A Novel Combinatorial Method for Estimating Transcript Expression with RNA-Seq: Finding a Bounded Number of Paths

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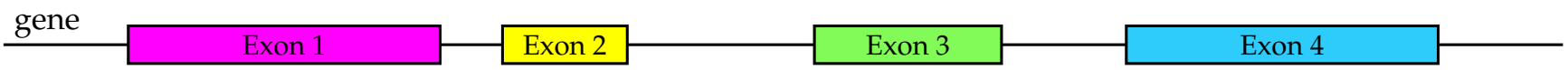
HiTSeq
July 20, 2013

THE BIOLOGICAL PROBLEM



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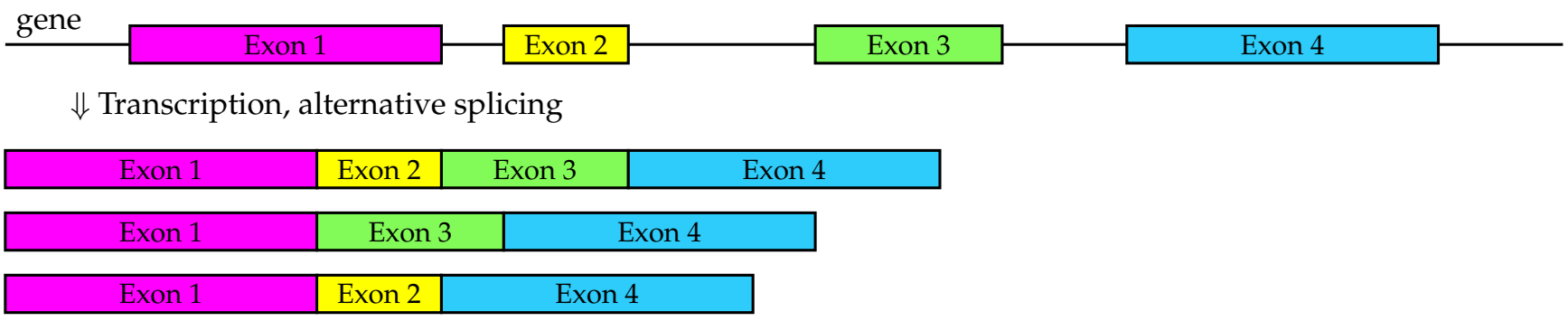

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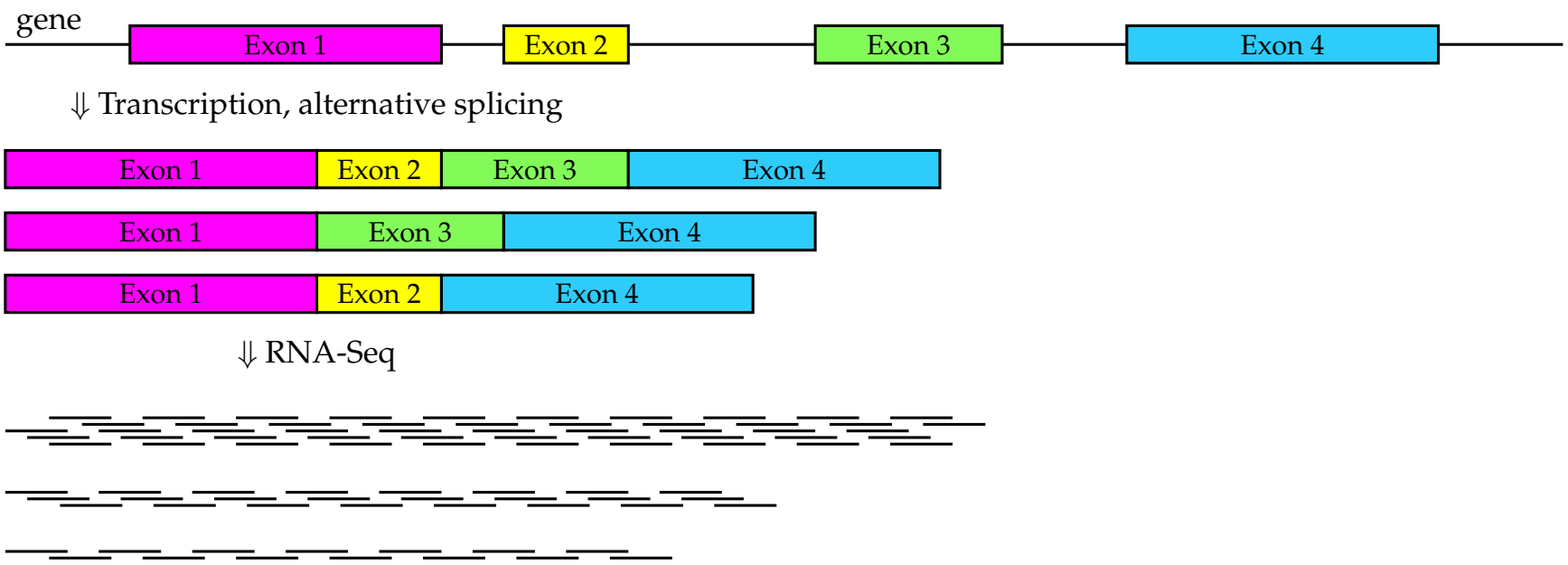

