Safety in multi-assembly via paths appearing in all path covers of a DAG

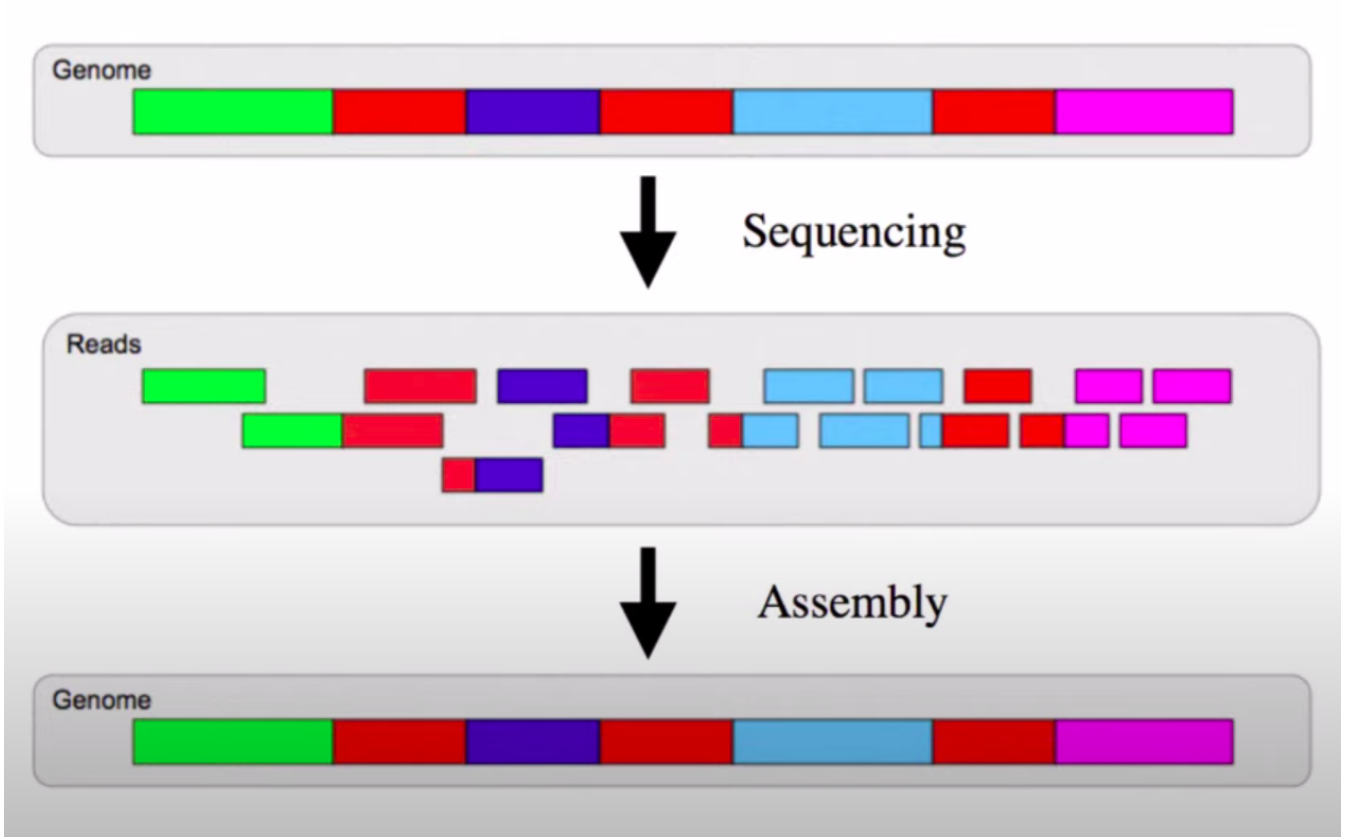
Manuel Cáceres, Brendan Mumey, Edin Husić, Romeo Rizzi, Massimo Cairo, Kristoffer Sahlin, Alexandru I. Tomescu

