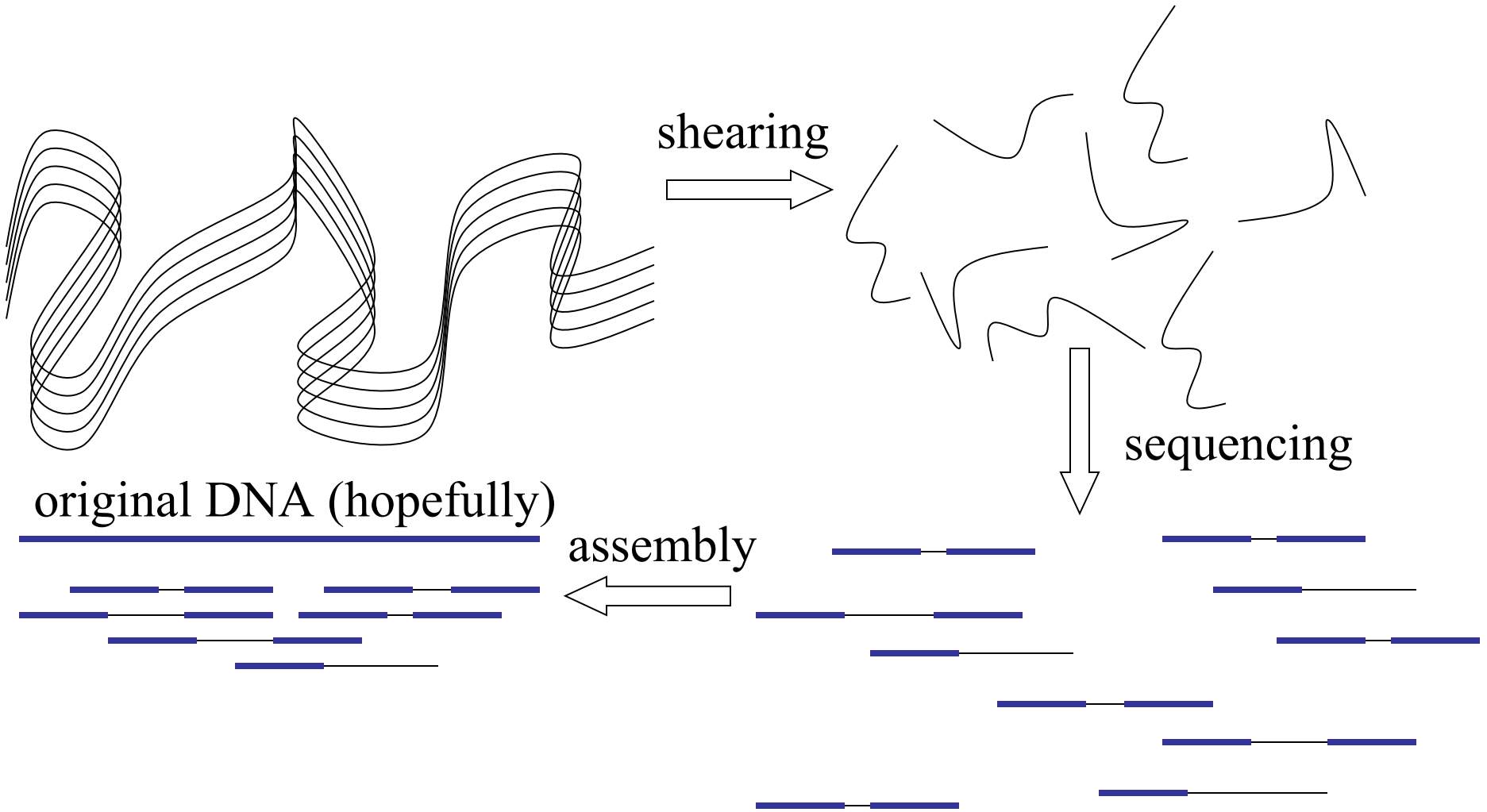
CMSC423: Bioinformatic Algorithms, Databases and Tools

Genome assembly

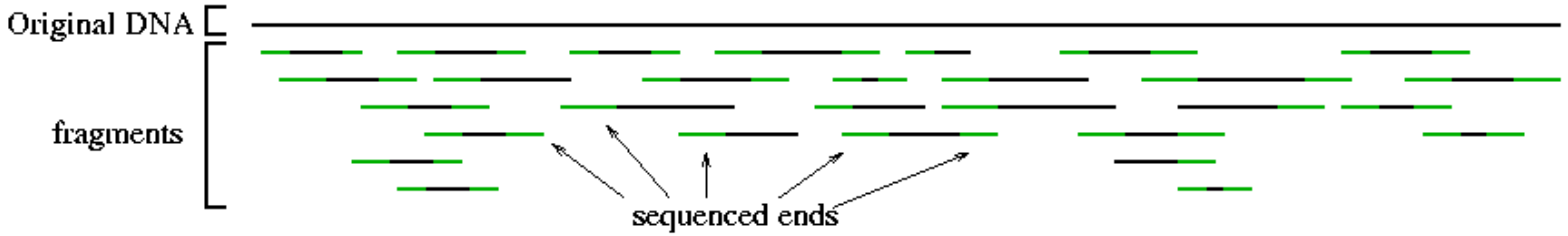
Reading assignment

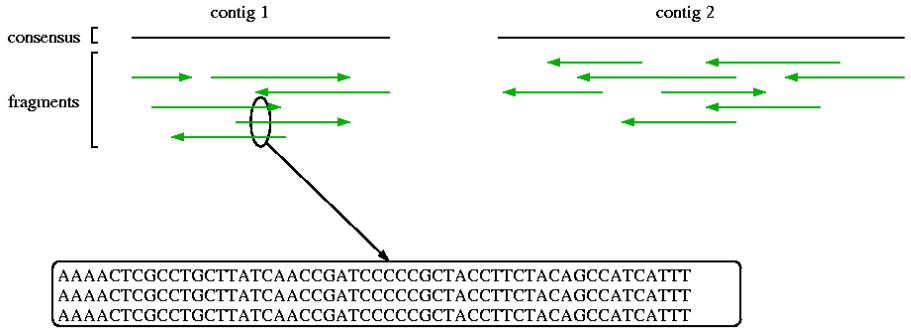
- http://www.cbcb.umd.edu/research/assembly_primer.shtml
- Chapter 4.5 – coverage statistics
- Chapter 8 – genome assembly
- http://amos.sourceforge.net

Shotgun sequencing



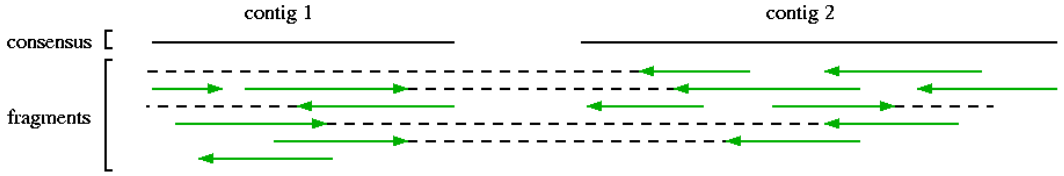
Overview of terms





Assembly

Scaffolding



Assembly Glossary

- Read – small (50-2000bp) segment of DNA "read" by a sequencing instrument
- Mate-pair, paired ends – pair of reads whose distance from each other within the genome is approximately known
- Contig – contiguous segment of DNA reconstructed (unambiguously) from a set of reads
- Scaffold – group of contigs that can be ordered and oriented with respect to each other (usually with the help of mate-pair data)

So...

