# P&S Mobile Genomics

## Lecture 8: Genome Assembly

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# Agenda for Today

- Genome Assembly
  - Basics
  - Overlap-Layout-Consensus

# Recall: Caveats of Sequencing Technologies

Small pieces of a puzzle
**short reads (Illumina)**

Large pieces of a puzzle
**long reads (ONT & PacBio)**

Which sequencing technology is the best?

- ❑ 100-300 bp
- ❑ 500-2M bp
- ❑ low error rate (~0.1%)
- ❑ high error rate (~15%)

https://www.pacb.com/smrt-science/smrt-sequencing/hifi-reads-for-highly-accurate-long-read-sequencing/

# Recall: A Dream

Looking forward,

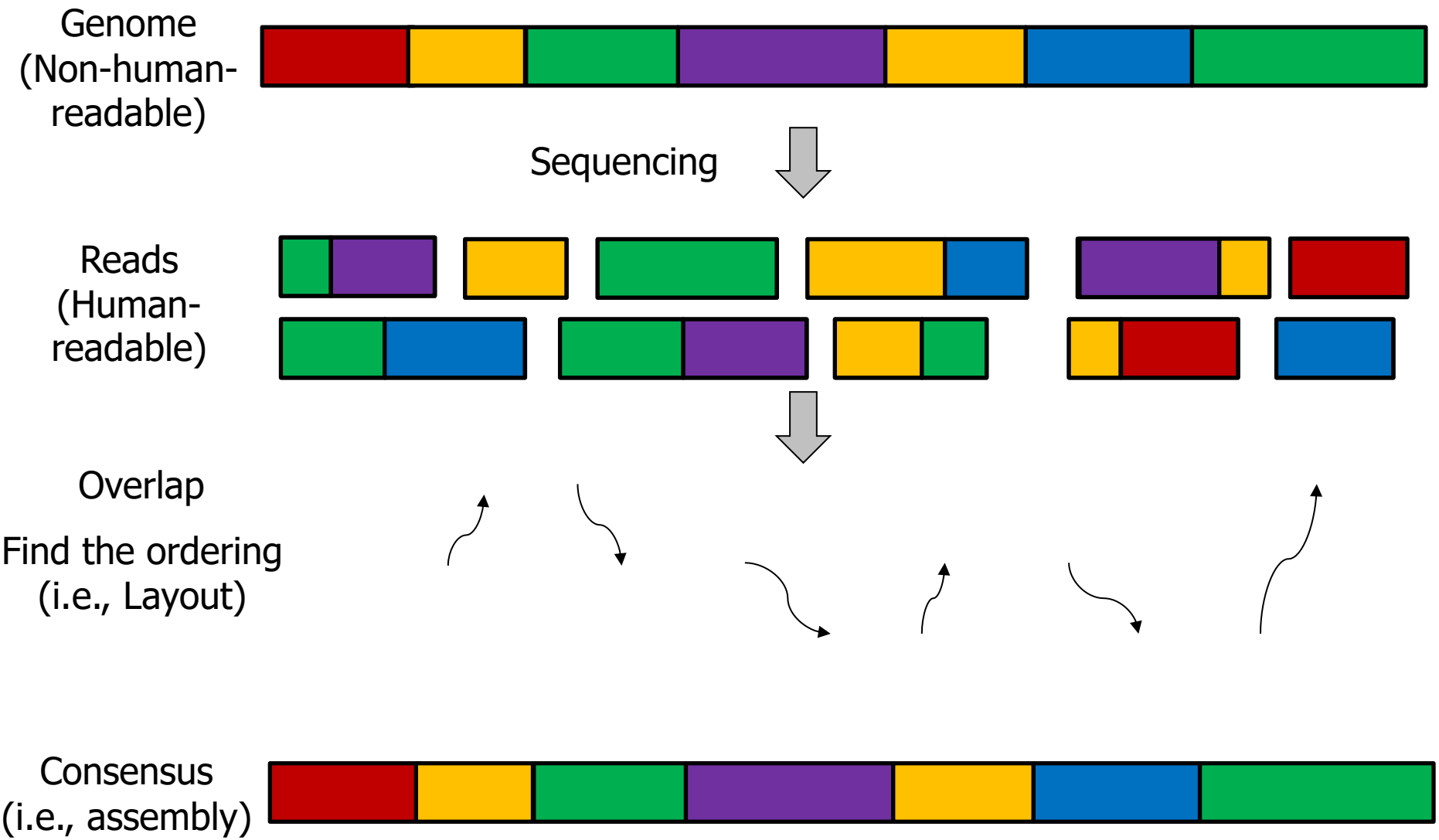
Will we be able to read

the entire genome sequence?

