MetaPathway Hunter (MPH) – a Tool for the Alignment of Metabolic Pathways

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Metabolic Pathways

- Metabolic pathways – detailed chemical reactions and catalyzing enzymes
  - a product of one reaction is a substrate of another
- Metabolic network of an organism – a set of metabolic pathways
- Insight into
  - cellular responses to environmental changes
  - evolutionary processes
Metabolic Networks
Data and Analysis

• Current efforts to reconstruct genome-scale metabolic networks
  – E. coli, S. cerevisiae, human
  – in vivo and in silico [Palsson, UCSD]

• Metabolic pathways databases
  – MetaCyc, KEGG, SGD
  – tools for pathway visualization, queries on pathway components

• Dearth of tools for comparison between pathways
Goal

• Develop a pathway *alignment tool* for the detection of
  – a novel pathway against a database of pathways
  – a pathway fragment in a network
  – pathway conservation between species
  – pathway evolution within a species

• Pathway *similarity* (not necessarily identity!) should rely on
  – pathway structure
  – enzyme similarity

• A good abstraction is needed…
Representation

Metabolic pathway => labeled (line) graph:

nodes = enzymes
	node labels = Enzyme Commission (EC) classification numbers (functionality)

edges = between two enzymes if one’s product is the other’s substrate
Computational Problem

• Search for highly similar substructures in larger ones
• Gives rise to numerous pattern matching problems on labelled graphs
  – Recall that subgraph isomorphism is NP-hard [Garey and Johnson, 1979]
• Our approach: approximate labeled subtree homeomorphism (ALSH)
Subtree Homeomorphism

Pattern

Text

2 deletions

1 deletion
Best Homeomorphism

Pattern

Text

2 deletions

1 deletion
Best Homeomorphism

Pattern

Text

2 deletions

1 deletion
Best Homeomorphism

Pattern

Text

2 deletions

1 deletion
Approximate Labelled Subtree Homeomorphism (ALSH)

\[ LSH(M) = D \times (|T_2| - |T_1|) + \sum_{i,j} \Delta[i, j] \]
Subtree Homeomorphism Complexity

Rooted trees: $m^{1.5}n/\log n$ [Shamir and Tsur, 1999]

Unrooted trees: $m^{1.5}n/\log n$ [Shamir and Tsur, 1999]

$m$ - # of vertices in $P$

$n$ - # of vertices in $T$
## Our Results [CPM’04, JDA’08]

<table>
<thead>
<tr>
<th>ALSH</th>
<th>Rooted</th>
<th>Unrooted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unordered Trees</td>
<td>$O(nm^2 + nm \log n)$</td>
<td>$O(nm^2 + nm \log n)$</td>
</tr>
<tr>
<td>Ordered Trees</td>
<td>$O(nm)$</td>
<td>$O(nm)$</td>
</tr>
</tbody>
</table>

$m$ - # of vertices in $P$

$n$ - # of vertices in $T$
Related Work (Algorithmic)

Related Work (Biological)

ALSH on Rooted Unordered Trees
ALSH on Rooted Unordered Trees

\[
\begin{array}{cccccc}
  & x_1 & x_2 & \ldots & u & \ldots \\
y_1 & w_{11} & w_{12} & \ldots & w_{1u} \\
y_2 & w_{21} & w_{22} & \ldots & w_{2u} \\
y_3 & w_{31} & w_{32} & \ldots & w_{3u} \\
\ldots & \ldots & \ldots & \ldots & \ldots \\
v & \text{sun} & \text{sun} & \text{sun} & \text{sun} & \text{sun} \\
\ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\
\end{array}
\]
ALSH on Rooted Unordered Trees

Weighted Assignment on G

$$RScore(v,u) = \max \left\{ \Delta[v,u] + AssignmentScore(G), \text{BestChild} (v,u) + D \right\}$$
The algorithm computes an assignment for each pair $(u_i \in P, v_j \in T)$, where $i = 1 \ldots m$ and $j = 1 \ldots n$.

The assignment for $u_i$ and $v_j$ is computed using the bipartite graph $G(V = X \cup Y, E)$, where $|X| = k_i \leq |Y| = l_j$.