- Sequencing technologies only "read" small chunks of DNA, yet genomes are substantially larger
- The shotgun sequencing approach generates many random fragments from the original DNA
- The task of the assembly program is to stitch together the many small pieces into a reconstruction of the genome

- Essentially..... a huge jigsaw puzzle

- Think: shred a collection of Harry Potter books at random then try to rebuild the original without any additional information.

Shortest common superstring problem

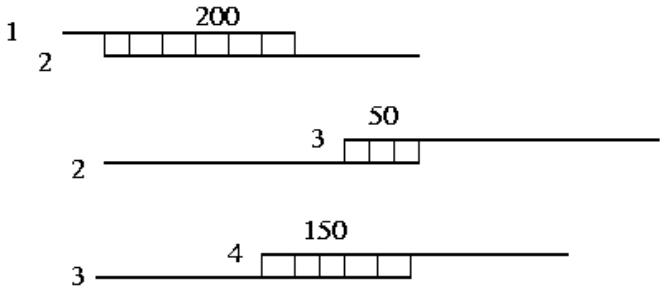
Given a set of strings, $\Sigma=(s_1, ..., s_n)$, determine the shortest string S such that every s_i is a sub-string of S.

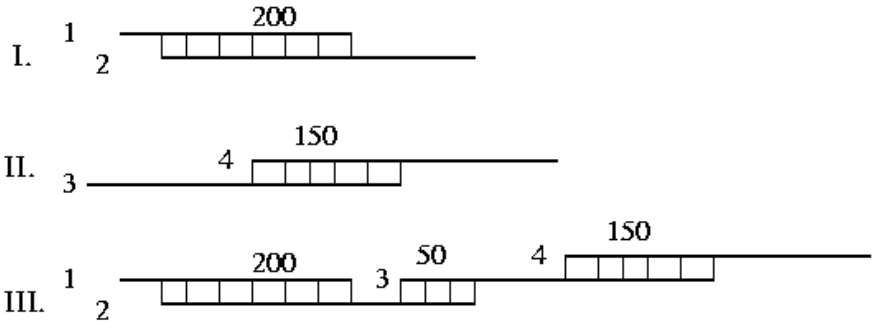
NP-hard

approximations: 4, 3, 2.89, ...

```
...ACAGGACTGCACAGATTGATAG
          ACTGCACAGATTGATAGCTGA...
```

Greedy algorithm (4-approximation)





phrap, TIGR Assembler, CAP

Greedy algorithm details

Compute all pairwise overlaps

*Pick best (e.g. in terms of alignment score) overlap

Join corresponding reads

Repeat from * until no more joins possible

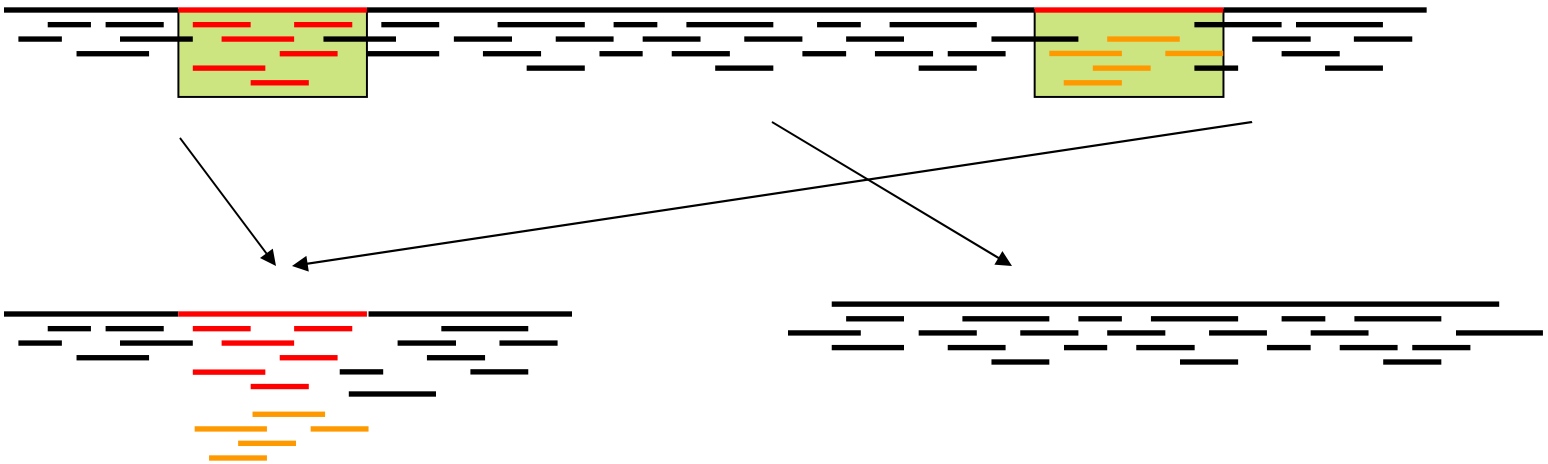
- How do you compute an overlap alignment?
- Hint: modify Smith-Waterman dynamic programming algorithm

Repeats (where greedy fails)