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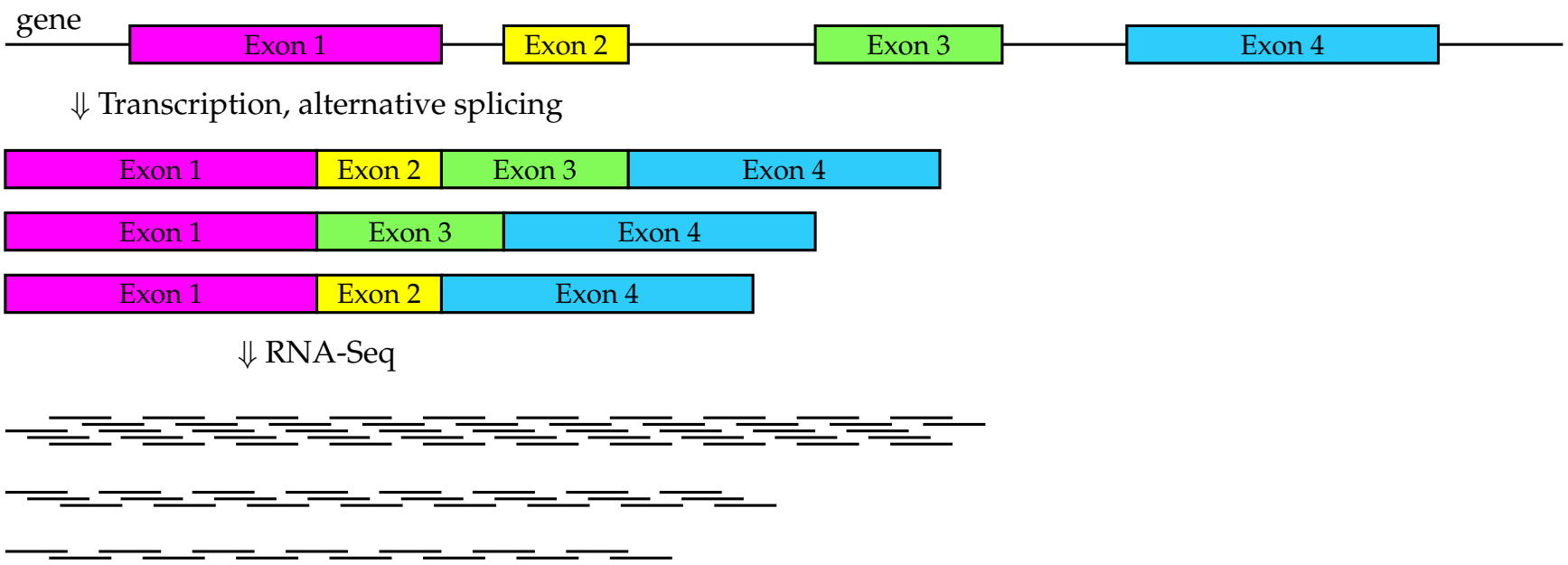

The problem: Assemble the transcripts and estimate their expression levels using only the RNA-Seq reads

Existing methods

Genome-independent:

- TransABySS ('10), Trinity ('11), Oases ('12)