[Fredman & Tarjan 87] show how to compute $AssignmentScore(G)$ in $O(X (E + V \log V)) = O(k_i^2 l_j + k_i l_j \log l_j)$.
Time Complexity of ALSH Rooted Unordered Trees

• Observation 1: \( \sum_{i=1}^{m} k_i = m - 1 \) and \( \sum_{j=1}^{n} l_j = n - 1 \)

Summing up all \((u_i, v_j)\) node pairs:

\[
O\left(\sum_{i=1}^{m} \sum_{j=1}^{n} k_i^2 l_j + k_i l_j \log l_j\right) = O\left(\sum_{i=1}^{m} k_i^2 n + k_i n \log n\right) = O(m^2 n + mn \log n)
\]

Under the similarity assumption weighted assignment can be solved in \(O(V^{0.5}E \log(VC))\) [Gabow and Tarjan, 89] and ALSH can be solved in \(O(m^{1.5}n \log(nC))\).
A Word on Unrooted Trees

• Less important for this application
• Can still be done with same complexity
  – Requires \textit{decremental analysis} of weighted matching
• Different technique in the ordered case
  – Involves \textit{cyclic string comparison}
More on Complexity

• For unordered trees (rooted and unrooted): can be reduced to
  \[ O\left( \frac{nm^2}{\log m} + nm \log n \right) \]
  using compressed graphs

• Rooted, ordered trees
  – No cross-edges in the bipartite graph
  – Gives rise to dynamic programming like algorithm

• Algorithms and results: Pinter et al., Combinatorial Pattern Matching (CPM), 2004, (JDA 2008)
Why is ALSH Suitable?

- The replacement can take place if
  - the replacing enzyme is multifunctional
    - can thus catalyze several consecutive reactions, or
  - the enzyme uses an alternative catalysis
    - leads directly from the initial substrate to the final product

- Translating this into graph terms degree-2 nodes may be deleted from the graph: perfectly captured by subtree homeomorphism
Everything extends to **multi-source** trees:
Beyond Trees (2)

• Breaking cycles
  – generate alternatives
  – surprisingly rare

• Handling DAGs
  – duplicate and split merge nodes
  – fits well with biology: *alternative pathways*
Similarity Measures

- Sequence similarity (Alignment)
- Reaction similarity (EC hierarchy)
- Semantic similarity (Gene Ontology)
- Cluster based similarity (ProtoNet)
Similarity Measures

- Sequence similarity (Alignment)
- Reaction similarity (EC hierarchy)
- Semantic similarity (Gene Ontology)
- Cluster based similarity (ProtoNet)
Enzymes Similarity Scoring

- **Functional similarity** based on EC classification
  - Hierarchical scheme developed by the IUBMB
  - Consists of 4 levels + a root level
  - Each enzyme has an EC number, e.g. [2.7.3.112]

- Similarity score of $e_1,e_2$ ~ the size of the subtree under the lowest common ancestor of $e_1,e_2$
Similarity Measures

\[ I(h) = -\ln E(h) \]

where \( E(h) \) is the number of enzymes under the class \( h \).

\[ \text{sim}(e_1, e_2) = I(lca(e_1, e_2)) \]
The *MetaPathwayHunter* Tool

[Bioinformatics 2005]

- Searches a pattern $P$ against a database of text trees ($Ts$)
- Each alignment is given a score $s$ and statistical significance $p$
- Statistical significance computation:
  - Executes $P$ against a database of 1000 randomized $Ts$
  - $p(P)$: the fraction of randomized $Ts$ whose alignment with $P$ scored $\geq s$
- Enables visualization of the alignment, which is color-coded by similarity
Experimental Evaluation: Data

• Biocyc
  – *E. coli*: 113 pathways

• SGD
  – Yeast: 151 pathways

• Pathways based on genome annotations + manual curation

• <10 cycles per organism
Executions

• Aligned all possible pairs of analogous metabolic pathways in *E. coli* and *S. cerevisiae* (budding yeast)
• All-against-all intra-species alignment
• Labels: EC numbers; matches are color coded
Inter-Species Alignments (1)

- Metabolic pathways are conserved: the alignments between 62 out of 80 pairs of analogous pathways were significant.
- Conservation is not limited to small pathways, e.g.

