Genome sequence assembly

Assembly concepts and methods

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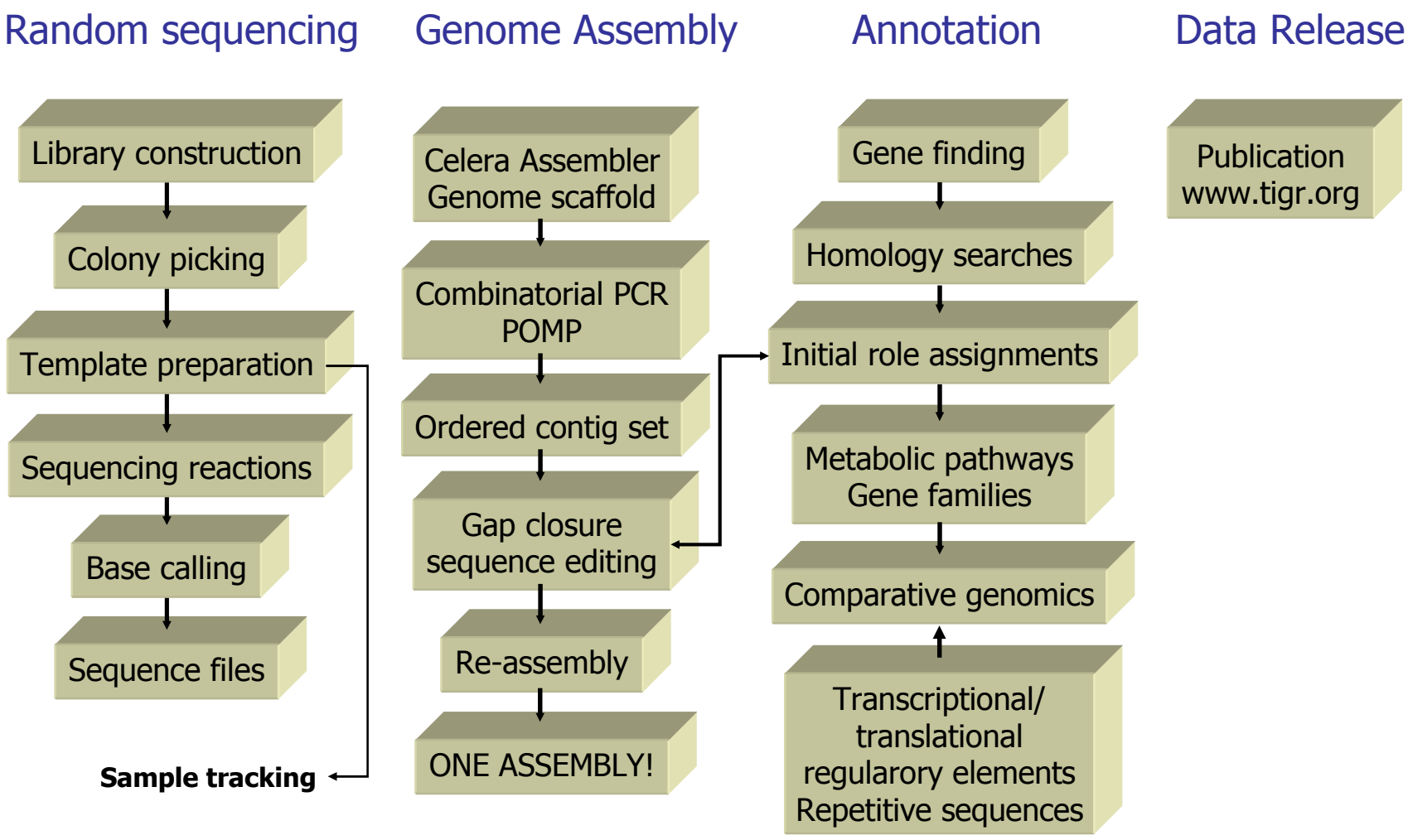
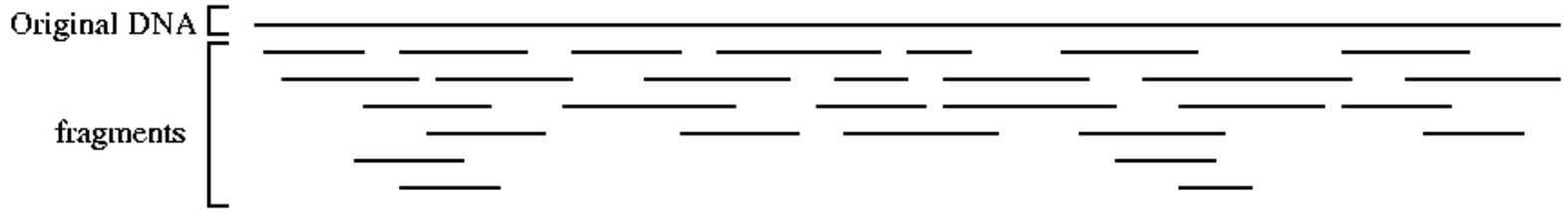
Outline

- Shotgun sequencing overview
- Shotgun sequencing statistics
- Theoretical Foundations
- Assembly algorithms
- Scaffolding

A Genome Sequencing Project

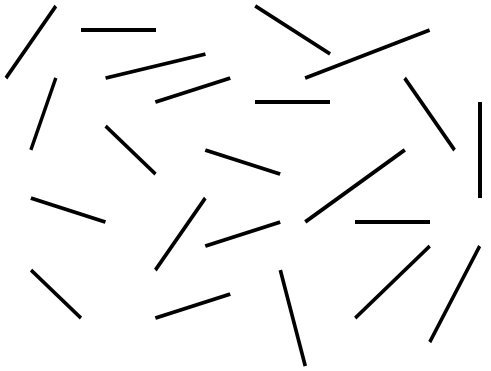


Building a library



- Break DNA into random fragments (8-10x coverage)

Actual situation



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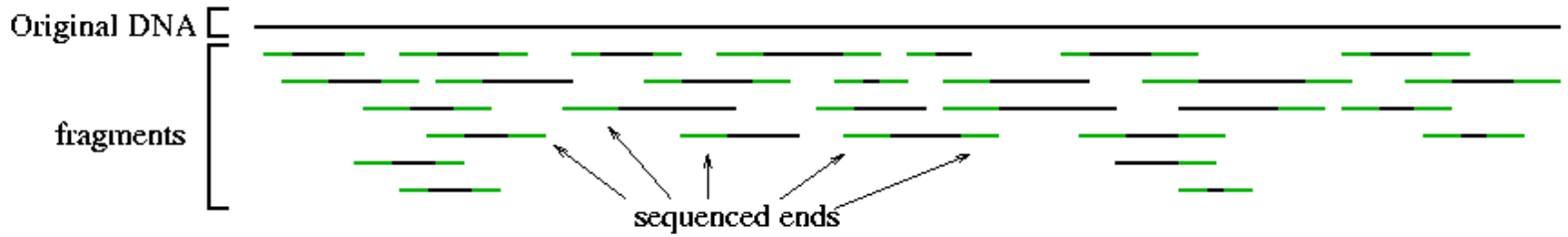
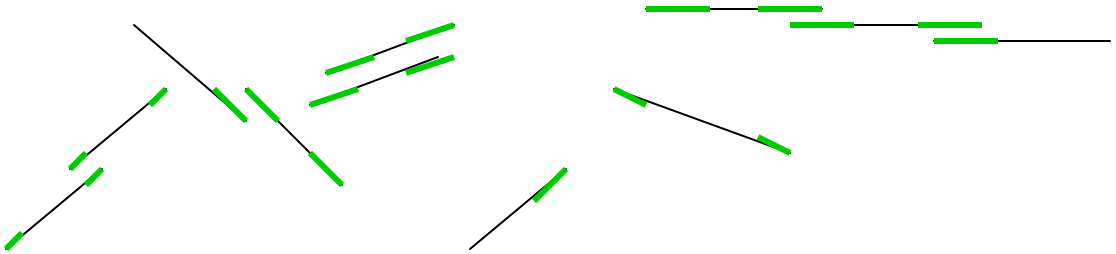
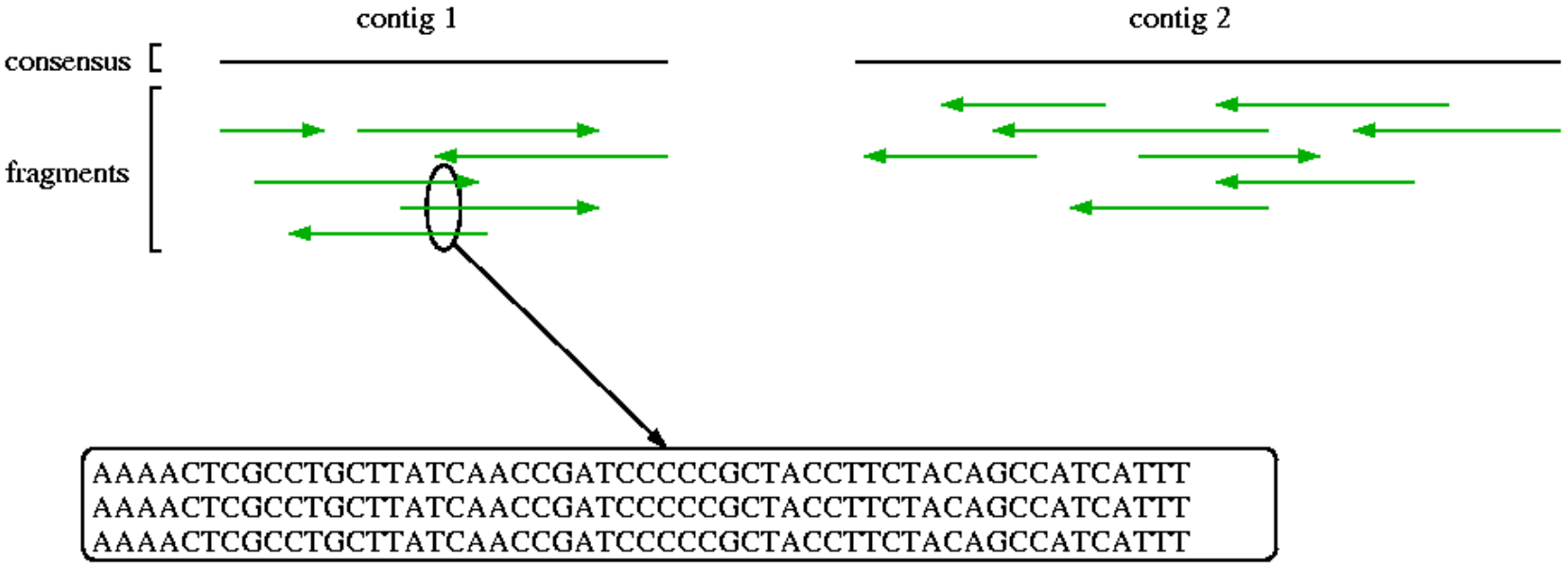