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   - detect exon boundaries from the spliced alignments
   - every node stands for an exon
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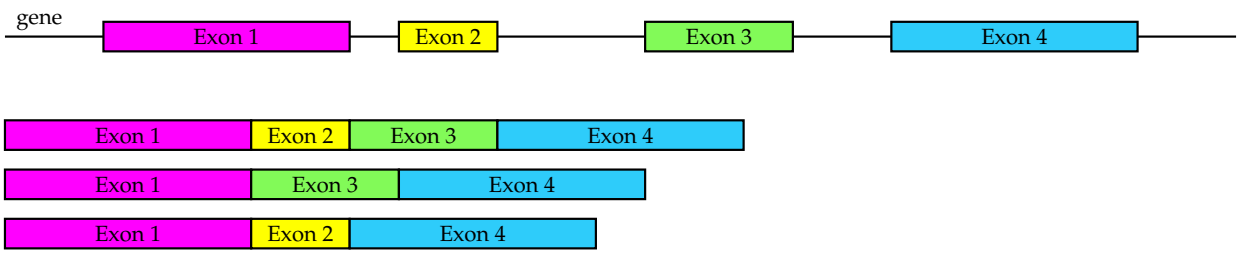

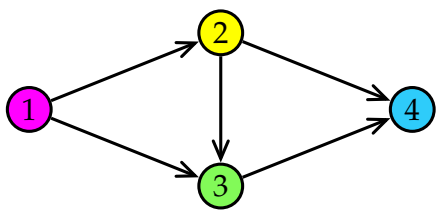

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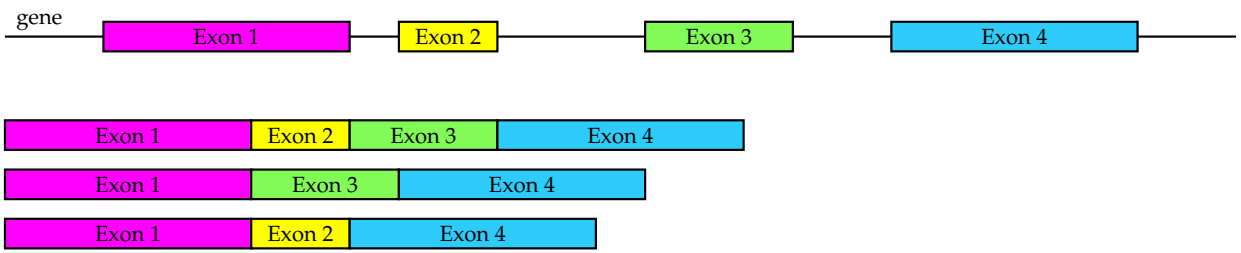

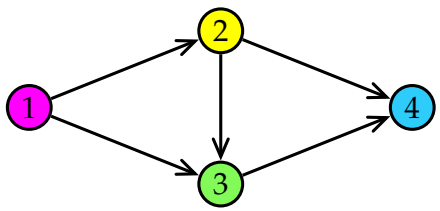

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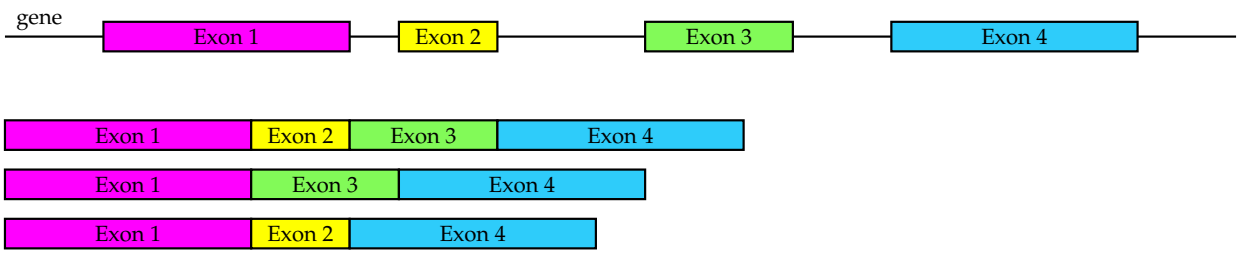

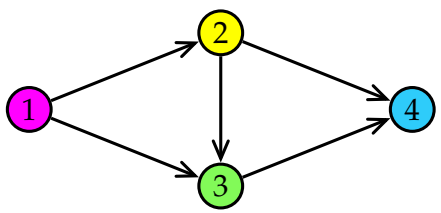

   - the splicing graph is a DAG
   - nodes and edges have observed coverages
   - exhaustively enumerate all possible paths
   - choose the most likely ones based on their coverage using an ILP, QP, QP + LASSO, statistical methods