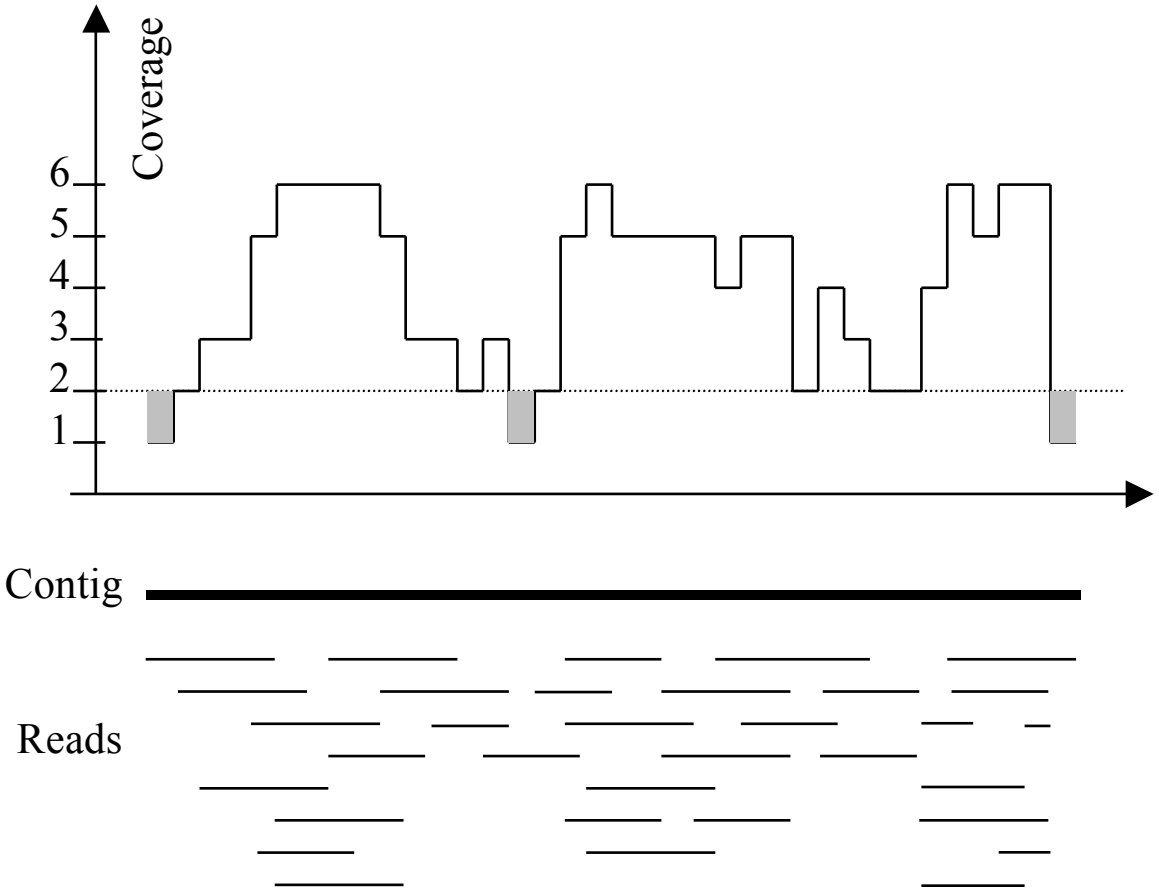
AAAAAAAAAAAAAAAAAAAA

AAAAAA AAAAAA AAAAAA
AAAAAA AAAAAA
AAAAAA AAAAAA

AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA
AAAAAA



Impact of randomness – non-uniform coverage



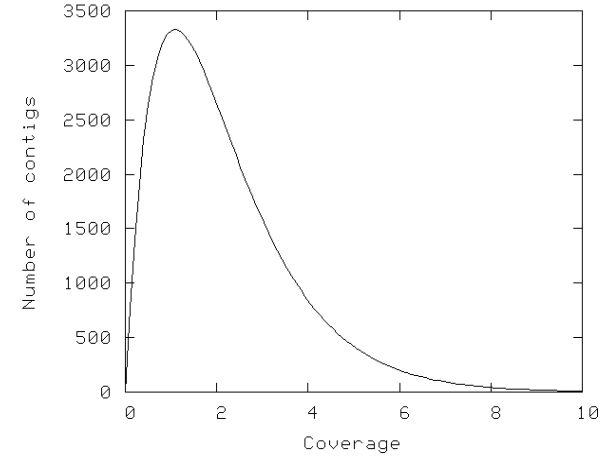
Imagine raindrops on a sidewalk

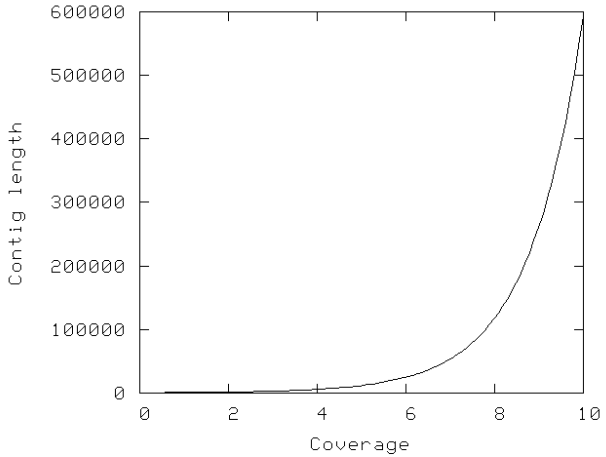
Lander-Waterman statistics

L = read length
T = minimum overlap
G = genome size
N = number of reads
c = coverage (NL / G)
$\sigma = 1 - T/L$

$E(\#islands) = Ne^{-c\sigma}$
$E(island\ size) = L(e^{c\sigma} - 1) / c + 1 - \sigma$
contig = island with 2 or more reads

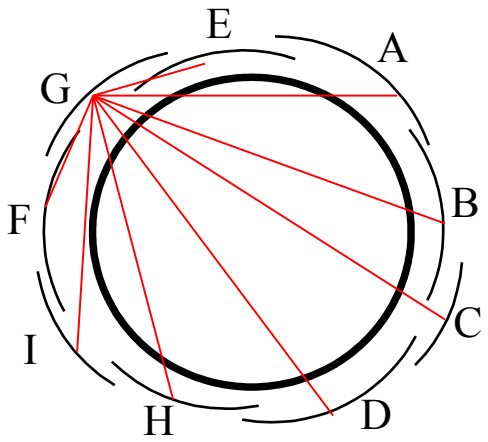
See chapter 4.5

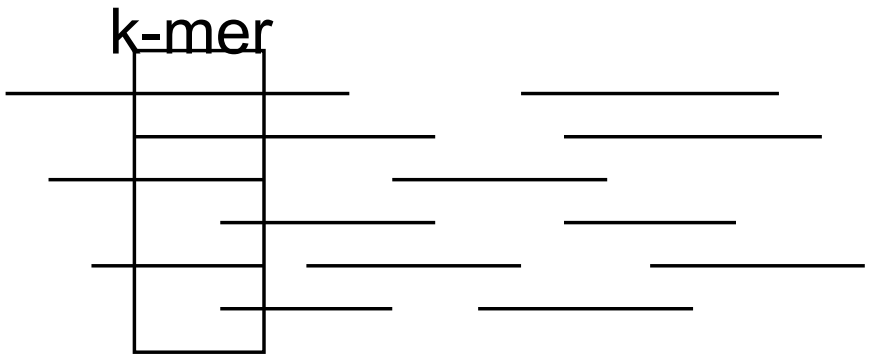




All pairs alignment

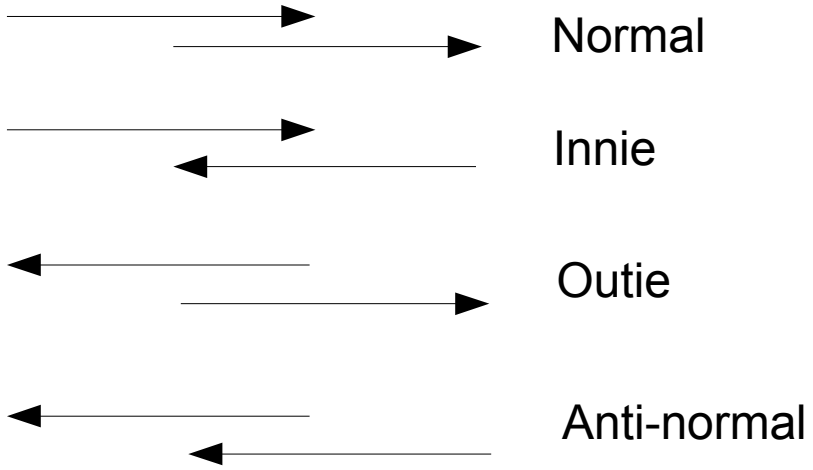
- Needed by the assembler
- Try all pairs – must consider ~ n^2 pairs
- Smarter solution: only n x coverage (e.g. 8) pairs are possible
  - Build a table of k-mers contained in sequences (single pass through the genome)
  - Generate the pairs from k-mer table (single pass through k-mer table)





Additional pairwise-alignment details

- 4 types of overlaps
- Often – assume first read is “forward”



- Representing the alignment

- Why not store length of overlap?