Phenylalanine, tyrosine, and tryptophan biosynthesis, \(E. \, coli\) vs. yeast: \(s=-4.28; \, p<0.01\)

- TyrA (\(E. \, coli\)) and Tyr1 (yeast) –
  - differ in the acceptor (NAD+ vs. NADP+)
  - functional homologs - no sequence similarity
Inter-Species Alignments (2)

Alignments with gaps:

- Met17 in yeast does the work of MetB and MetC in *E. coli*
- All Met* genes bear sequence homology

Potential gene fusion (*E. coli* to yeast)

Homoserine & methionine biosynthesis, *E. coli* vs. yeast:
\[ s = -13.15; p < 0.01 \]
Intra-Species Alignments

Hints to evolution of paralogous pathways

Amino acid biosynthesis pathways are conserved

• Valine, Leucine, Isoleucine (hydrophobic aa’s) have similar biosynthesis pathways

  • Valine and isoleucine employ the same pathway but on different substrates
    \[ s = 0, \ p < 0.01 \]

  • Leucine employs a homologous pathway
    \[ s = -19.75, \ p < 0.03 \]
MetaPathway Queries

Flexible searching – possible queries:

- Pathway endpoints (source and product)
- Common pathway core

E. coli alantoine degradation: \( s=0; \ p < 0.01 \)

Yeast ureide degradation \( s=0; \ p<0.01 \)
Status and Availability

- Engine in C++, GUI in Java
- Available upon request (download)
  - whole or client only
- Requires preprocessing (to trees)
Future Work

• Modeling
  – affine deletion scores
  – hypergraphs

• Algorithms
  – extensions to e.g. bounded tree-width graphs
  – multiple alignment

• Applications
  – compare different similarity metrics
  – apply to other data types (e.g. phylogenies)
  – other domains (semantic queries, NLP, etc.)
Thanks

• Elad Ben-Yosef
• Moshe Itzhaki
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• Hanah Margalit
• Roded Sharan
• Michael Waterman
Similarity Measures

EC hierarchy

1st level:
- six broad classes of enzymatic activity:
  1. Oxidoreductase
  2. Transferase
  3. Hydrolase
  4. Lyase
  5. Isomerase
  6. Ligase
Similarity Measures

EC hierarchy

2nd level:

- Identifies the subclass.
- For oxidoreductases the second level specifies the kind of donor which is oxidized.
- For example, sub-class [1.1] means that the enzyme acts on the CH-OH group of donors.
EC hierarchy

3rd level:
• Specifies the kind of acceptor.
• For example, the sub-sub-class [1.1.1] means that NAD+ or NADP+ are the acceptors.
Similarity Measures

**EC hierarchy**

4\(^{th}\) level (leafs):

- Identifies the particular reaction.
- For example, the donor of [1.1.1.1] and [1.1.1.2] is CH-OH and their accepters are NAD+ and NADP+, respectively.
Unrooted Unordered Trees (naïve approach)

Pattern (P)  

Text (T)

Weighted Assignment 2x4 ...
Unrooted Unordered Trees (naïve approach)

Pattern (P)                  Text (T)

Weighted Assignment 2x4 …
Unrooted Unordered Trees

Pattern (P)

Text (T)

Weighted Assignment $2 \times 4$ ...
Unrooted Unordered Algorithm

Pattern (P)

Text (T)

Weighted Assignment 3x4 …
Unrooted Unordered Algorithm

Pattern (P)

Text (T)

X

Y
Unrooted Unordered Algorithm

Pattern (P)

Text (T)

One augmentation!
Unrooted Unordered Algorithm

Pattern (P)

Text (T)

One augmentation!
Unrooted Unordered Algorithm

Pattern (P)

Text (T)

X

Y
One augmentation!

\[ UScore(v, u, x_q) = \max \left\{ \Delta[v, u] + AssignmentScore(X \setminus \{x_q\}, Y), \text{BestChild} (v, u) + D \right\} \]
Decremental Property of Weighted Assignment

Lemma:

Let

- $G(X \cup Y, E)$ be a bipartite graph
- $k_i = |X| \leq l_j = |Y|$
- $G_q = (X_q \cup Y, E)$ for $X_q = X \setminus \{x_q\}$

Computing the weighted assignments for the series of bipartite graphs $G_q$, for $q = 1 \ldots k_i$ can be done in time $O(k_i^2 l_j + k_i l_j \log l_j)$
Time Complexity of ALSH
Unrooted Unordered Trees

Correction path
Dijkstra’s algorithm
\(O(E + V \log V)\)
For all neighbors of \(u:\)
\(O(k_i^2 l_j + k_i l_j \log l_j)\)

→ ALSH on Unrooted Unordered trees can be computed in \(O(m^2 n + mn \log n)\)
Decremental Property of Weighted Assignment (proof)
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Theorem [Ford & Fulkerson 62, Edmonds & Karp 72, Tarjan 83]:
A flow $f$ is minimum cost iff its residual graph $R$ has no negative cycles.
Decremental Property of Weighted Assignment (proof)