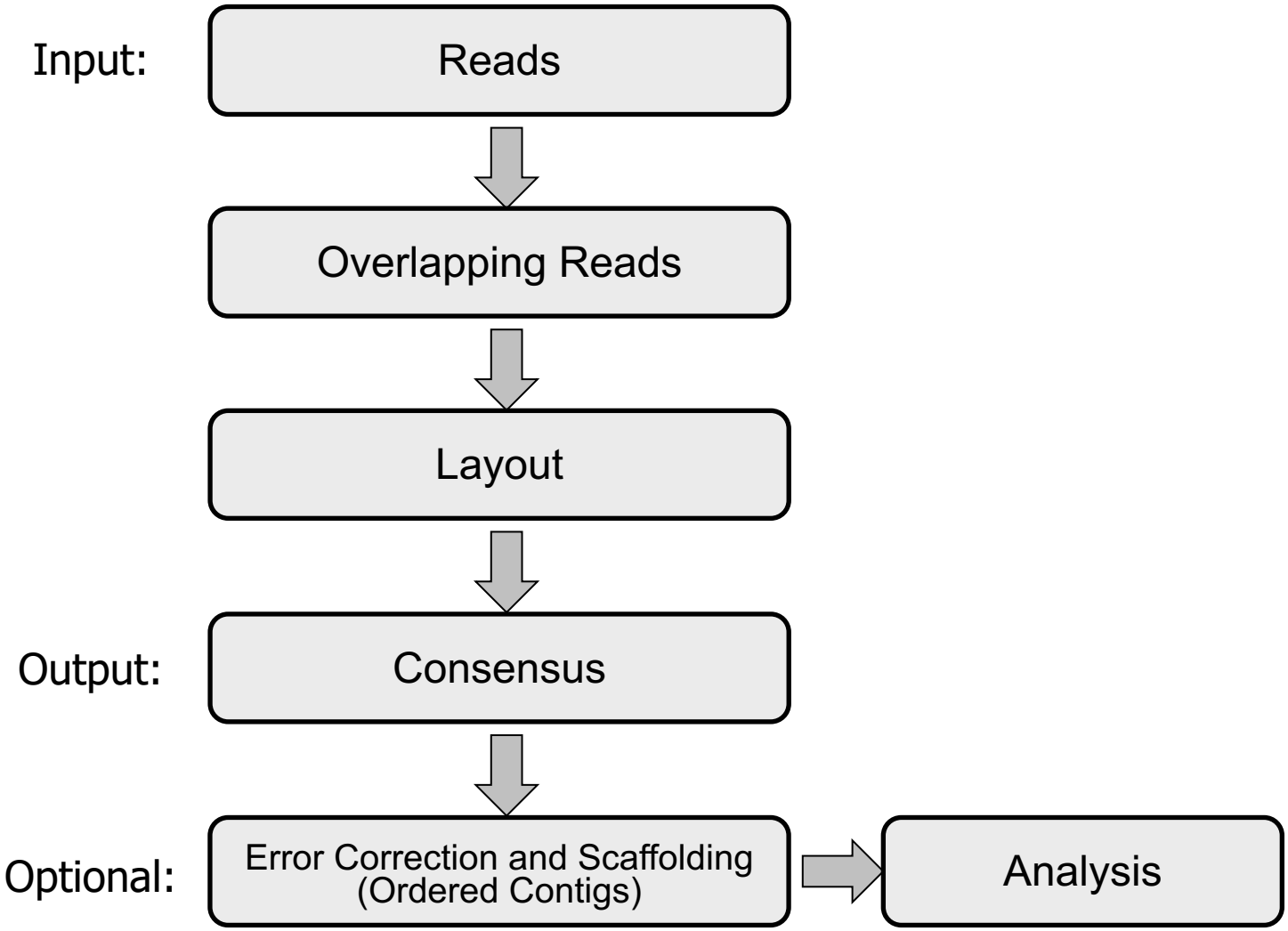
# Genome Assembly Basics

- There is no sequencing technology that can read an entire chromosome from start to end
  - Rather we have short fragments of genome: **Reads**
- Reconstruct the actual genome from its pieces to
  - Compare two genomes to reveal large structural variations as well as small mutations to **pinpoint diseases** and **study certain phenotypes** (e.g., eye color, hair color)
  - Map known genes
  - Use it as a reference to map reads from the same species
  - …
- Two major approaches to reconstruct a genome
  - Hierarchical sequencing
    - Human Genome Project
    - Slow, expensive, but highly accurate and contiguous assembly
  - Whole genome shotgun (WGS) sequencing
    - Fast, cheaper, but less accurate and less contiguous

# Genome Assembly from WGS Sequencing



# A Common Assembly Pipeline



# Overlapping Reads

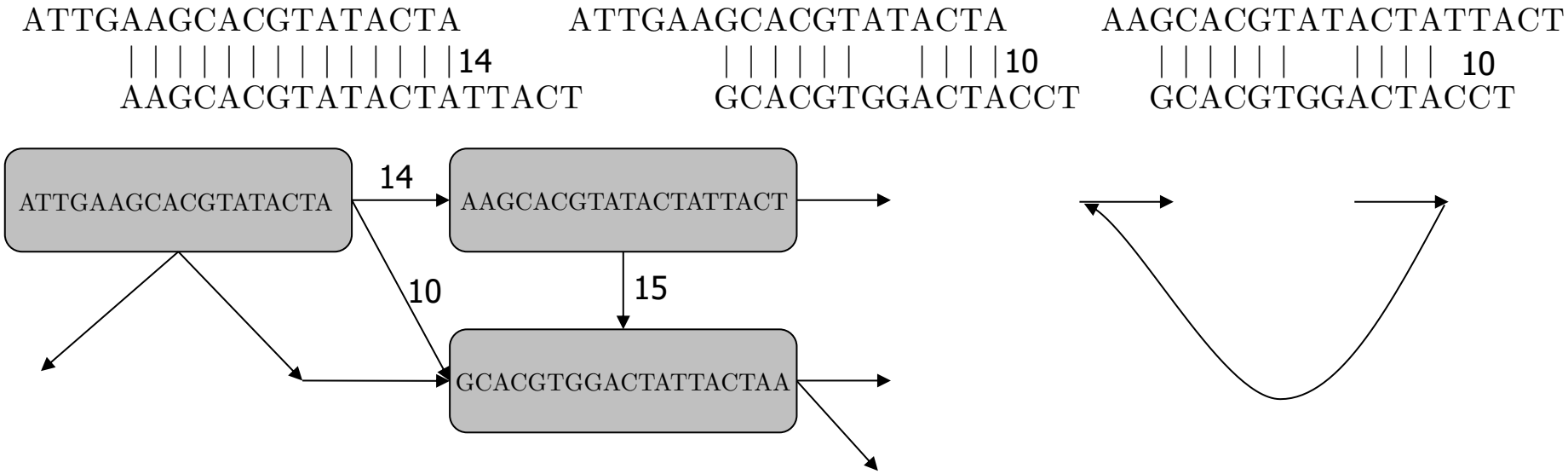
- Find matching blocks between **all pairs of reads** using
  - Exact matching short subsequences between reads
  - Suffix Tree
  - Alignment
- Suffix of a read overlaps prefix of another read


Overlapping Reads:

```
ATTGAAGCACGTATACTA
||||||||||||||||
AAGCACGTATACTATTACT

ATTGAAGCACGTATACTA
||||||   ||||
GCACGTGGACTATTACTAA

AAGCACGTATACTATTACT
||||||   ||||||||||
GCACGTGGACTATTACTAA

TACCGATTGGACTATCCATTTAC
||||||||||||||||||
GGACTATCCATTTACACCTGGAT
```

# Storing Overlaps in Graphs

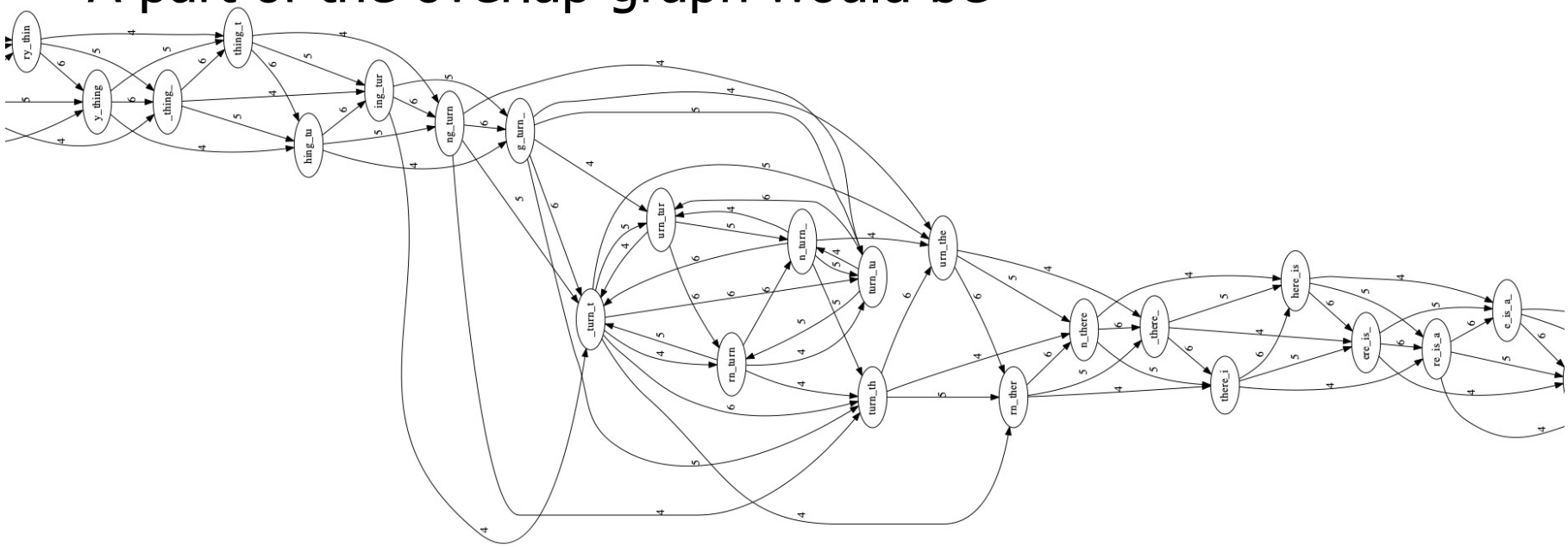
- Nodes: Reads/Chunks of reads
- Directed Edges: When suffix of one read overlaps prefix of another read
  - Label: Number of matches between overlapping reads