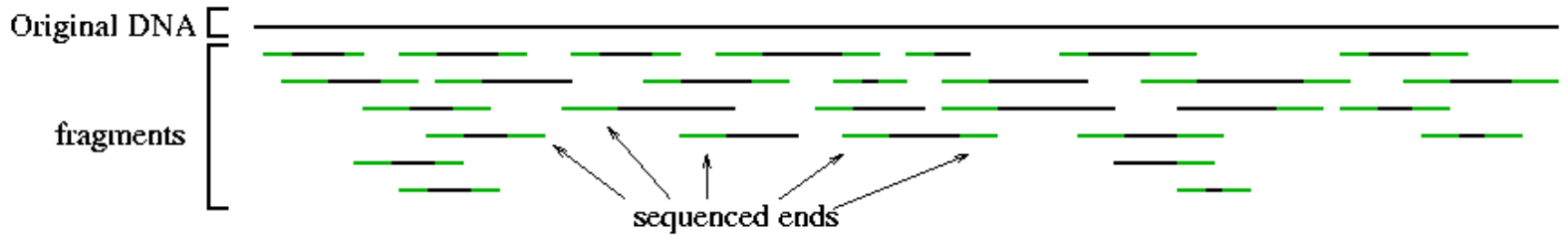
- Break DNA into random fragments (8-10x coverage)
- Sequence the ends of the fragments
  - Amplify the fragments in a vector
  - Sequence 800-1000 (500-700) bases at each end of the fragment

Assembling the fragments

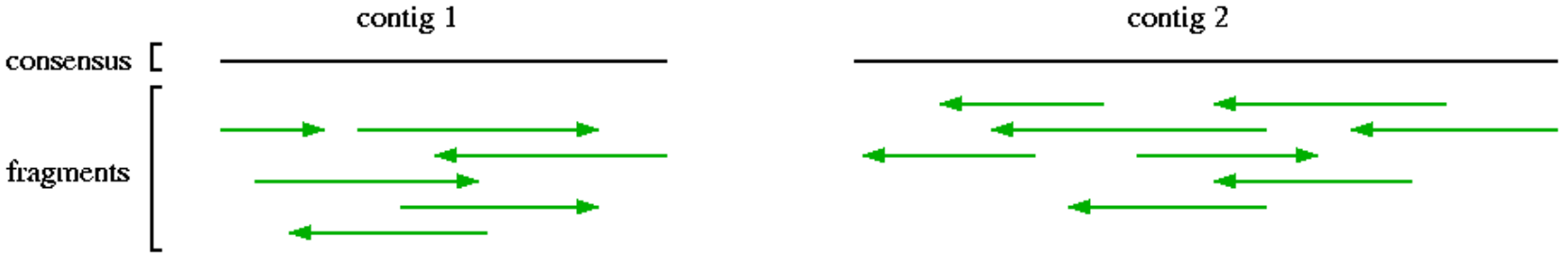




Assembling the fragments

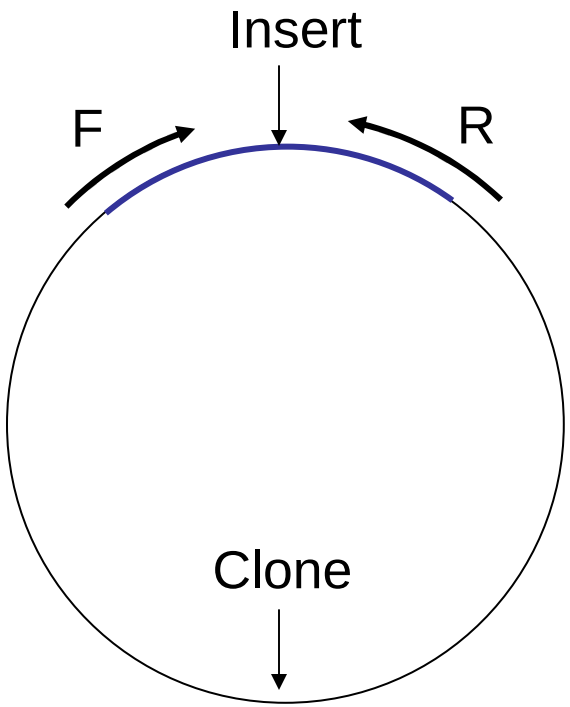


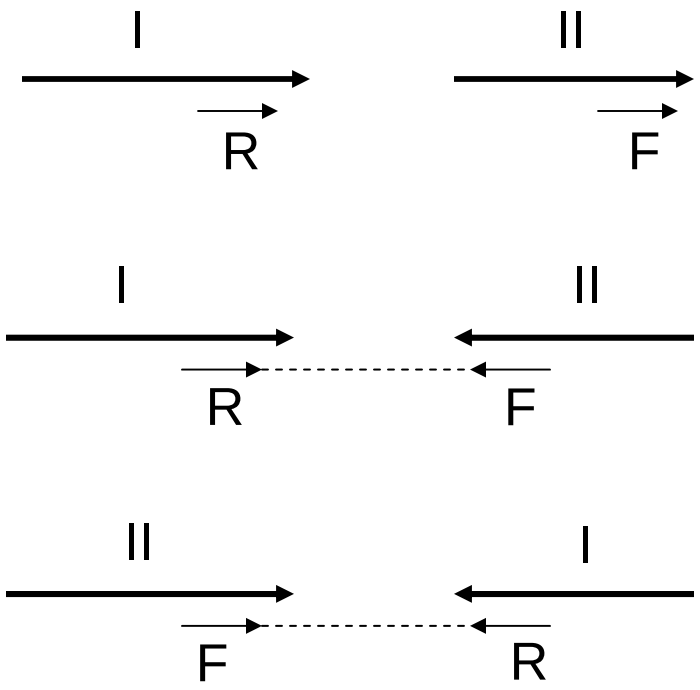
- Break DNA into random fragments (8-10x coverage)
- Sequence the ends of the fragments
- Assemble the sequenced ends



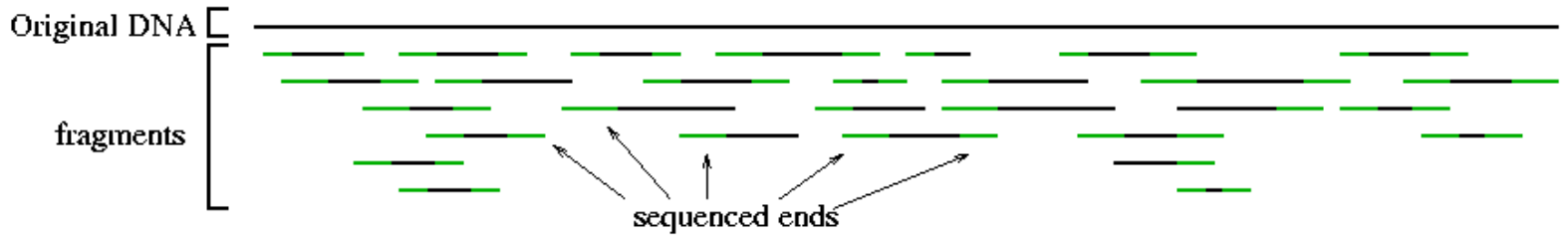
Forward-reverse constraints

- The sequenced ends are facing towards each other
- The distance between the two fragments is known (within certain experimental error)