Correction path $p$

Theorem [Ford & Fulkerson 62, Edmonds & Karp 72, Tarjan 83]: A flow $f$ is minimum cost iff its residual graph $R$ has no negative cycles.
Decremental Property of Weighted Assignment (proof)

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A flow $f$ is minimum cost *iff* its residual graph $R$ has no negative cycles.
Decremental Property of Weighted Assignment (proof)

Theorem [Ford and Fulkerson 62, Edmonds and Karp 72, Tarjan 83]:

A flow $f$ is minimum cost iff its residual graph $R$ has no negative cycles.

$Cost(p \oplus c) = Cost(p) + Cost(c) < Cost(p)$
Decremental Property of Weighted Assignment (proof)

Possible combination of $p$ and $c$: 

Diagram showing possible combinations of $p$ and $c$. Each combination is represented by a network of nodes connected by arrows, indicating the direction of the property.

- Diagram on the left: A network with multiple loops and connections, illustrating one possible combination.
- Diagram on the right: A vertical network with a hose-like structure, showing another possible combination.
Compressed Graph

- Motivation: 
  
  \[ O(nm^2 + nm \log n) \]
  
  \[ O\left(\frac{nm^2}{\log m} + nm \log n\right) \]

- Assuming a constant-sized label alphabet
- Using the notion of clique partition of a bipartite graph [Feder and Motwani 1991, Shamir and Tsur 1999]
• Each node in bipartite graph represents the whole subtree.

• Bounded alphabet the number of distinct trees is bounded.

• Lemma (a la [Shamir and Tsur, 1999]):
  – The number of distinct labeled rooted trees in a forest of \( n \) vertices is: \( O\left(\frac{n}{\log n}\right) \)
Compressed Graph

Graph G

\[ s \rightarrow 0 \rightarrow 0 \rightarrow 0 \rightarrow s \]

X

-7 -4 -2 -4 -2 -4 -3

Y

-7 -4 -2 -4 -5 -3

t

0 0 0 0 0 0
Compressed Graph

Graph $G$

Graph $G^*$
Compressed Graph

• Lemma:
  – The assignment between \( u \in P \) and \( v \in T \) can be computed in time:
    \[
    O(d(u)(D(u)c(v) + c(v)\log c(v)))
    \]
    where\[\begin{align*}
    E & + V \log V \\
    \end{align*}\]
  – \( d(u) \) is the number of neighbors of node \( u \)
  – \( D(u) \) is the number of distinct trees in the forest of trees rooted at neighbors of node \( u \)
  – \( c(v) \) is the number of children of node \( v \)
Compressed Graph

- Observation 2:
The sum of vertex degrees in an unrooted tree $P$ is:

$$
\sum_{u=1}^{m} d(u) = 2m - 2
$$

- Summing up the work over all $(v, u)$ pairs we get:

$$
O\left(\sum_{u=1}^{m} \sum_{v=1}^{n} (d(u)(clusters_u c(v) + c(v) \log c(v))) \right) =
$$

$$
O\left(\sum_{u=1}^{m} (d(u) \times clusters_u \times n + d(u)n \log n) \right) =
$$

$$
O(n \sum_{u=1}^{m} d(u)D(u) + mn \log n)
$$
Time Complexity

• Lemma:
\[ \sum_{u=1}^{m} d(u)D(u) = O\left(\frac{m^2}{\log m}\right) \]

• Thus the algorithm computes the optimal ALSH solution for two rooted unordered trees in
\[ O\left(\frac{m^2 n}{\log m} + mn \log n \right) \]
ALSH on Rooted Ordered Trees

\[ x_1 \quad y_1 \]
\[ x_2 \quad y_2 \quad y_3 \]
ALSH on Rooted Ordered Trees

\[ x_1 \quad x_2 \]
\[ y_1 \quad y_2 \quad y_3 \]

\[ P \quad T \]

\[ u \quad v \]
\[ x_1 \quad x_2 \quad y_1 \quad y_2 \quad y_3 \]
ALSH on Rooted Ordered Trees

The main property: **NO CROSS-EDGES** in the bipartite graph !!!
ALSH on Rooted Ordered Trees

