Floria: metagenome strain haplotyping with short/long reads

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* Equal contribution

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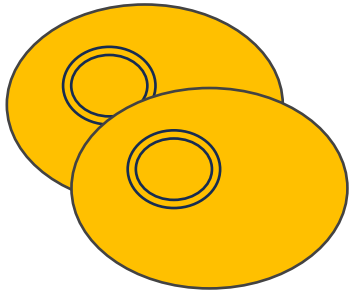
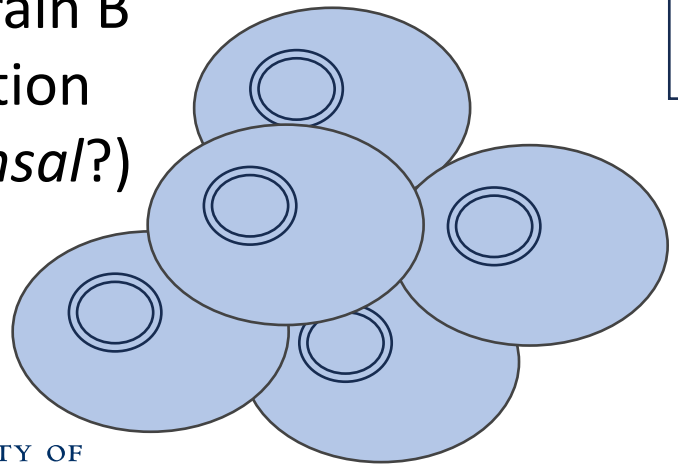
Strain-level heterogeneity in metagenomes is important!

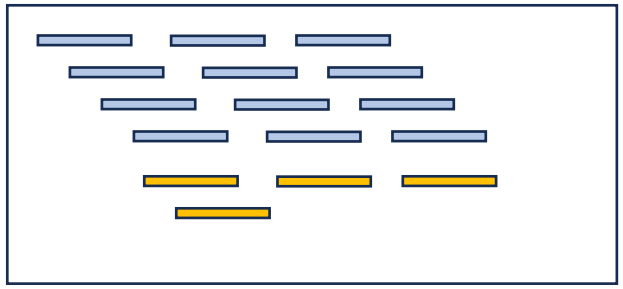
E. coli strain A population (*pathogenic*?)



E. coli strain B population (*commensal*?)



Strain-mixed metagenomic reads

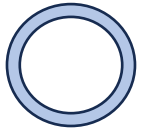


Assembly

What is assembled?

A) only

B) only



C) both

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Answer: depends on technology + algorithms

Technology	Resulting assembly
Short reads (algorithm: SPAdes)	**One** strain (lost information)
Low-fidelity long-reads (algorithm: metaFlye)	**One** strain (lost information)
High-fidelity long-reads (algorithm: hifiasm or metaMDBG)	**Both** strains

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Presentation focus

Introduction:
Haplotyping and phasing

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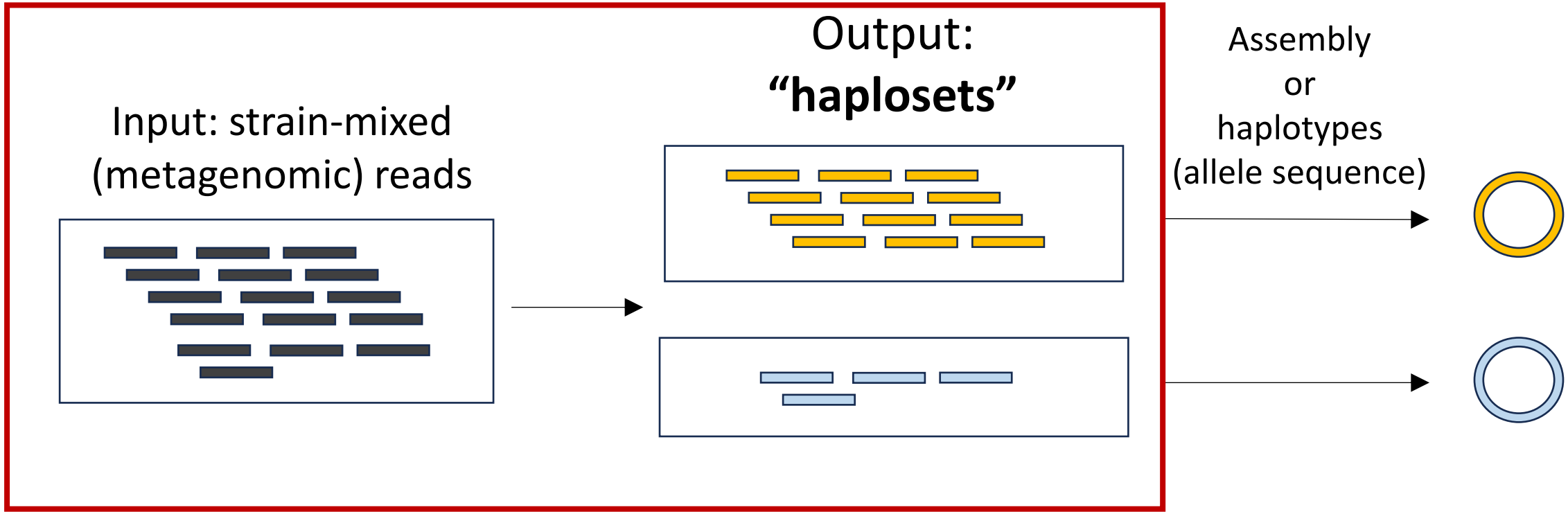
Computational goal:
reads → **strain-level** "haplosets"



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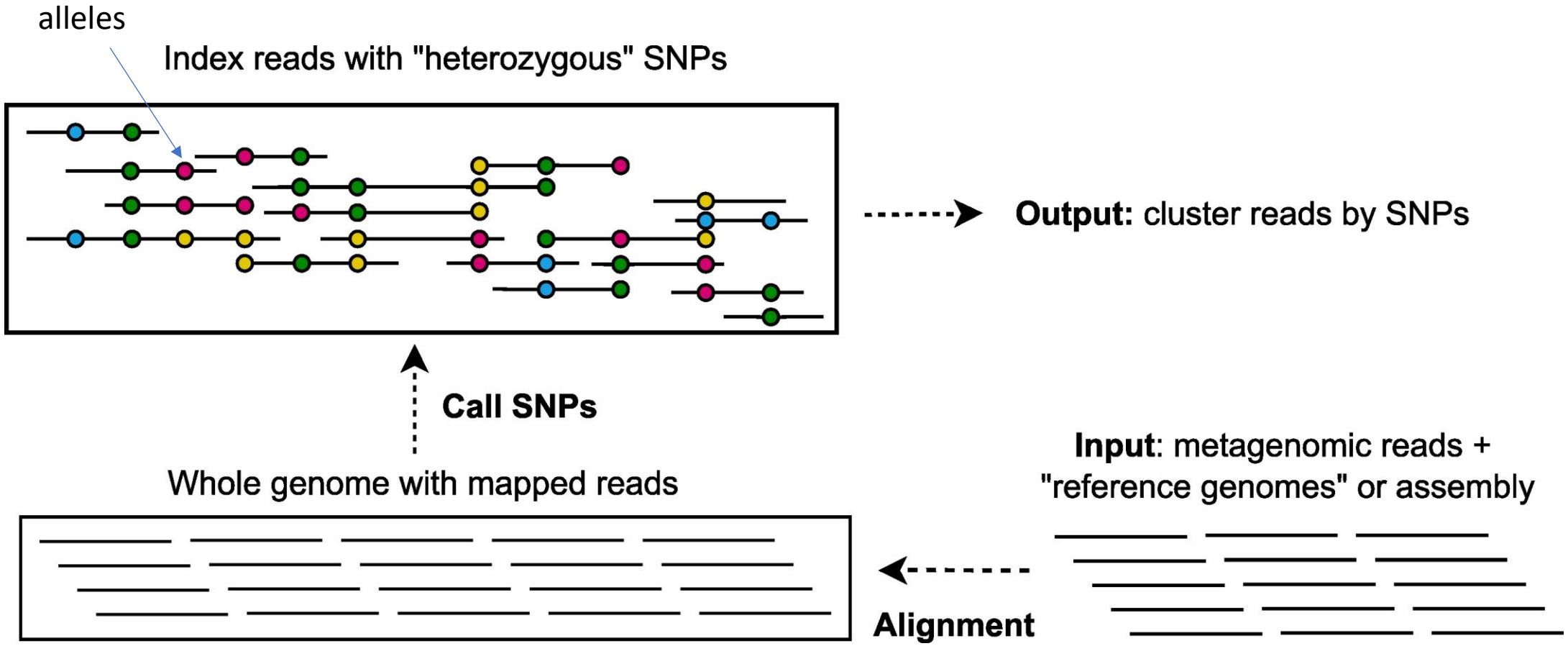
Presentation focus

Main idea: computational phasing (haplotyping) using SNPs + read-linkage



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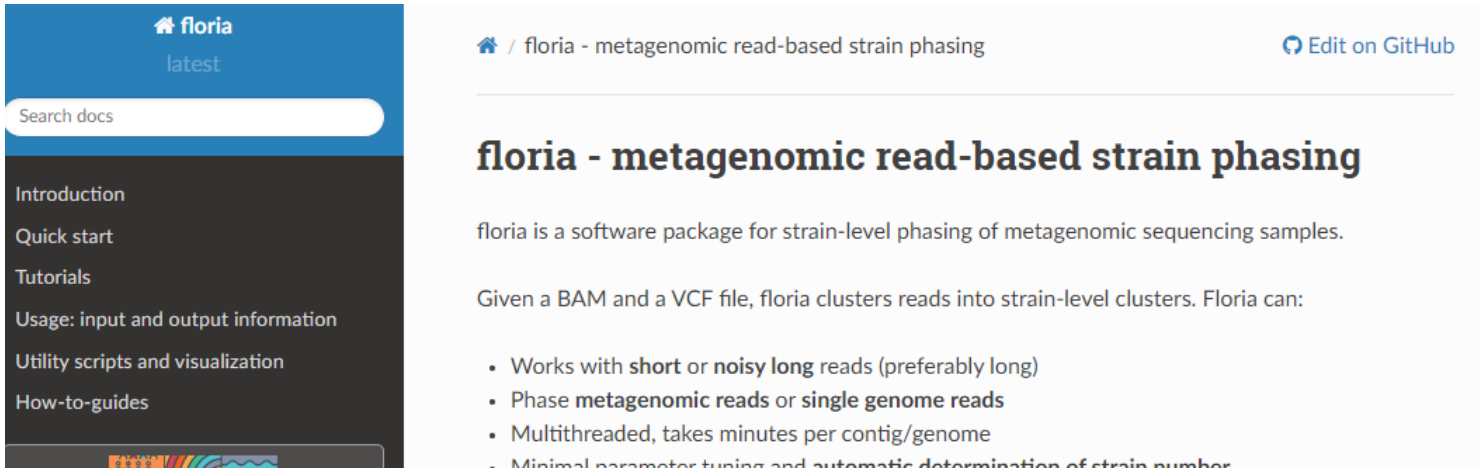
Contributions (Shaw and Gounot et al. 2024)