11.02.2021, DSB

Many problems in bioinformatics can be seen as puzzles

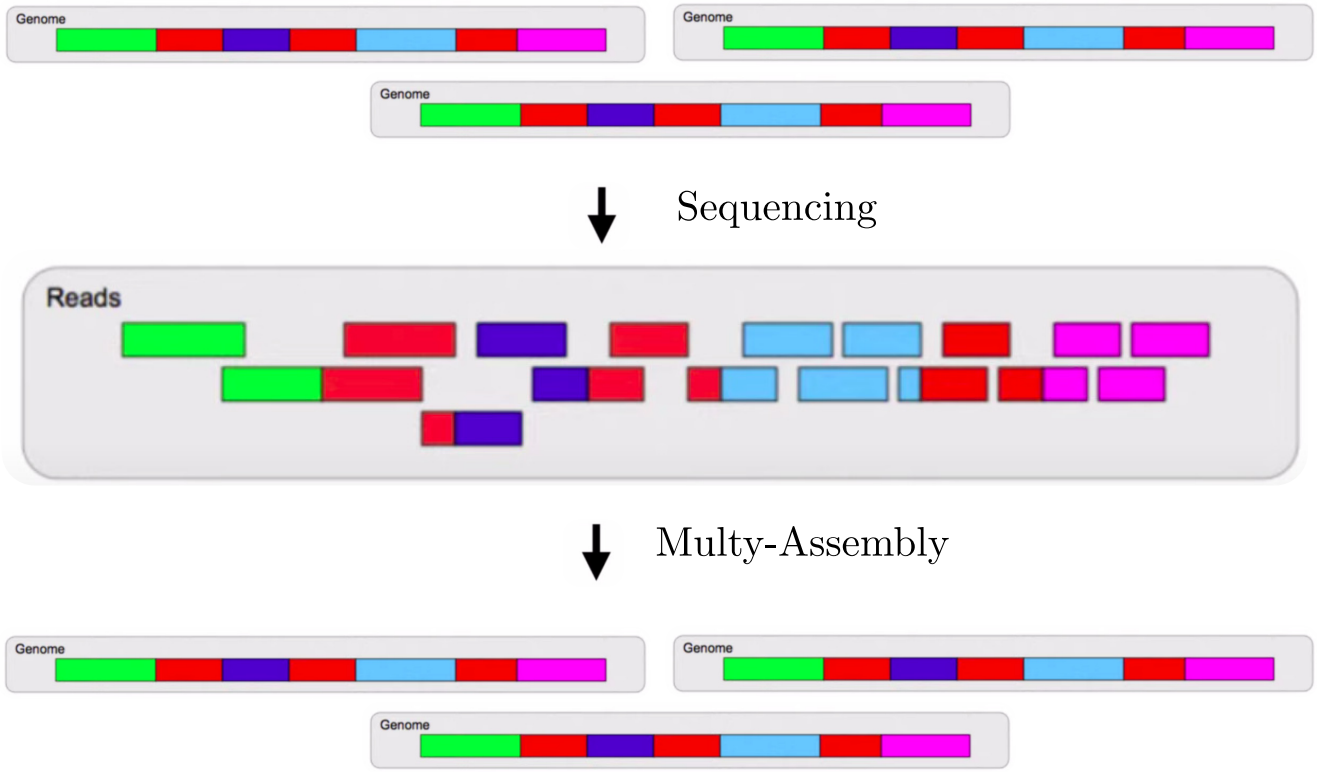
Genome Assembly

Reconstruct a genomic sequence based on *reads* obtained from it.

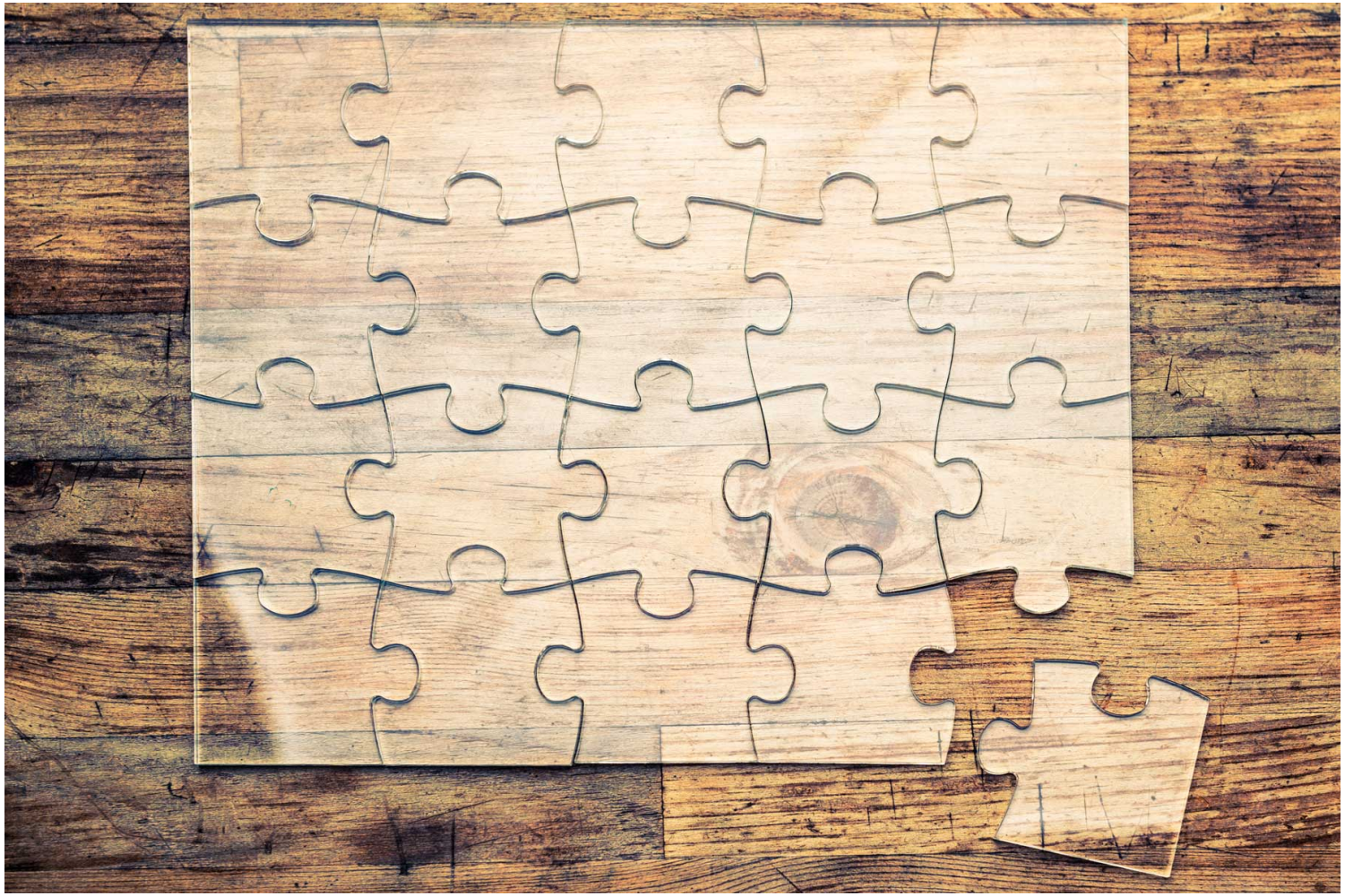


Multi-Assembly

Reconstruct multiple sequences based on *mixed-reads* obtained from all of them.