The main property: **NO CROSS-EDGES in the bipartite graph !!!**
ALSH on Rooted Ordered Trees
ALSH on Rooted Ordered Trees

\[ \sum_{i=1}^{m} \sum_{j=1}^{n} O(k_i \times l_j) = \sum_{i=1}^{m} O(n \times k_i) = O(nm) \]
ALSH on Unrooted Ordered Trees
(Cyclic order)
Cyclic String Comparison

T: BCBADEDCD

P: ABCDE

Source

Destination: Column n's Maxima
Cyclic String Comparison
Cyclic String Comparison
Cyclic String Comparison
Cyclic String Comparison

Diagram showing a cyclic string comparison with nodes A, B, C, D, E and paths labeled P and T.
Cyclic String Comparison
Cyclic String Comparison
Time Complexity

Real numbers score metric: $O(kl\log k)$
- [Maez 1990]

Rational numbers score metric: $O(kl)$
- [Schmidt 1998]
Time Complexity

Real numbers
score metric:
[Maez 1990]

Rational numbers
score metric:
[Schmidt 1998]

\[ O(kl \log k) \]

\[ O(kl) \]

**Observation 1**

\[
\sum_{i=1}^{m} \sum_{j=1}^{n} O(k_i l_j \log k_i) = \sum_{i=1}^{m} O(n \times k_i \log k_i) = O(nm \log m)
\]

\[
\sum_{i=1}^{m} \sum_{j=1}^{n} O(k_i \times l_j) = \sum_{i=1}^{m} O(n \times k_i) = O(nm)
\]
ALSH on Unrooted Ordered Trees (Linear order)
ALSH on Unrooted Ordered Trees
(Linear order)

\[ y_1 \quad y_2 \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad y_n \]

A
B
C
D
E

BACKWARD
ALSH on Unrooted Ordered Trees

(Linear order)

searching the best $[i-1, j] + [i+1, j+1]$
ALSH on Unrooted Ordered Trees
(Linear order)

A

B

C

D

E

y_1  y_2  \ldots  y_j  y_{j+1}  \ldots  y_n

Forward

Backward

searching the best [i-1,j] + [i+1,j+1]
Time Complexity

- Weighted assignment for each pair \((u, v)\) can be computed in:

\[
O(kl) + O(kl) + k \times O(l) = O(kl)
\]

- And summing up the work for all pairs \((u, v)\):

\[
\sum_{i=1}^{m} \sum_{j=1}^{n} O(k_i \times l_j) = \sum_{i=1}^{m} O(n \times k_i) = O(nm)
\]
Why is ALSH Suitable?

• A single enzyme in one pathway may replace a few consecutively acting enzymes in another pathway.

• The replacement can take place if
  – the replacing enzyme is multifunctional
    ▪ *can thus catalyze several consecutive reactions*, or
  – the enzyme uses an alternative catalysis
    ▪ *leads directly from the initial substrate to the final product*.

• Enzymes that catalyze just a single reaction are more likely replaced, for both biochemical and parsimony reasons.

• Translating this into graph terms, degree-2 nodes may be deleted from the graph: perfectly captured by subtree homeomorphism.
Lemma:

A min-cost max flow in $f^*$ corresponds directly to a min-cost max flow in $f$. 

<table>
<thead>
<tr>
<th></th>
<th>Graph $G^*$</th>
<th>Graph $G$</th>
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<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Graph G*" /></td>
<td><img src="image2" alt="Graph G" /></td>
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<tr>
<td>2</td>
<td><img src="image3" alt="Graph G*" /></td>
<td><img src="image4" alt="Graph G" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Graph G*" /></td>
<td><img src="image6" alt="Graph G" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Graph G*" /></td>
<td><img src="image8" alt="Graph G" /></td>
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<tr>
<td>5</td>
<td><img src="image9" alt="Graph G*" /></td>
<td><img src="image10" alt="Graph G" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image11" alt="Graph G*" /></td>
<td><img src="image12" alt="Graph G" /></td>
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</table>
The Sequence Alignment Problem

A = c t a c g a g a c
B = a a c g a c g a t

The Scoring Matrix $\delta$

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</table>

Measure the similarity of A and B by finding an alignment of the strings which optimizes the similarity score.
The Sequence Alignment Problem

Optimal Global Alignment.

A = c t a c g a g a c
B = a a c g a c g a t
Similarity Score: 2

Optimal Local Alignment.

A = c t a c g a g a c
B = a a c g a c g a t
Similarity Score: 5

The Scoring Matrix $\delta$

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