- **(1) Floria**: read clustering (phasing) algorithm from **alignments + SNPs**
  - Written in Rust; documentation + conda install
- **(2) Floria-PL**: end-to-end pipeline (fastq -> assemblies)
  - Written in Snakemake - **integrating** floria



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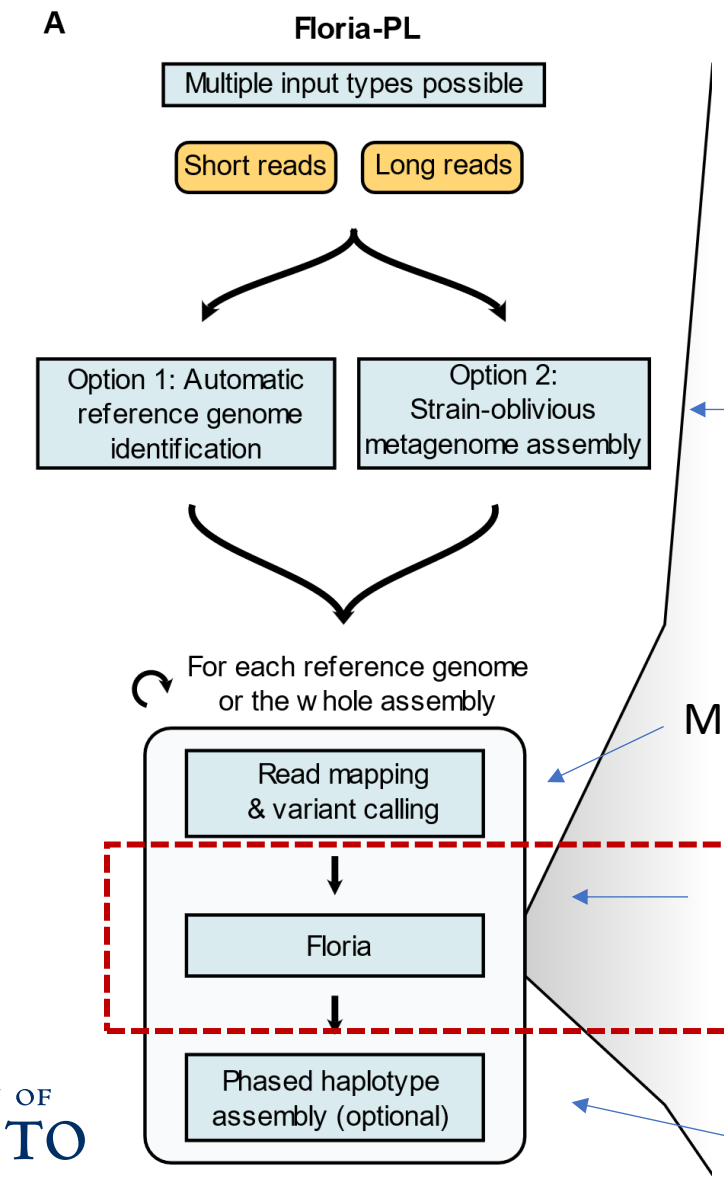
Floria-PL: pipeline integrating floria



Generate "reference" genomes (profiling or assembly)

Map reads to genomes + call variants

New algorithm: floria

Generate **haplosets from BAM + VCF**

Assemble haplosets - **OPTIONAL**

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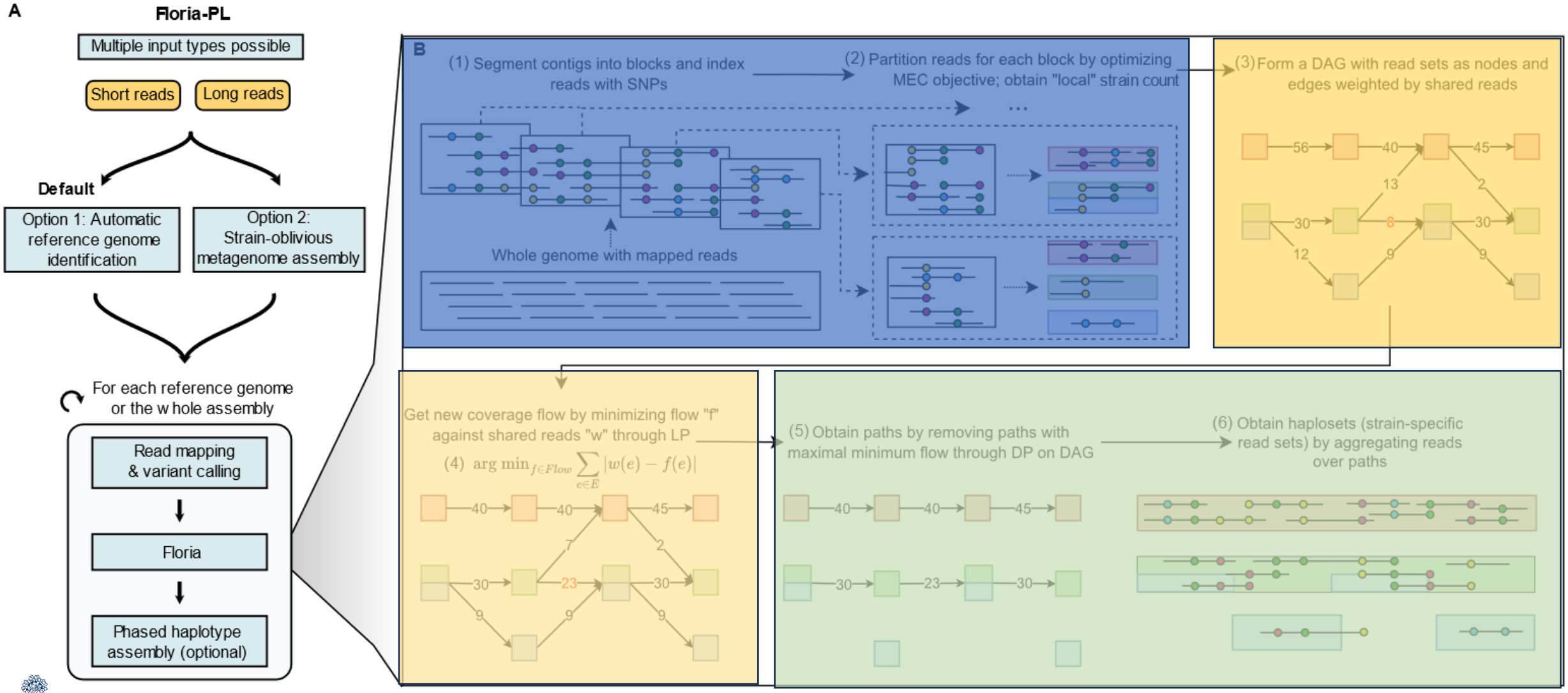
Floria: read clustering by optimization + network flows



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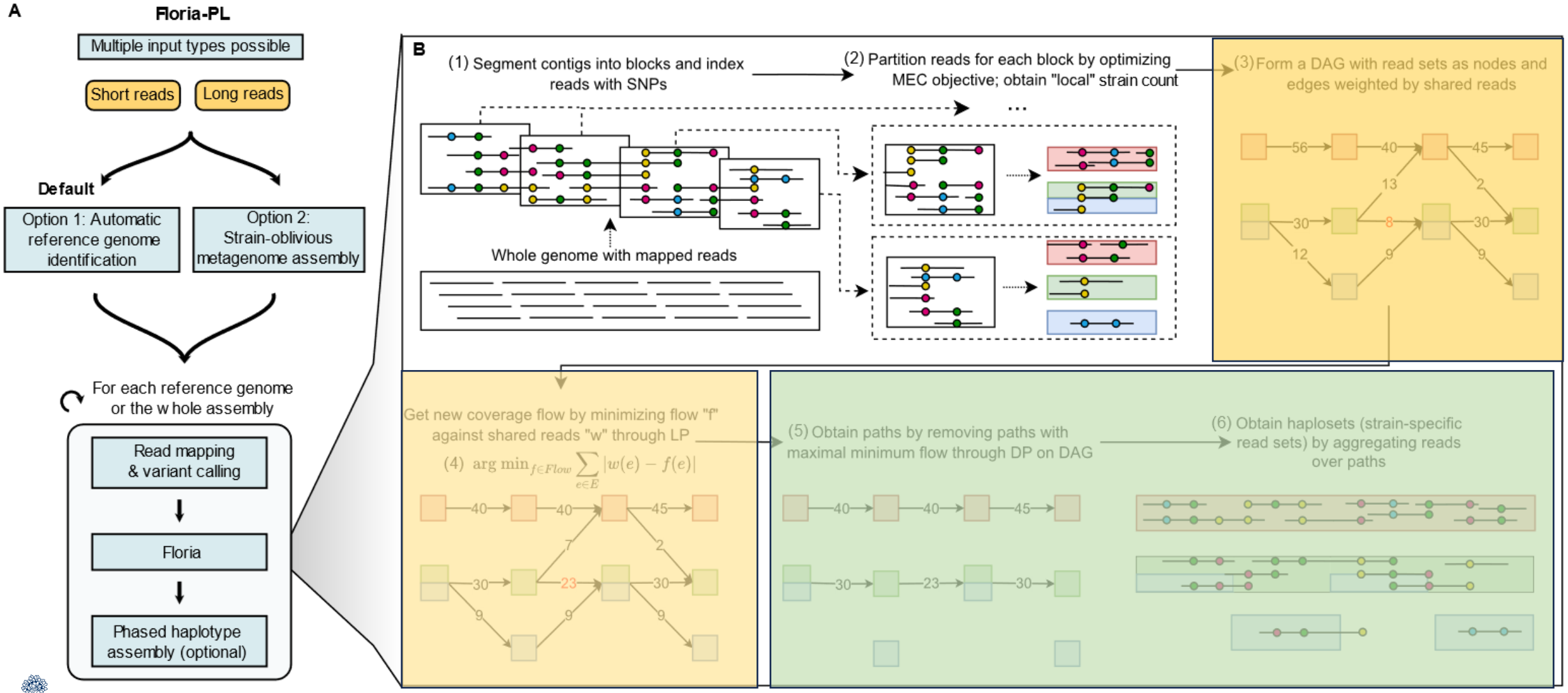
Step 1: local clustering