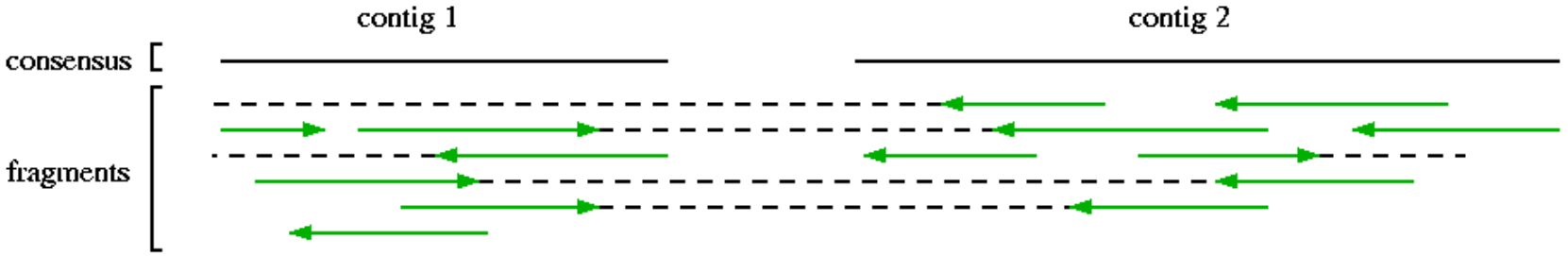




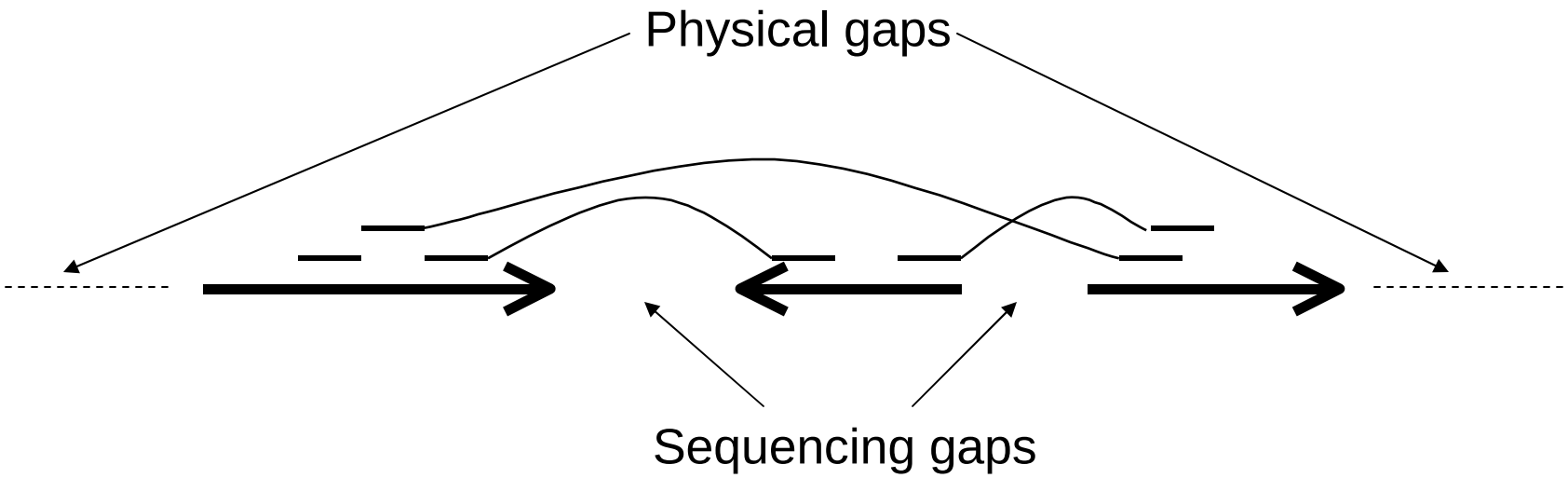
Building Scaffolds



- Break DNA into random fragments (8-10x coverage)
- Sequence the ends of the fragments
- Assemble the sequenced ends
- Build scaffolds



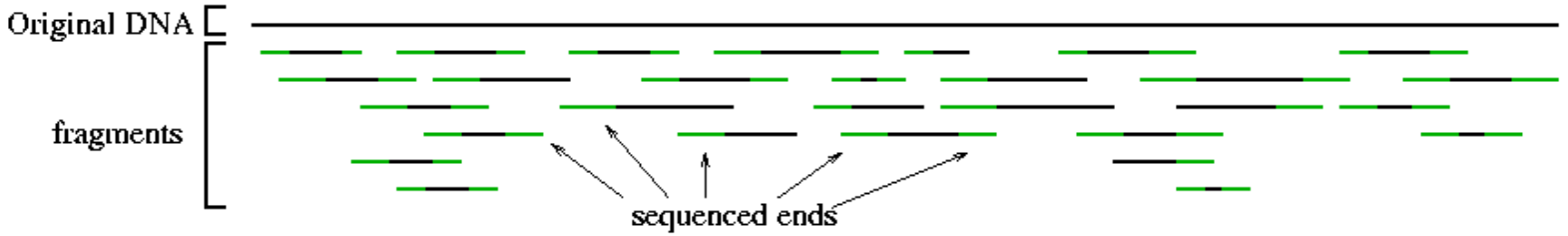
Assembly gaps



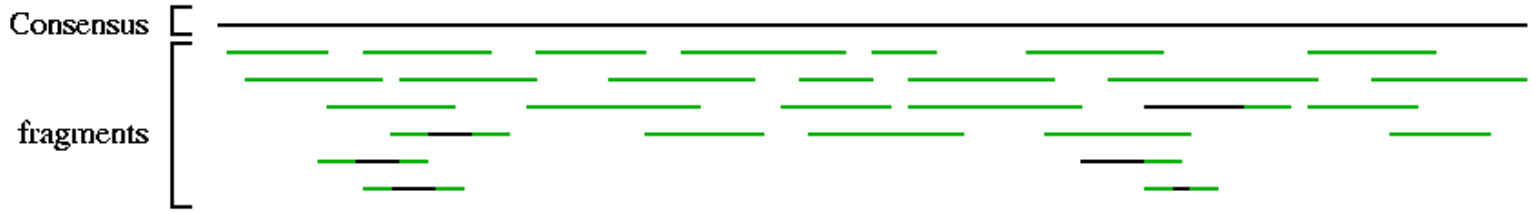
sequencing gap - we know the order and orientation of the contigs and have at least one clone spanning the gap

physical gap - no information known about the adjacent contigs, nor about the DNA spanning the gap

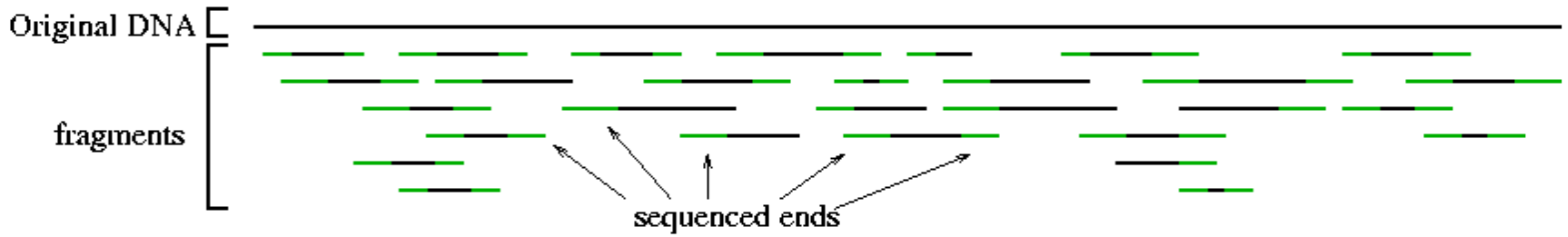
Finishing the project

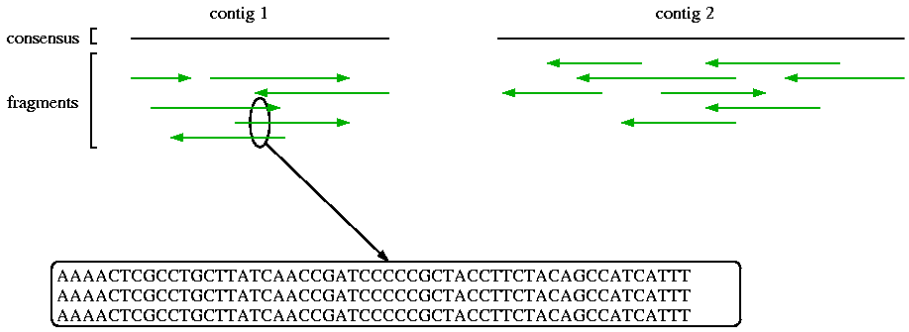


- Break DNA into random fragments (8-10x coverage)
- Sequence the ends of the fragments
- Assemble the sequenced ends
- Build scaffolds
- **Close gaps**



