Generating Peptide Candidates from Protein Sequence Databases for Protein Identification via Mass Spectrometry

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Informatics Research

Applied Biosystems

Protein Identification

- Turns mass spectrometry into proteomics
- Sequence is link to identity, annotation, literature, genomics
  - Proteomics workflows interrogate more than mass
  - Quality of AA sequence databases sequence & annotation varies wildly
- Protein identification is not BLAST!

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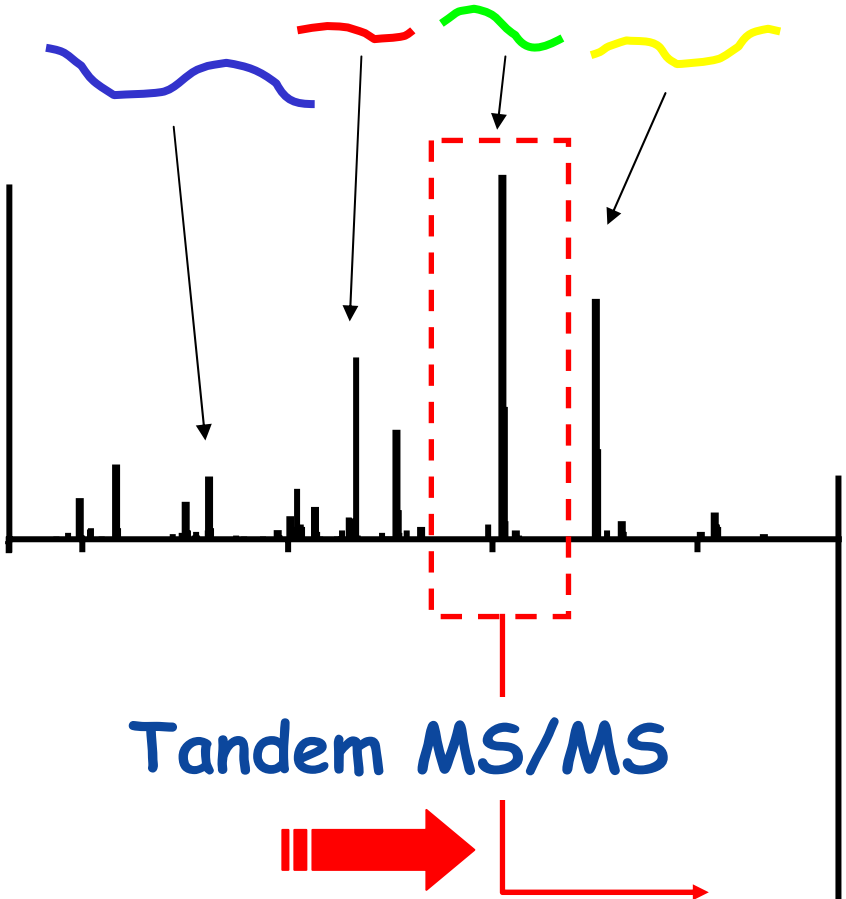
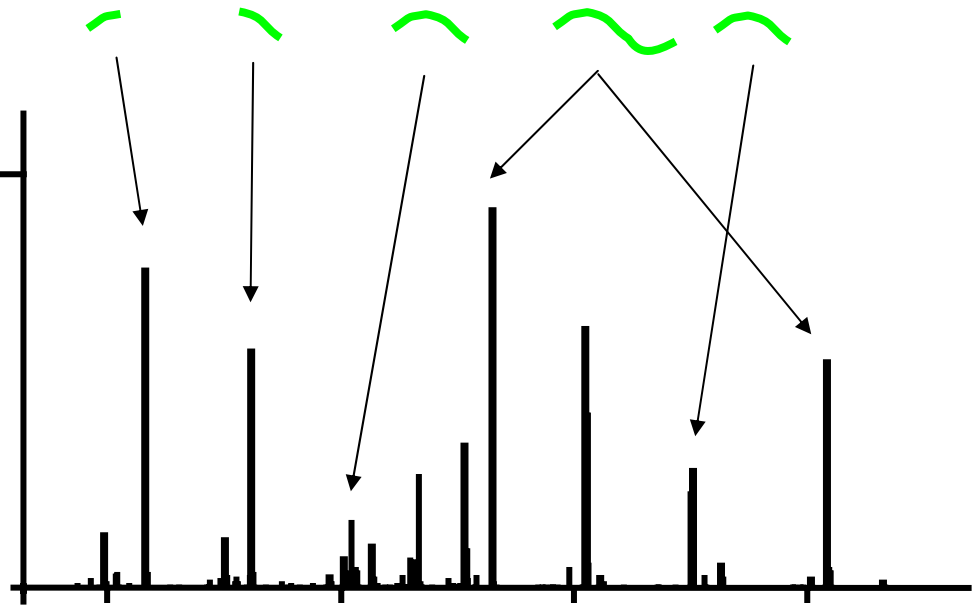
LC-MS/MS for Protein Id



LC-MS/MS: 1 MS spectrum followed by 2-5 Tandem MS/MS spectra every 5-10 sec.



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LC-MS/MS for Protein Id

- 1 experiment produces 1000's of MS/MS spectra
- Suitable for complex mixtures
- 100's-1000's of proteins identified from a single experiment
- **High-throughput protein identification!**

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Sequence Database Search Engines

Input: Set of MS/MS spectra and associated parent ion masses

Output: Peptide sequence for each spectrum

1. Generate peptide candidates from a protein or genomic sequence database
2. Score and rank the peptide candidates

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Peptide Candidate Generation

Input: Sequence σ (length n), from alphabet **A**
(Additive) mass μ(a) for a **2 A**
Query masses $M_1,\ldots,M_k$

Output: All (distinct) pairs of query masses i and subsequences ω with

$$\sum_{j=1}^{|\omega|} \mu(\omega_j) = M_i$$

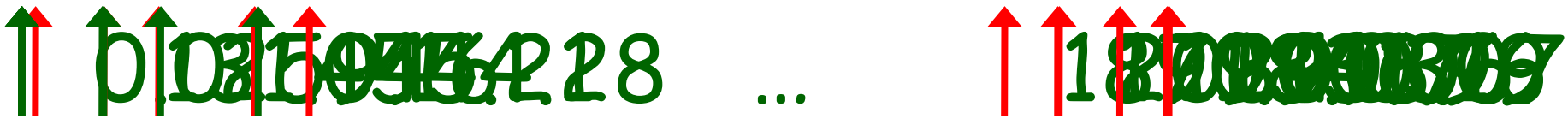
Peptide Candidate Generation and Peptide Id

- Sequence databases contain many individual proteins
- Must avoid redundant scoring
- Protein context is important

Simple Linear Scan

Query Mass = 2018.07

MKWVTFISLLFLFSSAYSRGV…



Output: WVTFISLLFLFSSAYSR

Sequential Linear Scan

- O(nk) time
- Simple to implement
- Easy to track protein context
- Poor data locality
- Redundant candidates
- String scanning problem

Simultaneous Linear Scan

Max Query Mass = 2018.07

Lookup each candidate mass in turn.

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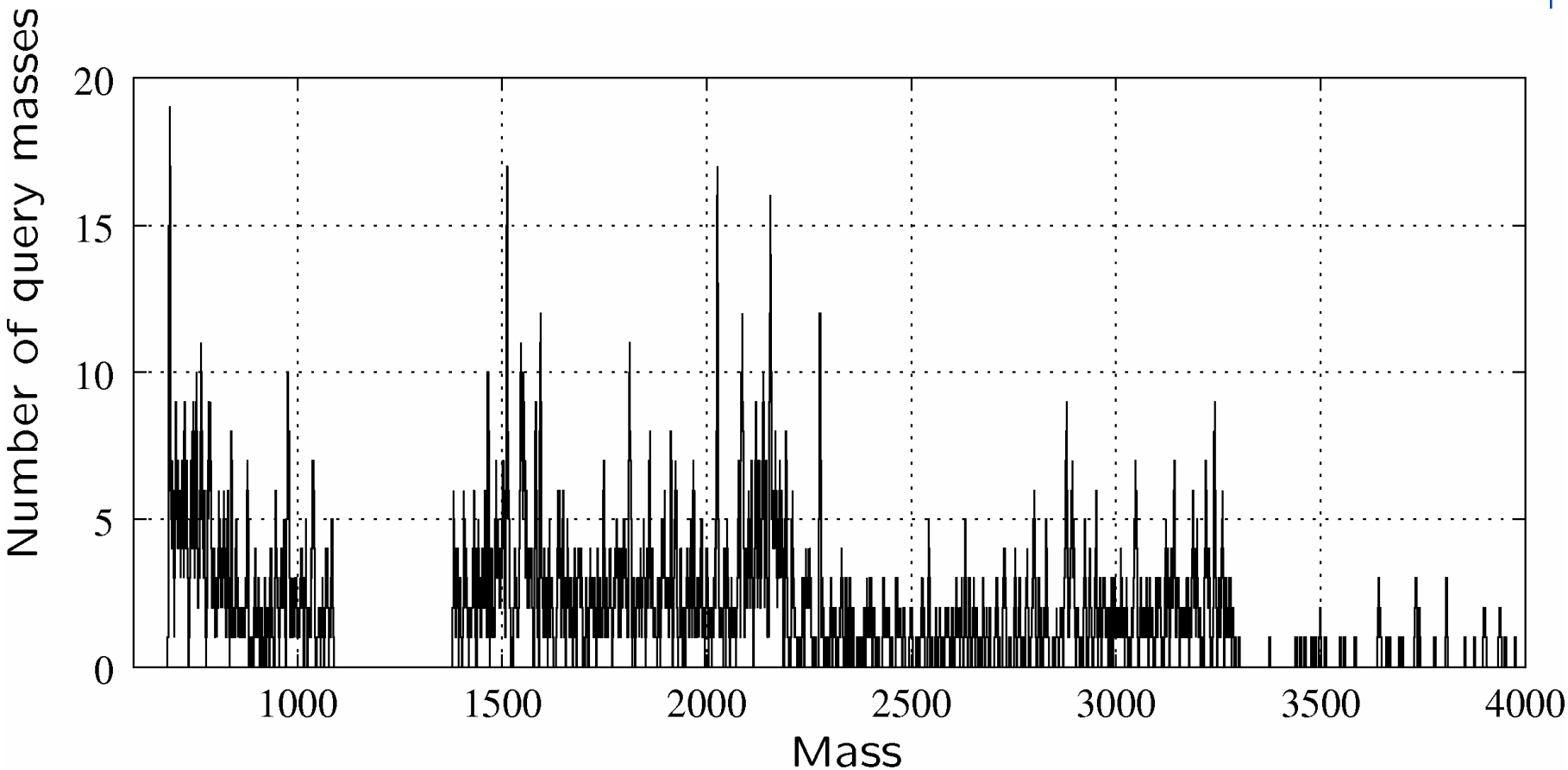
Simultaneous Linear Scan

- O(k log k + n L log k) time
- Simple to implement
- Easy to track protein context
- Better data locality
- Redundant candidates
- Now a query mass lookup problem!