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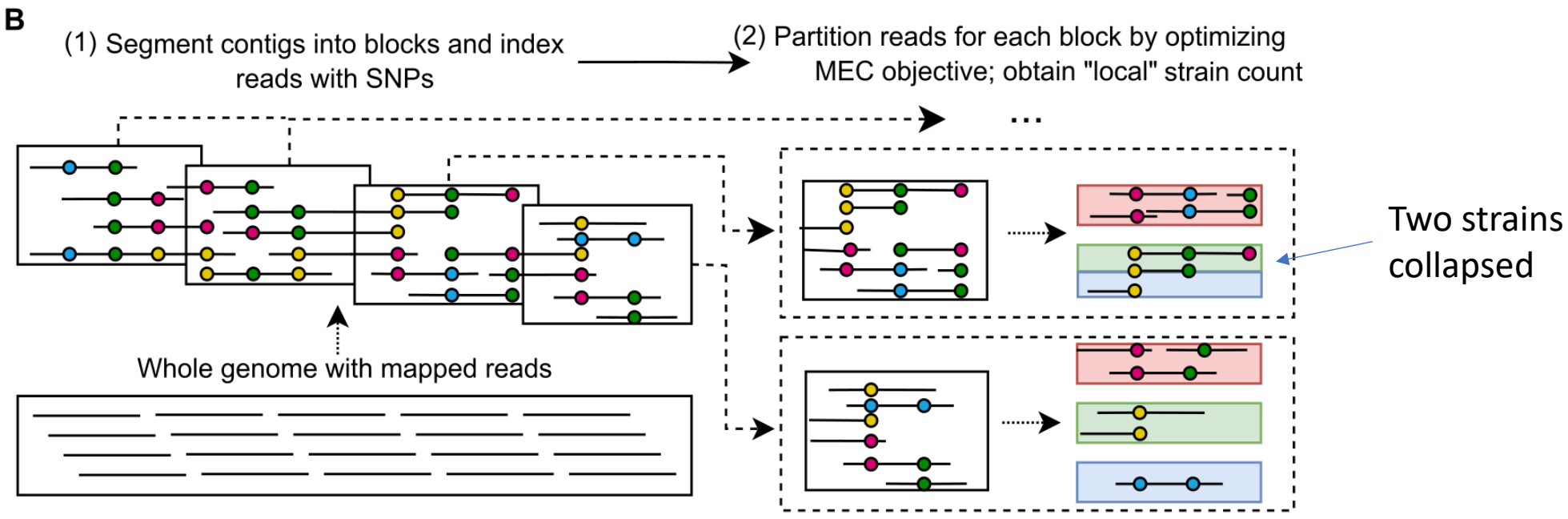

1. Clustering objective: **minimum error correction** (**MEC**) score
   - NP-Hard (Lancia et al., 2001)
   - **Floria:** beam search heuristic
     - often used in Natural Language Processing (NLP)
2. **# of strains?** → iteratively cluster until MEC score plateaus

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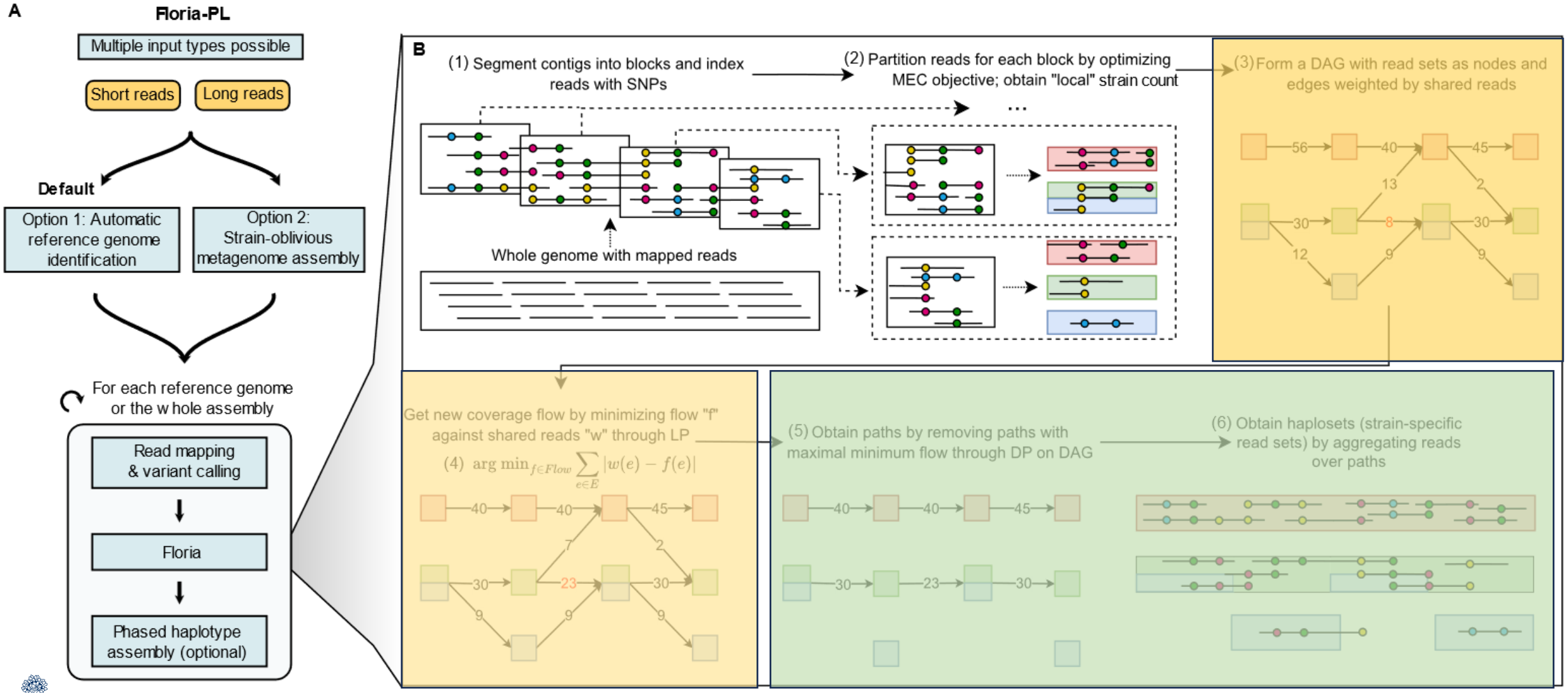
Step 1: local clustering



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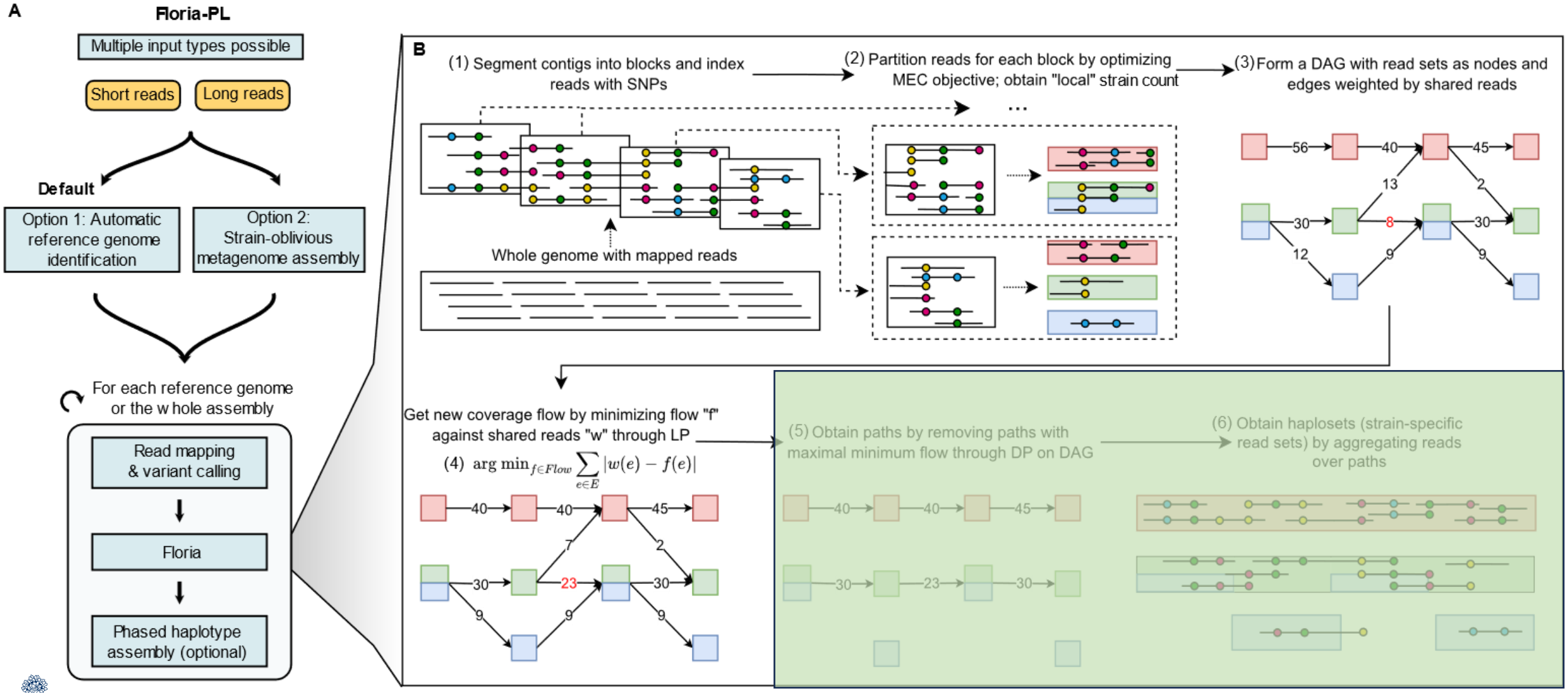
Step 2: **network flows**



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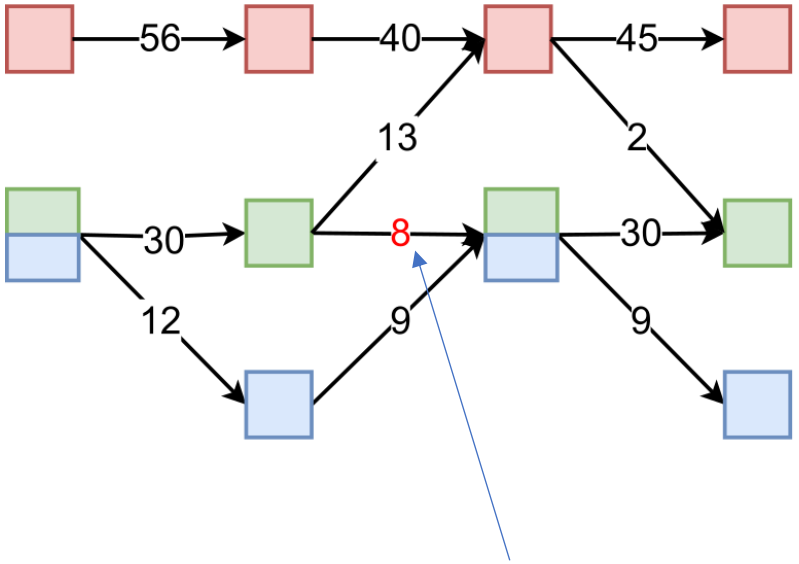
(DAG = Directed acyclic graph)