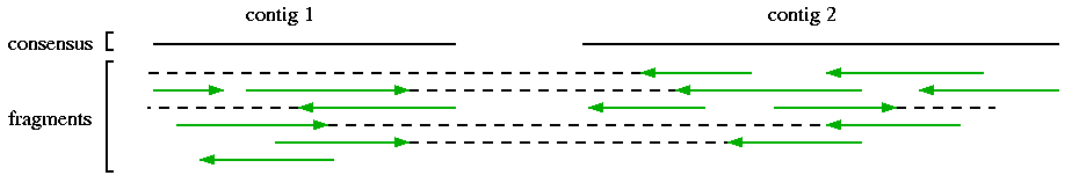
Unifying view of assembly





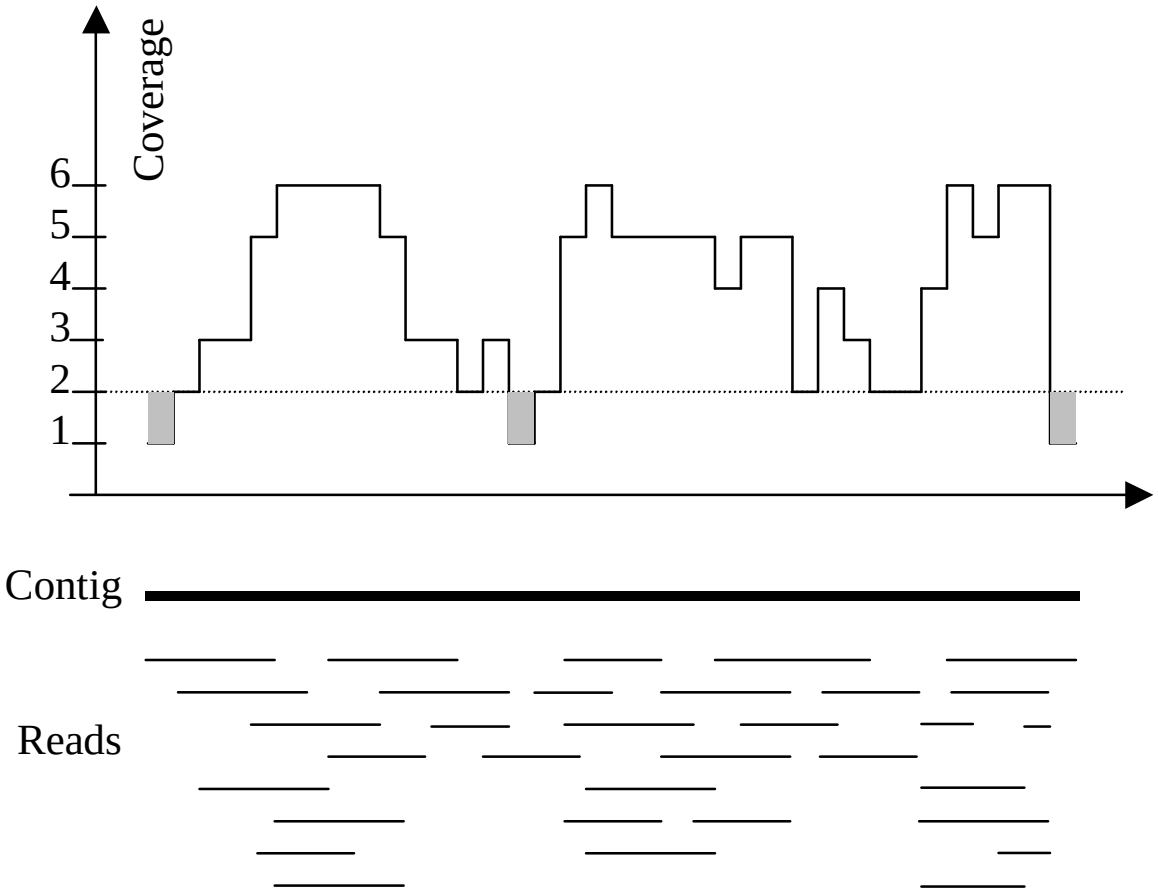
Contigs

Scaffolding



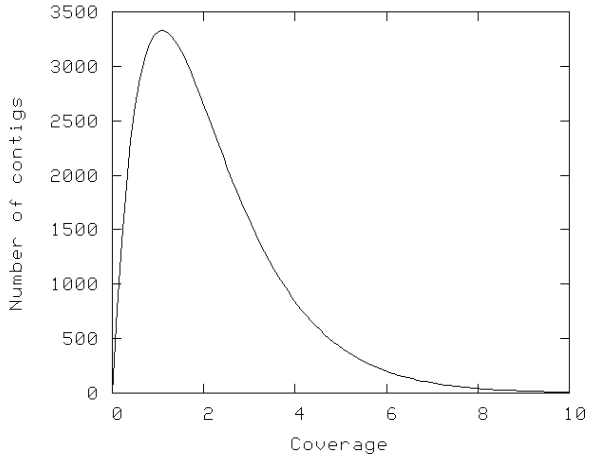
Shotgun sequencing statistics

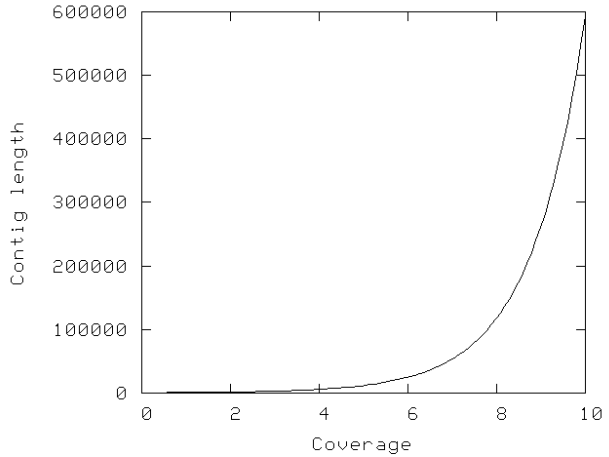
Typical contig coverage



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)

$\sigma = 1 - T/L$

$E(\#islands) = Ne^{-c\sigma}$

$E(island\ size) = L(e^{c\sigma} - 1) / c + 1 - \sigma$

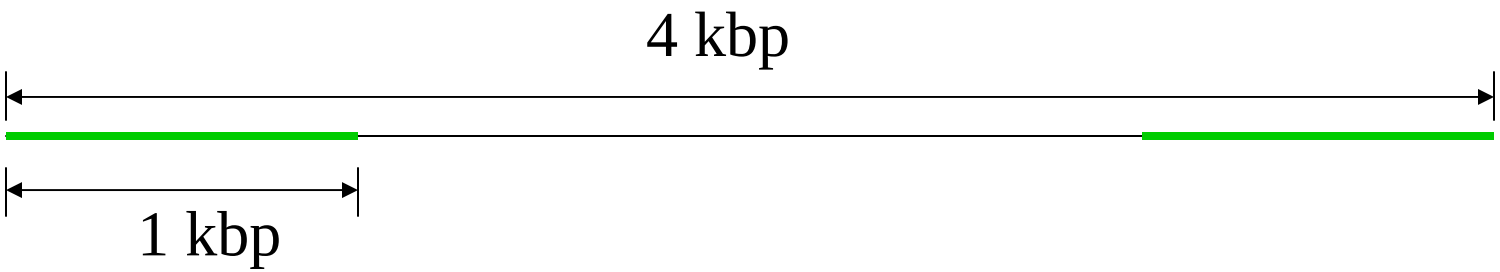
contig = island with 2 or more reads

Example

Genome size: 1 Mbp Read Length: 600 Detectable overlap: 40

c	N	#islands	#contigs	bases not in any read	bases not in contigs
1	1,667	655	614	698	367,806
3	5,000	304	250	121	49,787
5	8,334	78	57	20	6,735
8	13,334	7	5	1	335

Read coverage vs. Clone coverage



Read coverage = 8 x

Clone (insert) coverage = ? 16

BAC-end 2x coverage implies 100x coverage by BACs

(1 BAC clone = approx. 100kbp)

Theoretical Foundations

Shortest Common Superstring

Given: $S = \{s_1, \ldots, s_n\}$

Problem: Find minimal superstring of S

s_1 CACCC

s_2 CCGGGTGC

s_3 CCACC

s_1, s_2, s_3 = CAC**CCGGGTGC**CACC	15
s_1, s_3, s_2 = CAC**CCACC**GGGTGC	14
s_2, s_1, s_3 = CCGGGTG**CACCC**ACC	15
s_2, s_3, s_1 = CCGGGTG**CCACC**C	13
s_3, s_1, s_2 = C**CACCC**GGGTGC	12
s_3, s_2, s_1 = CCA**CCGGGTGC**ACCC	15

NP-Complete by reduction from VERTEX-COVER and later DIRECTED-HAMILTONIAN-PATH

RECONSTRUCT

Given: $F = \{f_1, \ldots, f_n\}$, error rate ε

Problem: Find minimal sequence S over F such that for all f_i in F, there is a substring B of S such that:

$$\min(\text{ed}(f_i, B), \text{ed}(f_i^c, B)) \leq \varepsilon |f_i|$$

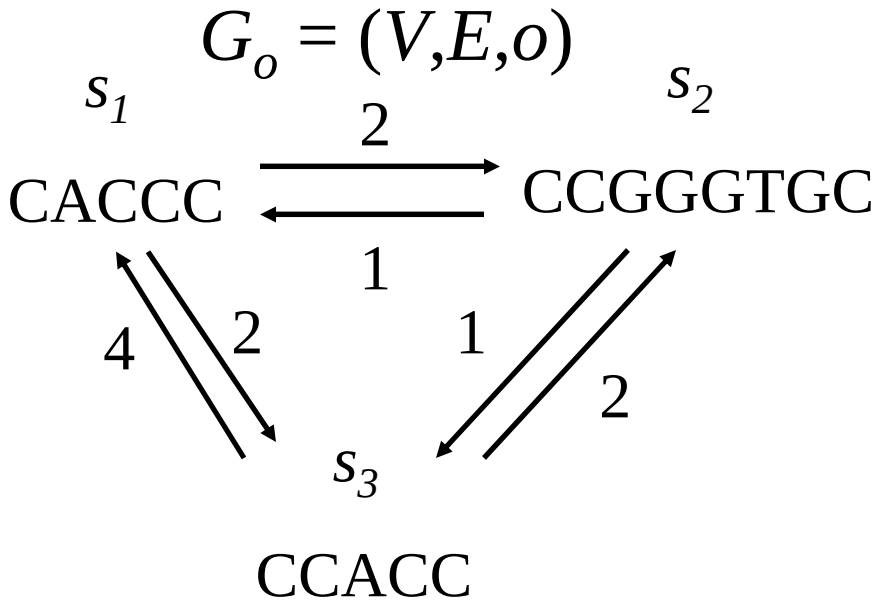
f_1^c GGGTG — ed(ACGTA, ACGGTA) =1

f_2^c GCACCCGG — ed(ACGGGTA, ACGGTA) =1

f_3^c GGTGG — ed(ACGCTA, ACGGTA) = 1

Also NP-complete: Take instance of SUPERSTRING, expand strings to force the original orientation, set $\varepsilon = 0$, and attempt to solve with RECONSTRUCT.

Overlap Graph



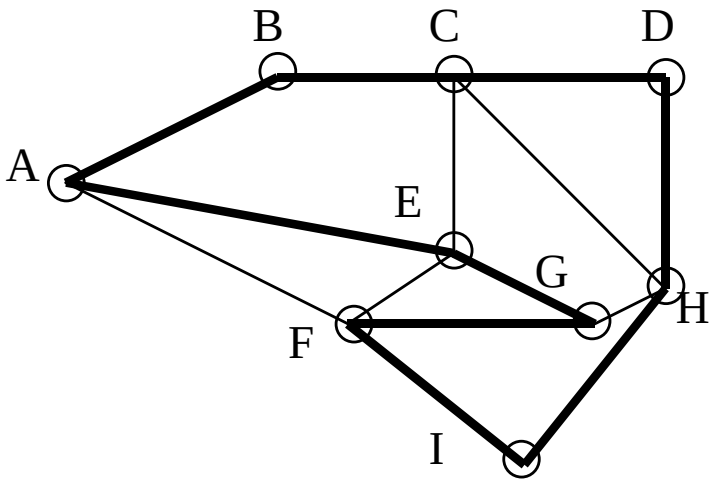
$V = \{s_1, s_2, s_3\}$ $E = \{s_i, s_j\}$

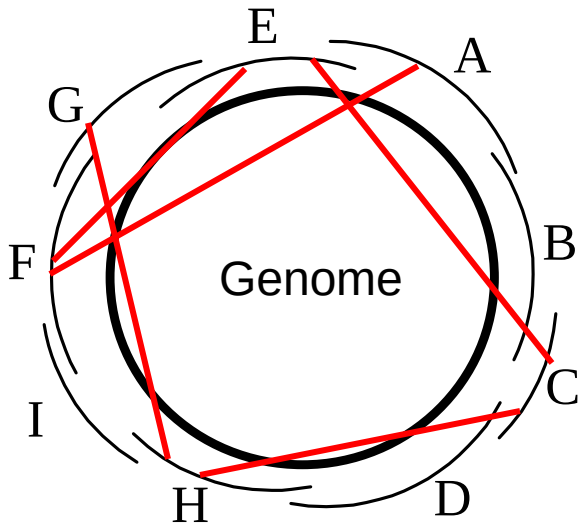
$o(s_i,s_j) = |v| \mid s_i = uv, s_j = vw$

The overlap graph, G_o, encodes the amount of overlap between all pair of strings.