Overlap Plot from a LC/MS/MS Experiment



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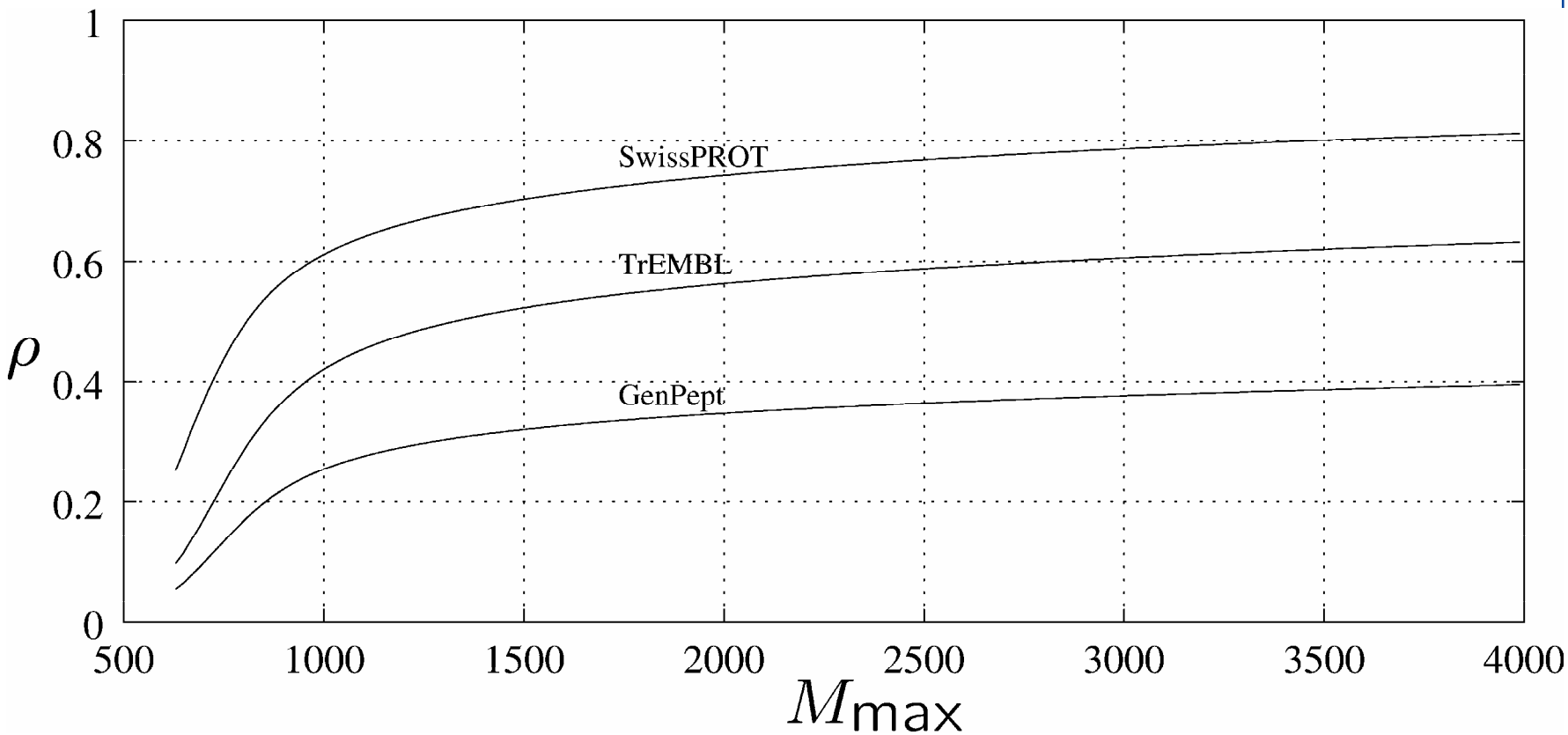
Redundant Candidate Elimination

- Must avoid repeat scoring of the same peptide candidate

- Want to avoid generating redundant candidates

- Non-redundant sequence databases contain lots of substring redundancy!

Substring Density (ρ)



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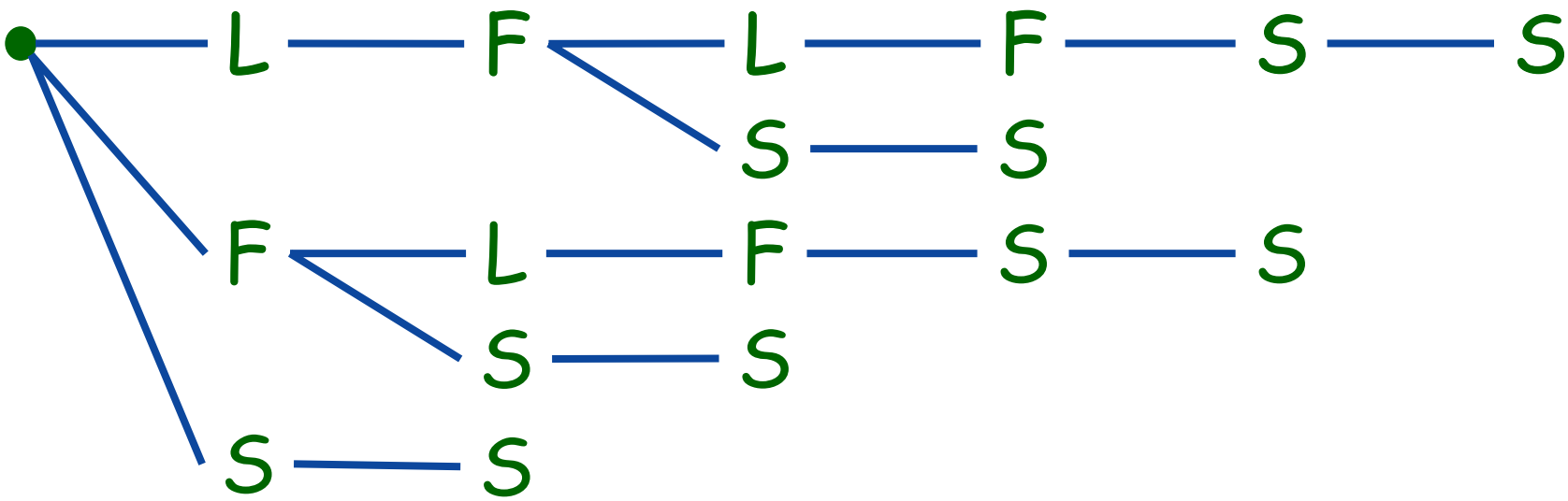
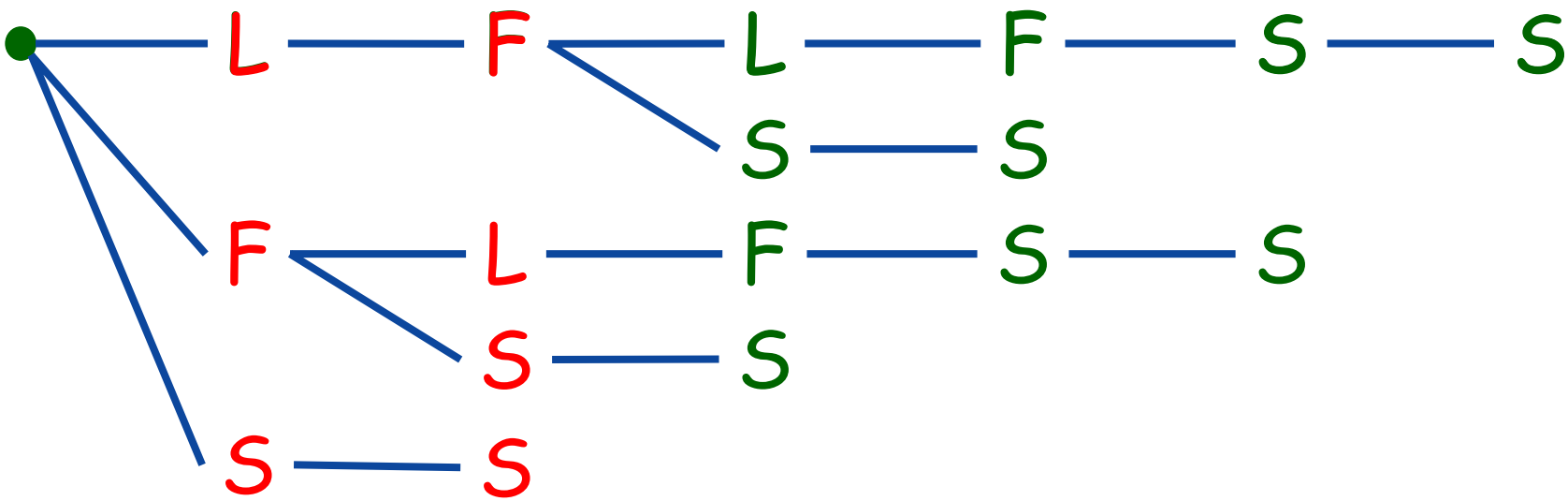
Redundant Candidate Elimination

- Suffix trees represent all distinct substrings of a string.



Redundant Candidate Elimination

- Suffix trees represent all distinct substrings of a string.



Suffix-Tree Traversal

- O(k log k + n L ρ log k) time
- Redundancy eliminated
- Tricky to implement well
- Memory overhead ¼ 5n
- Protein context more involved
- Data locality hard to quantify
- Must preprocess sequence db
- Still a query mass lookup problem!