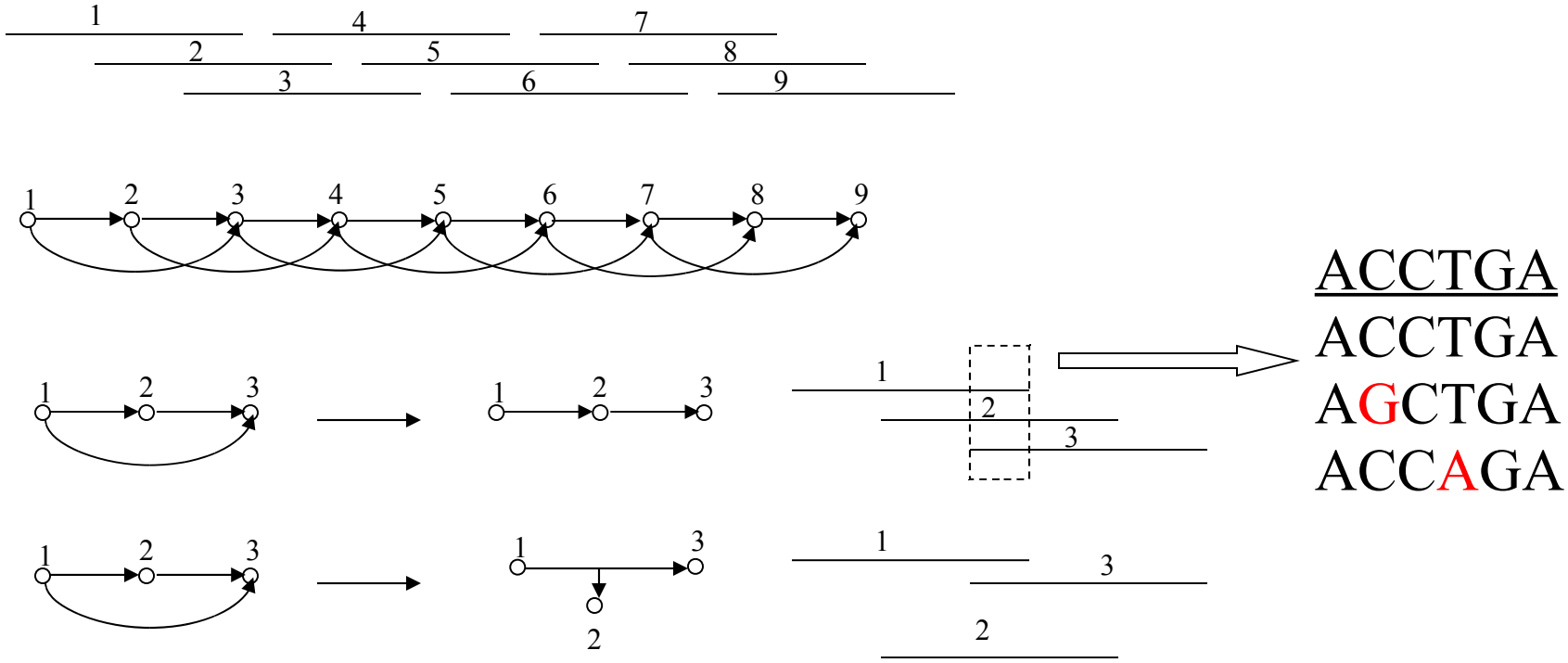
Brief aside (assembly paradigms)

- Greedy algorithm
  - easy to implement
  - relatively efficient
  - but... can make mistakes because it is greedy (only takes into account local information)
- How can you "reason" about repeats?
- Graph theory can help: 2 paradigms
  - Overlap-Layout-Consenusus: nodes=reads, edges= reads overlap
  - deBruijn/repeat graph: nodes = k-mers, edges = k+1-mers (extracted from the reads).
- Both translate into: find a constrained path within a graph

Overlap-layout-consensus

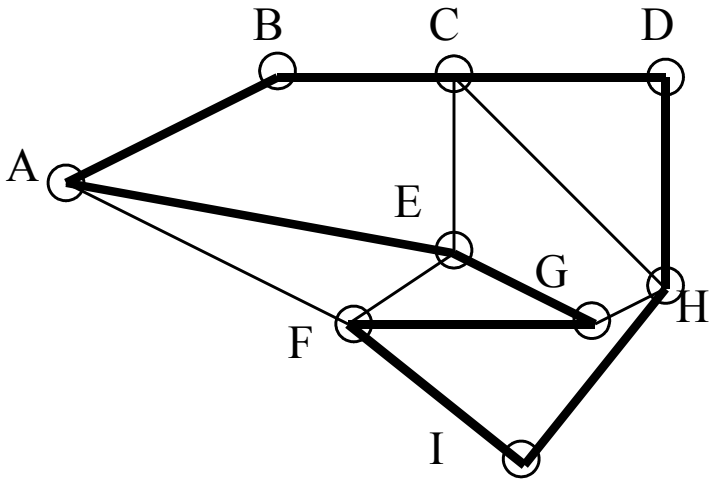
Main entity: read
Relationship between reads: overlap

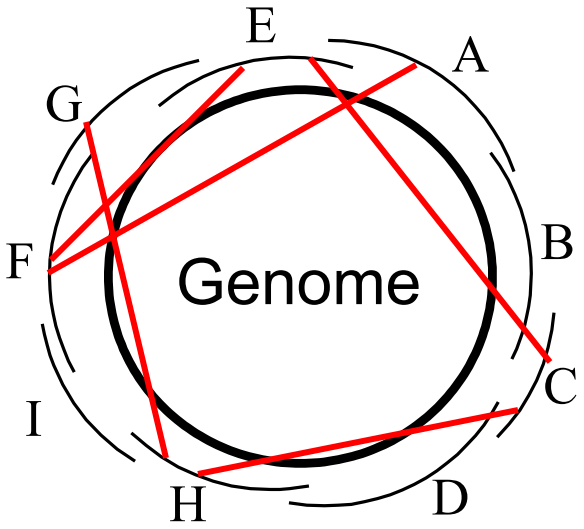
3 Stages: overlap (btwn reads) + layout (find placement of reads wrt each other) + consensus (multiple alignment of reads)