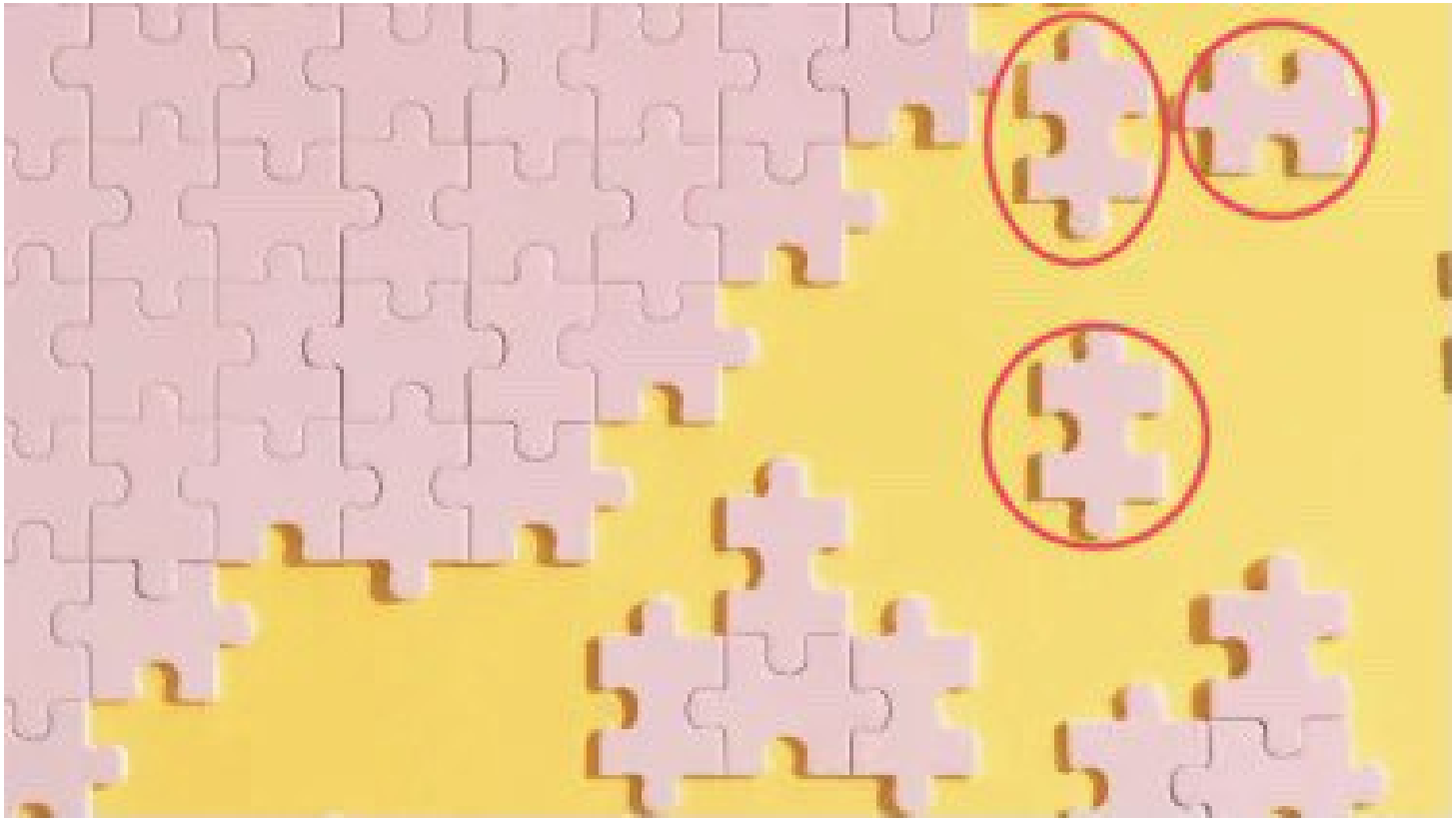


Puzzles solved perfectly

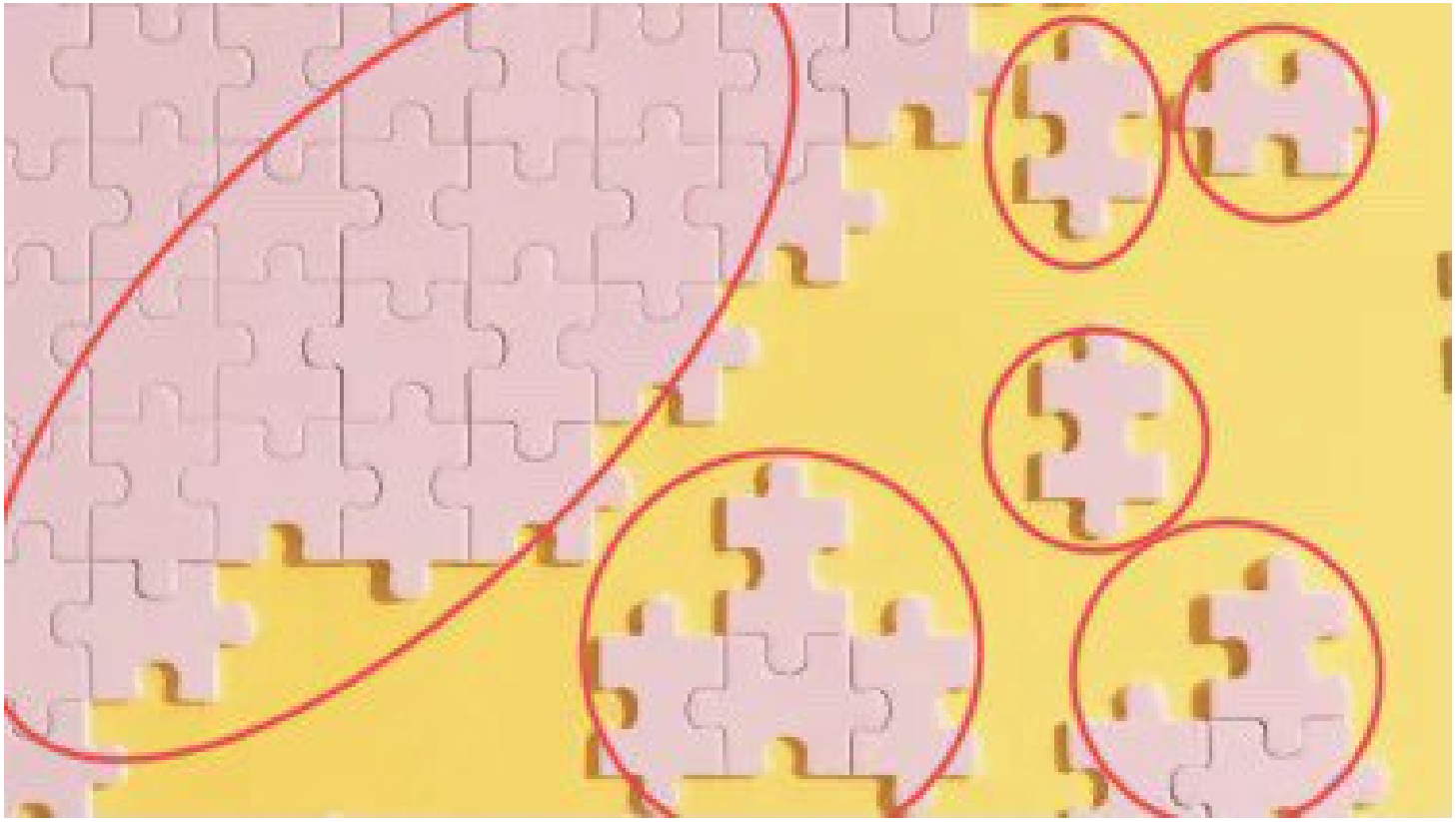


Unsolvable puzzles (multiple solutions)

Puzzles with multiple solutions



Safe parts common to *all* possible solutions



Contigs in Genome Assembly

Genomic fragments *promised* to occur in the original genome.

- Used and reported by practical assemblers [10, 11, 17, 20].
- Developed theoretically [24, 6, 7, 5].
  - *Completeness*

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Not used in multi-assembly!

Path cover in a DAG

$$G = (V, E)$$

Model for Multi-assembly

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- Set of paths $\mathcal{P}$ such that every vertex of the graph appears in some path.
- Directed acyclic graph.
- E.g.:
  - RNA transcript assembly [25, 21, 3, 13, 9, 16, 19, 4, 12, 23, 22, 14].
  - Viral quasi-species assembly [8, 27, 2, 1]

Two types of path covers

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- Minimum path cover (MPC).
  - Of size k, known as the width.
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  - Of size k, known as the width.
  - One can be computed in time $O(|V||E|)$ [18].
- Generalized path cover.
  - Of size $\leq \ell$ (parameter).
  - Paths starting at $S \subseteq V$ (parameter).
  - Paths ending at $T \subseteq V$ (parameter).

Our results

Theoretical results

Theoretical results

	Minimum path covers	Generalized path covers
Safe edges	$O(k\|V\|\|E\|)$	$O(k\|V\|\|E\|)$
Maximal safe paths	$O(k^2\|V\|\|E\|)$	$O(max(1, 2k - \ell)k\|V\|\|E\|)$

Recall k is the width of the graph (size of an MPC)

Practical results

Apply to RNA transcript assembly defining *RNA contigs*.