A unified problem formulation [RECOMB-Seq 2013]

Problem (Unannotated Transcript Expression Cover)

INPUT: *a splicing DAG* $G = (V, E)$*, and for all* $v \in V$ *and* $(u, v) \in E$*,*

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For example, if for all nodes v and edges (u, v),

- $f_v(x) = x, f_{uv}(x) = x \Rightarrow$ least sum of absolute differences model [CLIIQ]
- $f_u(x) = x^2, f_{uv}(x) = x^2 \Rightarrow$ least sum of squares model [IsoLasso, SLIDE]

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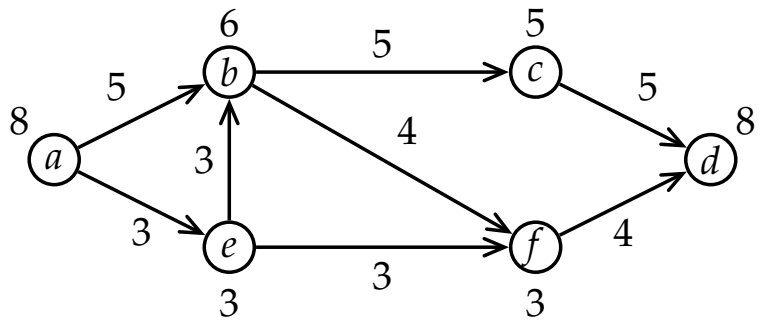
EXAMPLE $f_v(x) = x^2, f_{uv}(x) = x^2, k = 2$

$$\sum_{v \in V} \left(cov(v) - \sum_{P \in \mathcal{P} : v \in P} e(P) \right)^2 + \sum_{(u,v) \in E} \left(cov(u, v) - \sum_{P \in \mathcal{P} : (u,v) \in P} e(P) \right)^2$$



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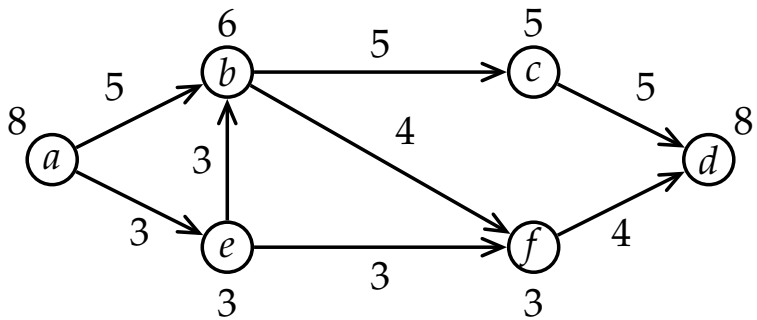

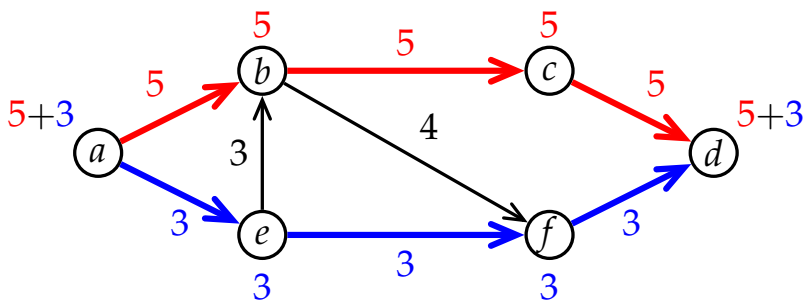

- [Left] A non-optimal tuple of 2 paths with cost $1 + 1 + 3^3 + 4^2 = 27$, from b, (f, d), (e, b), (b, f)

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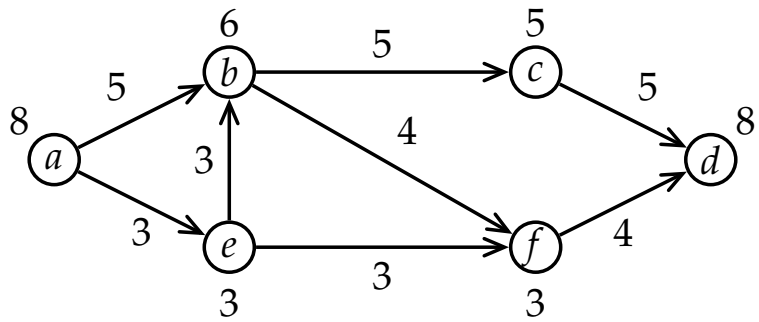