Paths through graphs and assembly

- Hamiltonian circuit: visit each node (city) exactly once, returning to the start
- I.e. use every read in the genome exactly once



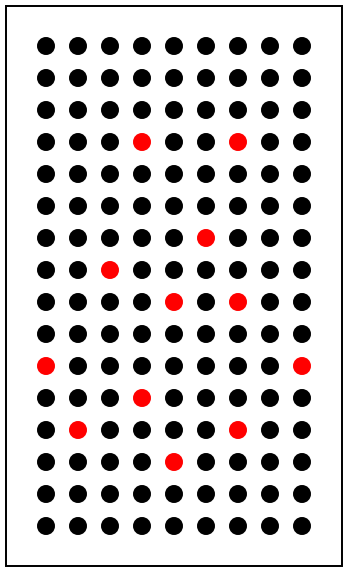


Aside: graph traversals

- Hamiltonian path: visit every single node of a graph EXACTLY once (NP-hard)
- Eulerian path: visit every edge of a graph EXACTLY once (polynomial time)
- Chinese Postman: find the shortest path in a graph that visits all the edges (i.e. Eulerian path where you allow a minimum number of edges to be reused)

- Note: a Hamiltonian path or an Eulerian path are not guaranteed to exist. A Chinese postman path can always be constructed

Sequencing by hybridization



AAAA
AAAC
AAAG
AAAT
AACA
AACG
AACT
AAGA
...

probes - all possible k-mers

AACAGTAGCTAGATG

```
AACA TAGC AGAT
 ACAG AGCT GATG
  CAGT GCTA
   AGTA CTAG
    GTAG TAGA
```

Assembling SBH data

Main entity: oligomer (overlap)
Relationship between oligomers: adjacency

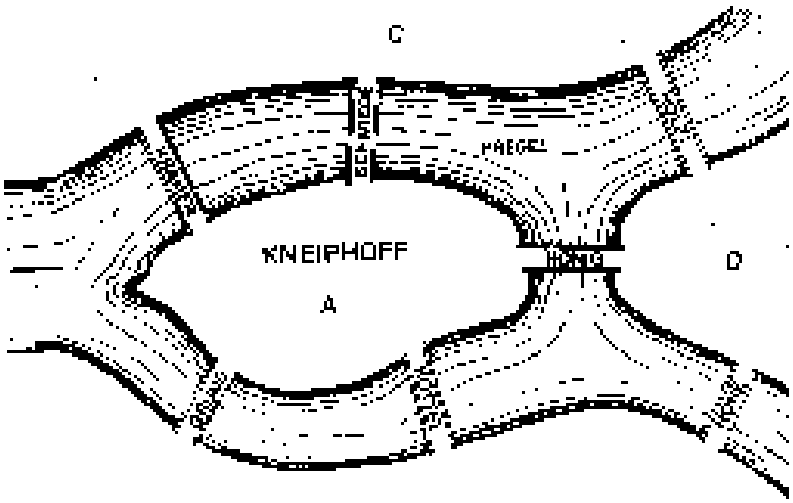
ACCTGATGCCAATTGCACT...

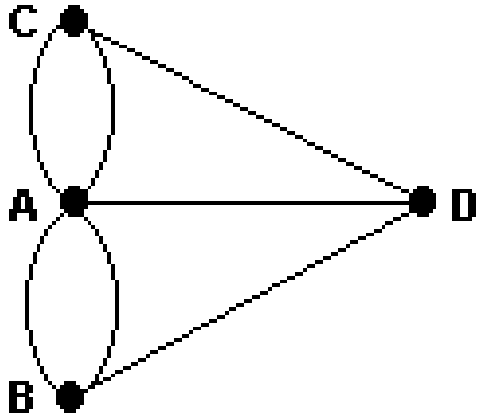
CTGAT follows CCTGA (they share 4 nucleotides: CTGA)

Problem: given all the k-mers, find the original string

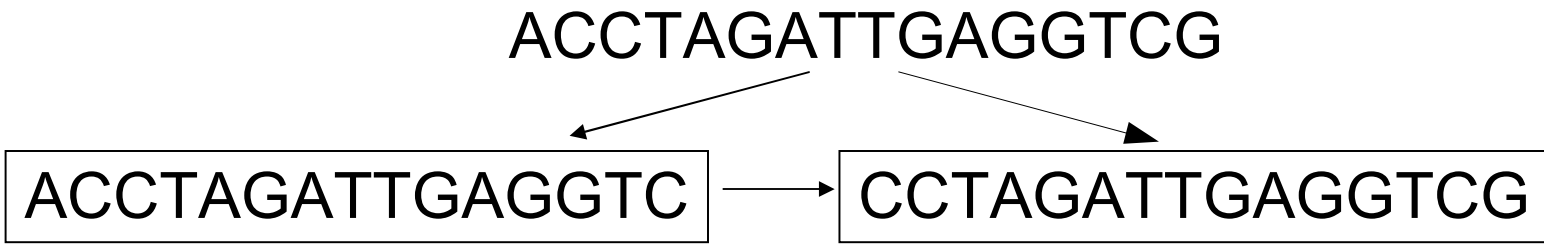
In assembly: fake the SBH experiment - break the reads into k-mers

Eulerian circuit





- Eulerian circuit: visit each edge (bridge) exactly once and come back to the start
- an edge (roughly) corresponds to a read