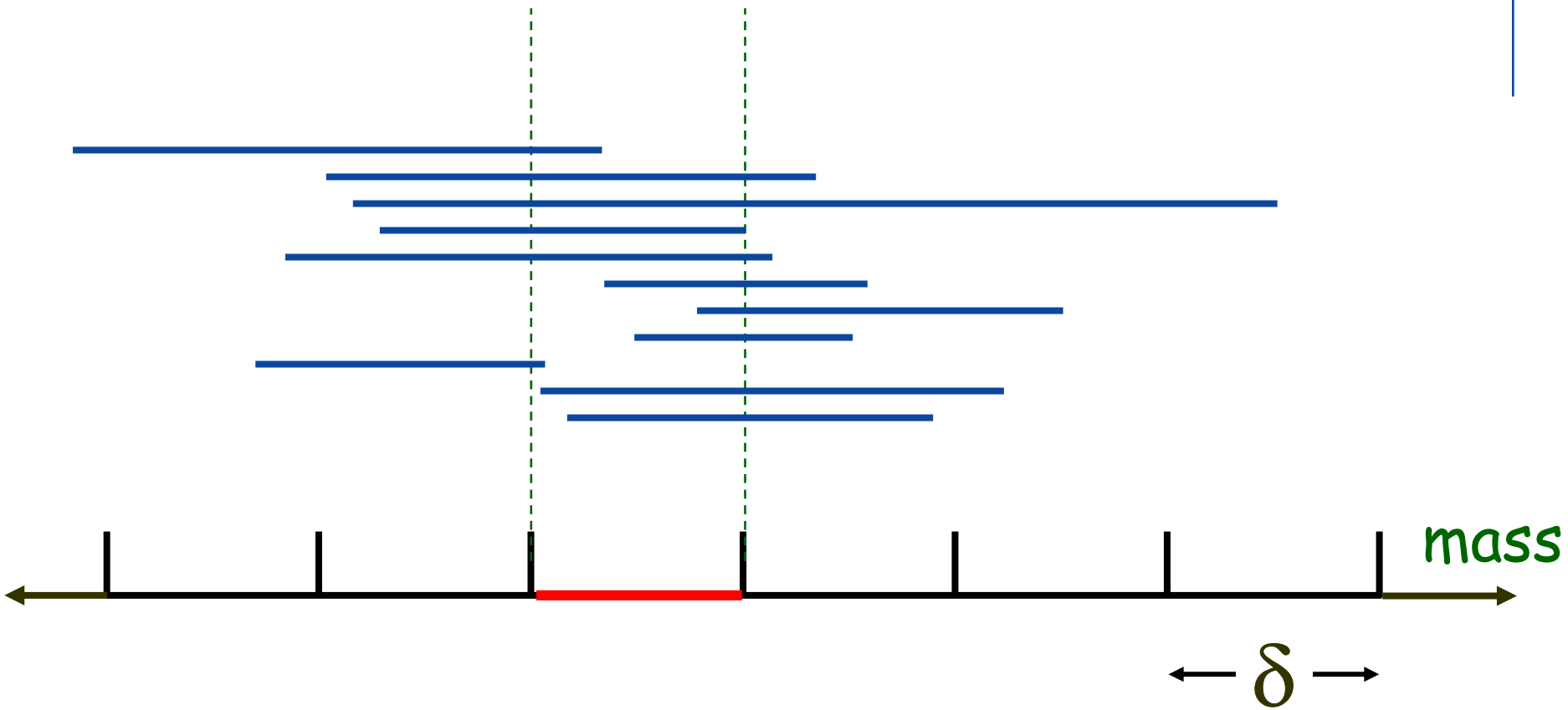
Fast Query Mass Lookup

- With (small) integer weights, $O(M_{max} + k + n\,L\,\rho\,O)$ time is possible
- Use a query mass lookup table!

- Can we achieve this for real weights and non-uniform tolerances?

YES!

Fast Query Mass Lookup



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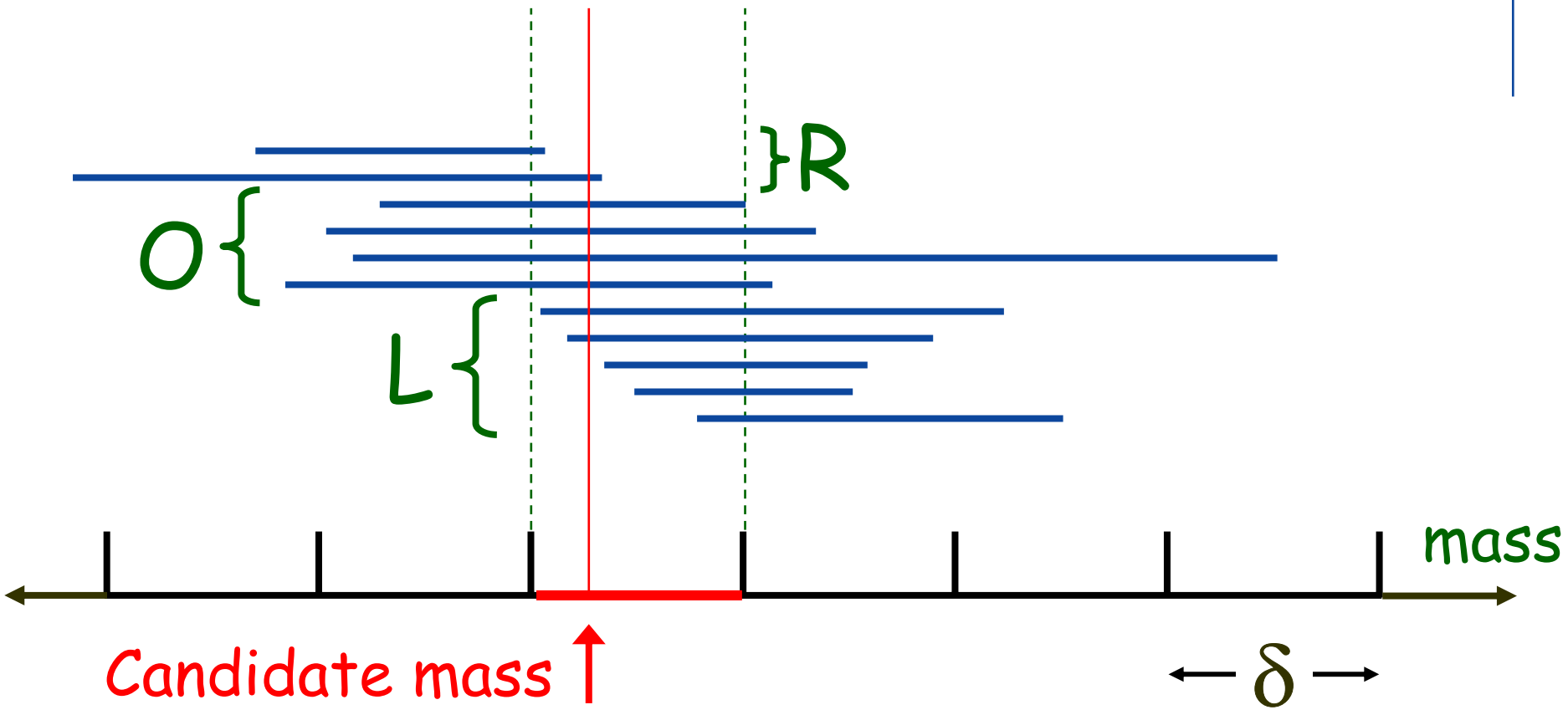
Fast Query Mass Lookup



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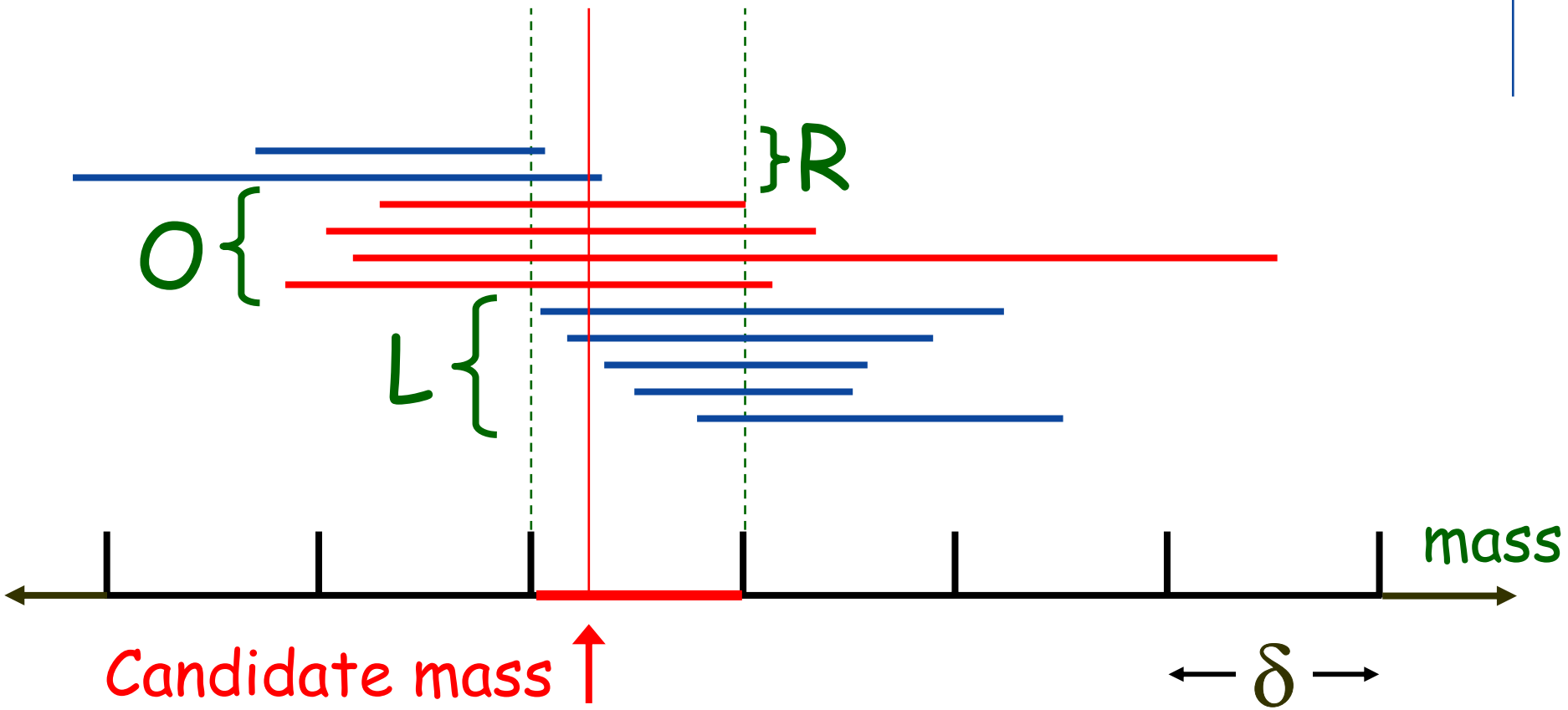
Fast Query Mass Lookup



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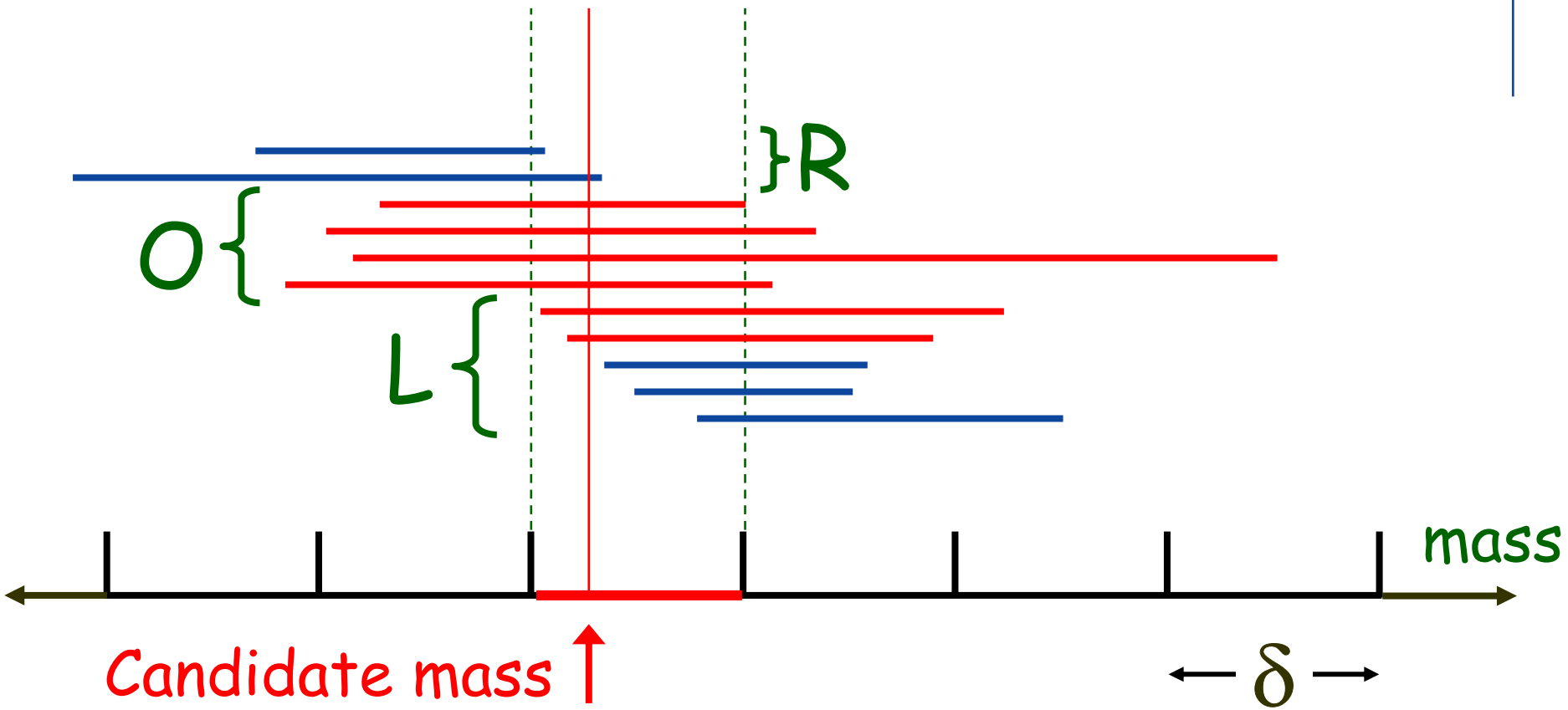
Fast Query Mass Lookup