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Proof-of-concept study

- Splicing graphs from human annotated transcripts.
- Double length compared to *unitigs*.
- Transcript coverage of 80%.
- Less than 15 seconds (all transcript annotation from Ensembl database [26]).

Theoretical results

(MPC only)

General Approach (Avoid-and-test)

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2. For each subpart $\rightarrow$ Every subpath P of a path of $\mathcal{P}$.
   - Test if P is safe $\rightarrow$ Compute the width of G^P.

Safe edges

Theorem (Safe edges MPC)

An edge e is safe if and only if $\mathrm{width}(G) < \mathrm{width}(G \setminus e)$.

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- But, we can do better.

Shrinking Primitive

Lemma (Shrinking [15])

Given a path cover $\mathcal{P} = P_1, \ldots, P_t$ of G, we can obtain a MPC of G in time $O(|E|(t - k + 1))$

Theorem (Safe edges MPC)

An edge e is safe if and only if $\mathrm{width}(G) < \mathrm{width}(G \setminus e)$.

- Naive algorithm: $O(k|V|^2|E|)$.
- But, we can do better.
  - $O(\sum_{e \in \mathcal{P}} \mu_e |E|) = O(k|V||E|)$

Maximal safe paths

Path Reduction

Definition

Given a path $P = x_1, \ldots, x_p$ of G, we define $G^P = (V, E^P)$, where

$$E^P = (E \setminus \{(x_{p-1}, x_p)\}) \cup \bigcup_{i=2}^{p} \left\{(u, x_p) \mid u \in N^-(x_i) \setminus \{x_{i-1}\}\right\}.$$

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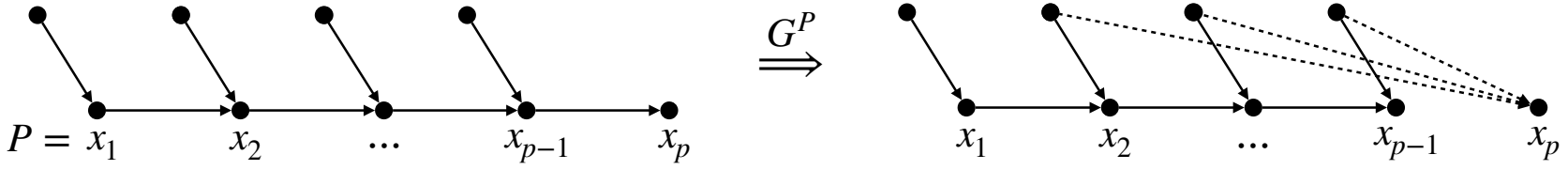

Theorem (Maximal safe paths MPC)

Let $P = x_1, \ldots, x_p$ *be a path of* G*, such that* $x_1, \ldots, x_{p-1}$ *is a safe path. It holds that* P *is safe if and only if* $\mathrm{width}(G) < \mathrm{width}(G^P)$*.*