- Graphs are useful to prevent storing redundant reads (i.e., one node per read) and to reveal unambiguous overlaps
- However, edges can get quite messy

# A Messy Overlap Graph

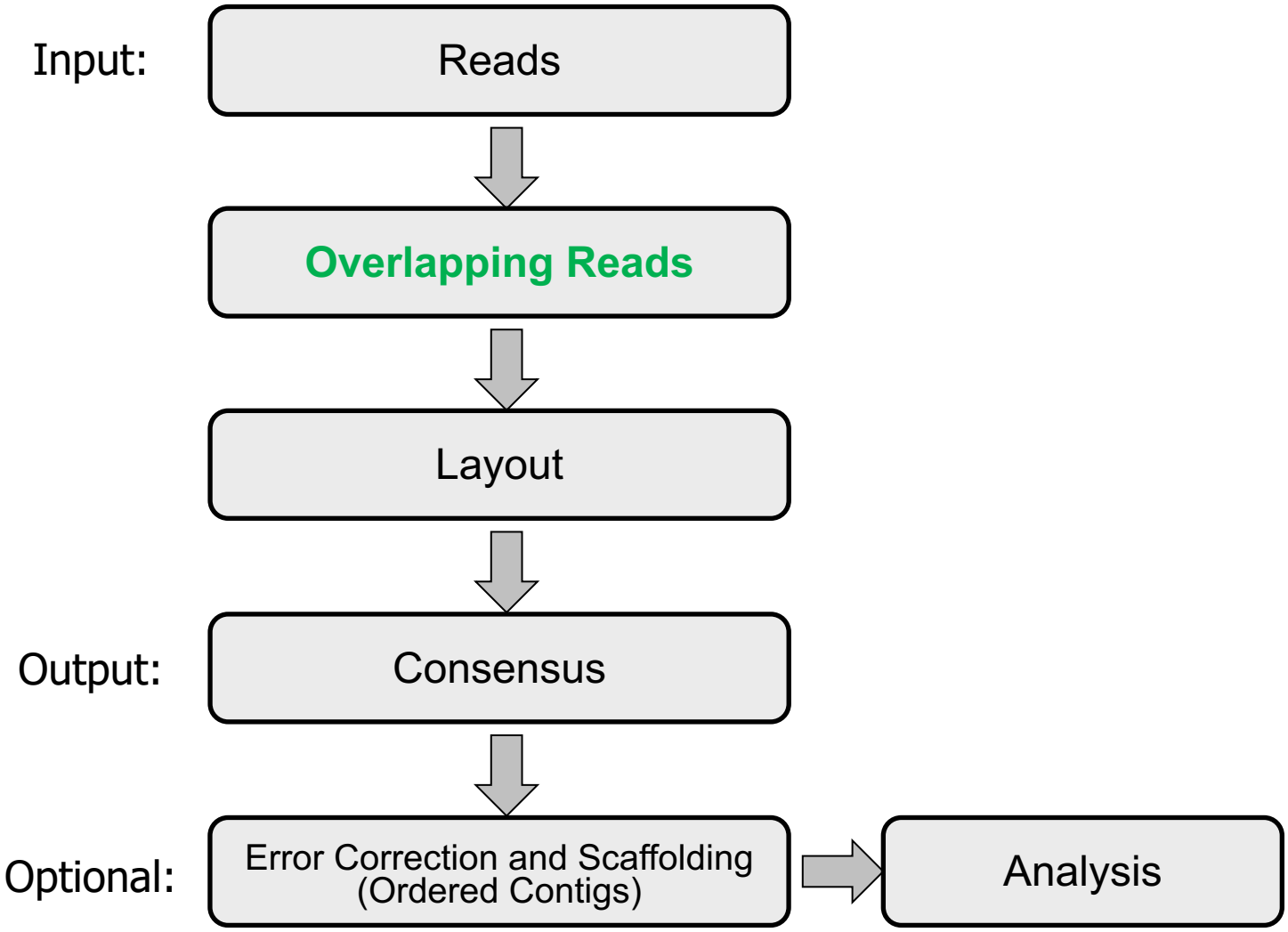
- Assume we generate an overlap graph using the all possible subsequences of fixed length 7 of the following string:
  - to_every_thing_turn_turn_turn_there_is_a_season
- A part of the overlap graph would be




- How to find a simpler ordering of reads relative to each other from the overlap graph?

Image source: http://www.cs.jhu.edu/~langmea/resources/lecture_notes/16_assembly_scs_v2.pdf

# A Common Assembly Pipeline



# Layout – Graph Cleaning

- Overlap graphs may contain **redundant information**
  - **Transitive (redundant) edges**: An edge from node v to node w (v -> w) is transitive if:
    - There exists v -> u and u -> w
    - We can remove the edge v -> w without losing the ability to visit w starting from v
  - **Bubbles**: A directed acyclic graph with sink and source nodes v and w such that
    - There exist at least two *isolated* paths from v to w
    - We want to collapse bubbles to simplify the overlap graph
  - **Tips:** Short branches in the graph that terminate very early

# Layout – Transitive Reduction

- Overlap graphs may contain **redundant edges**
  - Here the green edges are transitive edges because the blue edges can be used for the same connectivity
  - **Transitive edges** that can be removed without losing the connectivity information of the graph