(3) Form a DAG with read sets as nodes and edges weighted by shared reads



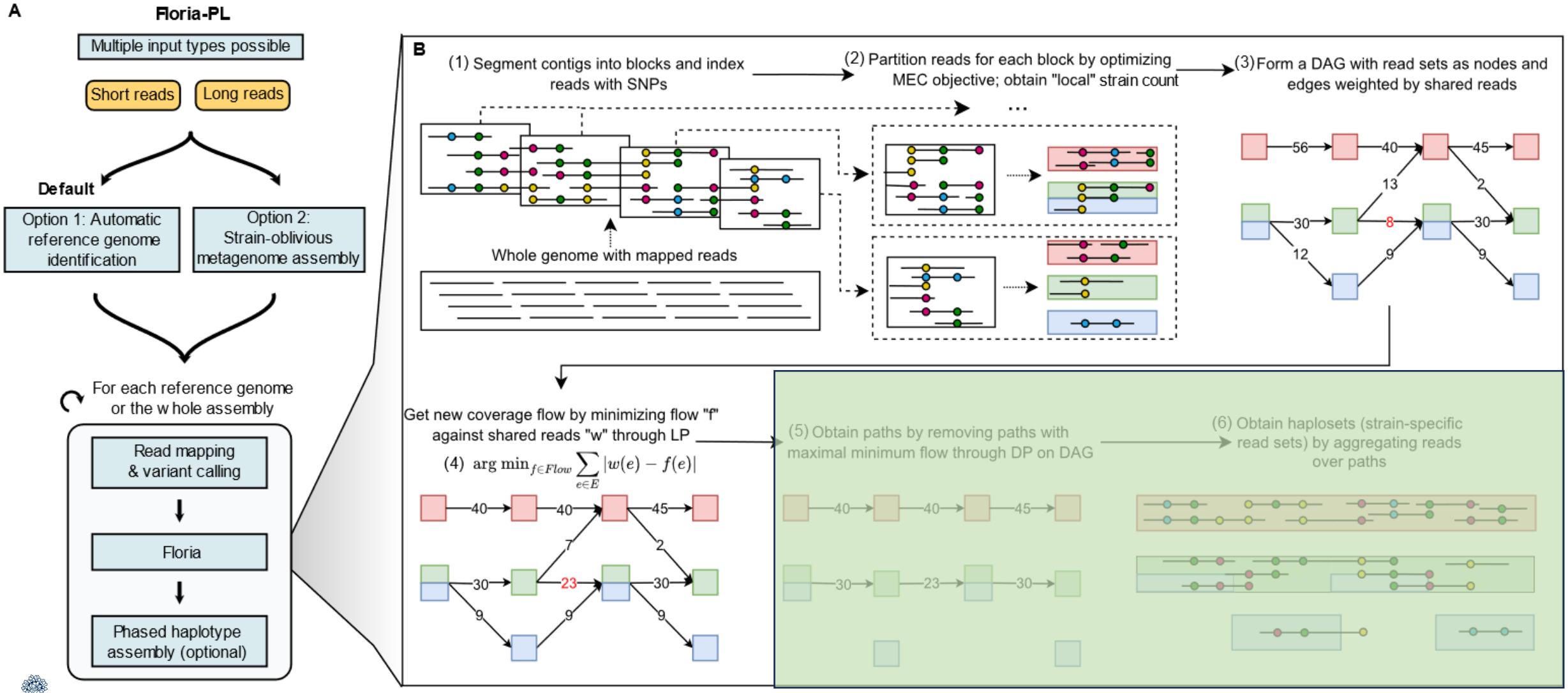
Error!
bad edge

By *linear programming:*

$$\arg\min_{f \in Flow} \sum_{e \in E} |w(e) - f(e)|$$

f – network flow
w – original weights

Step 2: **network flows**



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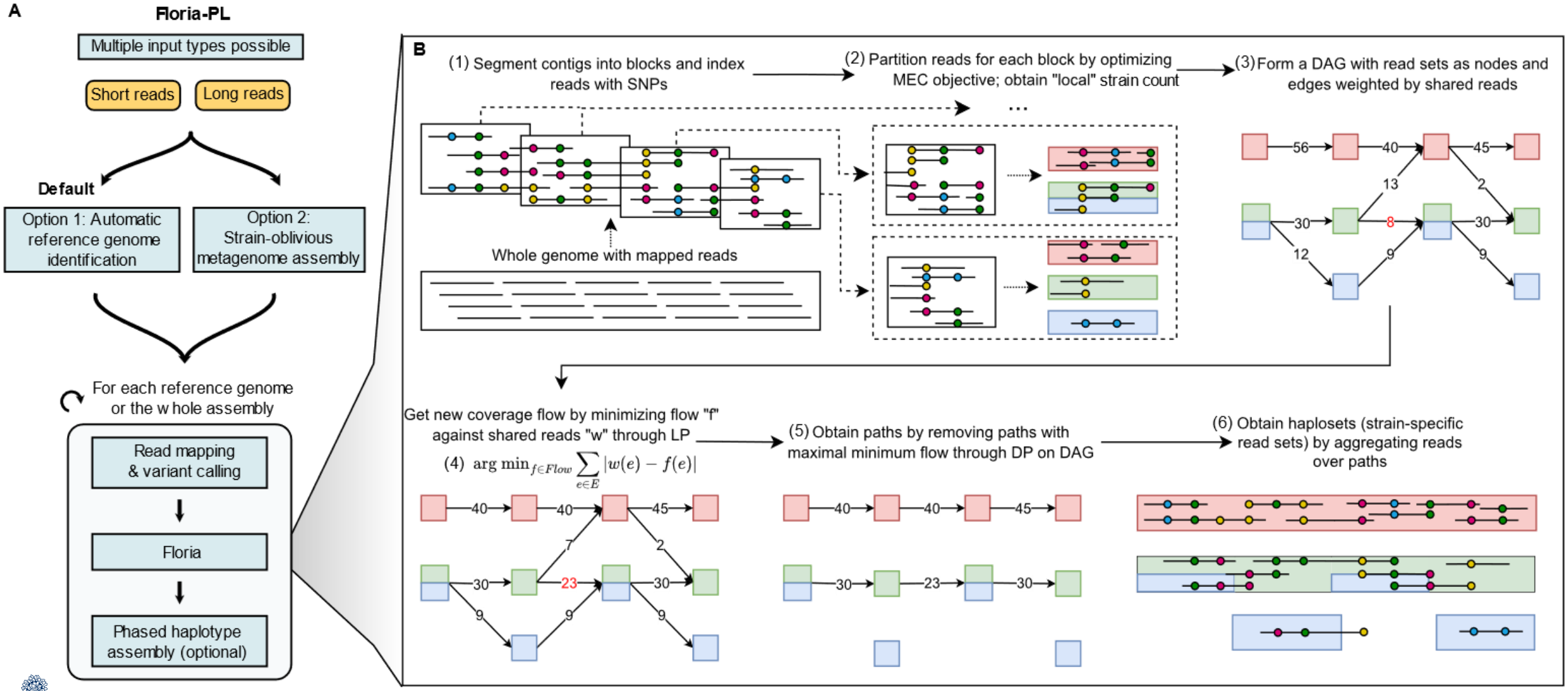
Step 3: Obtaining haploset paths



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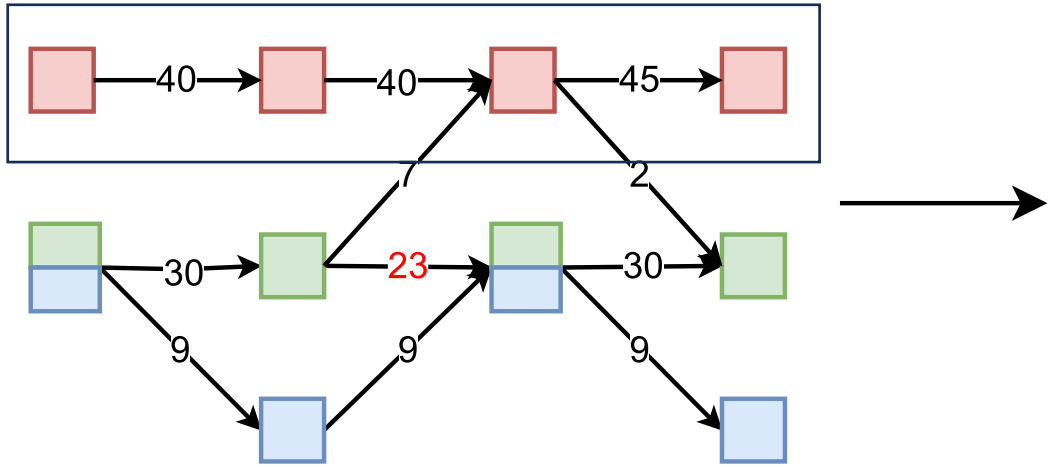
Largest *minimum* flow path
(via dynamic programming - DP)