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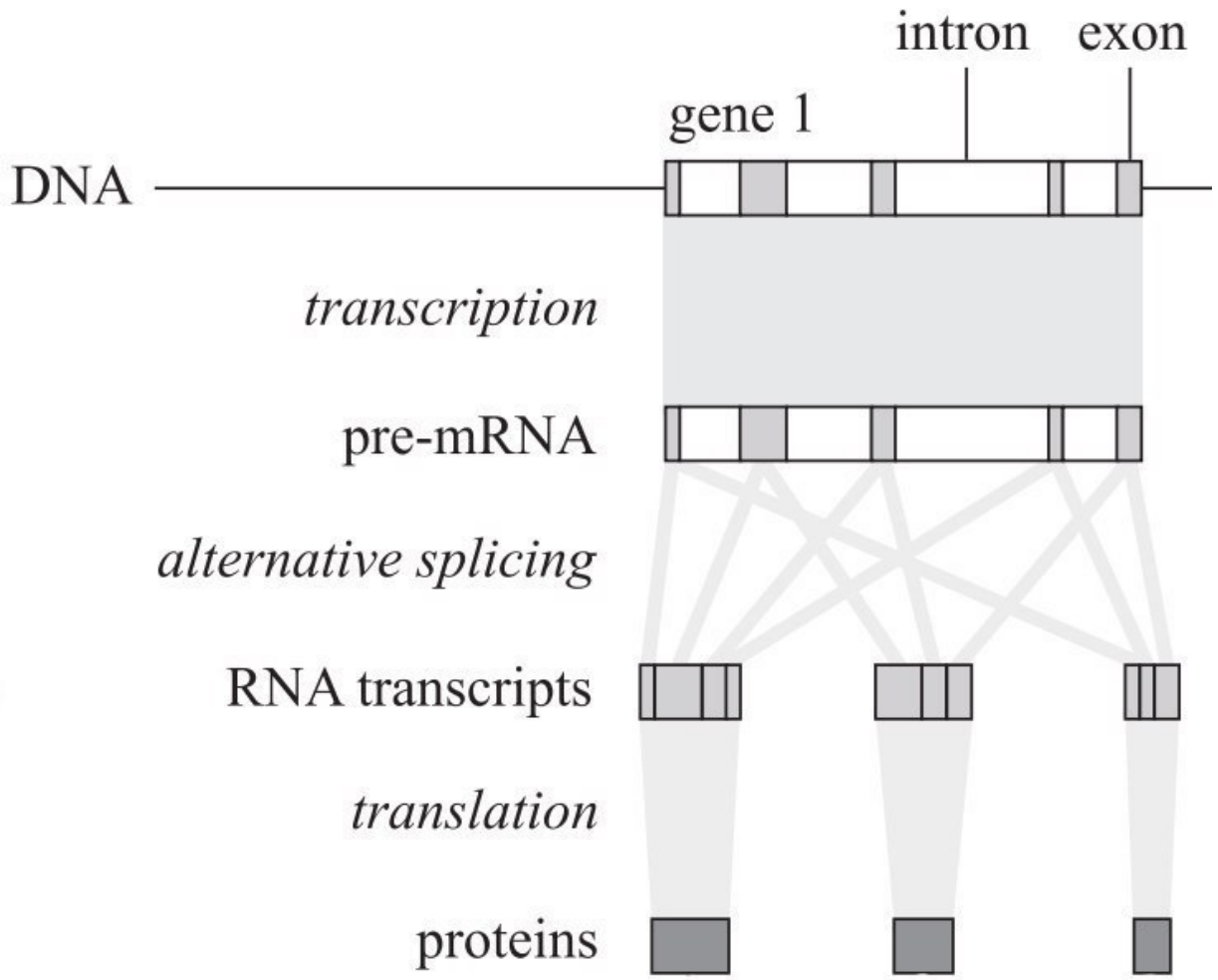
- Paths of increasing size algorithm: $O(k|V|^3|E|)$.
- By using shrinking, and two-finger on each path: $O(k^2|V||E|)$.

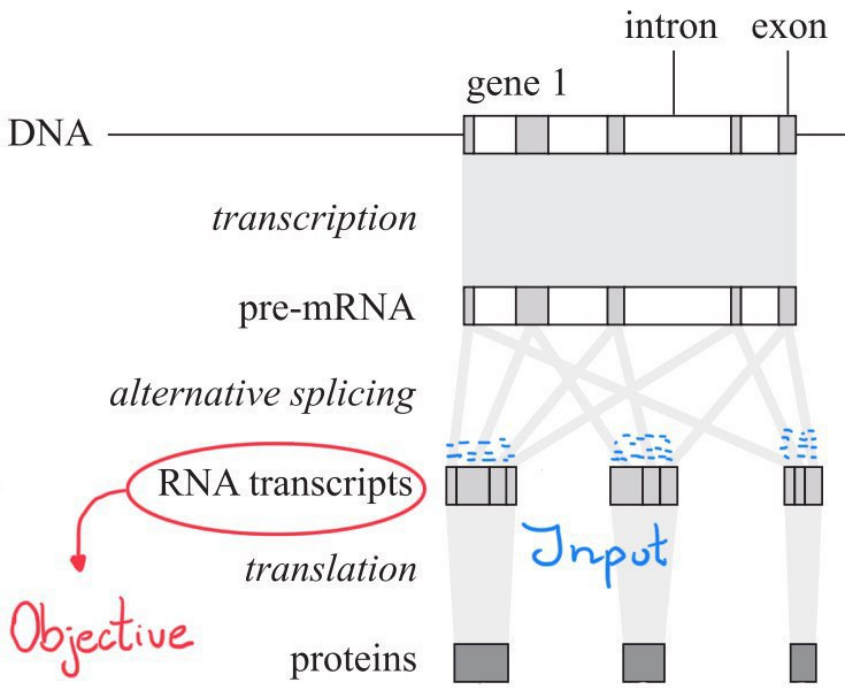
Practical results

(RNA contigs)