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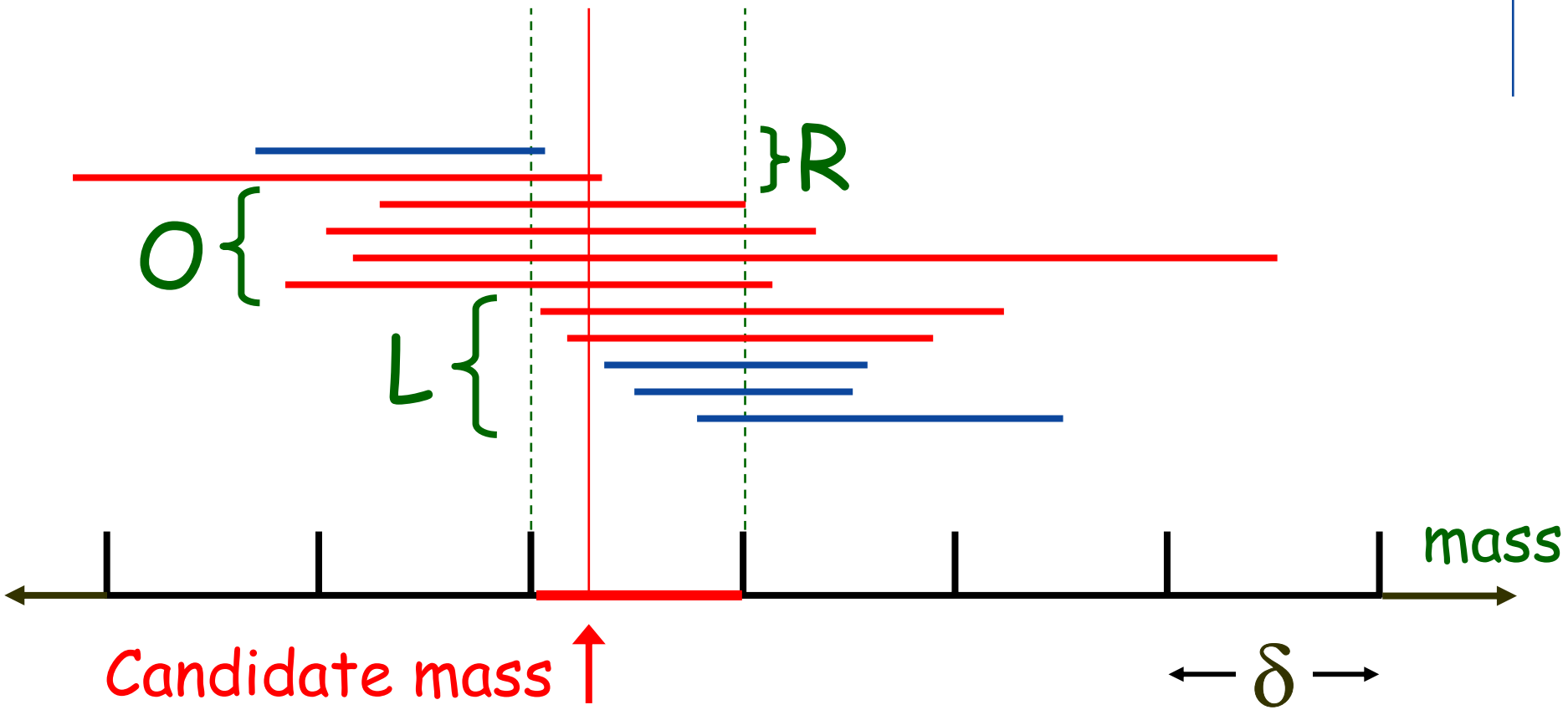
Fast Query Mass Lookup



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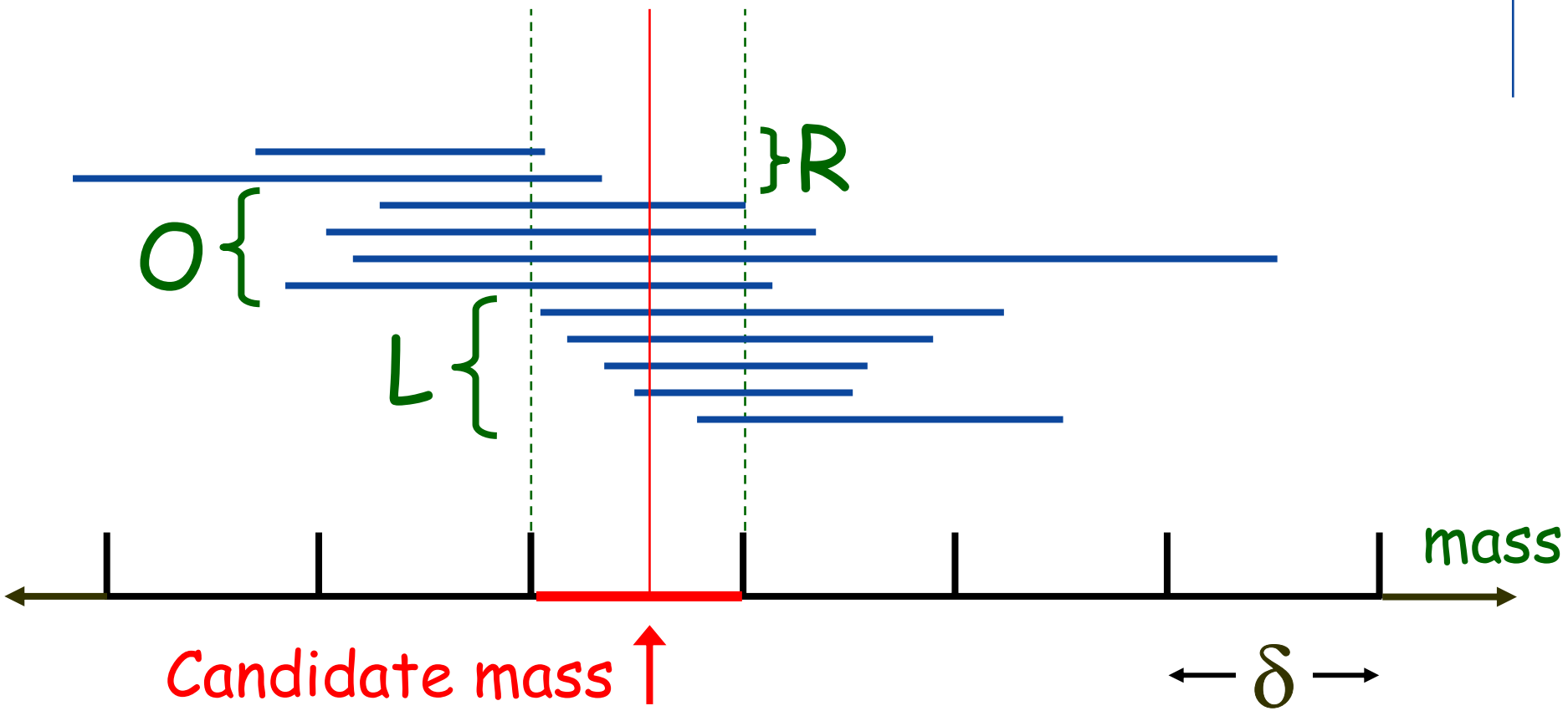
Fast Query Mass Lookup



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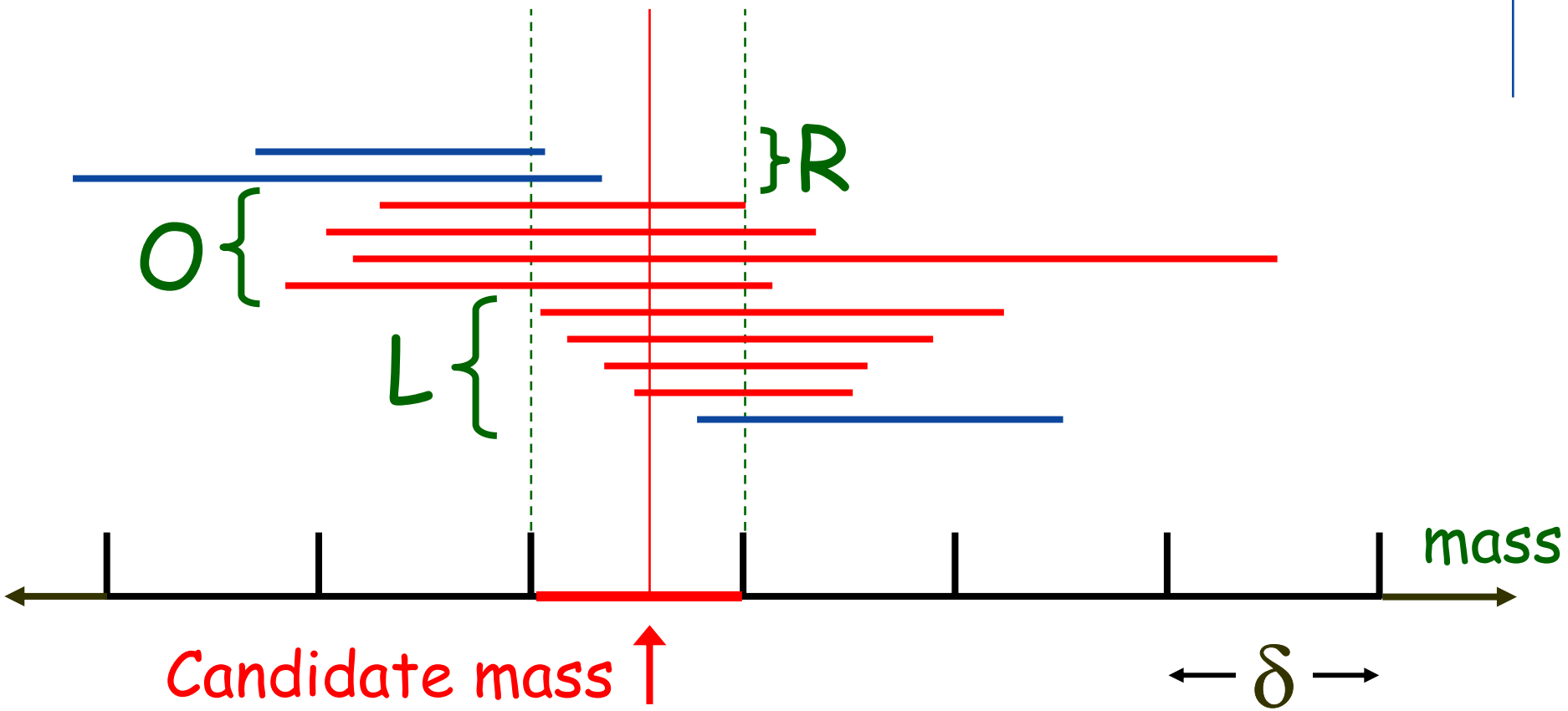
Fast Query Mass Lookup