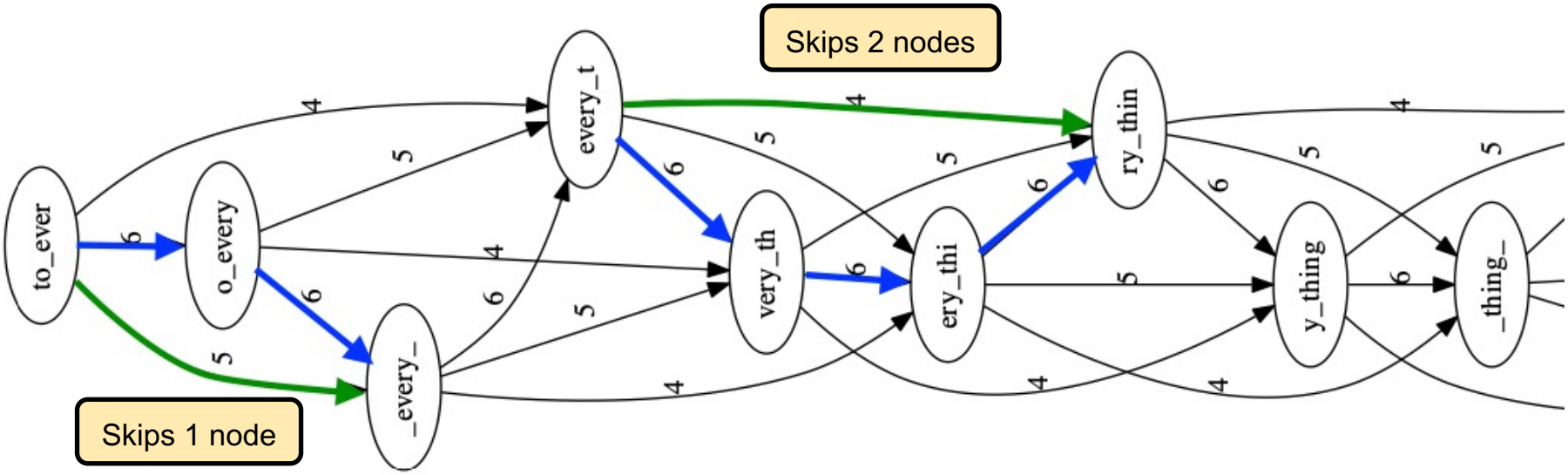
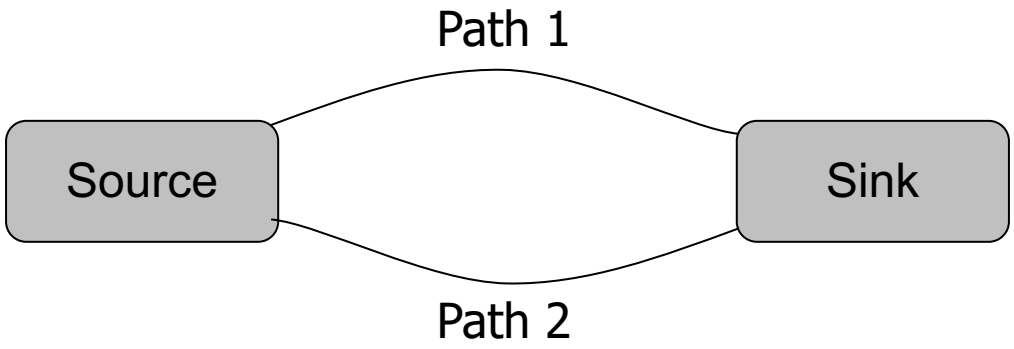



Image source: http://www.cs.jhu.edu/~langmea/resources/lecture_notes/16_assembly_scs_v2.pdf

# Layout – Bubble Collapsing (Popping)

- Overlap graph may contain bubbles that transitive edge removal do not detect
  - Usually shorter paths in bubbles are collapsed
  - Shorter paths may be due to **repeats** after transitive reduction
- We can collapse bubbles to
  - Reduce the complexity of the overlap graph
  - Improve the contiguity of the assembly inferred from the graph
- Why do we have bubbles?
  - Sequencing errors (missing overlaps)
  - Variants between parent genomes (diploid and polyploid genomes)



# Layout – Readings on Graph Cleaning

- Read the following paper if you are curious about
  - How the transitive reduction works:

BIOINFORMATICS

Vol. 21 Suppl. 2 2005, pages ii79–ii85
doi:10.1093/bioinformatics/bti1114

*Genes and Genomes*

**The fragment assembly string graph**

Eugene W. Myers
Department of Computer Science, University of California, Berkeley, CA, USA

  - How to collapse bubbles in overlap graphs:

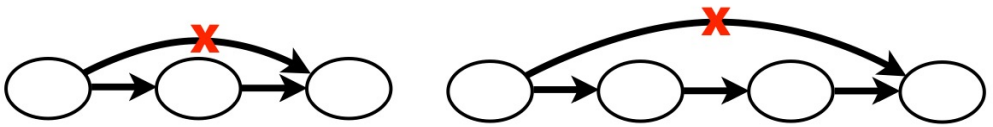
**Minimap and miniasm: fast mapping and de novo assembly for noisy long sequences** FREE

Heng Li Author Notes

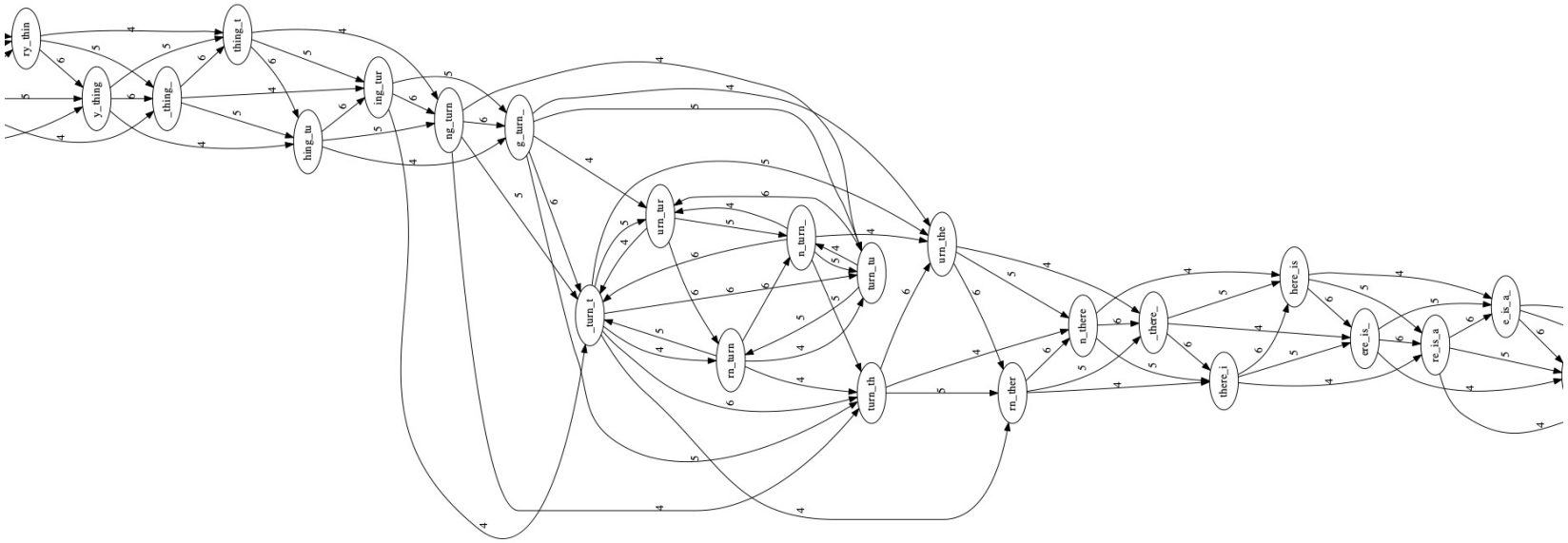
*Bioinformatics*, Volume 32, Issue 14, 15 July 2016, Pages 2103–2110,
https://doi.org/10.1093/bioinformatics/btw152
**Published:** 19 March 2016 **Article history** ▾

# Layout – Transitive Reduction Example

- Overlap graphs may contain **redundant edges**
  - Transitive edges that can be removed without losing the connectivity information of the graph
  - Let's remove the transitive edges that skip one or two nodes:




  - Remember the messy overlap graph?