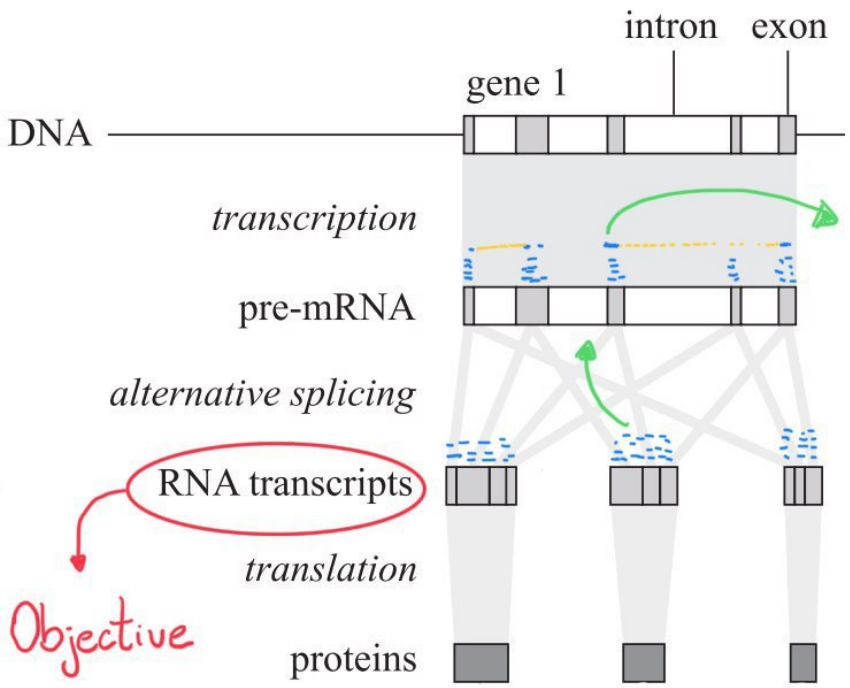
Central dogma of molecular biology



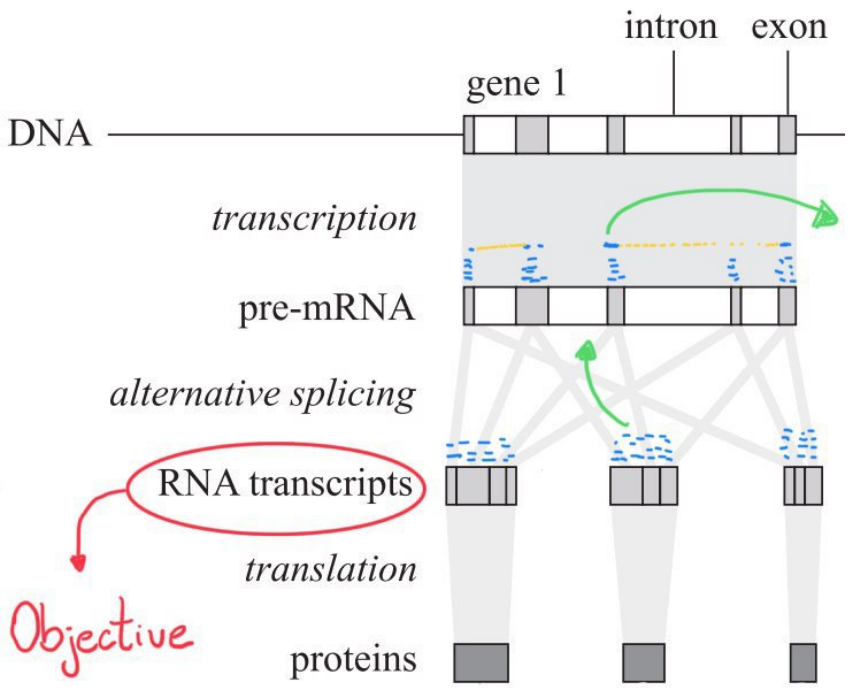
RNA transcript assembly

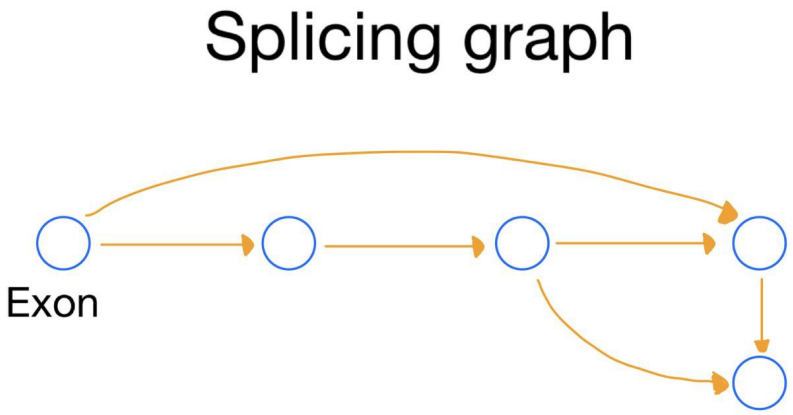


RNA transcript assembly

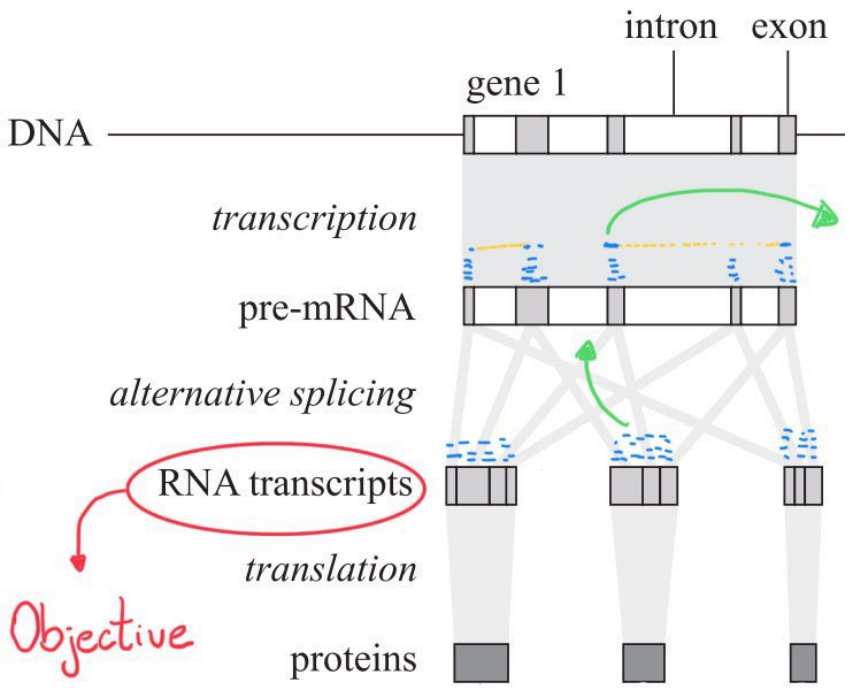


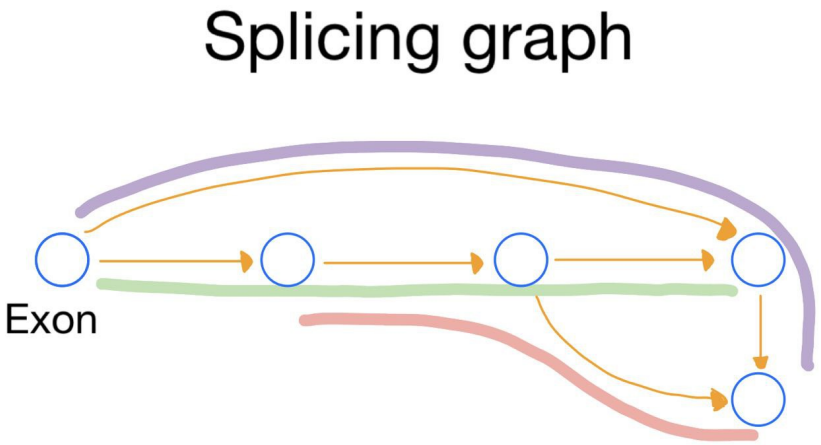
RNA transcript assembly



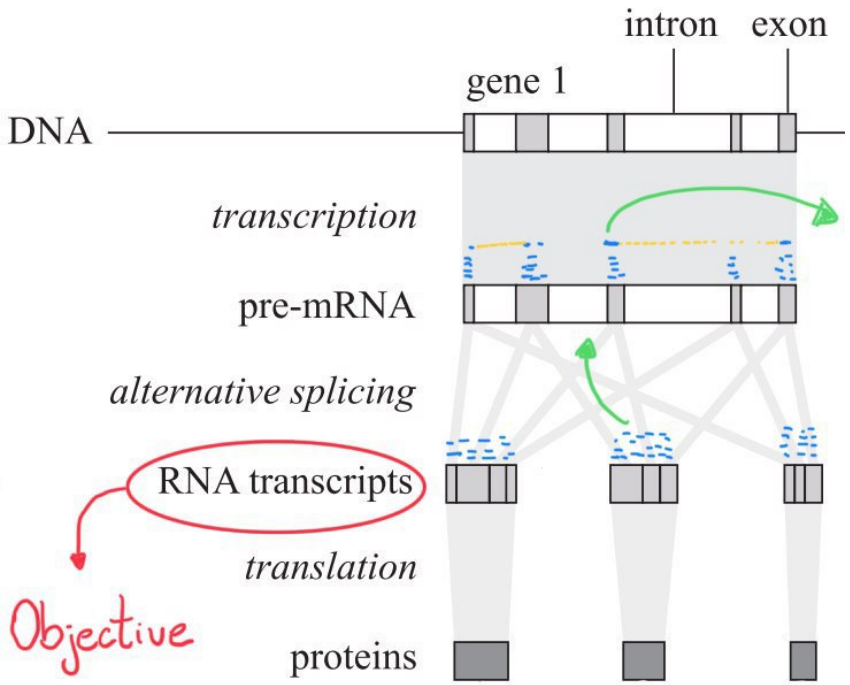


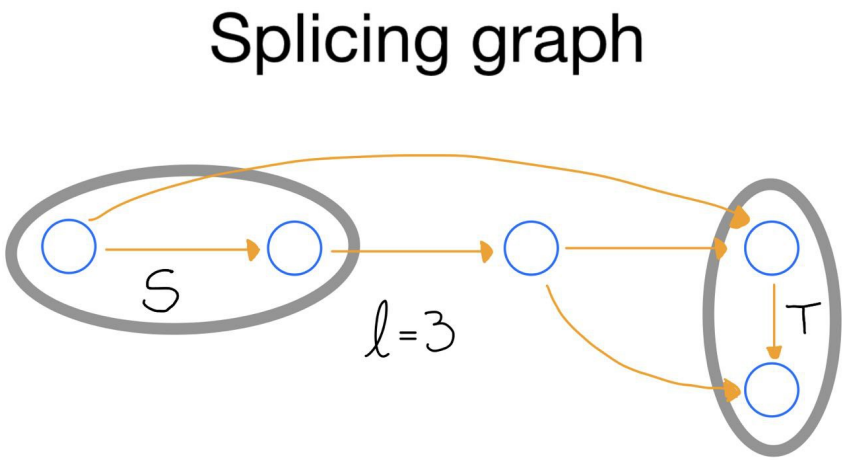
RNA transcript assembly





RNA contigs





RNA contigs = Maximal safe paths for generalized path cover

Experimental setup

- Human gene annotation (Ensembl [26],`GRCh38.p13`).
  - All transcripts in chromosomes 1 to 22, on the forward strand.
- Build splicing graph.
  - Perfect scenario.
  - Known answers.
- $\ell \in \{k, k+1, \ldots, 2k\}$.
- Baseline comparison with ST-unitigs.