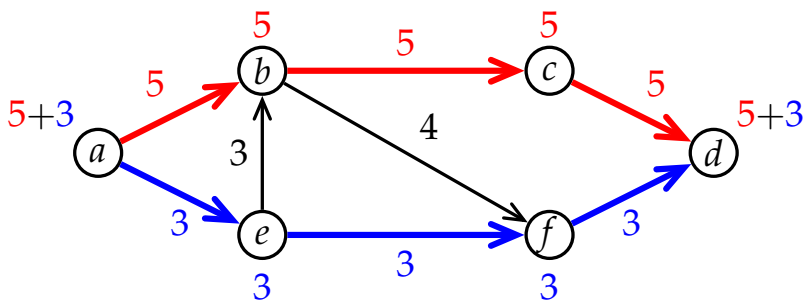

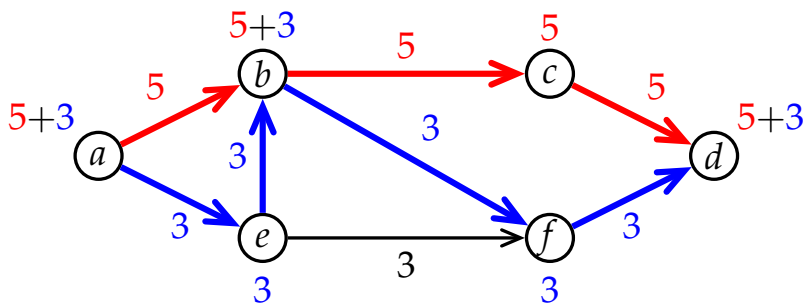

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- [Right] The optimal tuple of 2 paths with cost $2^2 + 1 + 1 + 3^2 = 15$, from b, and (b, f), (f, d), (e, f)

Computational Complexity

Theorem

If the penalty functions f_v and f_{uv} are such that $f_v(0) = 0$, $f_{uv}(0) = 0$, and $f_v(x) > 0$, $f_{uv}(x) > 0$ for all $x > 0$, then Problem k-UTEC is NP-hard in the strong sense.

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- The proof reduces from the 3-PARTITION Problem
- Our proof idea was already employed by [Li, Jiang, Zhang, arXiv, 2013] to show that the Isoform Reconstruction by Maximum Likelihood Problem, deployed in tools such as iReckon, NSMAP, Montebello, is also NP-hard

A dynamic programming algorithm

We can exploit the fact that the input splicing graph G is acyclic.

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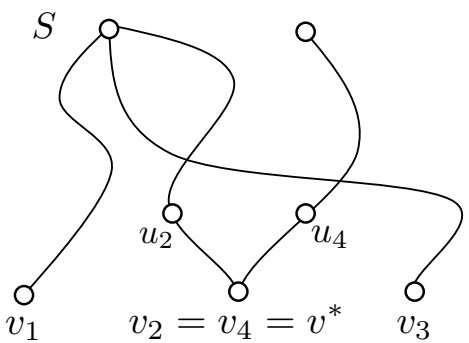

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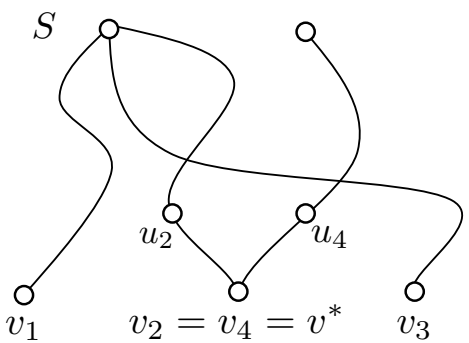