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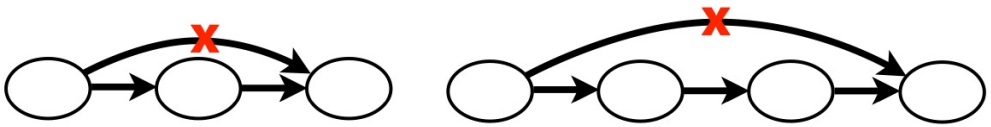
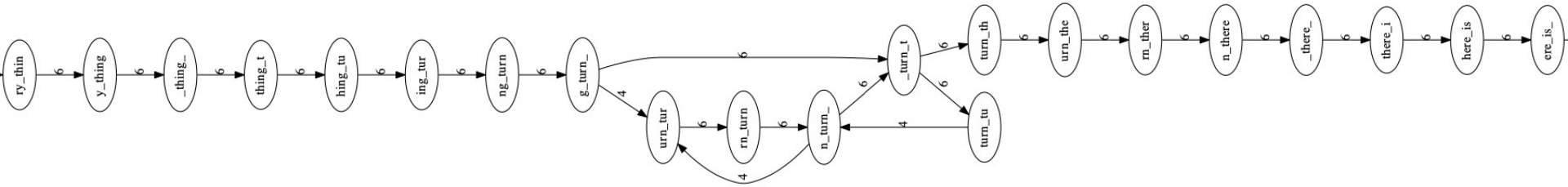


  - After the transitive reduction:




  - It is now much easier to infer the contigs (i.e., the pieces of assembly) from this graph

Image source: http://www.cs.jhu.edu/~langmea/resources/lecture_notes/16_assembly_scs_v2.pdf

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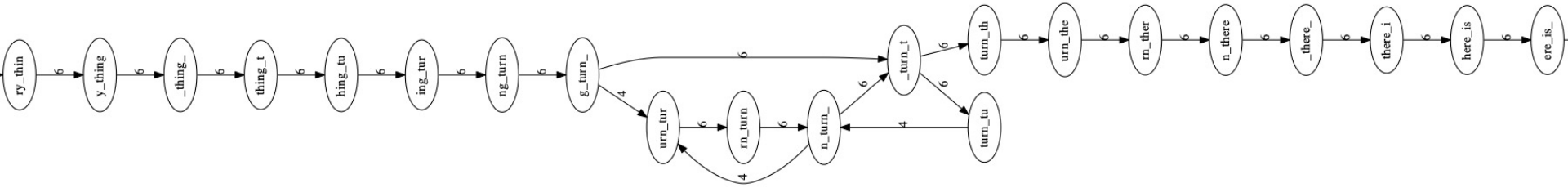
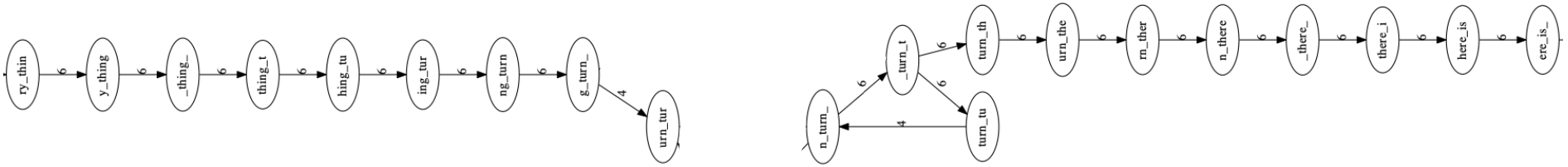



  - Bubble Collapsing

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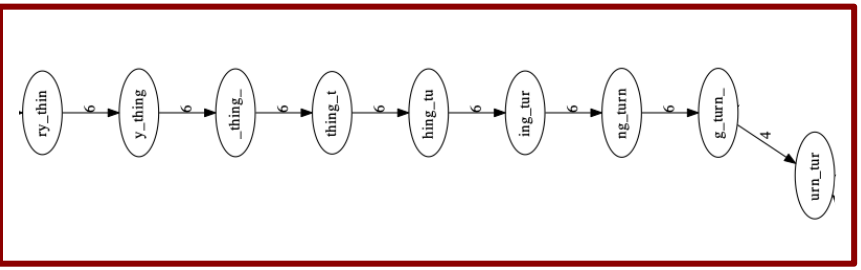
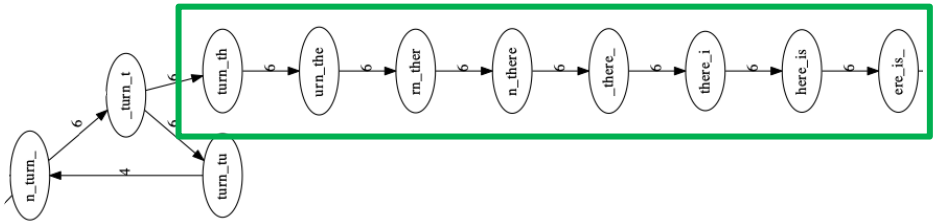
# Spelling out the Contigs

- Take all nodes with unambiguous branches (e.g., single branch, leading no cycles)
- "Spell out" the contig by following the unambiguous branches