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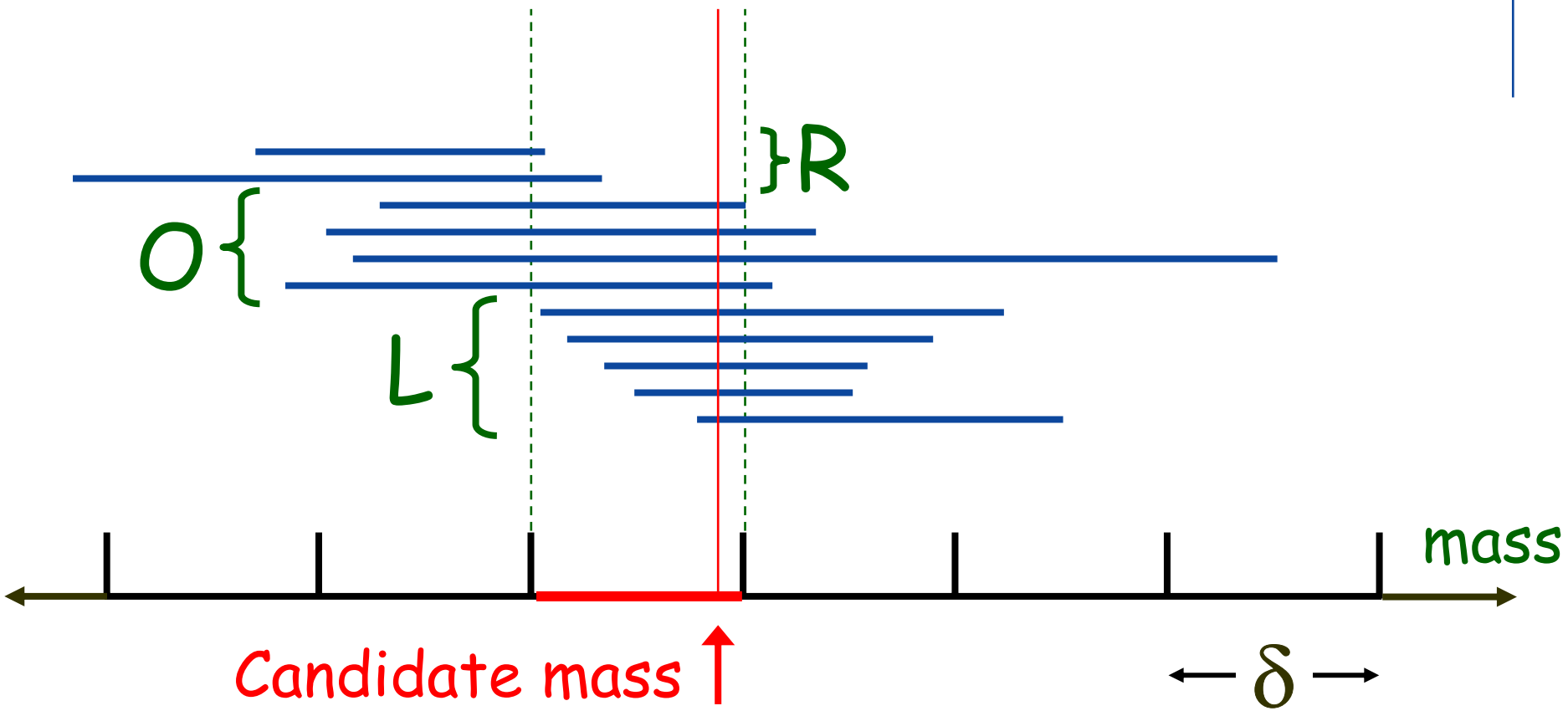
Fast Query Mass Lookup



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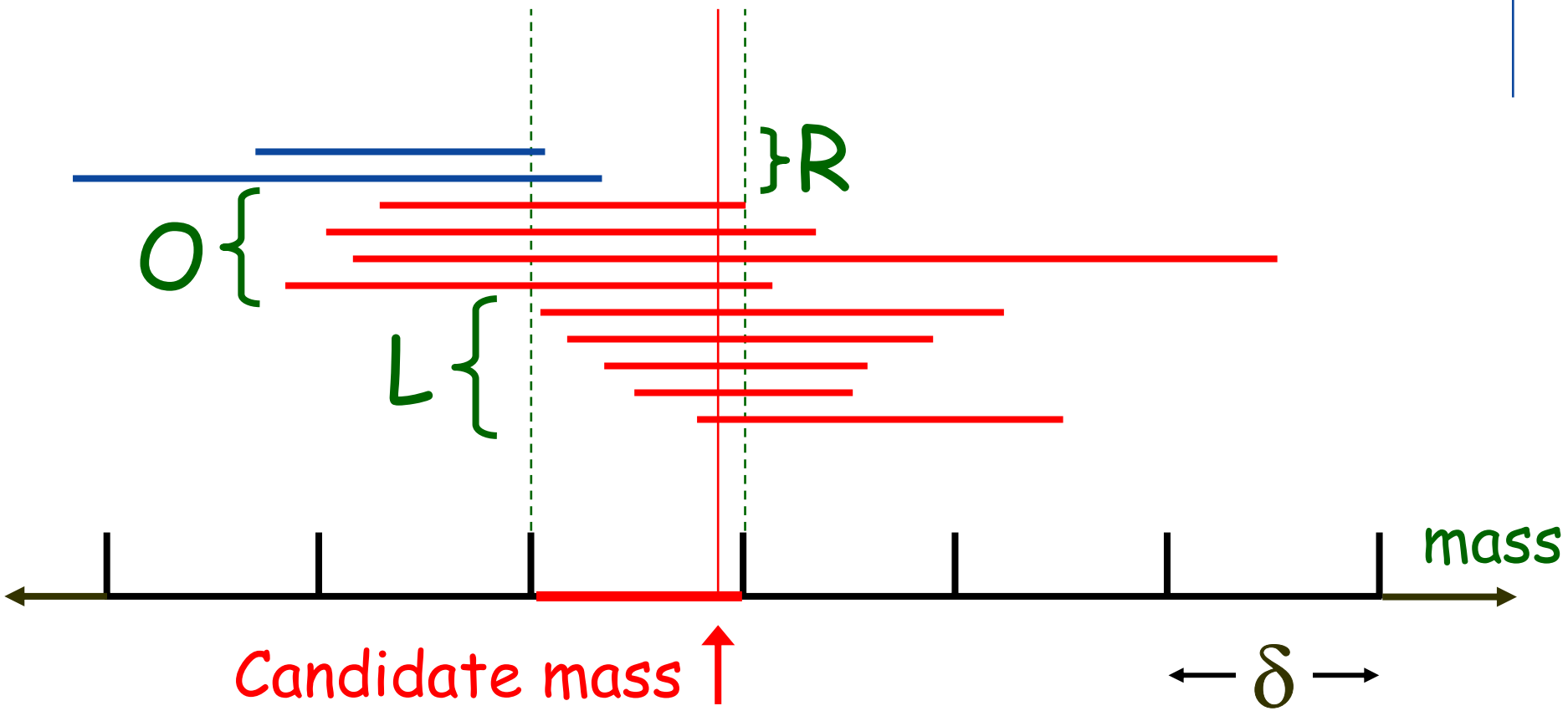
Fast Query Mass Lookup



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Fast Query Mass Lookup

- Must have $\delta \cdot I_{min}$
- Table size is $O(M_{max}/\delta + k\, I_{max}/\delta)$
- Practical for typical parameters

- Running time:

  Table construction + $O(n\, L\, \rho\, O)$

  is dominated by size of output

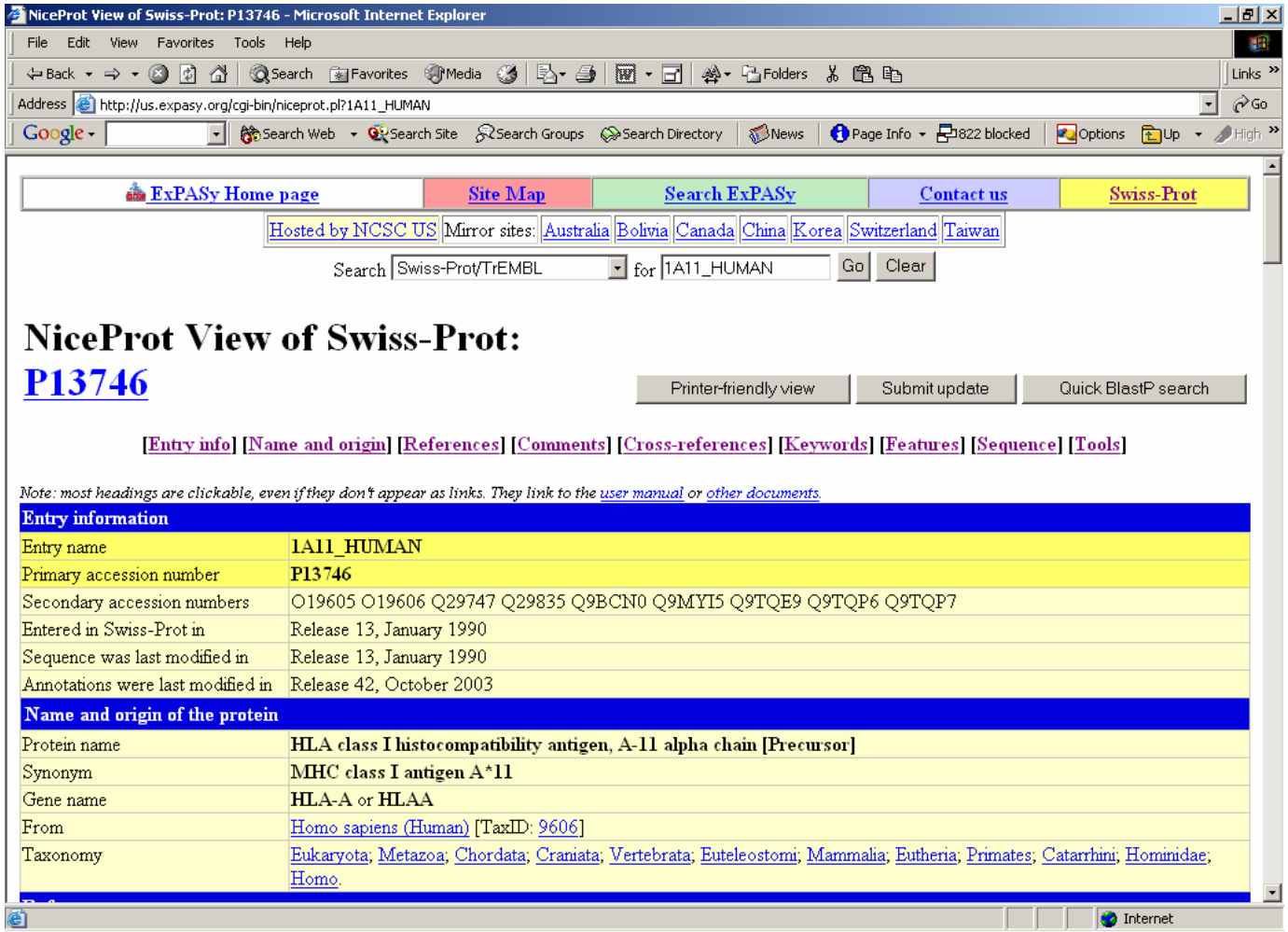
Observations

- Peptide candidate generation is a key subproblem.
- Must eliminate substring redundancy.
- As k increases, peptide candidate generation becomes an interval lookup problem.
- Run time dominated by output size.