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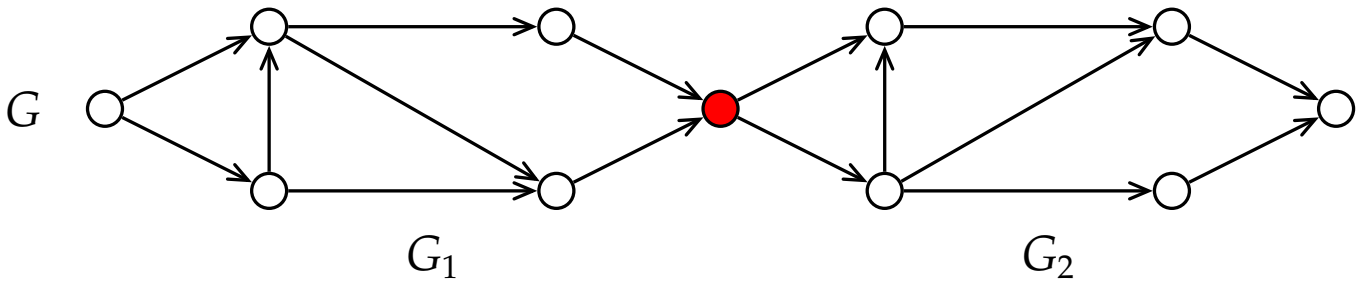
Theorem

*If the penalty functions positive-valued then Problem k-**UTEC** can be solved in time* $O(|M|^k \Delta^k n^k)$,
where $n := |V(G)|$, *we assume that* M *is the set of possible expression levels, and the maximum in-degree of* G *is* Δ.

Heuristics and optimizations

For a practical implementation, we

1. Decompose the problem along cut nodes



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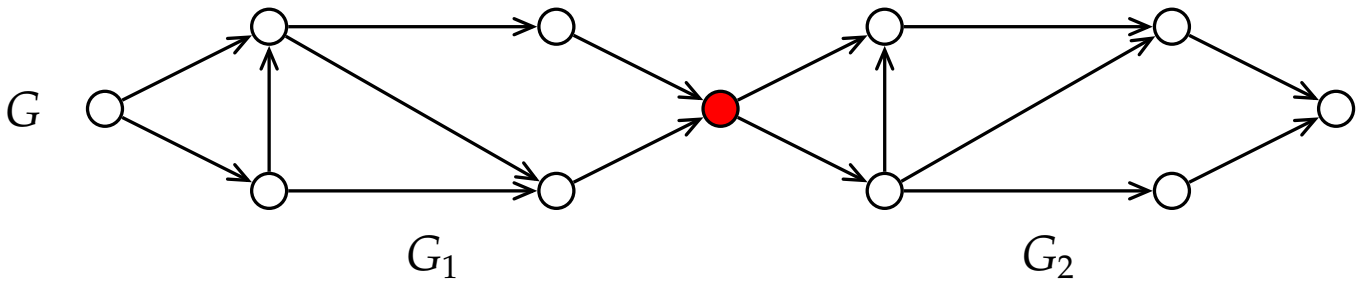

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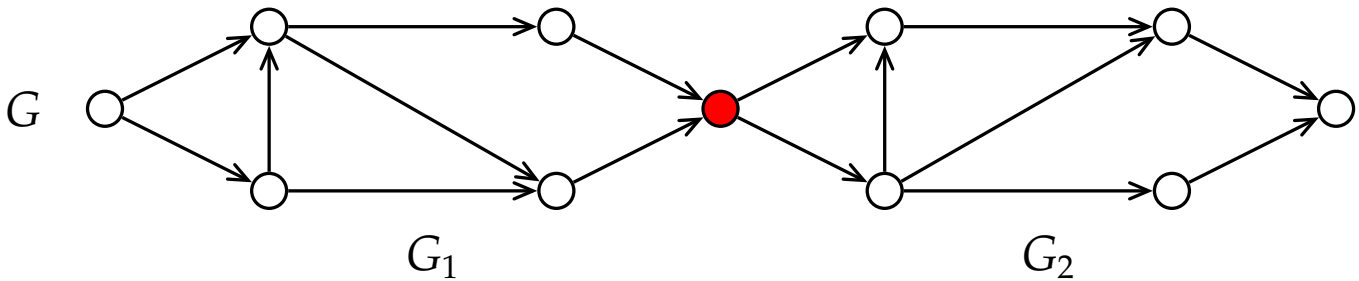
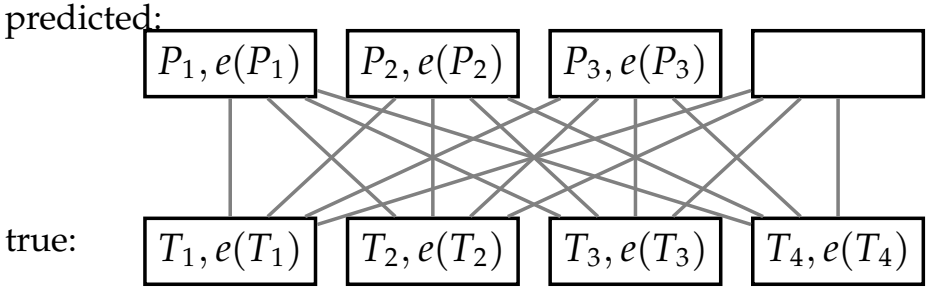