Results

(less than 15 seconds to run)

Results

ℓ	small graphs (3-21 vertices)		medium graphs (22-52 vertices)		large graphs (53-725 vertices)	
	prec	mcr	prec	mcr	prec	mcr
k	0.84	0.82	0.81	0.64	0.84	0.56
	1.00	0.86	0.92	0.62	0.89	0.53
$k+1$	1.00	0.79	0.99	0.61	0.99	0.53
	1.00	0.83	1.00	0.59	1.00	0.50
t	1.00	0.79	1.00	0.61	1.00	0.53
	1.00	0.83	1.00	0.59	1.00	0.50
$2k$	1.00	0.79	1.00	0.61	1.00	0.53
	1.00	0.83	1.00	0.59	1.00	0.50
ST-unitigs	1.00	0.64	1.00	0.49	1.00	0.42
	1.00	0.67	1.00	0.47	1.00	0.39

Precision and Relative Maximum Coverage (of transcripts) for RNA contigs and ST-unitigs.

Results

ℓ	small graphs (3-21 vertices)	medium graphs (22-52 vertices)	large graphs (53-725 vertices)
k	2.65×	3.54×	3.50×
$k+1$	1.43×	1.83×	1.97×
t	1.42×	1.82×	1.94×
$2k$	1.42×	1.82×	1.94×

Relative length of longest RNA contig containing a ST-unitig.

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- Proof-of-concept RNA contigs on human annotated transcripts.
  - Publicly available code, datasets, and manipulation scripts.
  - `https://github.com/elarielcl/SafePathsRNAPC`

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- Efficient algorithms obtaining all maximal safe paths for generalized path covers.
- Proof-of-concept RNA contigs on human annotated transcripts.
  - Publicly available code, datasets, and manipulation scripts.
  - `https://github.com/elarielcl/SafePathsRNAPC`
- Future developments of safe paths
  - Output of transcript assemblers.
  - Use as preprocessing step.
  - Validate transcript assemblies.
  - Apply to other multi-assembly problem.

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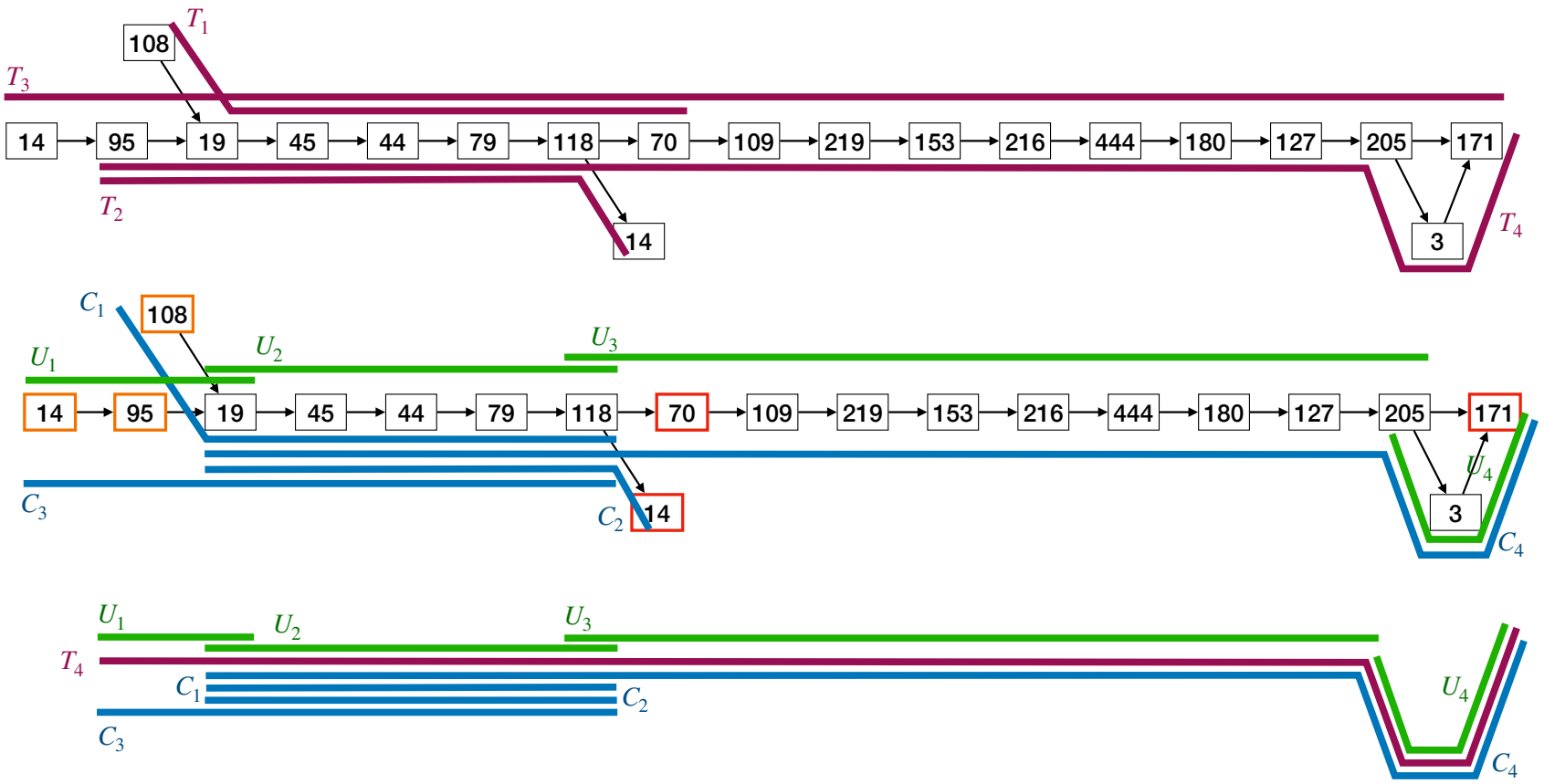
T_1
T_2
T_3
T_4
108
14
95
19
45
44
79
118
70
109
219
153
216
444
180
127
205
171
14
3
C_1
C_2
C_3
C_4
U_1
U_2
U_3
U_4

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