Sequence Database Search Engines

- What if peptide isn't in database?
- Need richer set of peptide candidates
  - Protein isoforms, sequence variants, SNPs, alternate splice forms
  - Some have phenotypic or clinical annotations

Swiss-Prot



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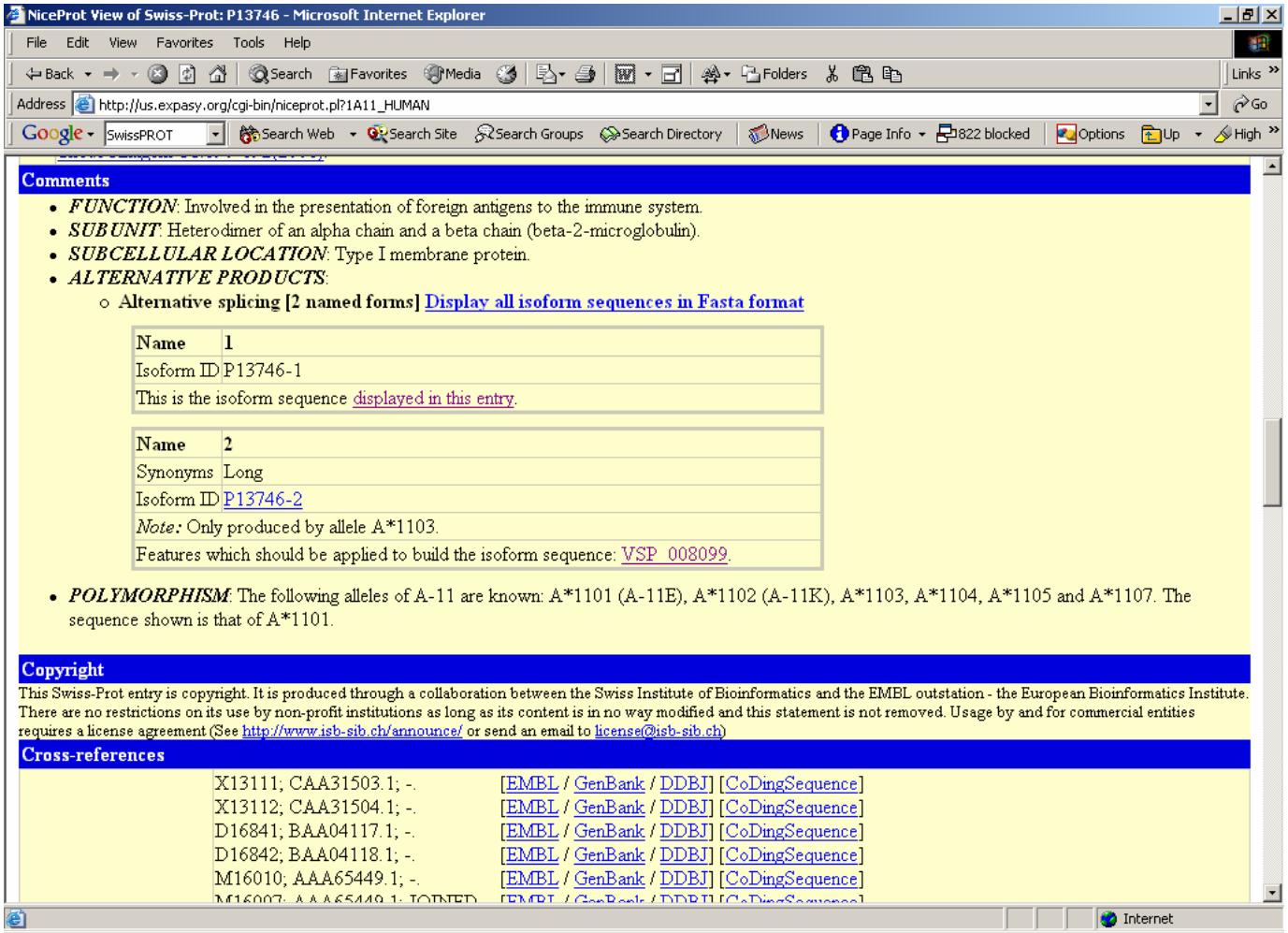
Swiss-Prot Variant Annotations



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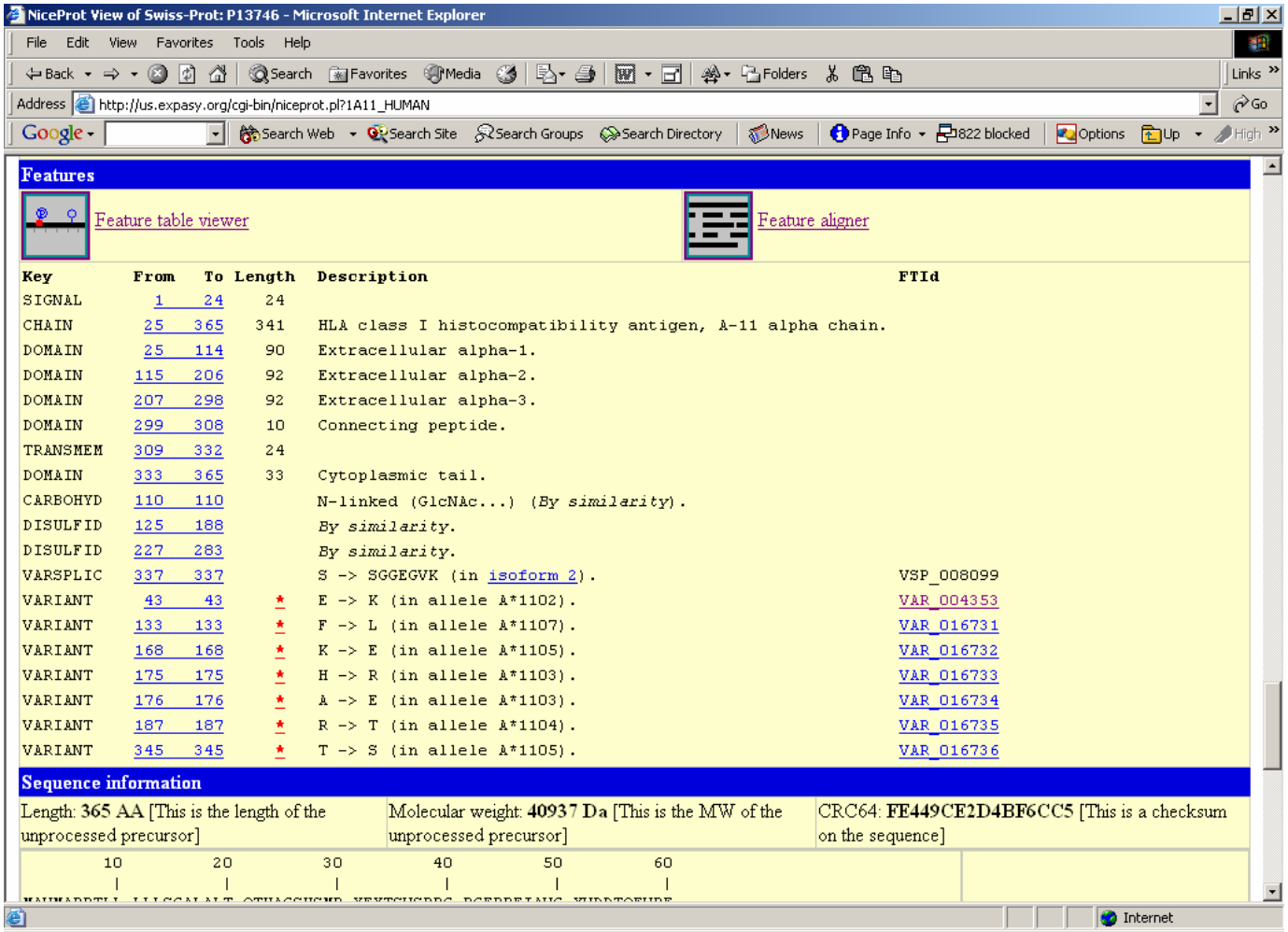