2. Employ a genetic algorithm for finding the optimal expression levels

3. Reduce the exponential dependency on k by iteratively looking for the optimal $k' < k$ paths and removing their coverage from the graph, until obtaining k paths

VALIDATION

- construct a bipartite graph with predicted and true transcripts



- the edge weight between $[P_i, e(P_i)]$ and $[T_j, e(T_j)]$ is a combined measure of
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  - *relative expression level difference* := $\frac{|e(T_j) - e(P_i)|}{e(T_j)}$

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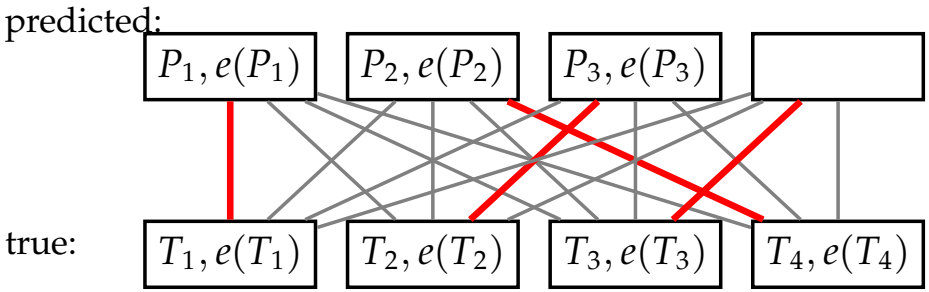

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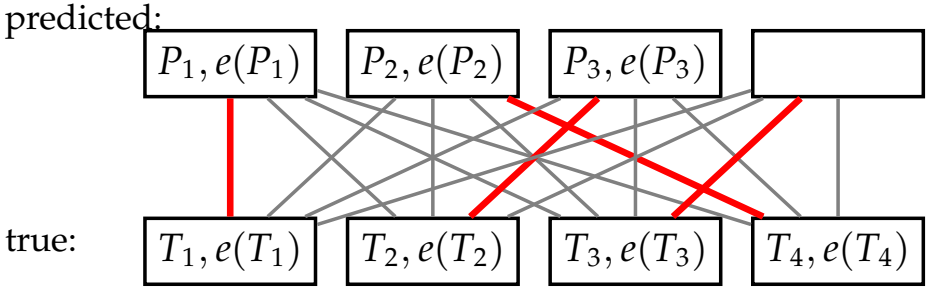