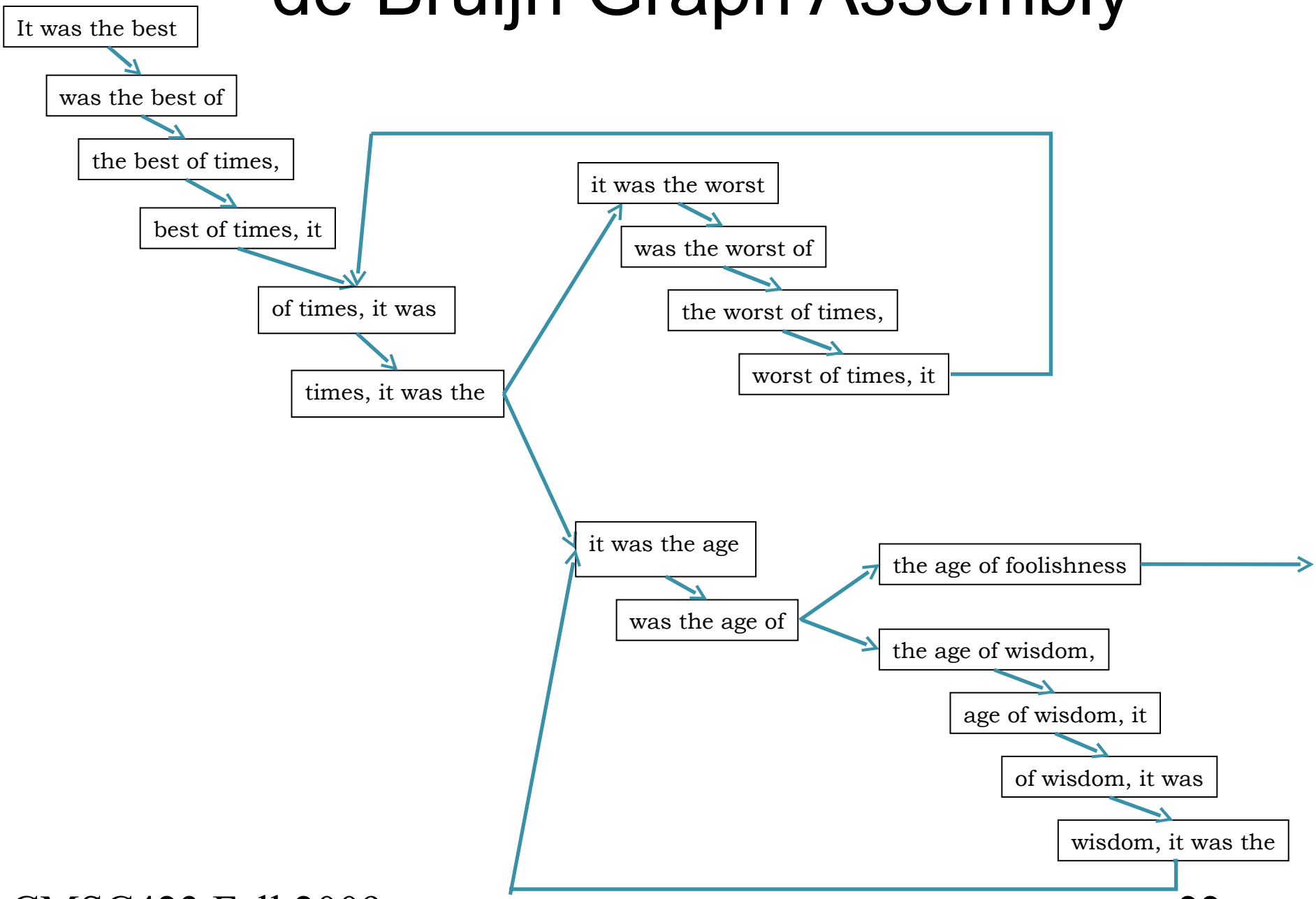
deBruijn graph

- Nodes – set of k-mers obtained from the reads
- Edges – link k-mers that overlap by k-1 letters

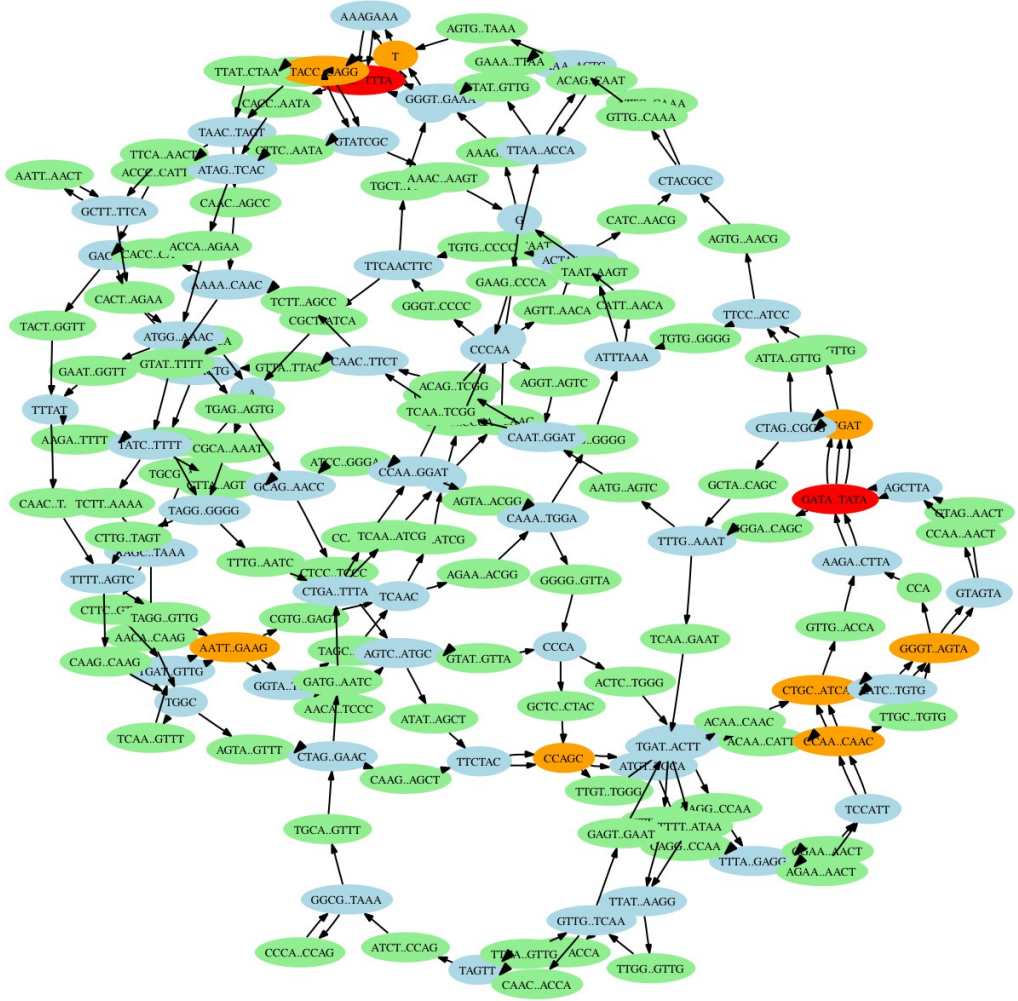
  ACCAGTGCA

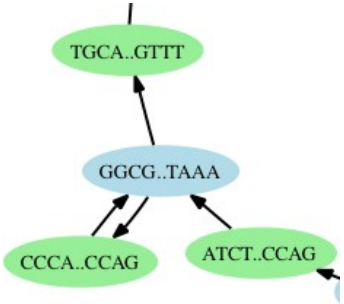
  CCAGTGCAT
- This formulation particularly useful for very short reads
- Solution – Eulerian path (actually Chinese postman) through the graph
- Note – multiple Eulerian paths possible (exponential number) due to repeats