Paths through graphs and assembly

- Hamiltonian circuit: visit each node (city) exactly once, returning to the start





Greedy Approximation

$$G_o = (V,E,o)$$

$$\text{GREEDY}(S) \leq 2.5\ \text{OPT}(S)$$

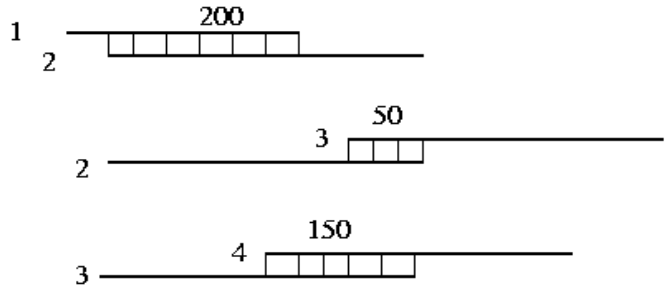
$$\text{Runtime } O(\binom{n}{2} l^2)$$

SUPERSTRING is MAX SNP-hard, so one of the best approximation algorithms possible.

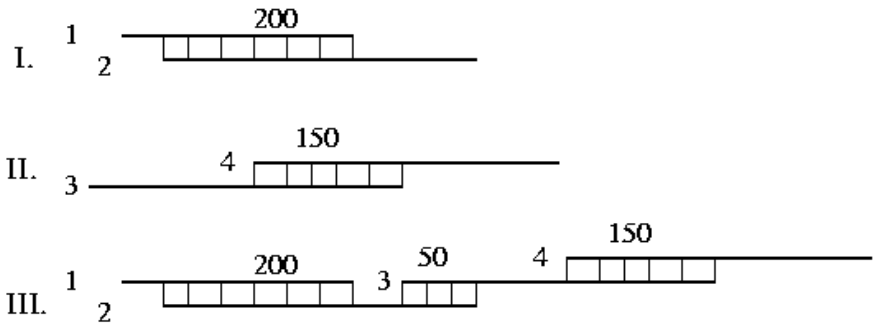
Greedy Assembly

Build a rough map of fragment overlaps

1. Pick the largest scoring overlap
2. Merge the two fragments
3. Repeat until no more merges can be done



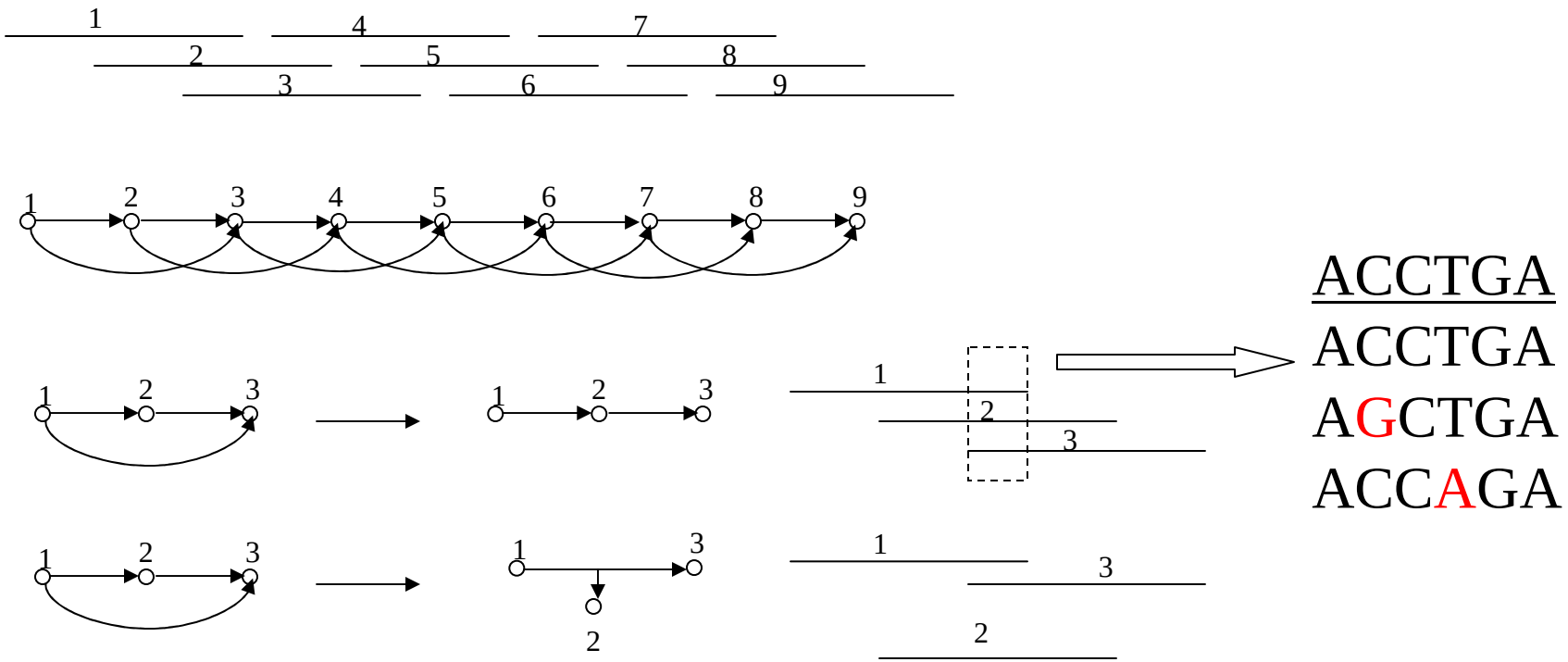
- TIGR Assembler
- phrap
- gap



Overlap-layout-consensus

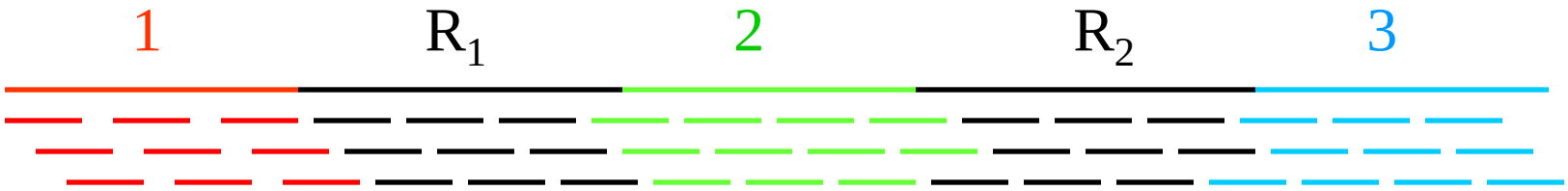
Main entity: read

Relationship between reads: overlap

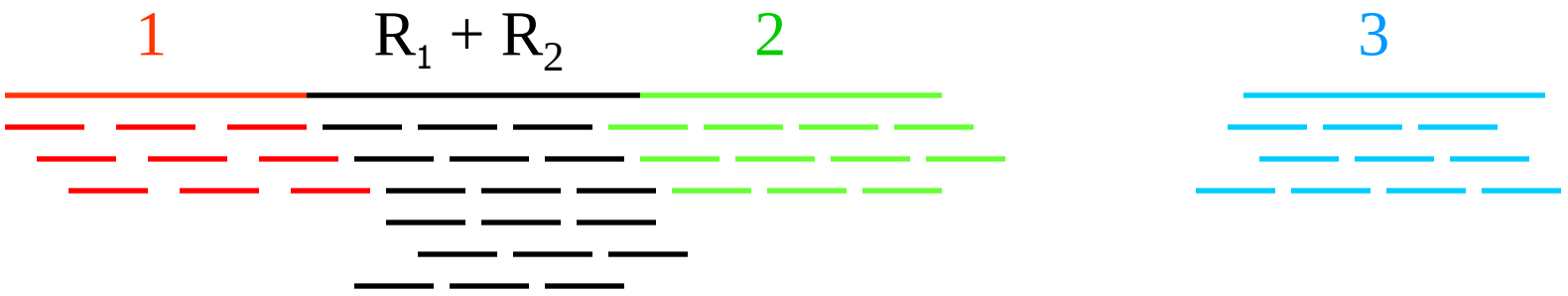


Repeats!