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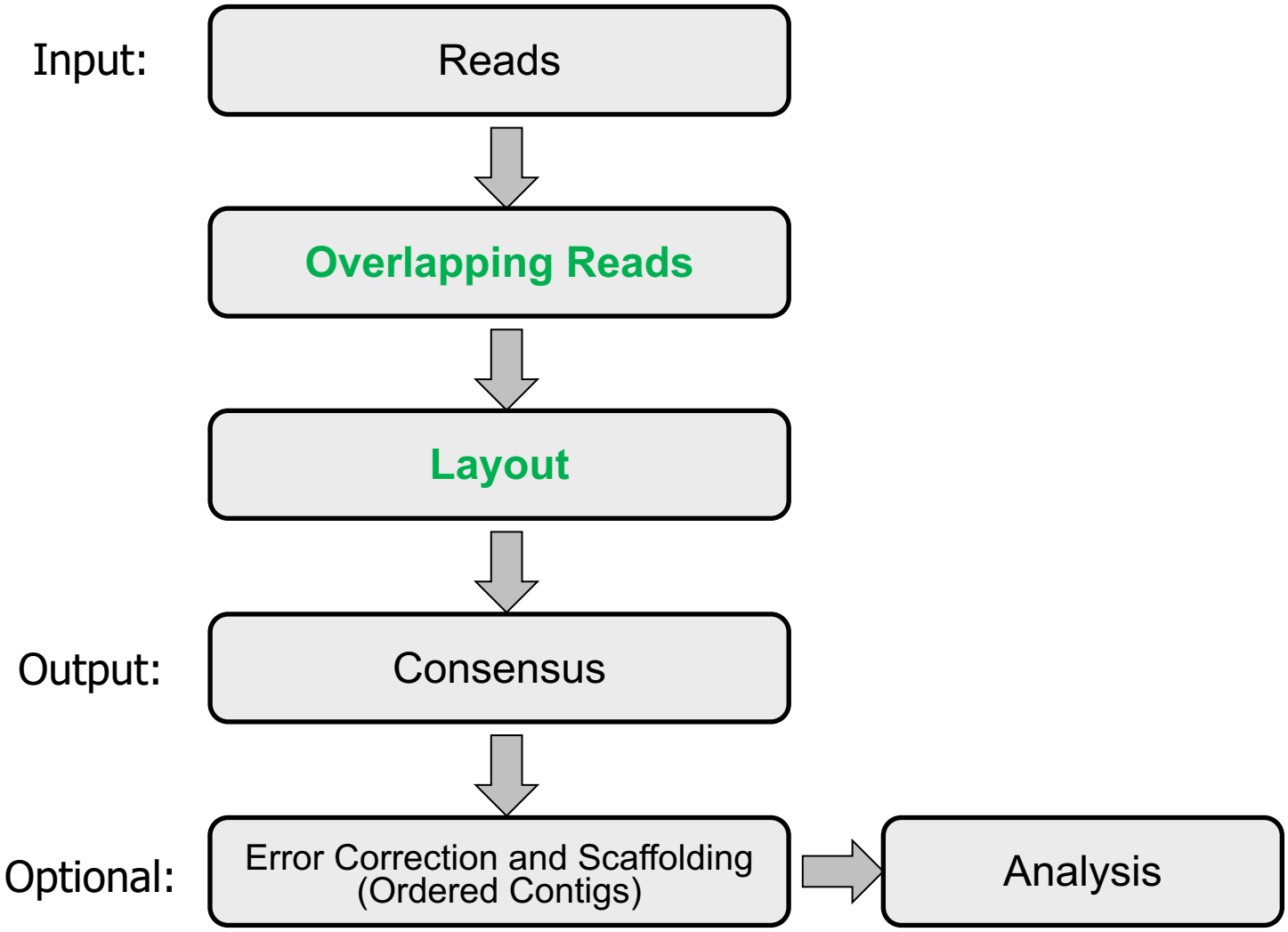
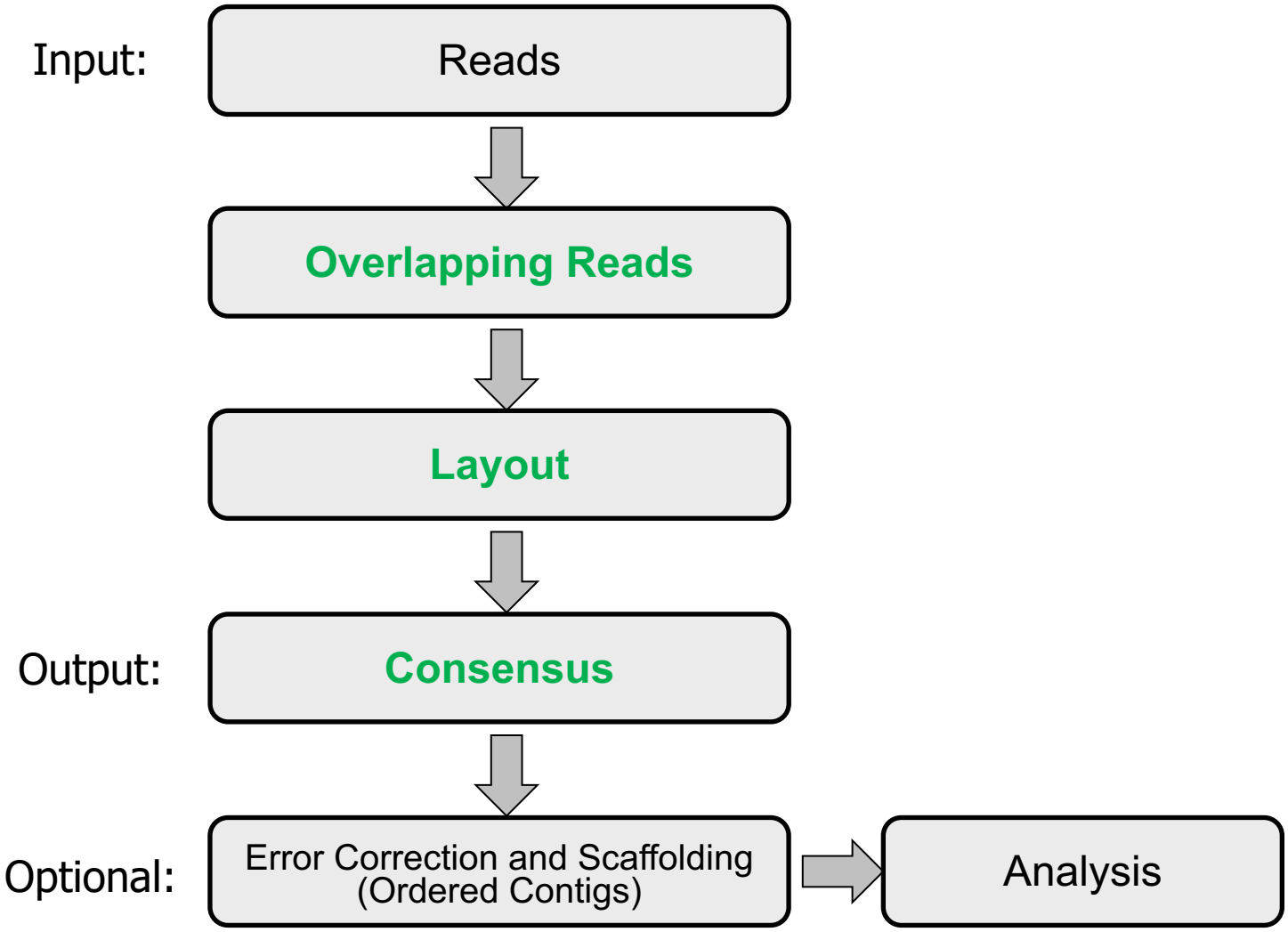
# A Common Assembly Pipeline



# Consensus of Overlapping Reads

- Layout the overlaps of reads from the overlap graph
- Take the consensus at each base to generate contigs

