum length (bp)	Coverage (percent >50 bp)	Coverage (percent >100 bp)
Initial	3,621,167	16	16	0	0
Simplified	2,222,845	16	44	0.1	0
Tips clipped	15,267	2195	7949	96.2	95.4
Tour Bus	3303	4334	17,811	96.8	96.4
Coverage cutoff	1496	8564	29,856	96.9	96.5
Ideal	1305	9609	29,856	97.0	96.8

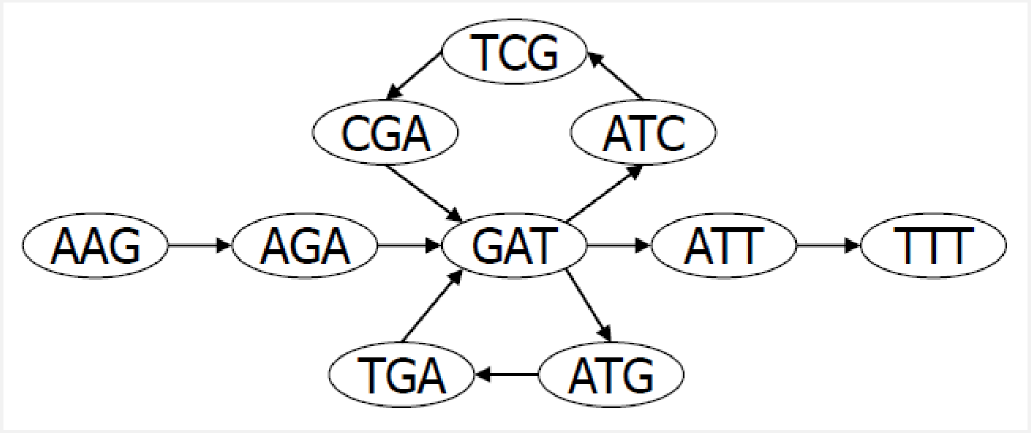
Zerbino & Birney, *Genome Research*, 18(5):821-829, 2008

Recall this example, where K = 3

Genome = AAGATCGATGATTT

$\mathcal{R}$ = { AAGATC, GATCGAT, CGATGA, ATGATT, GATTT }

DB$_3$($\mathcal{R}$):



Two possible Eulerian paths but can't tell which is real

AAGATCGATGATTT

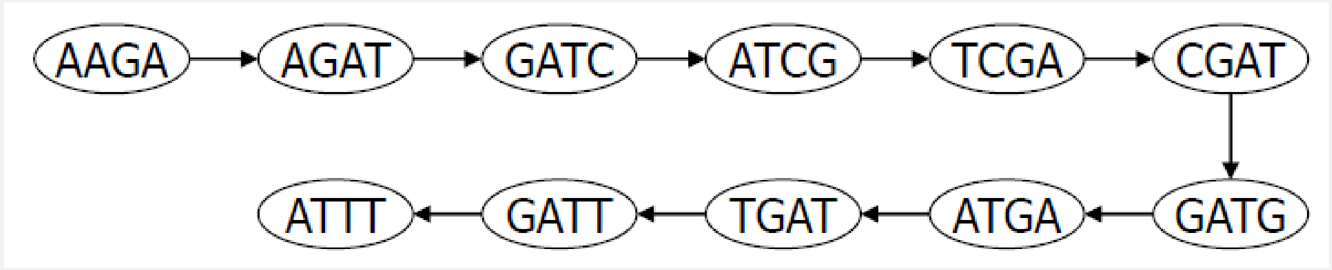
AAGATGATCGATTT

Consider K = 4

Genome = AAGATCGATGATTT

$\mathcal{R}$ = { AAGATC, GATCGAT, CGATGA, ATGATT, GATTT }

DB$_4$($\mathcal{R}$):



A unique Eulerian path:

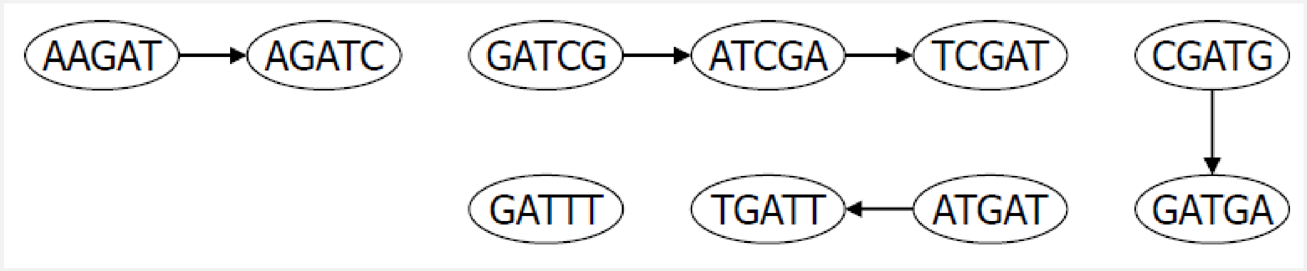
AAGATCGATGATTT

Consider K = 5

Genome = AAGATCGATGATTT

$\mathcal{R}$ = { AAGATC, GATCGAT, CGATGA, ATGATT, GATTT }

$DB_5(\mathcal{R})$:



No Eulerian paths; fragmented graphs give these strings:

AAGATC, GATCGAT, CGATGA, ATGATT, GATTT

How to choose suitable K

Large K

K-mers more likely to have errors

# of correct K-mers is reduced

More genome coverage gaps

Small K

More likely to be repeated

Short repeats create loops and branches in de Bruijn graph

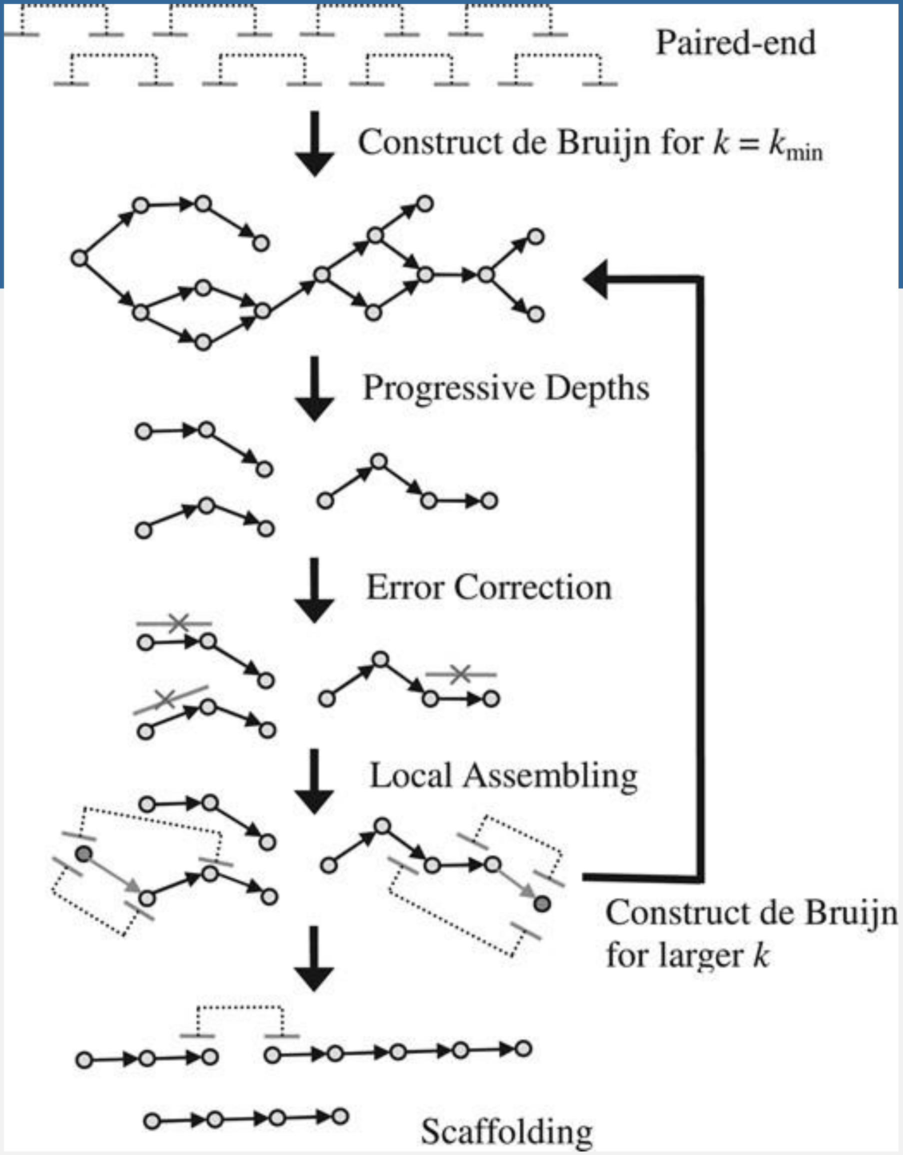
IDBA

Instead of a fixed K, try different values

When K is small, we get short but high-quality contigs

Use them to correct errors in reads

Then, increment K and try again

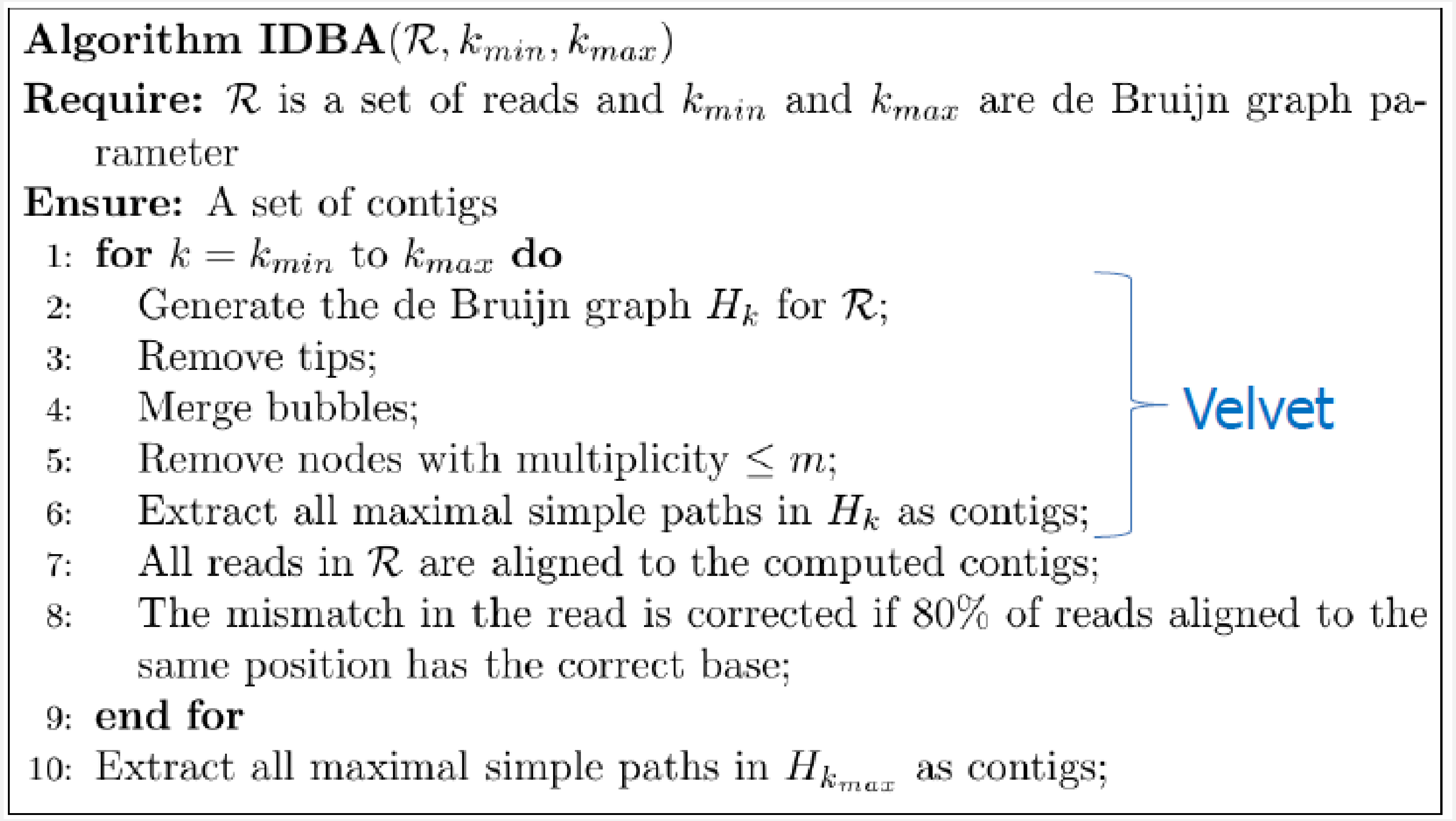


Paired-end
Construct de Bruijn for $k = k_{min}$
Progressive Depths
Error Correction
Local Assembling
Construct de Bruijn for larger k
Scaffolding