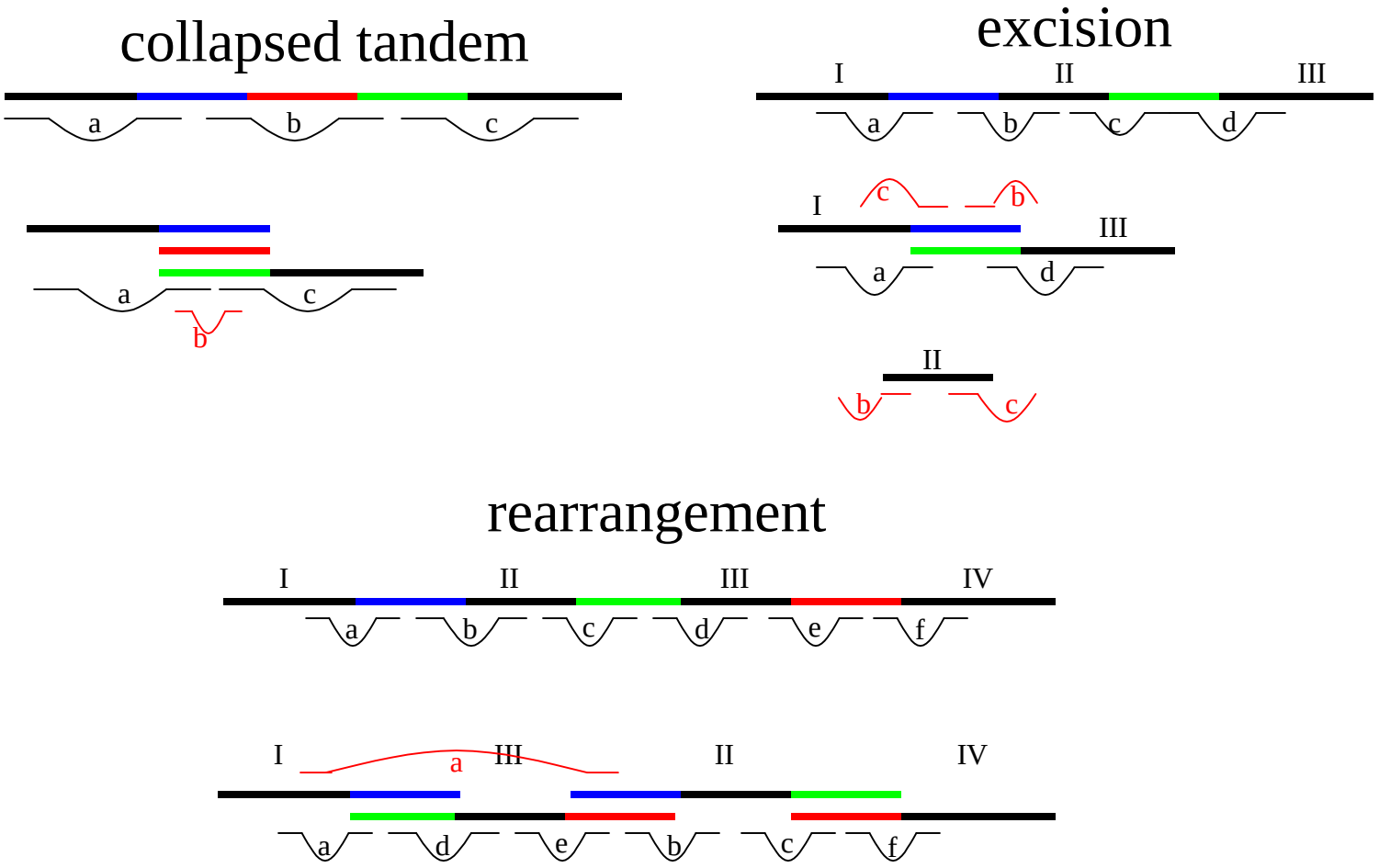
True Layout of Reads



Greedy Reconstruction



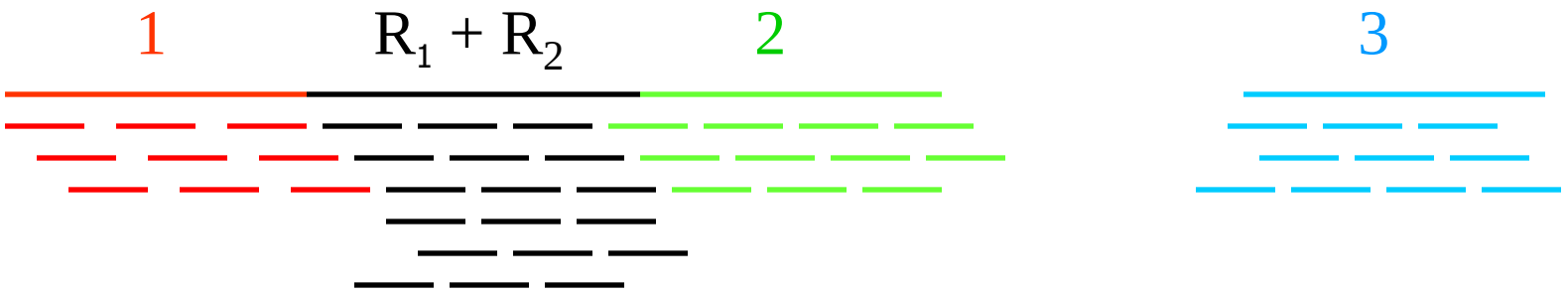
Mis-assembled repeats



Modern Assembly

Try to detect presence of repeats by

1. Unusual depth of coverage (arrival rate)
2. Mate Pair information
3. Forks in overlap graph



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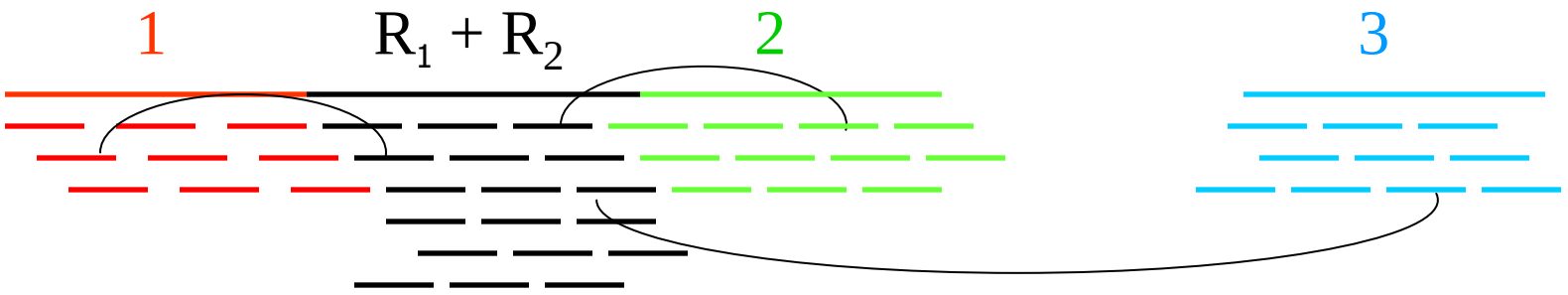

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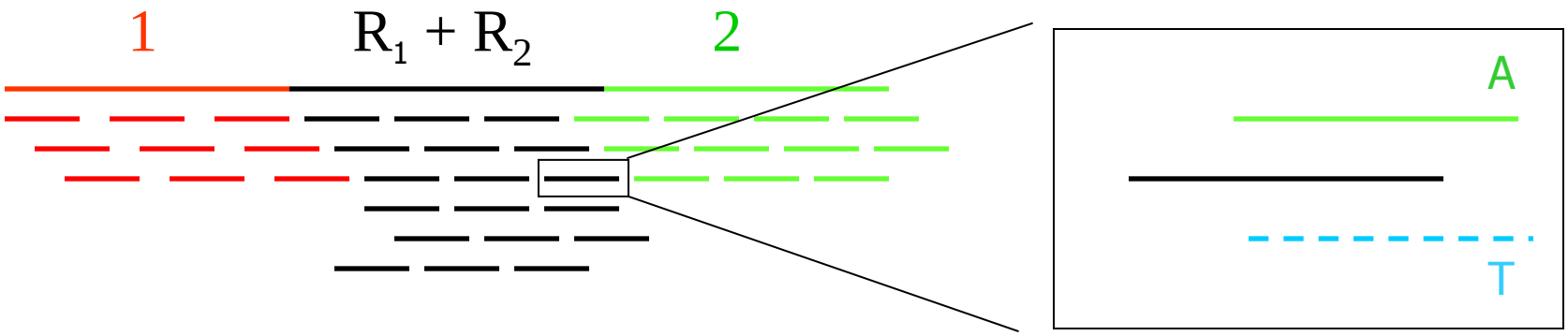
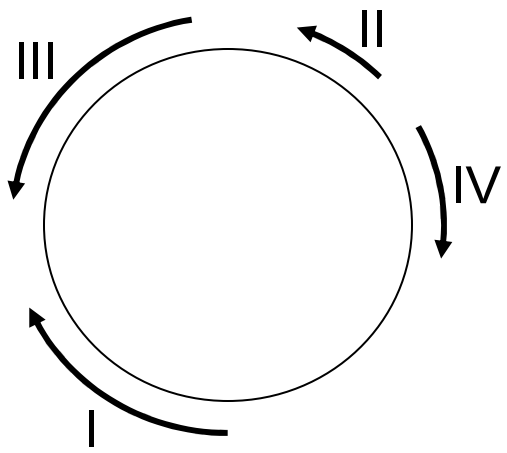


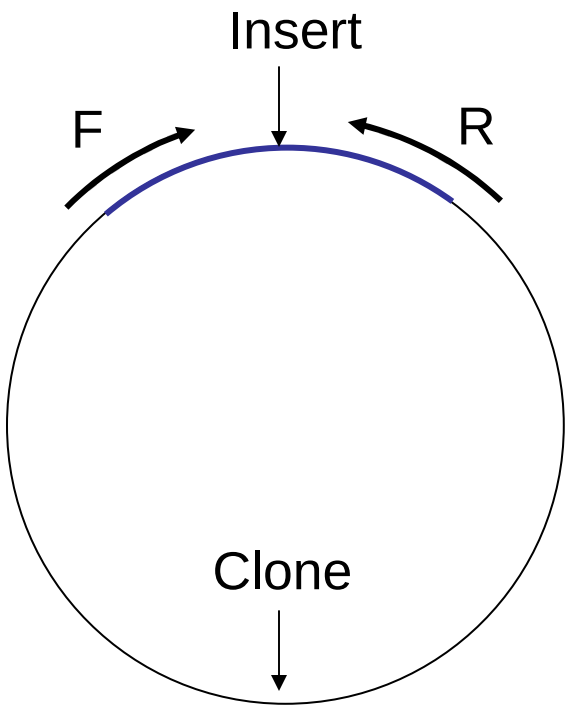
SCAFFOLDING

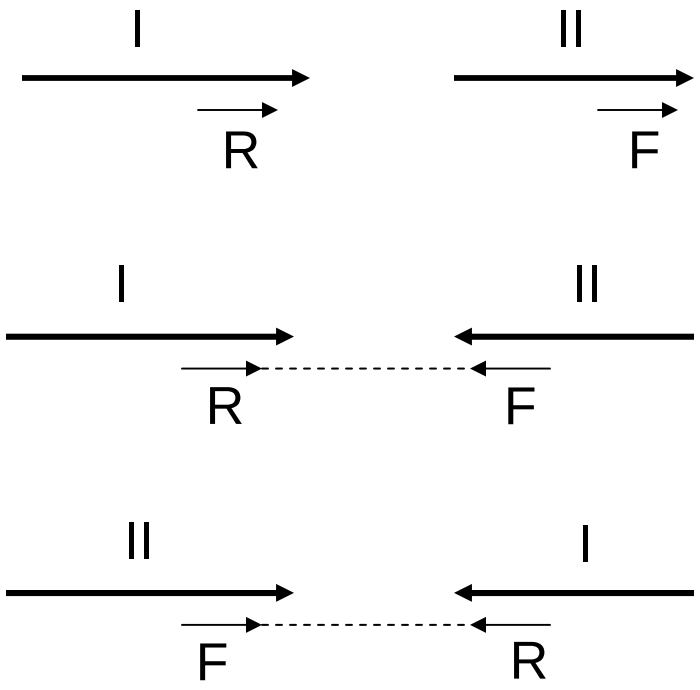
Scaffolding

- Given a set of non-overlapping contigs order and orient them along a chromosome