# A Common Assembly Pipeline



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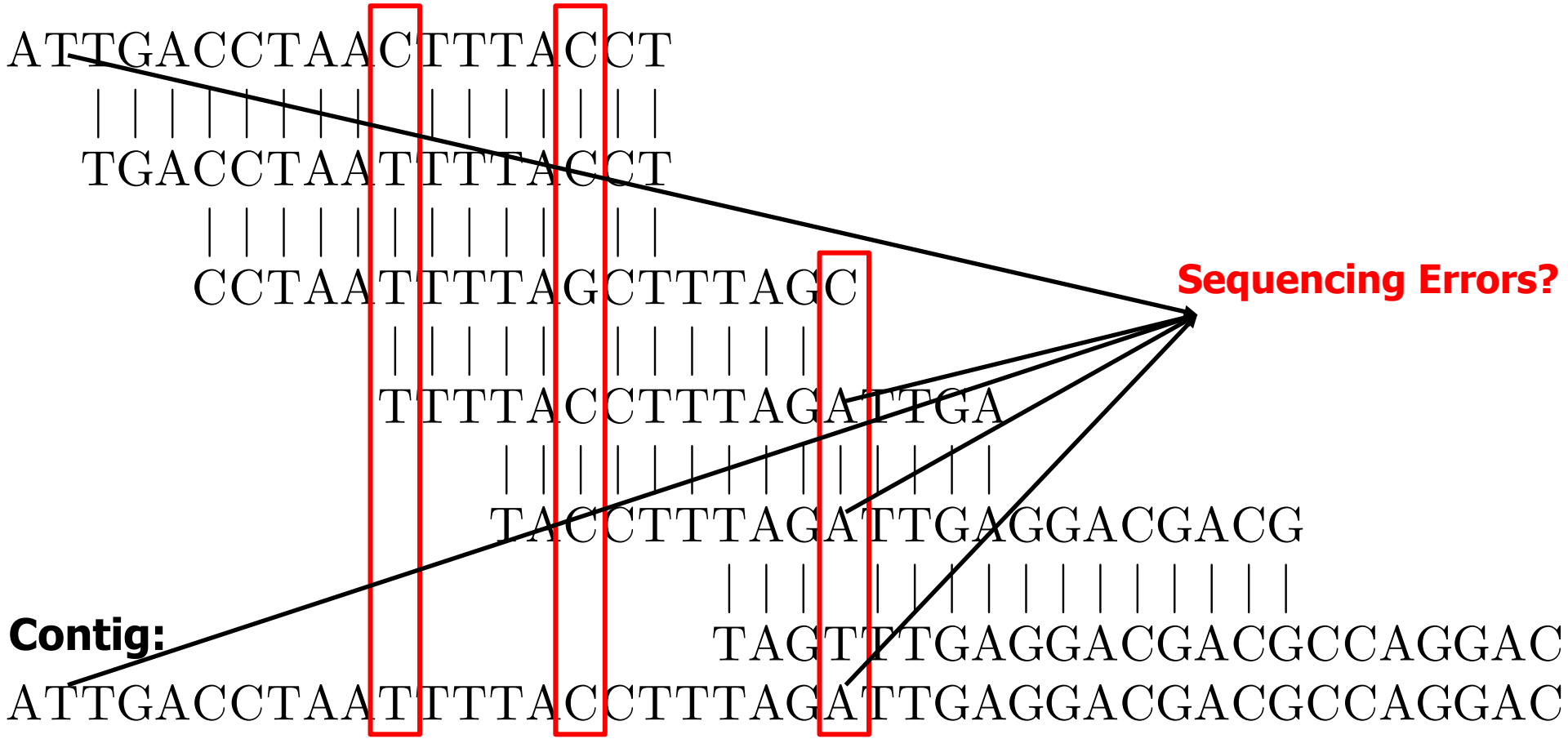
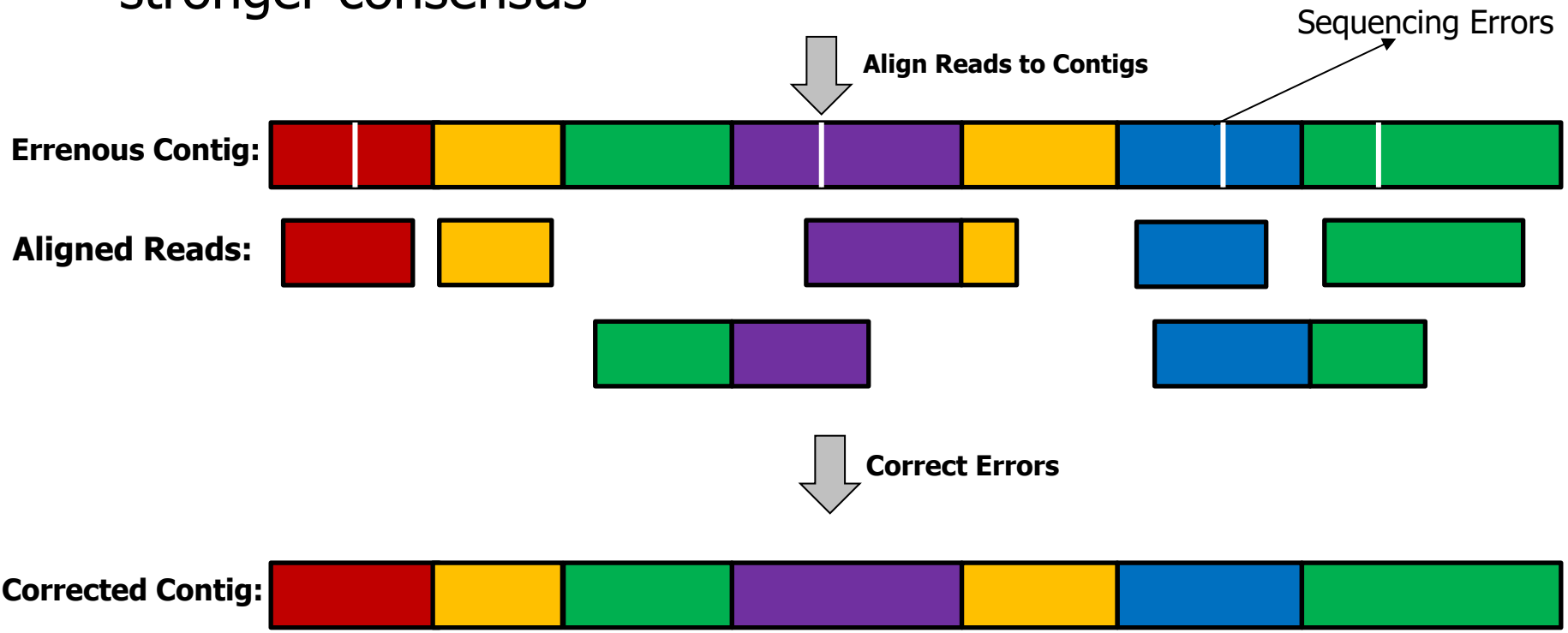


# Assembly Polishing (i.e., Error Correction)

- Sequencing errors on reads may propagate to contigs
  - Leading to inaccurate analysis on the assembly we just generated
- Idea: Align reads back to contigs again to generate a stronger consensus



# A Reading on Assembly Polishing

- Firtina et al., "Apollo: A Sequencing-Technology-Independent, Scalable, and Accurate Assembly Polishing Algorithm," Bioinformatics, June 2020.

**Apollo: a sequencing-technology-independent, scalable and accurate assembly polishing algorithm**

FREE

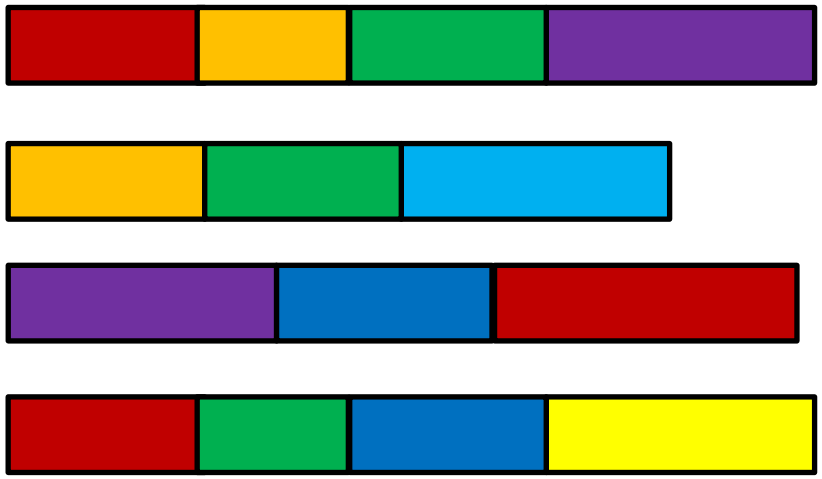
Can Firtina, Jeremie S Kim, Mohammed Alser, Damla Senol Cali, A Ercument Cicek, Can Alkan ✉, Onur Mutlu ✉

*Bioinformatics*, Volume 36, Issue 12, 15 June 2020, Pages 3669–3679,
https://doi.org/10.1093/bioinformatics/btaa179
**Published:** 13 March 2020 **Article history** ▾