Peng, et al., "IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth", Bioinformatics, 28(11): 1420-1428, 2010

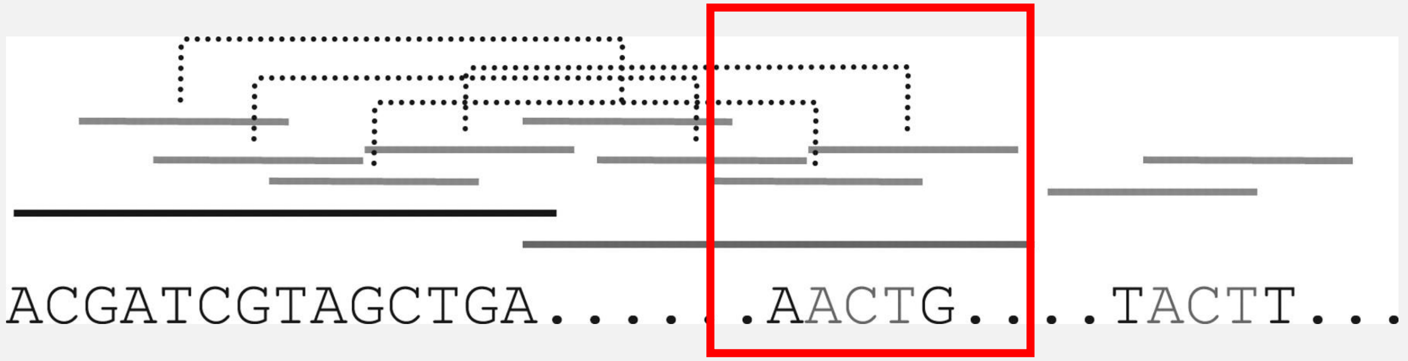
IDBA algorithm

Step 7 uses techniques similar to reference based genome assembly



```
Algorithm IDBA($\mathcal{R}, k_{min}, k_{max}$)
Require: $\mathcal{R}$ is a set of reads and $k_{min}$ and $k_{max}$ are de Bruijn graph parameter
Ensure: A set of contigs
 1: for $k = k_{min}$ to $k_{max}$ do
 2:     Generate the de Bruijn graph $H_k$ for $\mathcal{R}$;
 3:     Remove tips;
 4:     Merge bubbles;
 5:     Remove nodes with multiplicity $\leq m$;
 6:     Extract all maximal simple paths in $H_k$ as contigs;
 7:     All reads in $\mathcal{R}$ are aligned to the computed contigs;
 8:     The mismatch in the read is corrected if 80% of reads aligned to the
        same position has the correct base;
 9: end for
10: Extract all maximal simple paths in $H_{k_{max}}$ as contigs;
```

Low-coverage regions remain problematic



A region is covered by two reads ...AACT and ACTG...

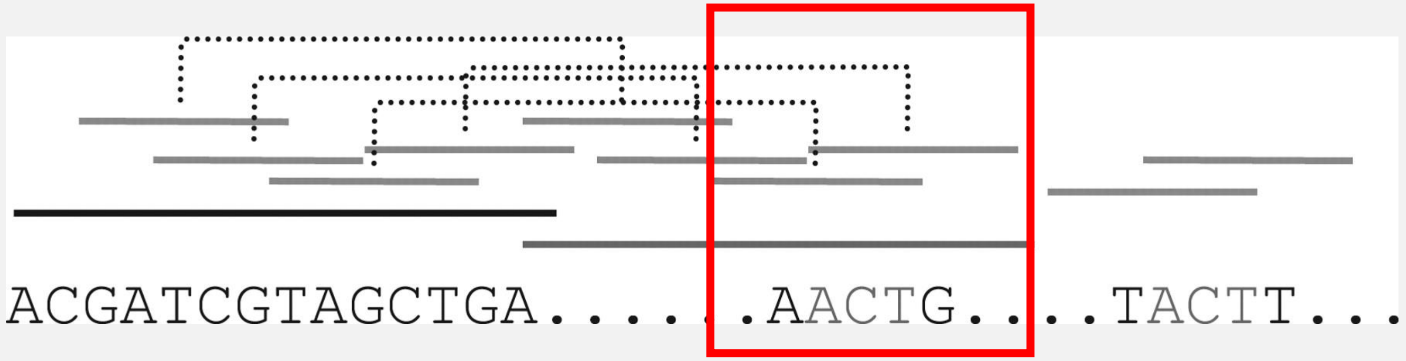
At K = 3, $DB_K(\mathcal{R})$ shows a path AAC → ACT → CTG but cannot output the contig … AACTG …