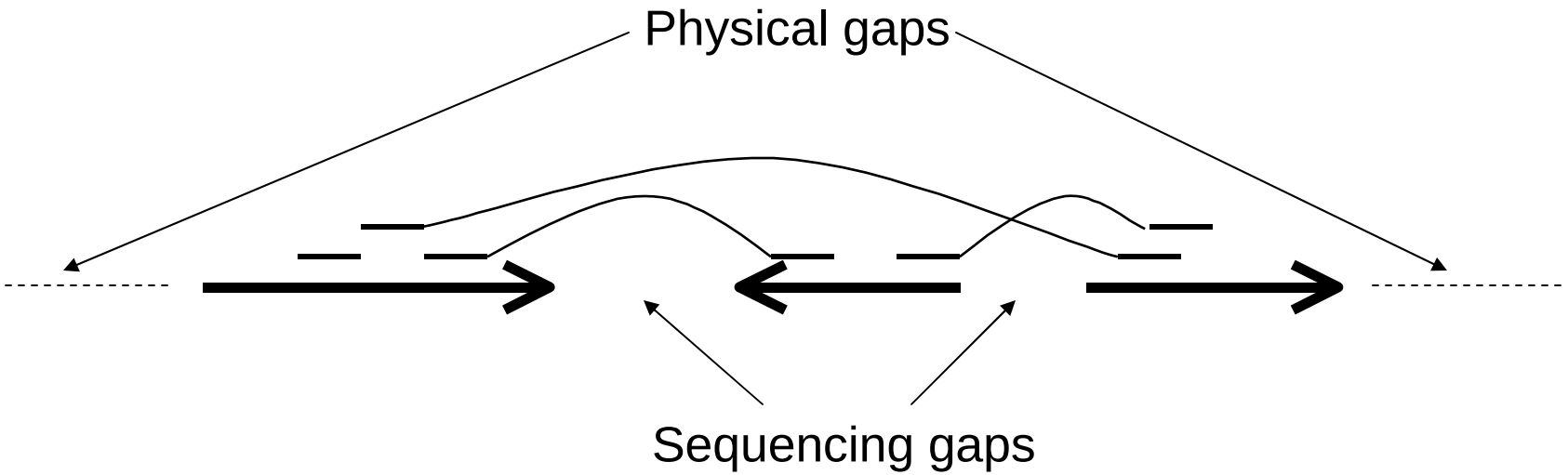


Clone-mates





Scaffolder output



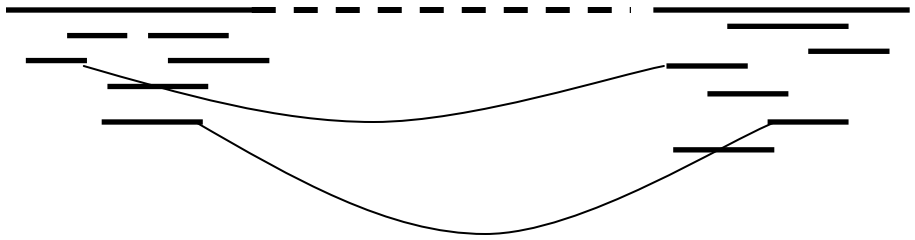
- order and orientation of contigs
- size of gaps between contigs
- linking evidence: mate-pairs spanning gaps

Problems with the data

- Incorrect sizing of inserts
  - cut from gel – sizing is subjective
  - error increases with size
- Chimeras (ends belong to different inserts)
  - biological reasons (esp. for large sized inserts)
  - sample tracking (human error)
- Software must handle a certain error rate.

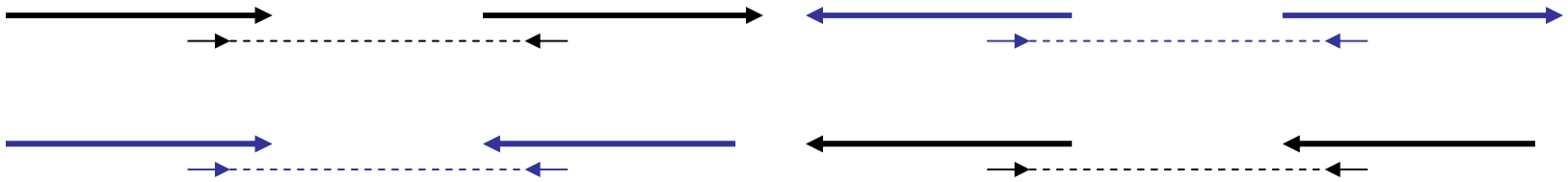
Theoretical abstraction

- Given a set of entities (reads/contigs) and constraints between them (overlaps/mate pairs) provide a linear/circular embedding that preserves most constraints.



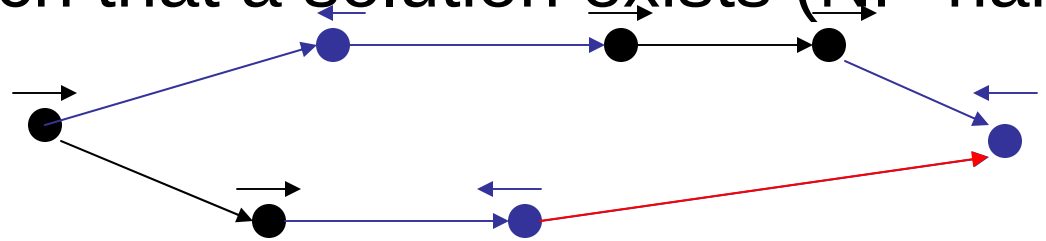
Graph representation

- Nodes: contigs
- Directed edges: constraints on relative placement of contigs – relative order and relative orientation
- Embedding: order (coordinate along chromosome) and orientation (strand sampled)



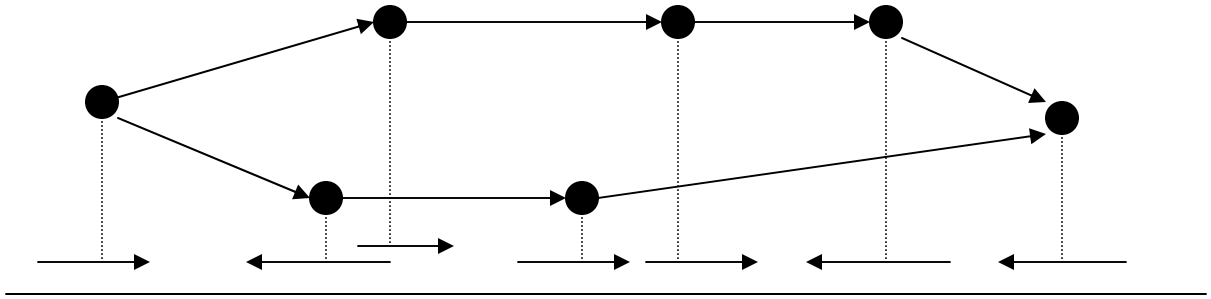
Challenges

- Orientation – node coloring problem (forward/reverse)
  - feasibility – no cycles with odd number of "reversal" edges
  - optimality – remove minimum number of edges
    such that a solution exists (NP-hard)