# Scaffolding – Ordering the Contigs

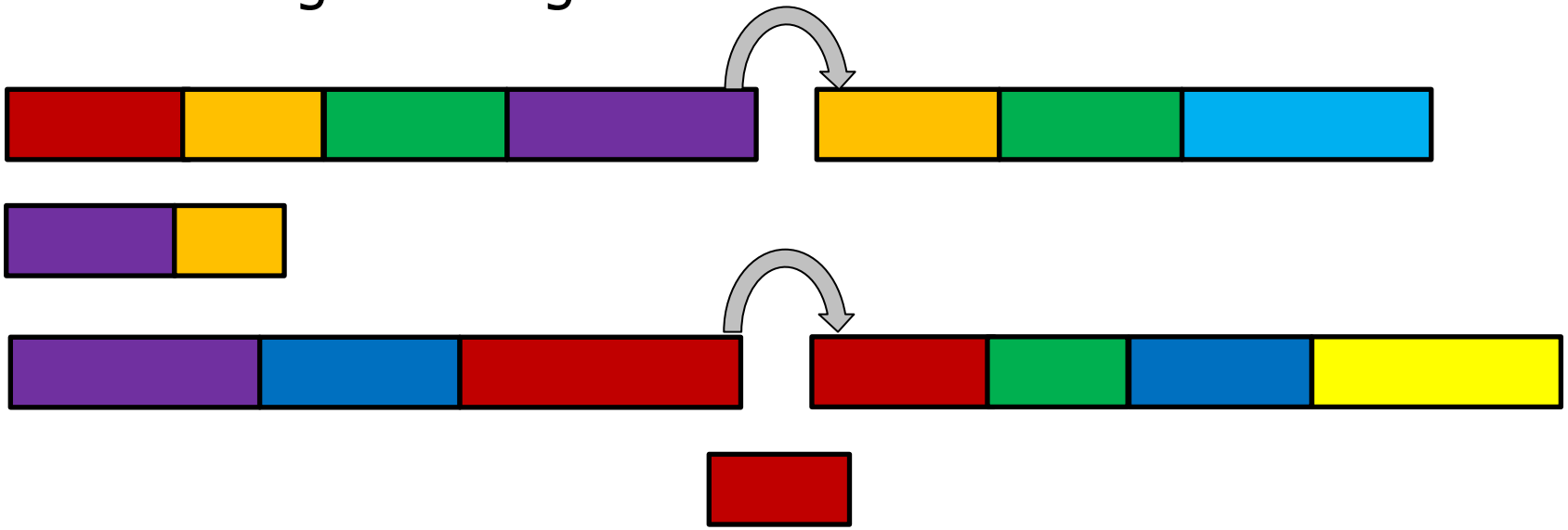
- Contigs are still usually not the complete sequence of genome
- A genome may potentially be represented by several gapped contigs
  - What is the relative order of contigs to represent the genome correctly?

**Unordered Contigs:**



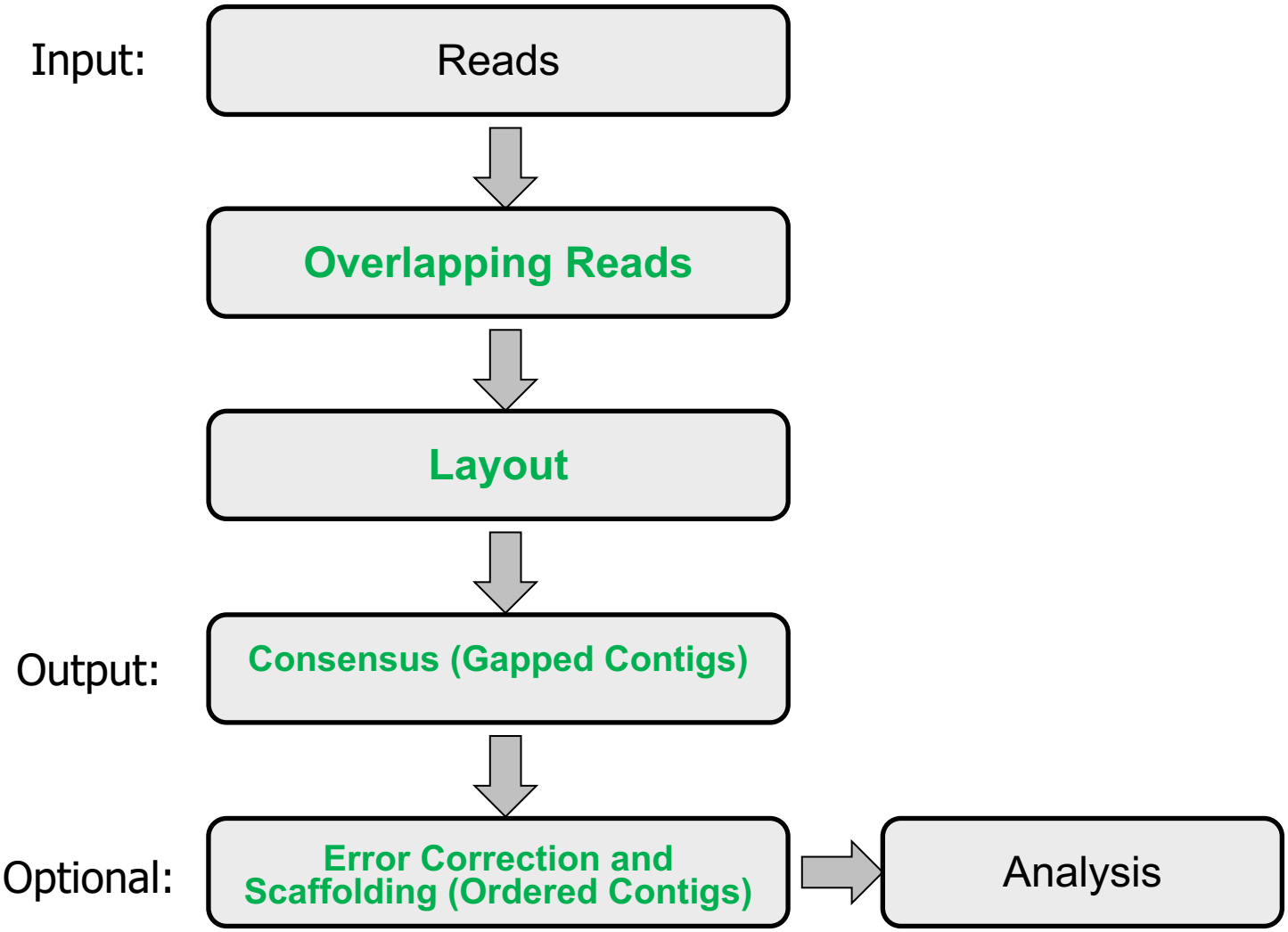
# Scaffolding – Ordering the Contigs (cont'd)

- Overlap parts of reads to contigs to find the pairwise ordering of contigs



- Ultra long reads, paired-end reads, optical mapping usually help scaffolding
  - These are good keywords to check if you are curious

# A Common Assembly Pipeline



# What Makes a Good Assembly?

- Accurate
  - Should be resolved from errors as much as possible
  - Solutions:
    - Long and accurate reads (e.g., PacBio HiFi reads)
    - Error correction tools
    - Accurate assemblers
- Contiguous
  - Gaps: Missing information on assembly
  - Solutions:
    - Long and accurate reads
    - Accurate assemblers
    - We need better tools to resolve repeats in overlap graphs
- Tools to generate overlaps: Minimap2, Canu
- Tools for assembly: Miniasm, Canu, Hifiasm, Flye
- Tools to assess the assembly quality: QUAST and the MUMmer package

# P&S Mobile Genomics

## Lecture 8: Genome Assembly

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