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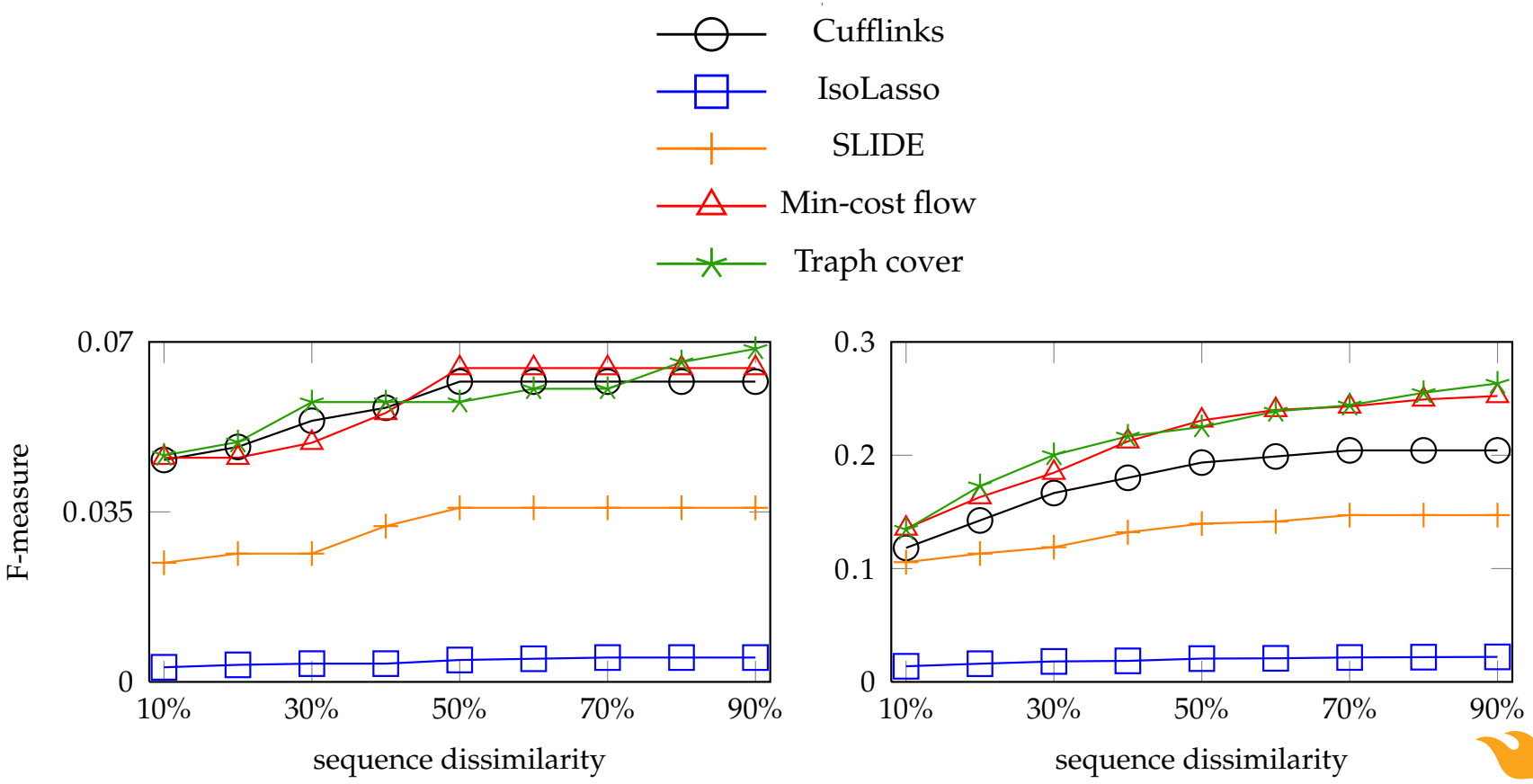
- compute minimum weight perfect matching
- a True Positive is a match with sequence dissimilarity and expression difference under given thresholds
- other events define False Positives and False Negatives
- compute precision, recall, F-measure

Experimental results on simulated data

- Simulated paired-end reads from the transcripts of 1,462 genes in HC 2
- Reads aligned with TopHat
- Alignments for all genes combined into one file, fed to the tools



(a) expr. difference threshold 10%

(b) expr. difference threshold 90%

Experimental results on real data

- 2,406,339 paired-end reads of length 75bp mapping to HC 2
- 735 genes where all tools made predictions
- 6,325 annotated transcripts in total

Tool	Total predicted	Shared with annotation at sequence dissimilarity under				
		10%	20%	30%	40%	50%
Cufflinks	1916	648	955	1171	1307	1413
IsoLasso	1468	589	782	923	1022	1100
SLIDE	2229	635	983	1242	1391	1474
Min-cost flow	2148	722	1000	1228	1341	1456
Traph cover	2109	**788**	**1063**	**1283**	**1407**	**1501**

Conclusions cs.helsinki.fi/gsa/traph/

- A unified problem formulation for transcript identification and quantification
- We replace the exhaustive enumeration of all (tuples of) paths by enumeration of all k-tuples of vertices
- We can increase the accuracy of the min-cost flow solution by tackling an NP-hard problem

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Future work:

- integrate paired-end information
- procure real ground-truth
- exploit graph-width measures (e.g. tree-width), write approximation algorithms
- apply to other multi-assembly problems