AACTG *is not in any read*

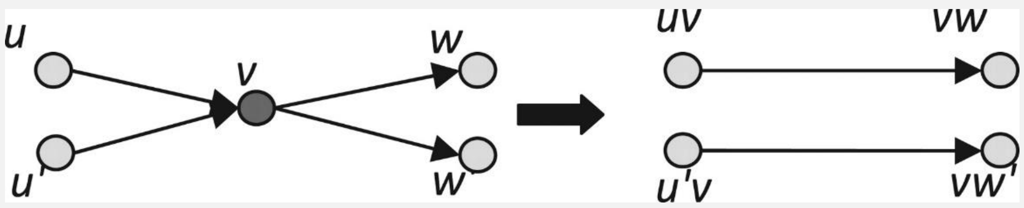
ACT *has 2 incoming & 2 outgoing edges due to* TACTT

Similar problems exist at K = 4, 5, …

IDBA-UD ≈ IDBA + local assembly



Using information from paired-end read mapping, the two copies of ACT can be separated



Then, …AACTG … can be produced

Performance

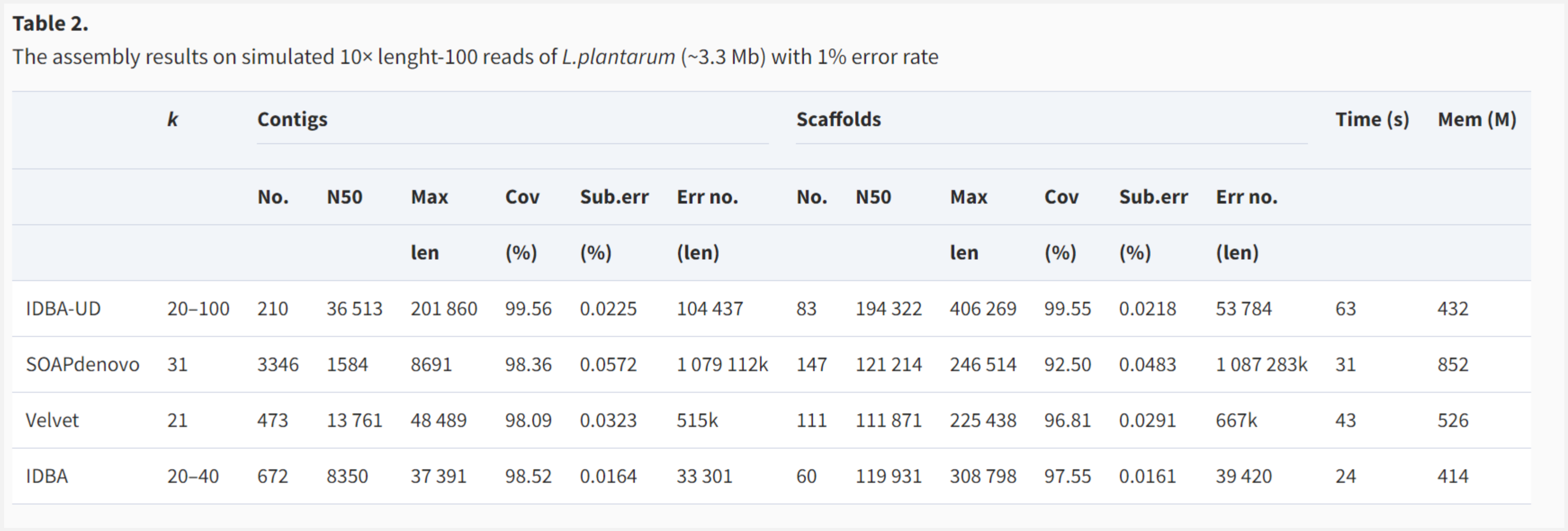


Table 2.
The assembly results on simulated 10× lenght-100 reads of *L.plantarum* (~3.3 Mb) with 1% error rate