Largest *minimum* flow path
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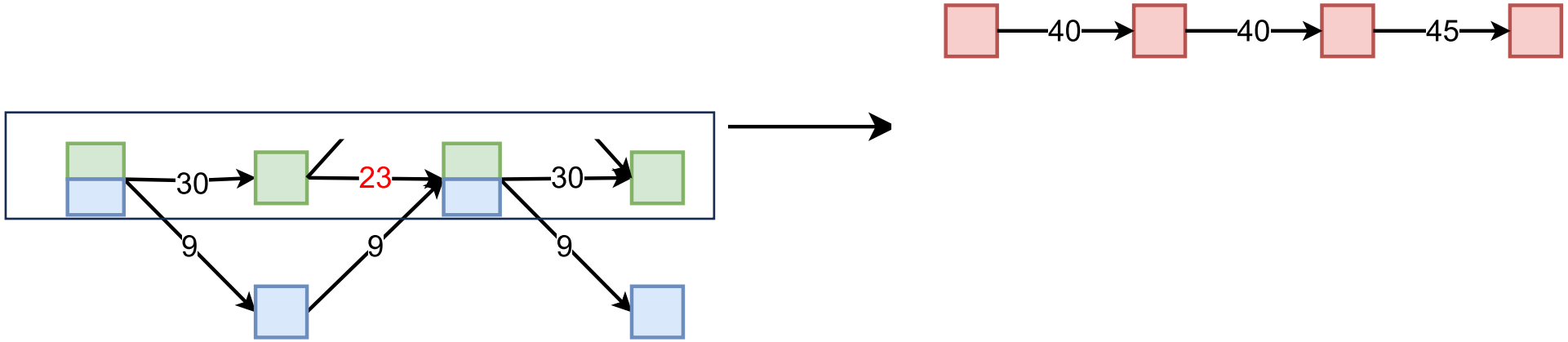
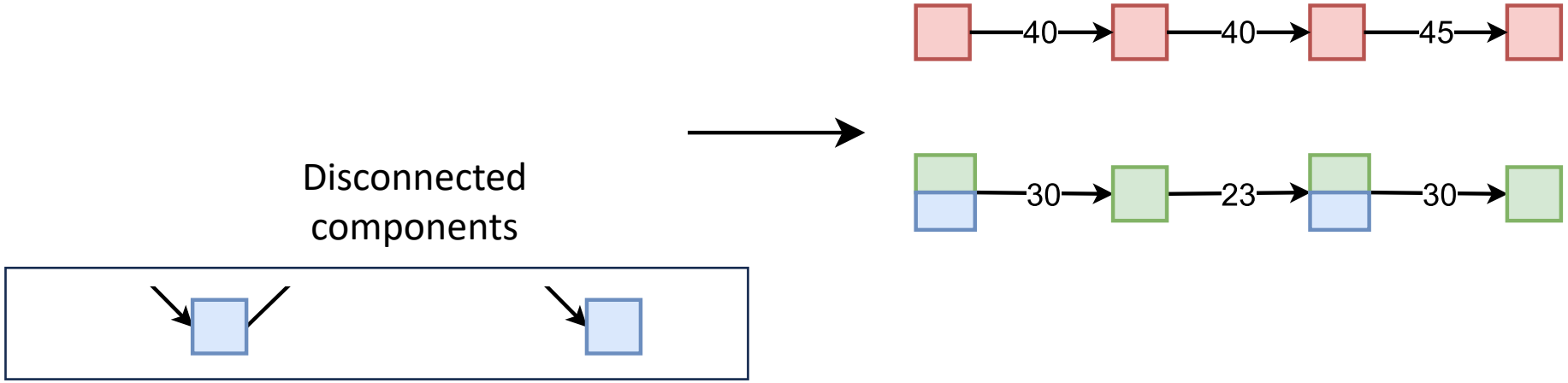
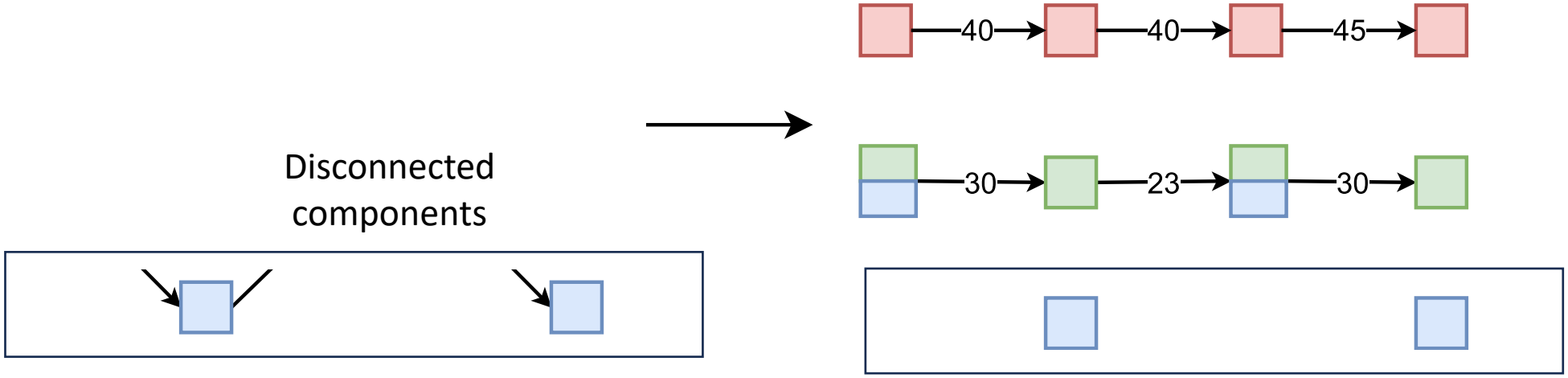
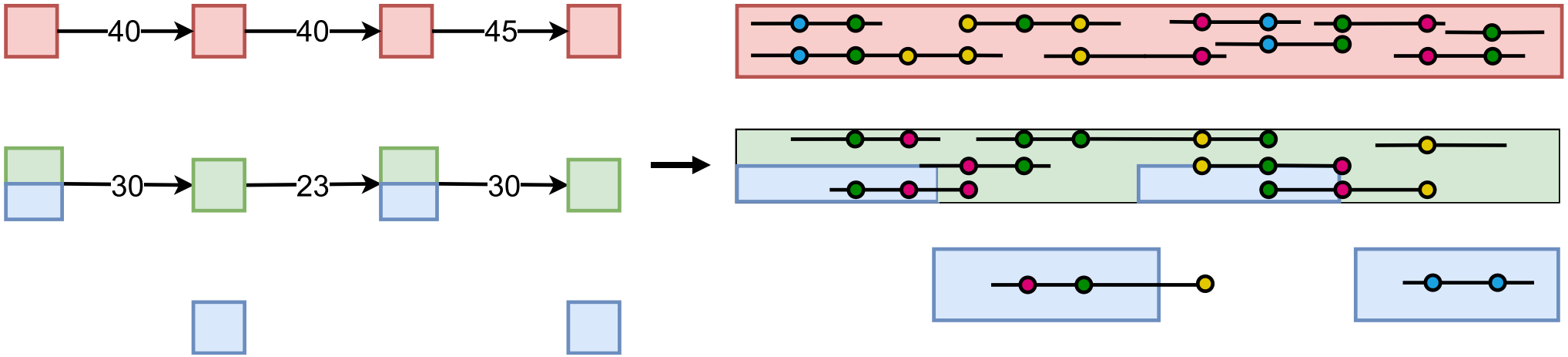


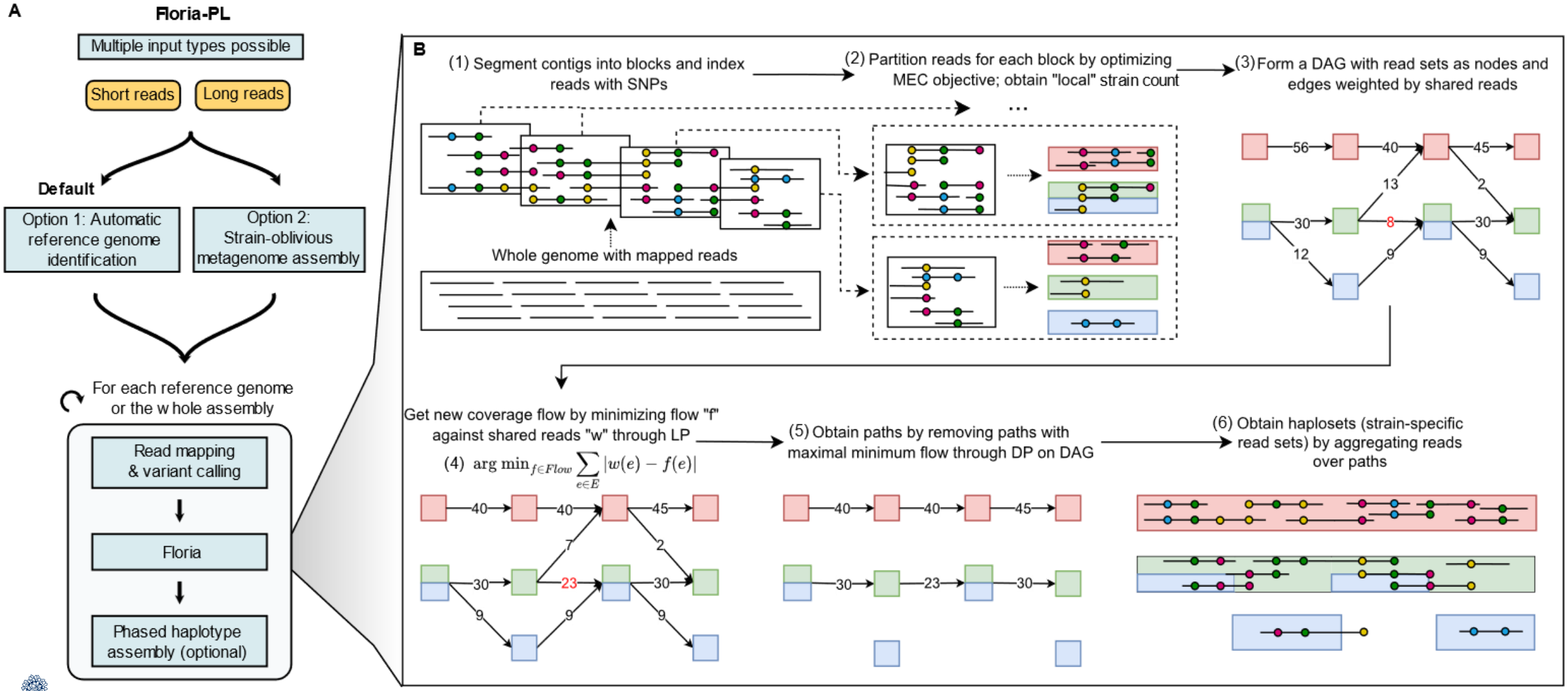

Disconnected
components
40
40
45
30
23
30


Disconnected
components
40
40
45
30
23
30

(6) Obtain haplosets (strain-specific read sets)
by aggregating reads over paths



Step 3: trim flow graph to obtain haplosets



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Benchmarking results

Benchmarking: simulated metagenome

Synthetic metagenomes:

- 40 common gut species
- 1-5 strain genomes per species
- Synthetic noisy nanopore reads **(88% identity)**
- Random strain coverages between [5,25]

Comparison against:

1. metaFlye – metagenome assembler (Kolmogorov et al. 2020)
2. Strainberry – strain assembler (Vicedomini et al. 2021)