de Bruijn Graph Assembly



deBruijn graph of *Mycoplasma genitalium*





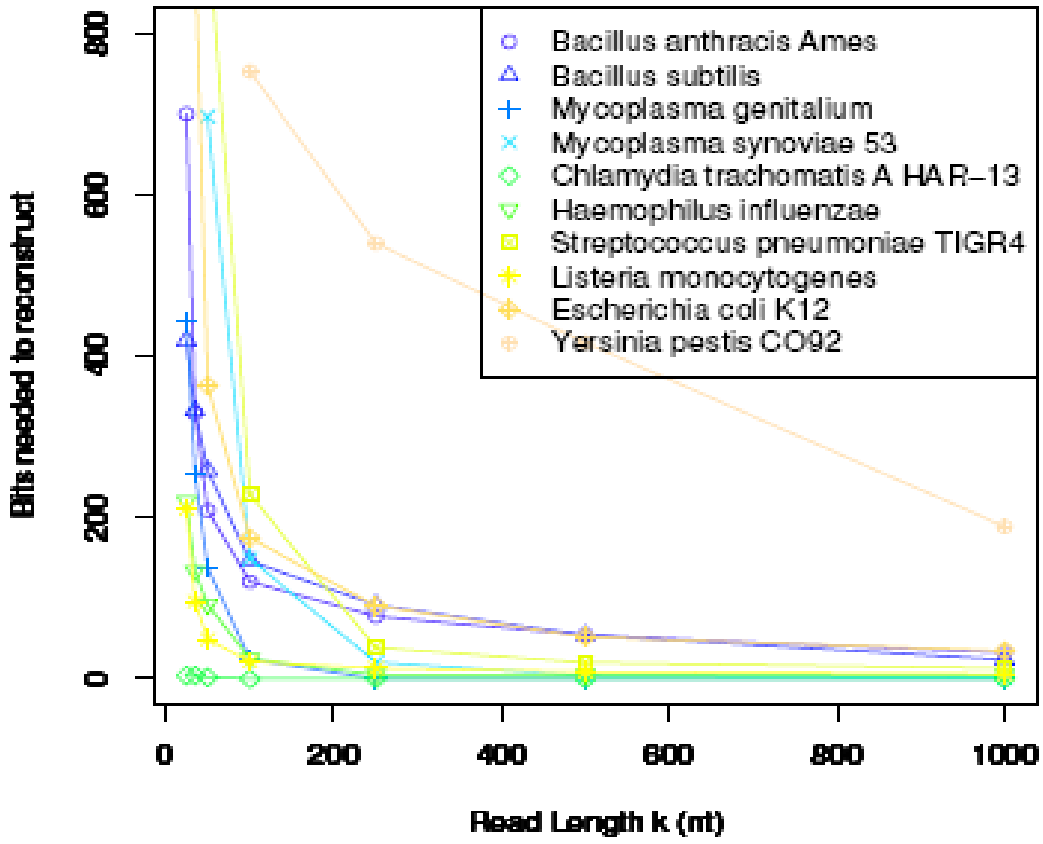
Assembly...parting thoughts

- The basic idea of both OLC and deBruijn approaches: identify sections of DNA that MUST be present in the actual genome:
  - OLC – each read must be used because it is a piece of the original genome
  - deBruijn – each edge must be used because the DNA string corresponding to it is a piece of the original genome

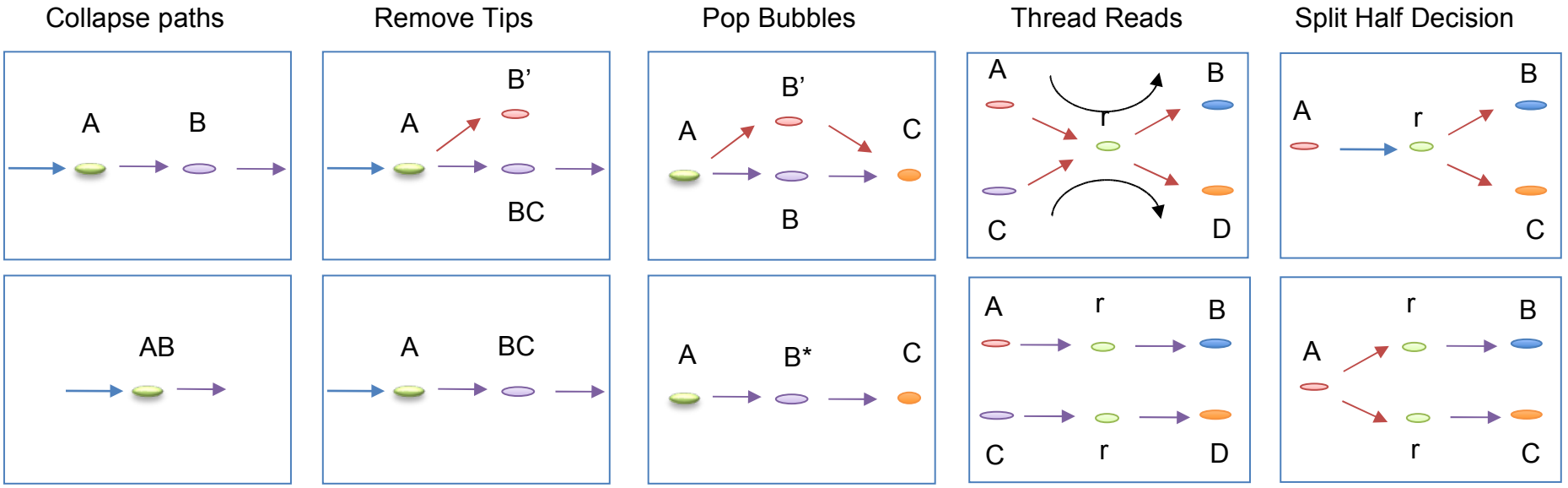
Assembly... recap

- Greedy algorithm... pretty good but gets stuck at repeats
- Overlap layout consensus – equivalent to Hamiltonian path (NP-hard)
- deBruijn graph – equivalent to Eulerian path (polynomial time)
- ... BUT – exponential # of Eulerian paths consistent with reads (because of repeats)
- Ultimately... still NP-hard