	k	**Contigs**						**Scaffolds**						**Time (s)**	**Mem (M)**
		No.	**N50**	**Max len**	**Cov (%)**	**Sub.err (%)**	**Err no. (len)**	**No.**	**N50**	**Max len**	**Cov (%)**	**Sub.err (%)**	**Err no. (len)**		
IDBA-UD	20–100	210	36 513	201 860	99.56	0.0225	104 437	83	194 322	406 269	99.55	0.0218	53 784	63	432
SOAPdenovo	31	3346	1584	8691	98.36	0.0572	1 079 112k	147	121 214	246 514	92.50	0.0483	1 087 283k	31	852
Velvet	21	473	13 761	48 489	98.09	0.0323	515k	111	111 871	225 438	96.81	0.0291	667k	43	526
IDBA	20–40	672	8350	37 391	98.52	0.0164	33 301	60	119 931	308 798	97.55	0.0161	39 420	24	414

Peng, et al., "IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth", *Bioinformatics*, 28(11): 1420-1428, 2010

Limitations

De Bruijn graph is big

Need lots of memory to run

Cannot use connectivity of paired-end reads before scaffolding

SPAdes uses paired de Bruijn graph to capture this info

If there are long repeats, this approach may fail to get long contigs

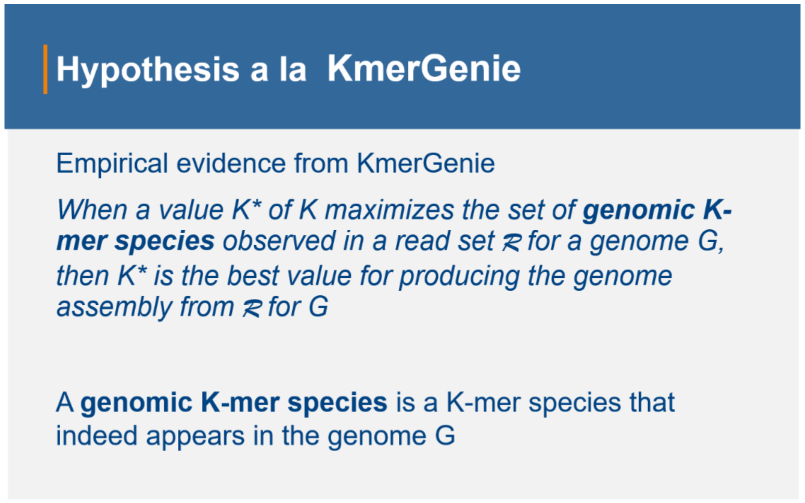
Exercise

IDBA tries increasingly bigger values for K

I.e., it must assemble the genome multiple times

Would it be possible to guess a good value for K by just taking a quick look at the read set?

How?



Hypothesis a la KmerGenie

Empirical evidence from KmerGenie

When a value K of K maximizes the set of **genomic K-mer species** observed in a read set $\mathcal{R}$ for a genome G, then K* is the best value for producing the genome assembly from $\mathcal{R}$ for G*

A **genomic K-mer species** is a K-mer species that indeed appears in the genome G

Good to read

Velvet

Zerbino & Birney, "Velvet: Algorithms for de novo short read assembly using de Bruijn graphs", *Genome Research*, 18(5):821-829, 2008

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2336801/

IDBA-UD

Peng, et al., "IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth", Bioinformatics, 28(11): 1420-1428, 2010

https://pubmed.ncbi.nlm.nih.gov/22495754/

Good to read

SOAPdenovo

Li et al., "De novo assembly of human genomes with massively parallel short read sequencing", *Genome research*, 20(2):265-272, 2010

https://pubmed.ncbi.nlm.nih.gov/20019144/

SPAdes

Bakevich et al. "SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing", *Journal of Computational Biology*, 19(5):455-477, 2012

https://pubmed.ncbi.nlm.nih.gov/22506599/

KmerGenie

Chikni & Medvedev, "Informed and automated k-mer size selection for genome assembly", *Bioinformatics*, 30(1):31-37, 2014

https://pubmed.ncbi.nlm.nih.gov/23732276/