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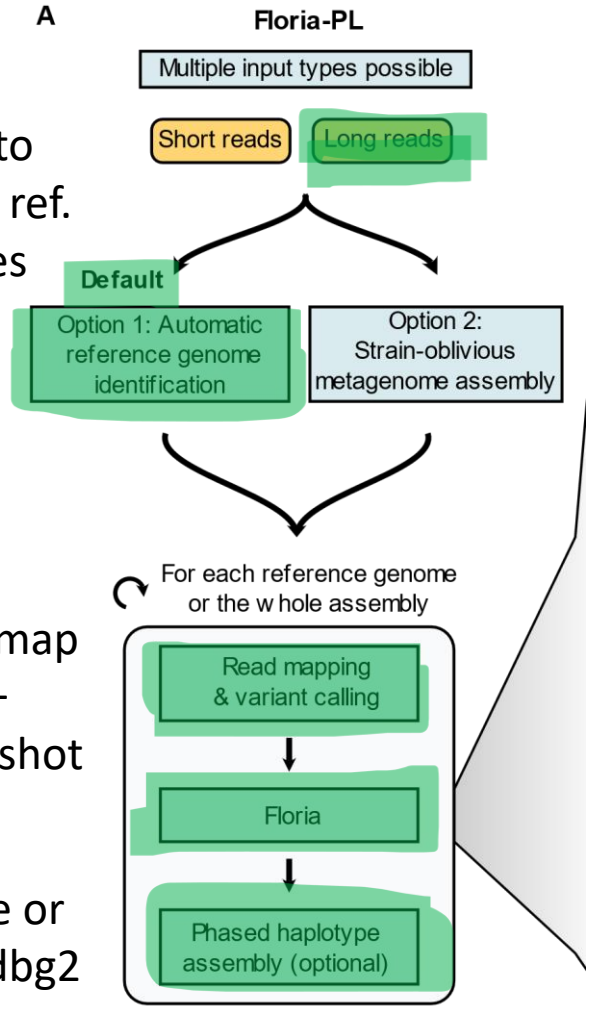
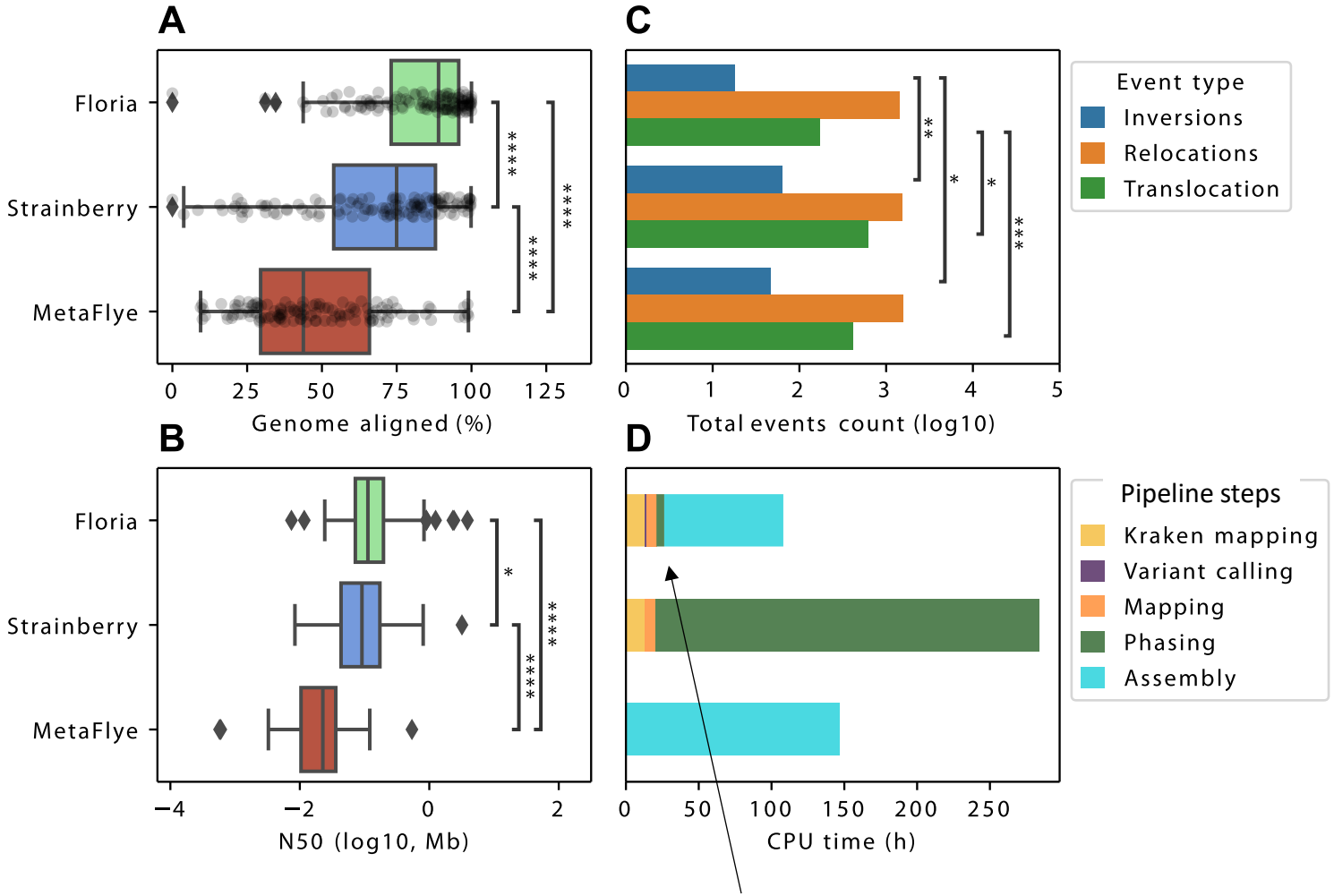
Assembly benchmarking

Kraken to identify ref. genomes

Minimap + Longshot

Flye or wtdbg2





Floria **phasing** time << **assembly** time!

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Results: **real** metagenomes

Long read **AND** short read

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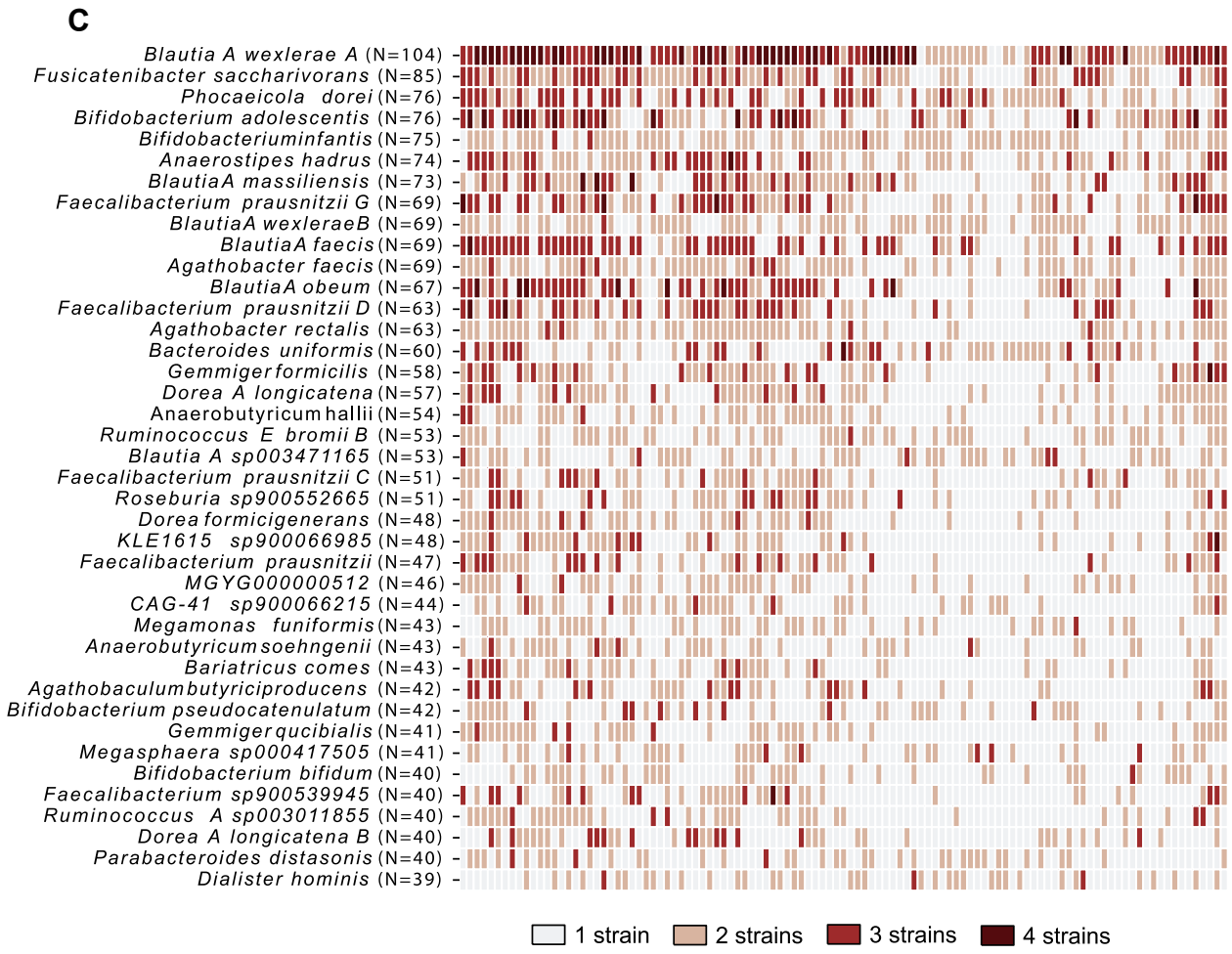
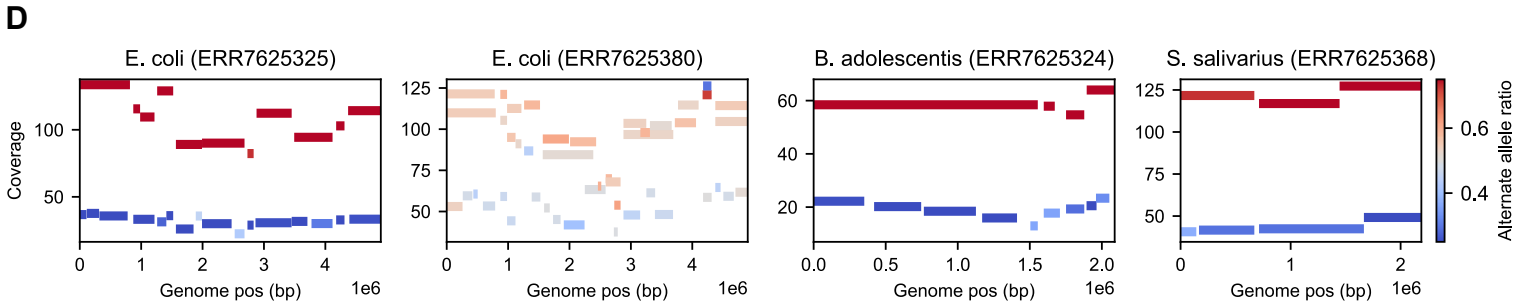
Floria on 109 gut nanopore samples!

(dataset: Gounot et al. 2022, Nat. Comms.)

< 15 mins per sample for phasing!