Challenges

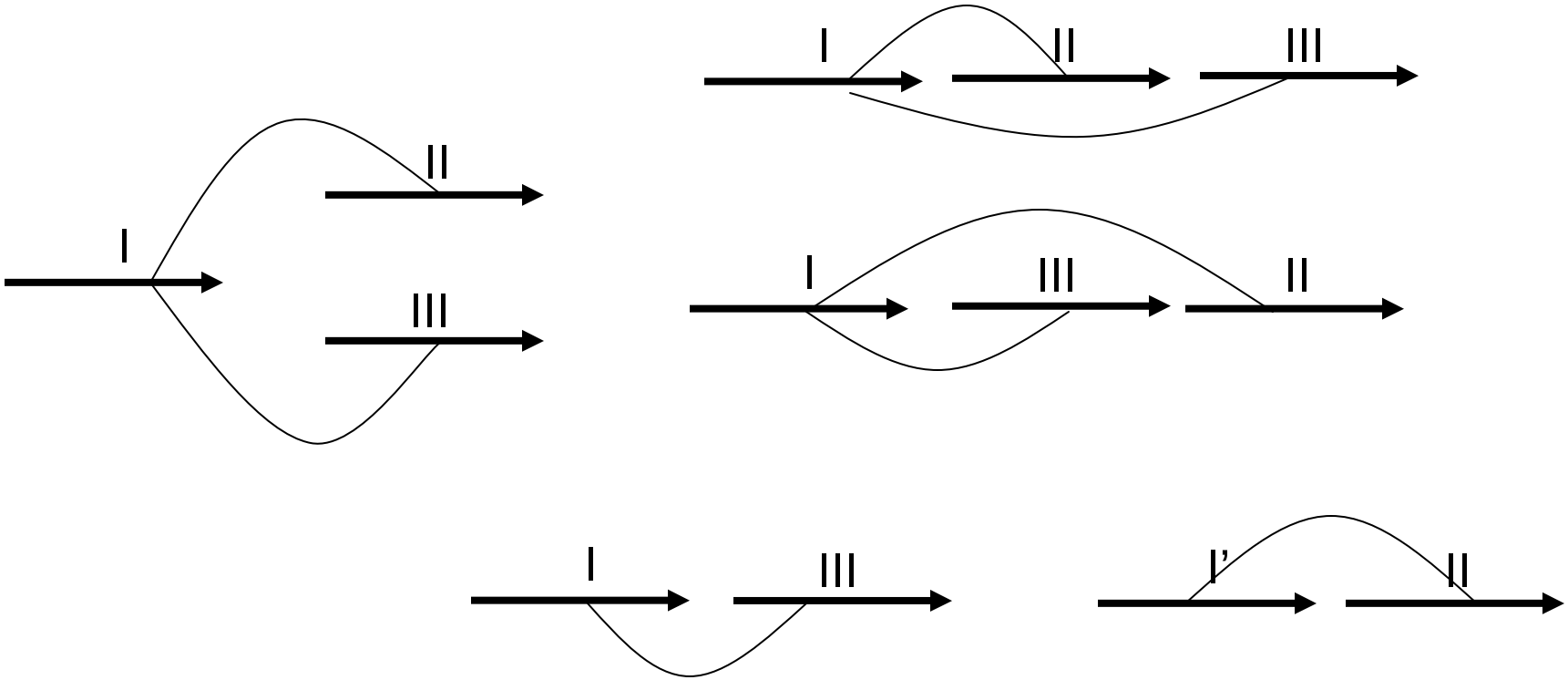
- Ordering – generate a linear embedding
  - feasibility – lengths of parallel DAG paths are consistent
  - optimality – remove minimum number of edges
    such that DAG is feasible (NP-hard)



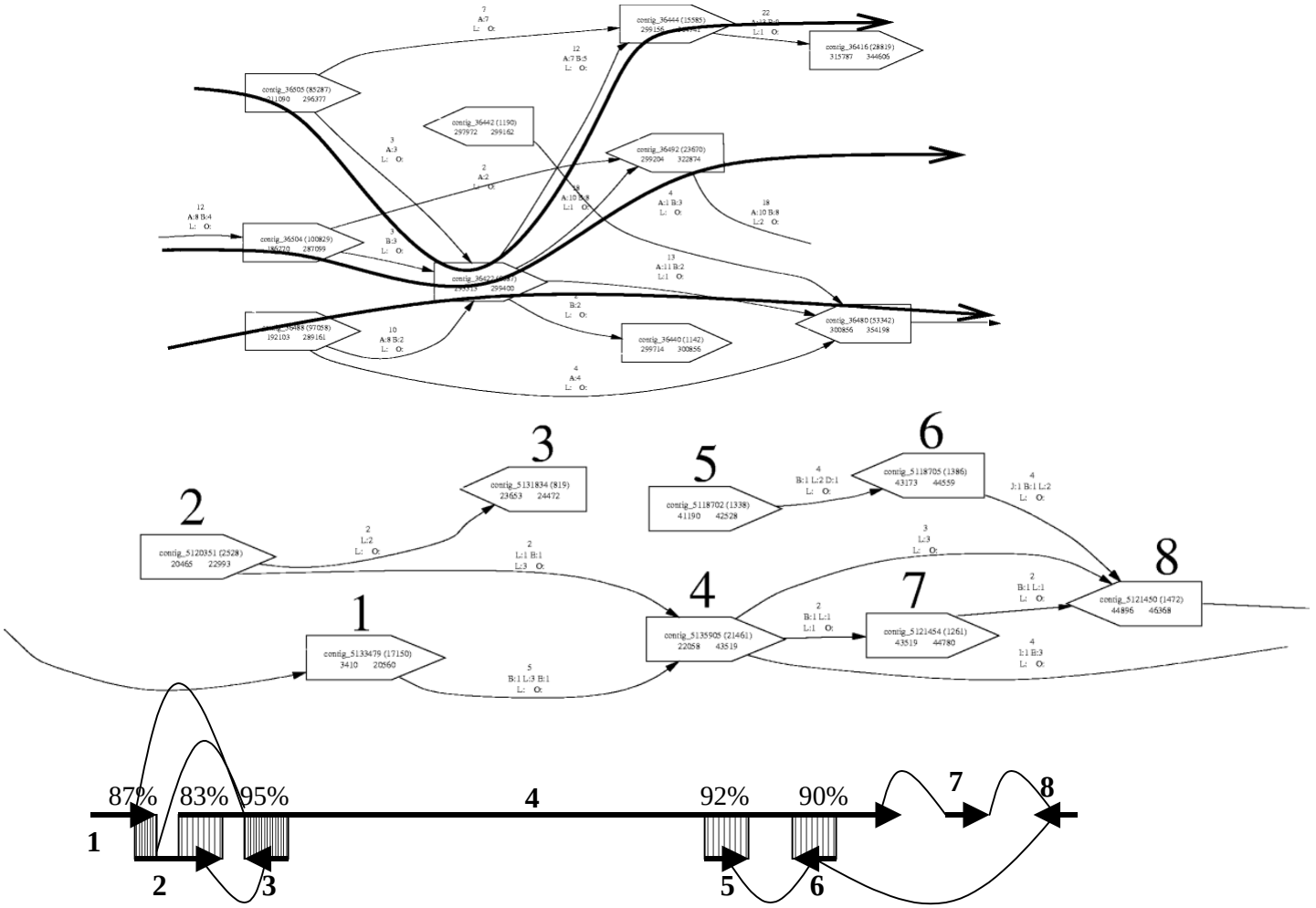
The real world

- Use of scaffolds
  - Analysis – longest unambiguous sub-graphs
  - Finishing – present all “reliable” relationships between contigs
- Sources of error
  - mis-assemblies
  - sizing errors (increases with library size)
  - chimeras

Ambiguous scaffold

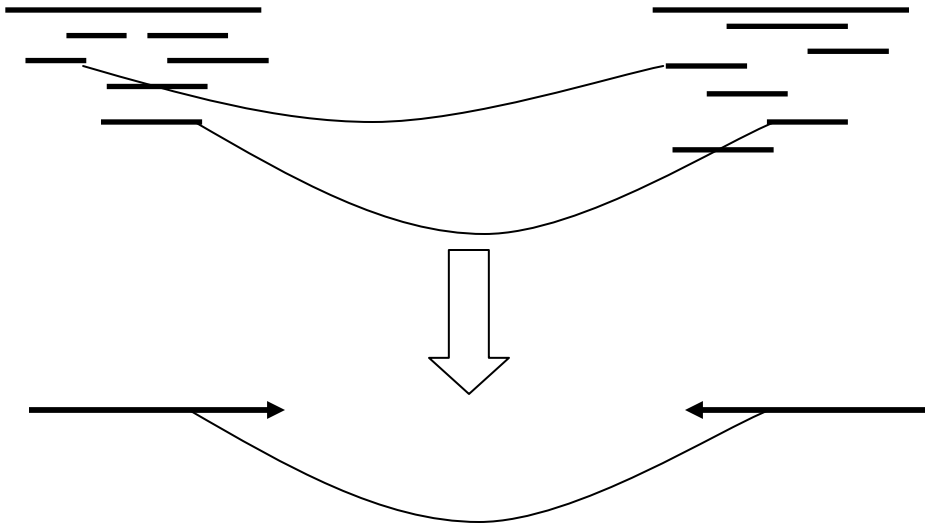


Repeats vs. Haplotypes



Hierarchical scaffolding

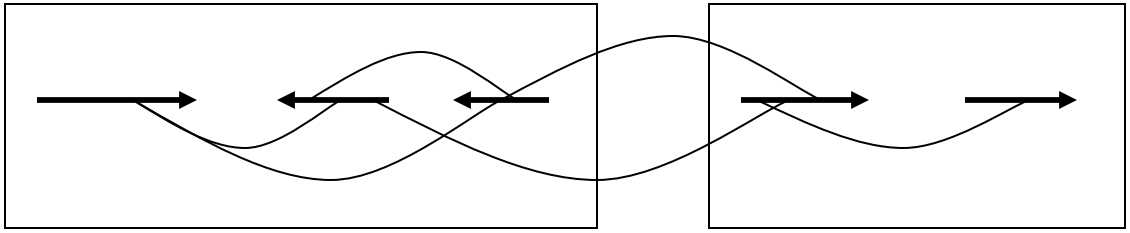
1. For each contig pair, consolidate all linking data into a single relationship – 2 correct links required



Hierarchical scaffolding

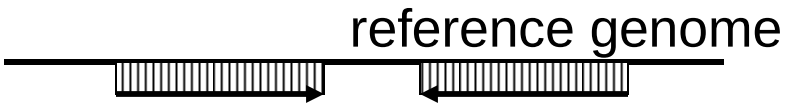
2. Use most reliable links to build scaffolds

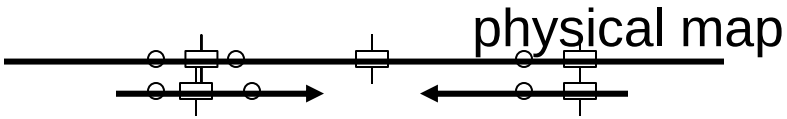
3. Repeatedly build super-scaffolds based on less reliable linking data

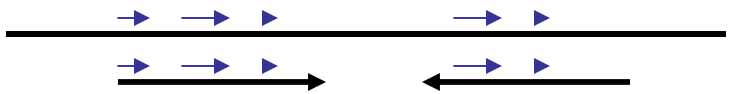


Linking information

- Overlaps
- Mate-pair links
- Similarity links
- Physical markers
- Gene synteny







BAMBUS
(bamboo)

Best effort **A**ttempt
Multiple **B**ranches allowed
Order, **O**rient

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