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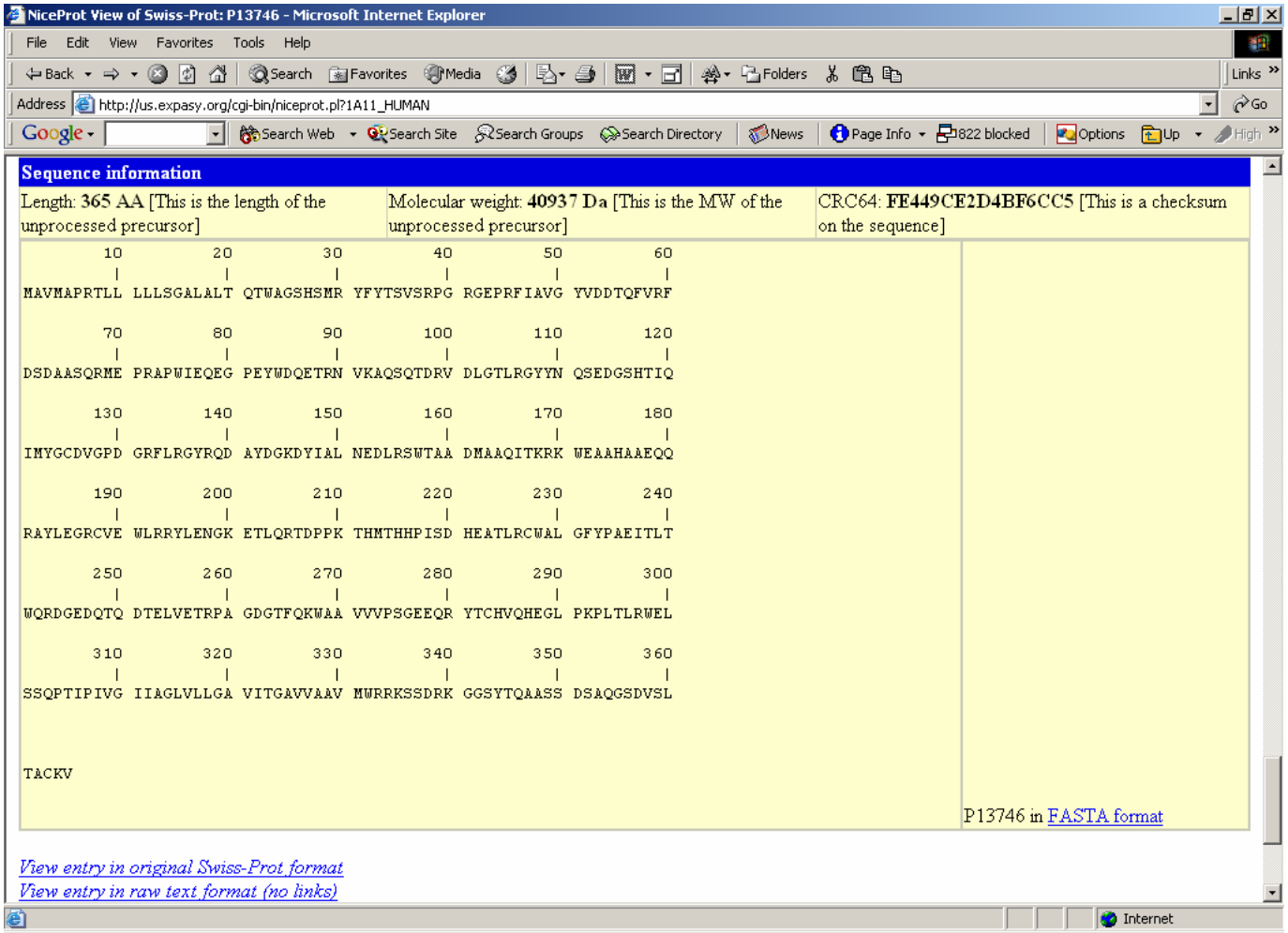
Swiss-Prot Sequence



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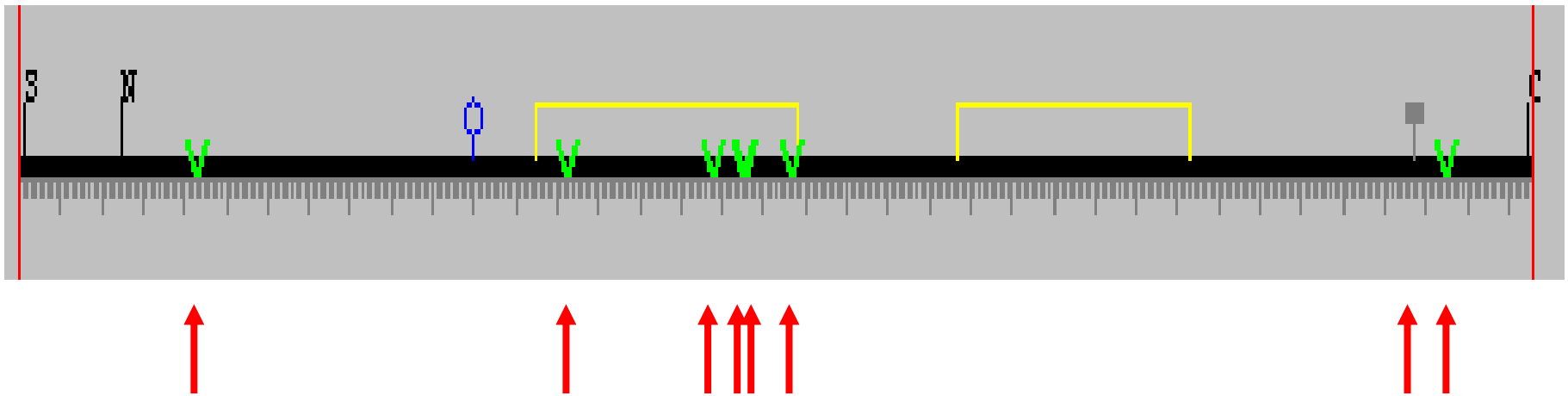
Swiss-Prot

- VarSplic enumerates all variants, conflicts, isoforms
- Swiss-Prot sequence size:
  - 56 Mb
- VarSplic sequence size:
  - 90 Mb
- How many more peptide candidates?

Swiss-Prot
Variant Annotations

Feature viewer



Variants

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Swiss-Prot VarSplic Output

```
P13746-00-01-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-01-01-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-00-00-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-00-03-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-01-03-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-00-04-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGKPRFIAVGYVDDTQFVRF
P13746-01-04-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGKPRFIAVGYVDDTQFVRF
P13746-00-05-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-01-05-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-01-00-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-00-02-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
P13746-01-02-00      MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRF
                     ****************************************:*****************
```

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Swiss-Prot VarSplic Output

```
P13746-00-01-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYTQAASSDSAQ
P13746-01-01-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYTQAASSDSAQ
P13746-00-00-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYTQAASSDSAQ
P13746-00-03-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYTQAASSDSAQ
P13746-01-03-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYTQAASSDSAQ
P13746-00-04-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYTQAASSDSAQ
P13746-01-04-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYTQAASSDSAQ
P13746-00-05-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYTQAASSDSAQ
P13746-01-05-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYTQAASSDSAQ
P13746-01-00-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYTQAASSDSAQ
P13746-00-02-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSS------DRKGGSYSQAASSDSAQ
P13746-01-02-00      SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSYSQAASSDSAQ
                     ************************************       *******:*********
```

Peptide Candidates

- Parent ion
  - Typically < 3000 Da
- Tryptic Peptides
  - Cut at K or R
- Search engines
  - Don't handle > 4+ well
  - Long peptides don't fragment well
- # of distinct 30-mers upper bounds total peptide content

Peptide Candidates

- At most 2% additional peptides in ~ 1.6 times as much sequence

Sequence Database	Swiss-Prot	VarSplic
Size	56 Mb	90 Mb
30-mers (N_{30})	44 Mb	45 Mb
Overhead	27%	97%

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Sequence Database Compression

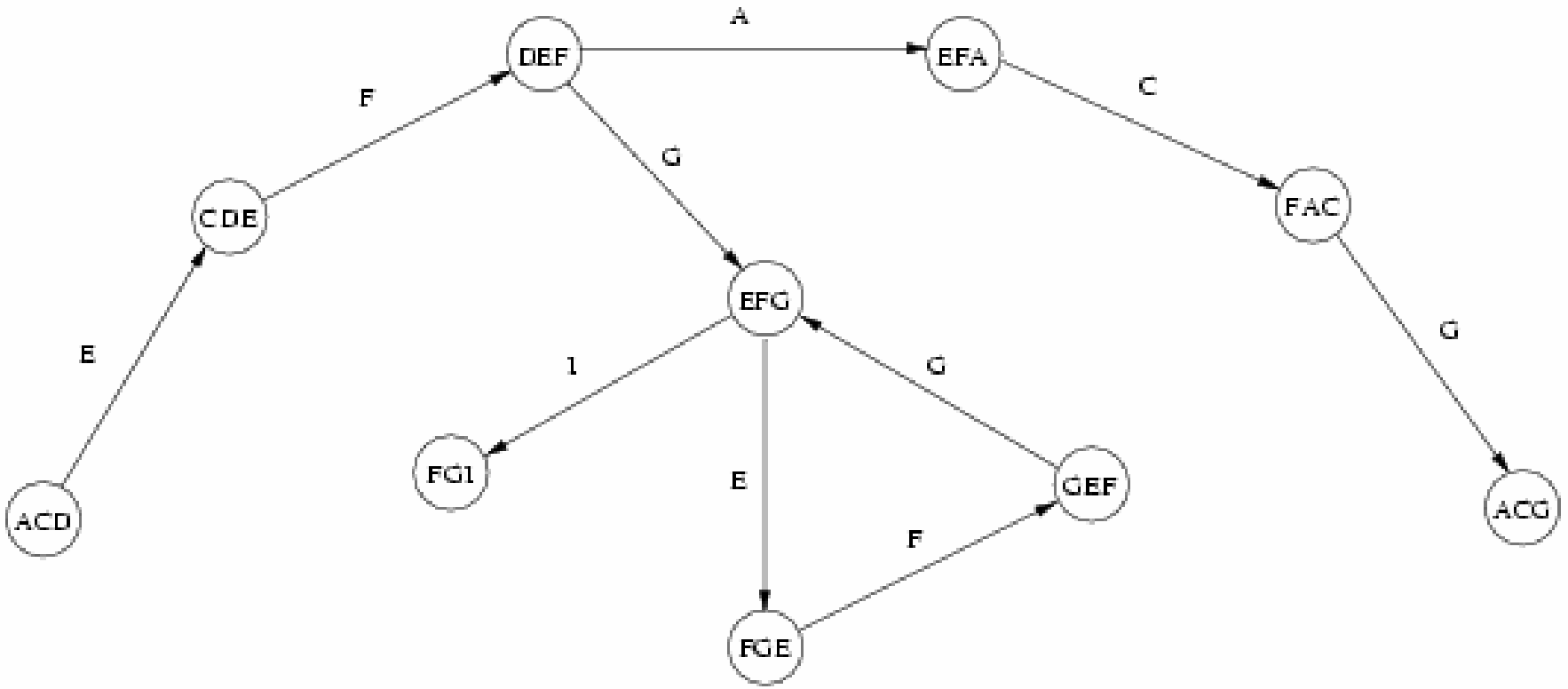
Construct sequence database that is

- Complete
  - All 30-mers are present
- Correct
  - No other 30-mers are present
- Compact
  - No 30-mer is present more than once

Sequence Database Compression

Sequence Database	Swiss-Prot	VarSplic
Original Size	55 Mb	90 Mb
Distinct 30-mers	44 Mb	45 Mb
Overhead	27%	97%
C^3 Size	53 Mb	54 Mb
C^3 Overhead	19%	20%
C^3 Compression	93%	61%
Compression LB	79%	51%