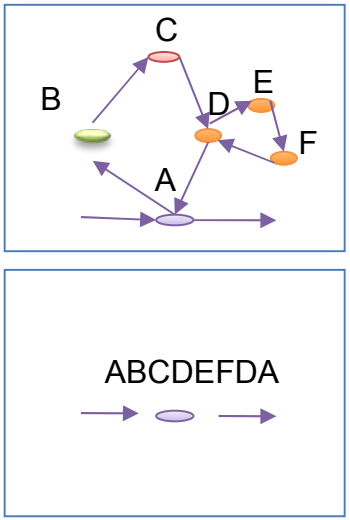
Read-length vs. genome complexity



In practice: graph simplifications



Collapse trees of cycles

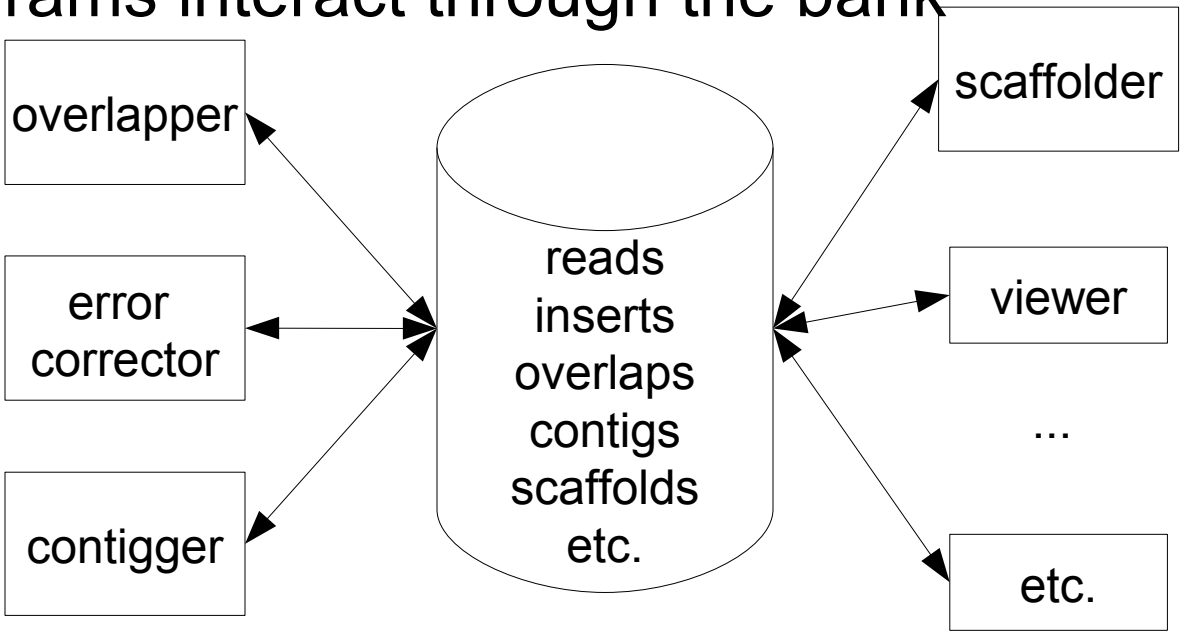


Thm: a graph has a unique Eulerian path if and only if its cycle graph is a tree.

Defn: cycle graph – each node is a cycle in the original graph, nodes are connected by an edge if the corresponding cycles intersect.

AMOS quick tour

- amos.sourceforge.net
- Basic workflow:
  - sequences are converted into the AMOS format (.afg)
  - an .afg file is loaded into a flat-file database (the "bank")
  - all programs interact through the bank



An AMOS pipeline

```
#!runAmos -C

#------------------------------------ USER DEFINED VALUES -----------------#
# allow input to be either <file>.afg or just <file>
REF = $(PREFIX).1con
TGT = $(strip .afg PREFIX).afg
#--------------------------------------------------------------------------#

BINDIR   = /usr/local/bin
NUCMER   = $(shell which nucmer)

SEQS     = $(PREFIX).seq
BANK     = $(PREFIX).bank
ALIGN    = $(PREFIX).delta
LAYOUT   = $(PREFIX).layout
CONFLICT = $(PREFIX).conflict
CONTIG   = $(PREFIX).contig
FASTA   = $(PREFIX).fasta

INPUTS   = $(TGT) $(REF)
OUTPUTS  = $(CONTIG) $(FASTA)


## Building AMOS bank
10: $(BINDIR)/bank-transact -c -z -b $(BANK) -m $(TGT)

## Collecting clear range sequences
20: $(BINDIR)/dumpreads $(BANK) > $(SEQS)

## Running nucmer
30: $(NUCMER) --maxmatch --prefix=$(PREFIX) $(REF) $(SEQS)

## Running layout
40: $(BINDIR)/layout-align -U $(LAYOUT) -C $(CONFLICT) -b $(BANK) $(ALIGN)

## Running consensus
50: $(BINDIR)/make-consensus -B -b $(BANK)

## Outputting contigs
60: $(BINDIR)/bank2contig $(BANK) > $(CONTIG)

## Converting to FastA file
70: $(BINDIR)/ctg2fasta < $(CONTIG) > $(FASTA)
```

Project

- You will need to modify the Minimus pipeline to use your own overlapper program (replacing the hash-overlap command with your own)
- Part of the project is figuring out how to do this (using the AMOS documentation)

AMOS interchange format

Based on Celera message format


```
{RED
act:A
iid:1
eid:nihaf5_10_a01.ab1
seq:
gggaattgctcgtttctggagccccgccagcgtctgcgctccgcctgtgcgcacagaaga
gaggtgtgagtaaagacagtgctgagtaccggcagaggagagagagaaacaacatcgccg
tcaggaagagtcgagataaagcgcggcgccgcatccagatgacccagcagagggcgctgc
agctgcaggatgagaatcaccggctgcaggtgcacatccagcgcctgctgcacgaggtgg
aggcgctcaggcattacctgtcccagcgtcacctgcaggacacatctgaggagcactgat
gagaatacacctggagaacacacacctgaagaaaaa
.
qlt:
7777777777777<?IMKD@988<?@C>>>HQQQUUUUXZhhhhhhhh[cXXXUUUUZZ_
ZUUUUUUXXXZZUSOPPSSZhhhhZZZXX]]ZZ\\\\\\h_hhhZZZ^^\ZZZUUU\\h\
h\\\\bbbbhh\\ZZZ[^Zhhhhhhhbbb\\_bbbb\\bZ[Z[^\hbbbbbhbhhhhhbh
hbbhhhZXXXXXZZZ[[ZZbbhhhhhbbbhhc[\ZZZb\ZZbbh\\\bbbb__\\\\\\h
\\hhhhhh\_____\\hhhhhhhhhhh[ZZZZZhhZXXXXXZ\\hhhhhhh[[ZXXXZZ
ZZZZZZZZZZhhZZZZZhhhhZUSSQOUULLAD998
.
frg:0
clr:14,333
}
```

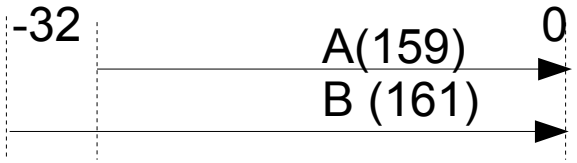
Basic flow...

- Start with an AMOS .afg file (I will provide one)
- Load it in the bank
  - bank-transact -cf -b mybank.bnk -m myfile.afg
- Dump the reads back out in a multi-fasta file
  - dumpreads mybank.bnk > myfile.fa
  - why? the IDs are now the internal IDs within the bank
- Use your program to compute overlaps (output an afg file)
  - myoverlapper myfile.fa > myoverlaps.afg
- Load the new overlaps in the bank
  - bank-transact -b mybank.bnk -m myoverlaps.afg
- Continue with standard Minimus pipeline

Overlap format

```
{OVL
adj:N
rds:159,161
scr:0
ahg:-32
bhg:0
}
{OVL
adj:N
rds:159,162
scr:0
ahg:-17
bhg:43
}
{OVL
adj:I
rds:159,163
scr:0
ahg:362
bhg:560
}
```

adj – "Normal", "Innie"
rds: iid1, iid2
ahg, bhg – ahang, bhang



Note: output is "redundant"
both A ovl B and B ovl A
are reported