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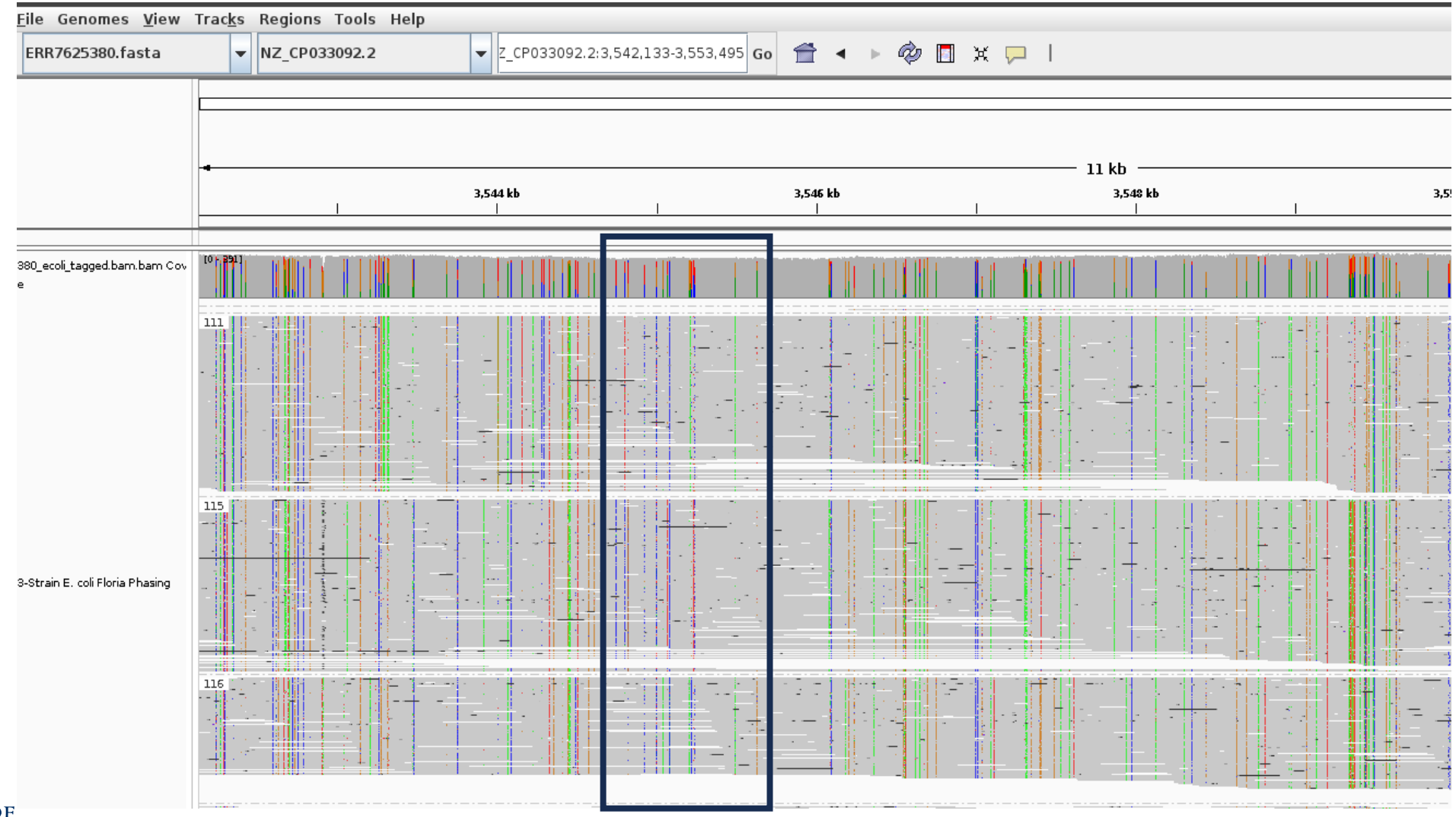
Floria allows for **visualization** – 3-strain *E. coli*



Consistently different alleles

Longitudinal strain tracking with floria

- 24 longitudinal **SHORT-READ gut samples (636 days)**
  - Dataset from *"**Metabolic independence drives gut microbial colonization and resilience in health and disease**"* by Watson et al. (Genome Biology 2023)
- Run floria → obtain haplosets → track across time

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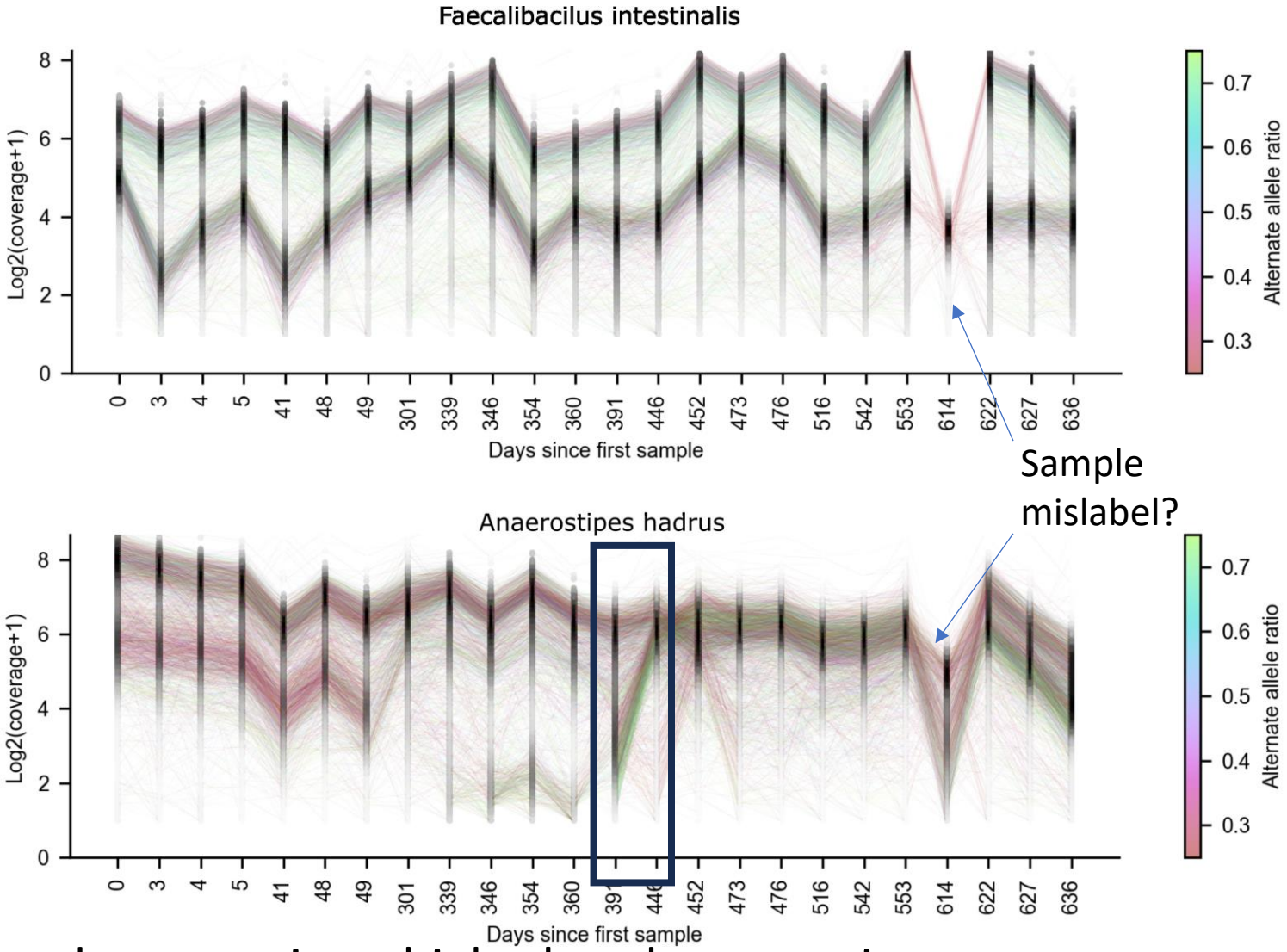
Line: haploset match across sample

Two species with interesting patterns:

F. intestinalis - ***stable***

A. hadrus – ***transient***



Low-abundance strain to high-abundance strain emergence? **Visualize!**

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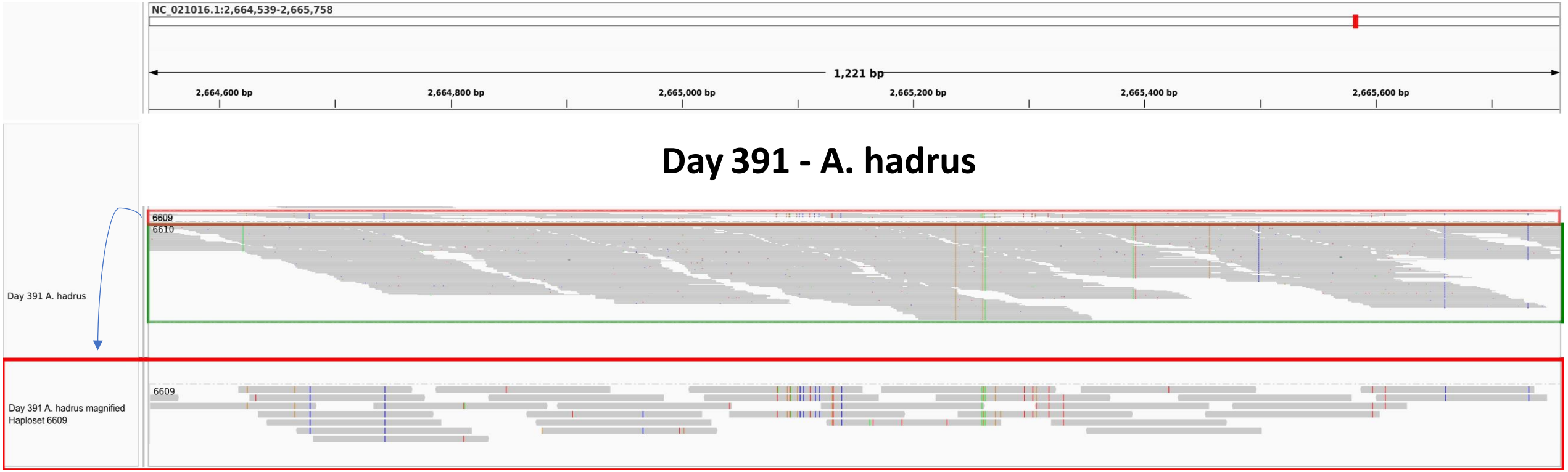

Day 391 - A. hadrus

Zoomed in on red haploset ("6609"):

LOW coverage

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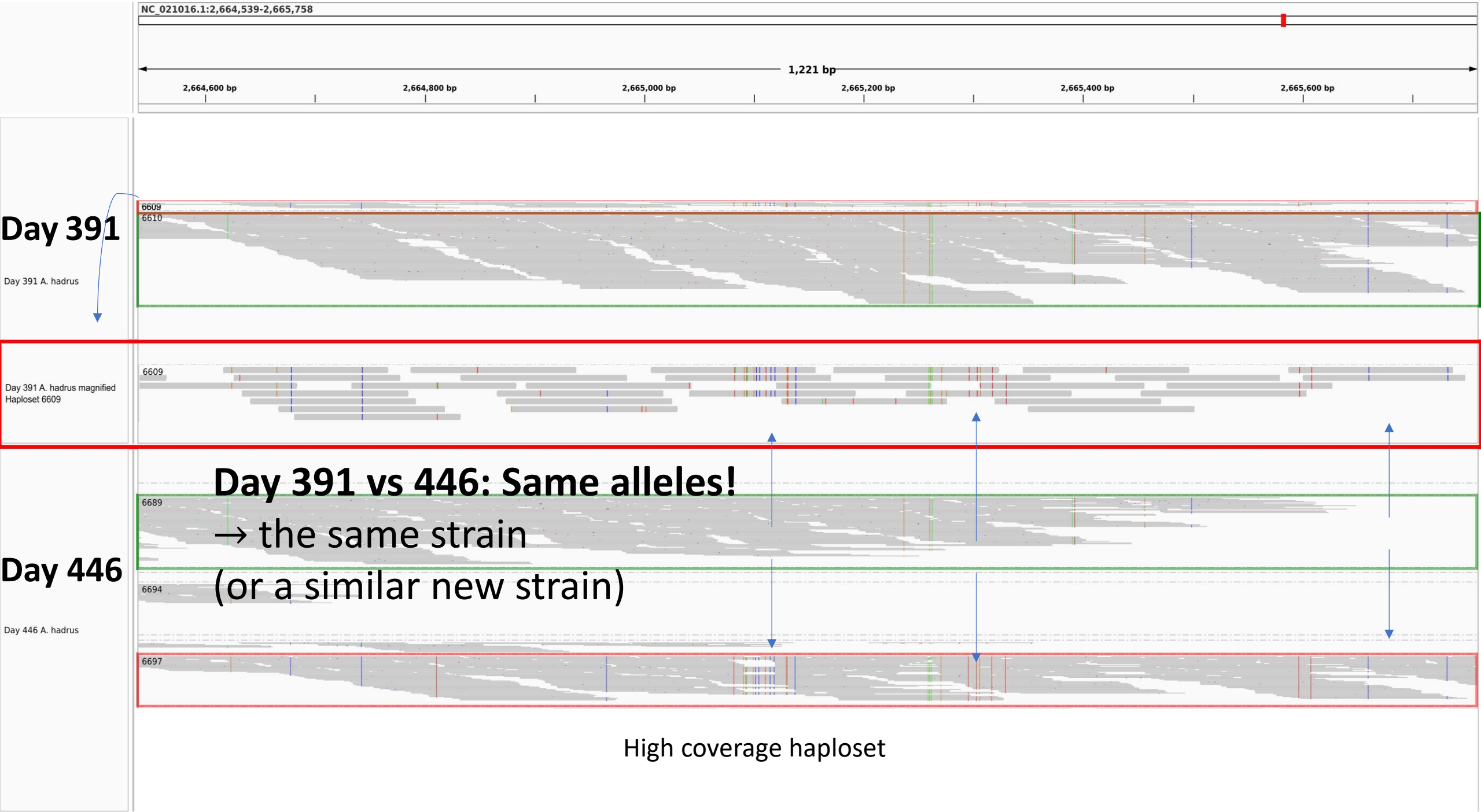
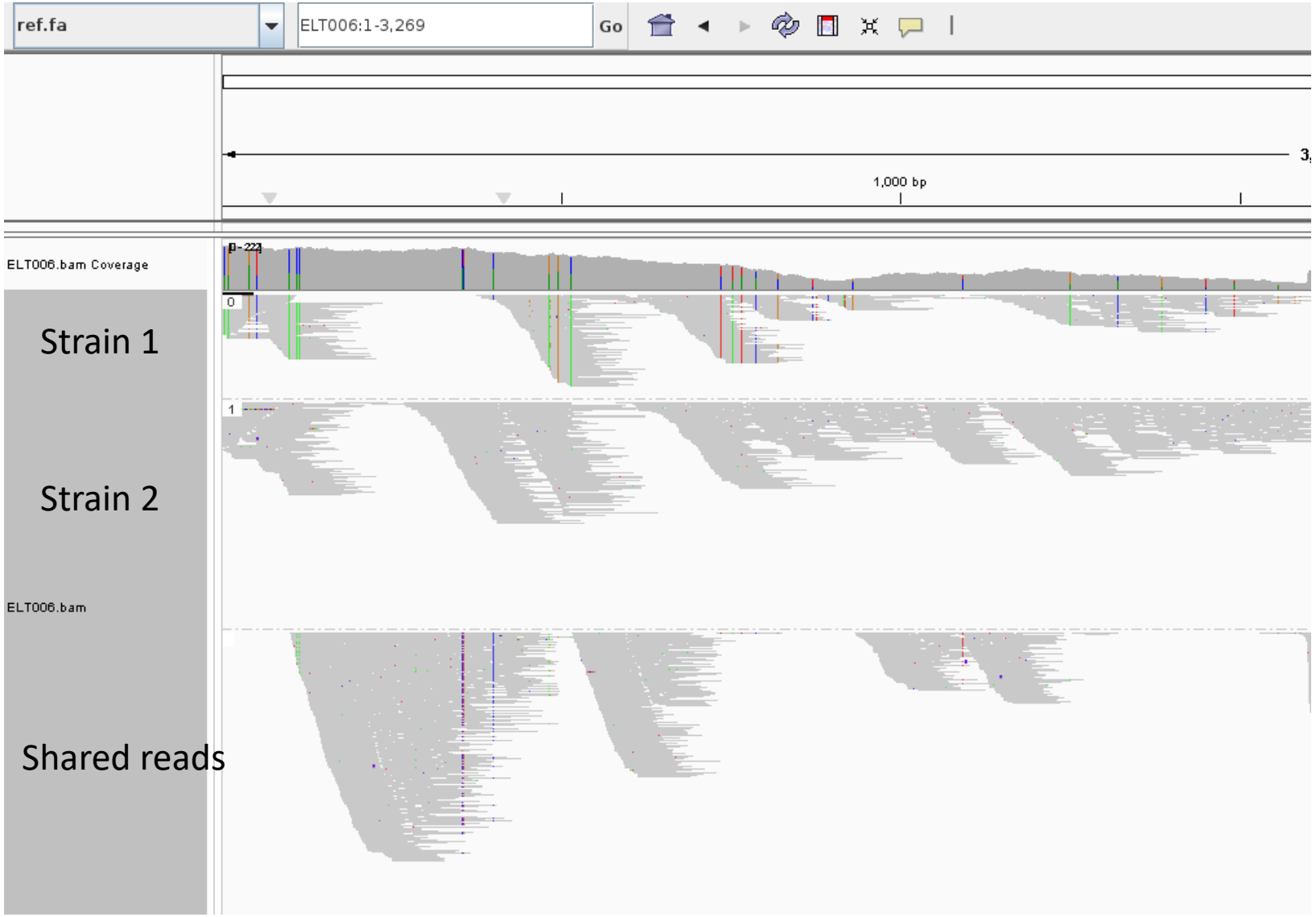

NC_021016.1:2,664,539-2,665,758
1,221 bp
2,664,600 bp
2,664,800 bp
2,665,000 bp
2,665,200 bp
2,665,400 bp
2,665,600 bp
Day 391
Day 391 A. hadrus
6609
6610
Day 391 A. hadrus magnified
Haploset 6609
6609
Day 391 vs 446: Same alleles!
→ the same strain
(or a similar new strain)
Day 446
Day 446 A. hadrus
6689
6694
6697
High coverage haploset

Floria works on **ancient viral metagenomes!?**

From Maxime Borry (Postdoc at Max Planck for Evo Anthro):

Ancient mixed infection of hepatitis B (Kocher et al., 2021, Science)



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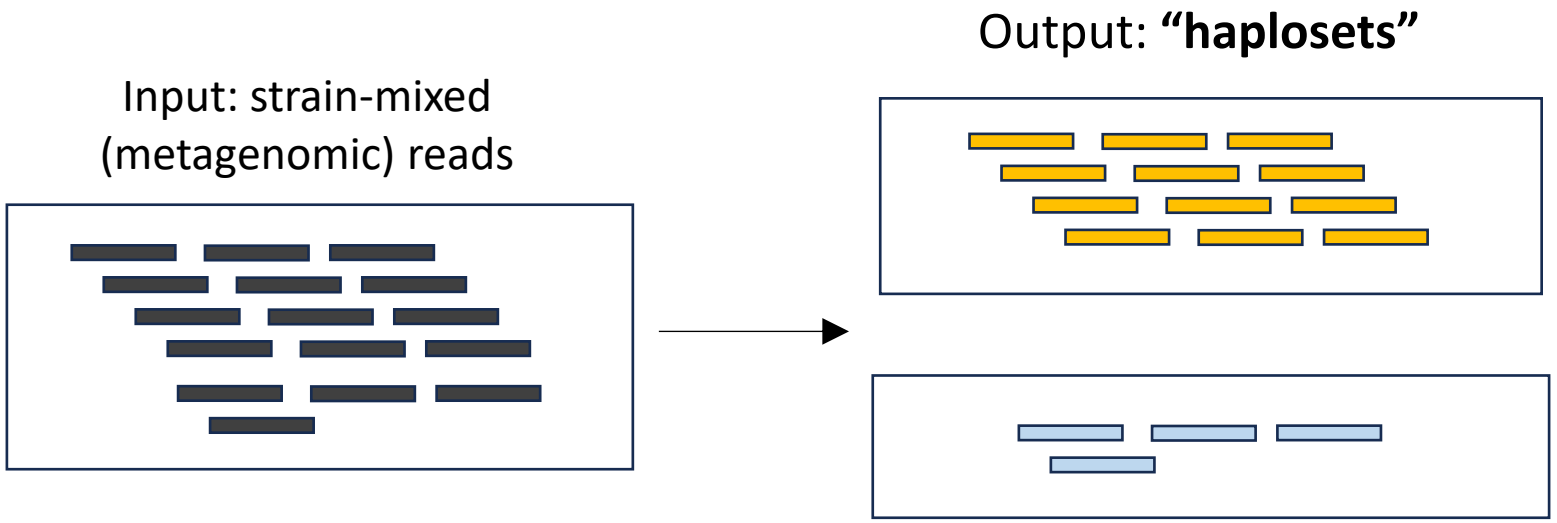
Conclusion



- Developed **floria**: a strain-level read clustering (phasing) algorithm
  - Short OR long reads
  - < 20 min per metagenome
  - assembly optional
- Fast, informative, and versatile
  - can do strain-level metagenomic assembly…
  - but even more useful for **hypothesis generation and data sleuthing**

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  - Research fellow at Genome Institute of Singapore
  - **co-lead author**

- Hanrong Chen
  - Postdoc at Genome Institute of Singapore

- Niranjan Nagarajan
  - Genome Institute of Singapore**, co-lead PI**

- Yun William Yu
  - Carnegie Mellon University, **co-lead PI**

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Floria: fast and accurate strain haplotyping in metagenomes

Jim Shaw, Jean-Sebastien Gounot, Hanrong Chen, Niranjan Nagarajan ✉, Yun William Yu ✉ Author Notes

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