SBH-graph



ACDEFGI, ACDEFACG, DEFGEFGI

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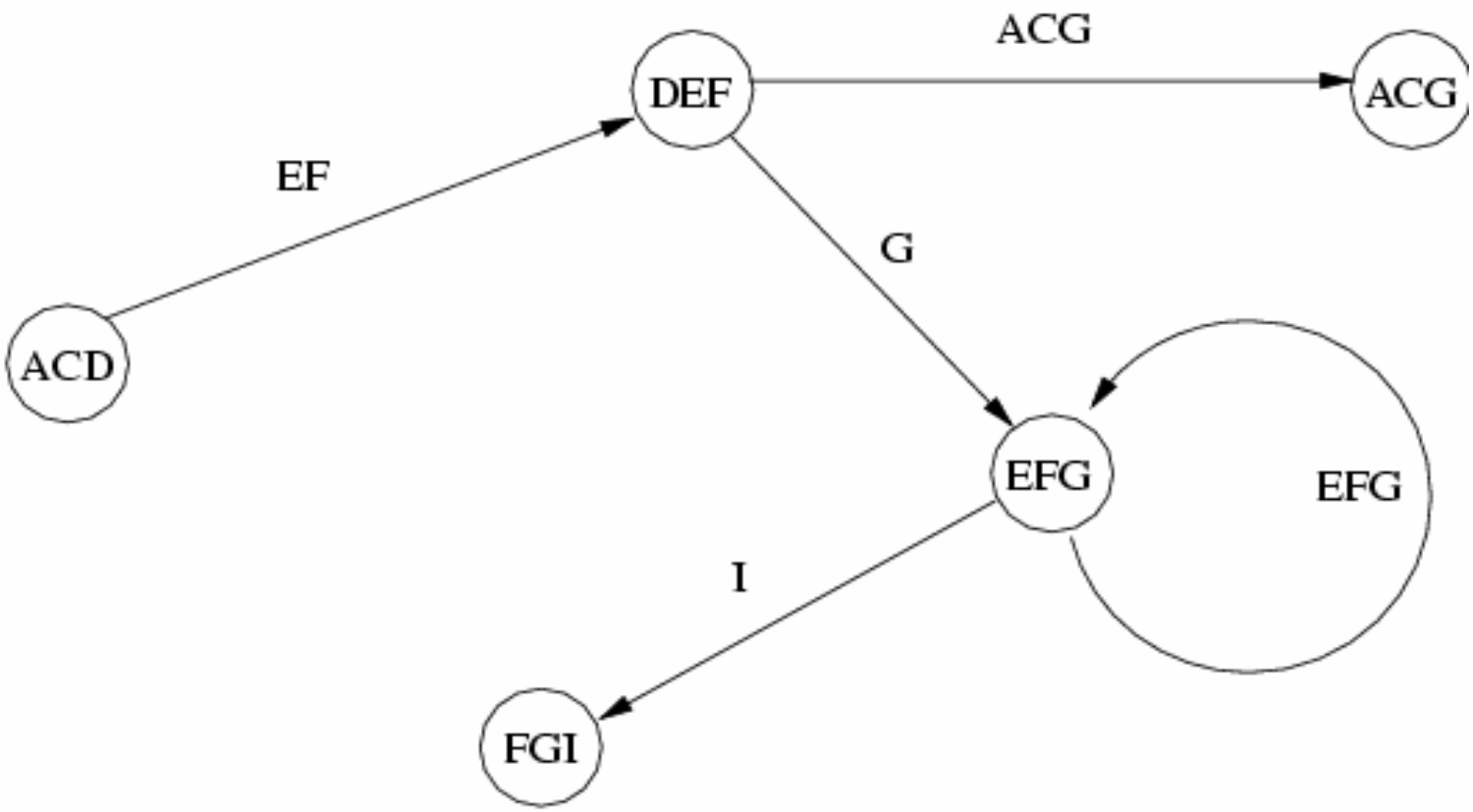
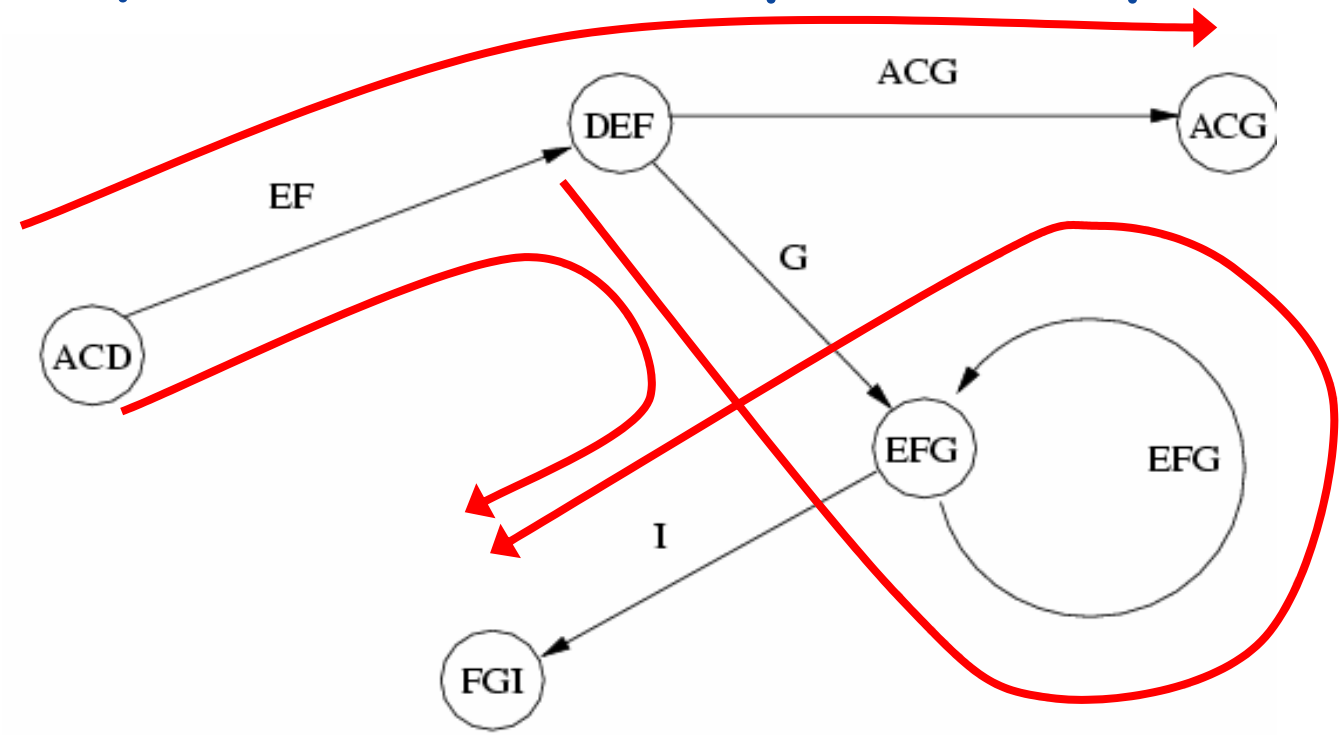
Compressed SBH-graph



ACDEFGI, ACDEFACG, DEFGEFGI

Sequence Databases & CSBH-graphs

- Sequences correspond to paths



ACDEFGI, ACDEFACG, DEFGEFGI

Sequence Databases & CSBH-graphs

- Complete
  - All edges are on some path
- Correct
  - Output path sequence only
- Compact
  - No edge is used more than once

- C^3 Path Set uses all edges exactly once.

Size of C^3 Path Set for k-mers

- Each path costs
  - (k-1)-mer + path sequence + EOS
- Sequence database with p paths
  - $N_k + p\ k$

- Minimize sequence database size by minimizing number of paths
  - subject to C^3 constraints

Best case senario…

…if CSBH-graph admits an Eulerian path.

Sequence database size

$$(k-1) + N_k + 1$$

How many paths are required if the CSBH-graph is not Eulerian?

Non-Eulerian Components

- Net degree
  - b(v) = # in edges - # out edges
- Total degree surplus
  - $B_+ = \sum_{b(v)>0} b(v)$
- For each path
  - Start node's net degree +1
  - End node's net degree -1
  - Otherwise, net degree: no change
- To reduce all nodes to net degree 0, must have at least B_+ paths.

Components w/ $B_+(C) == 0$

- Balanced component must have Eulerian tour, so require exactly one path.
- m balanced components

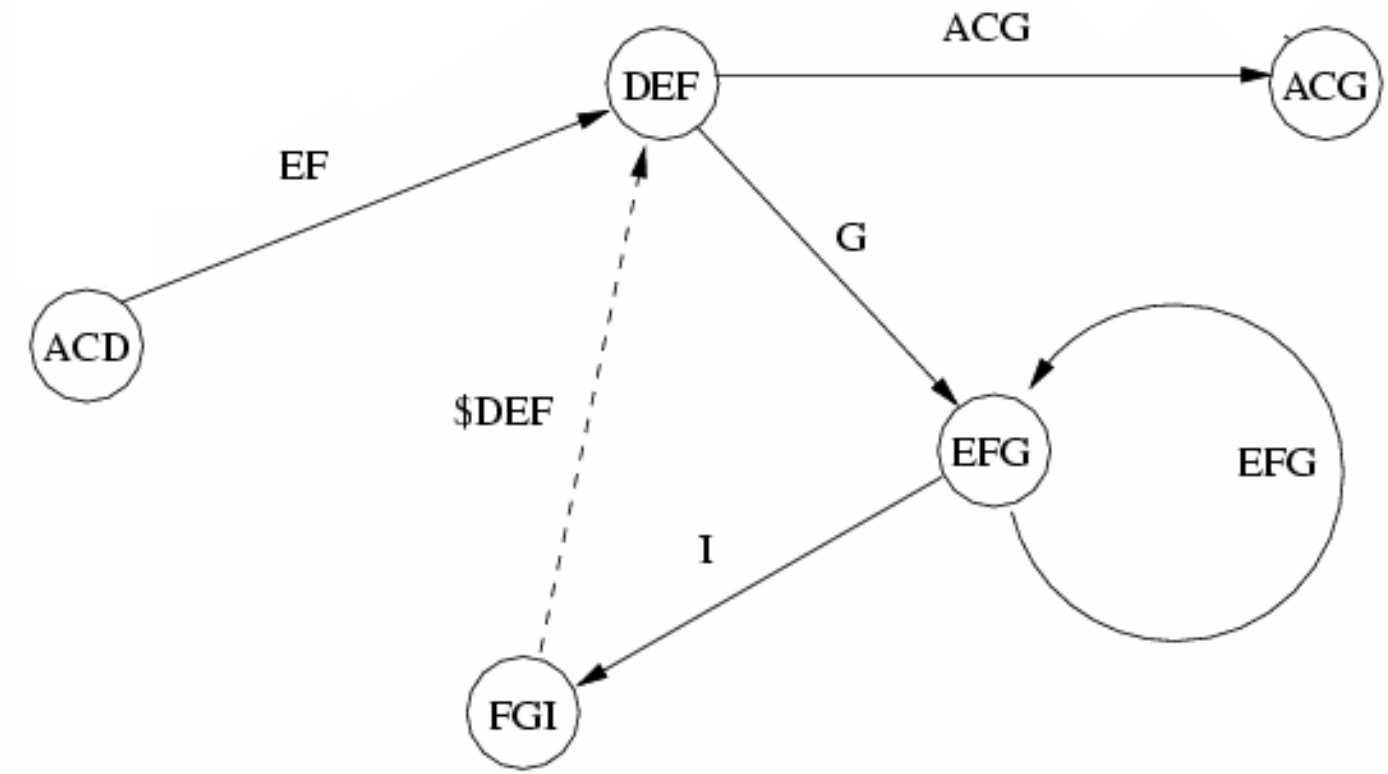
Paths Lower Bound

The C^3 path set must contain at least $B_+ + m$ paths.

This lower bound is achievable!

Just add $(B_+ - 1)$ "restart" edges to non-Eulerian components

Achieving Path Lower Bound



AA Sequence Databases

Sequence Database	Sequence Length	Distinct 30-mers	Overhead
IPI-HUMAN	20358846	12115520	68%
IPI	54145883	29769766	81%
Swiss-Prot	56454588	44374286	27%
Swiss-Prot-VS	89541275	45307827	97%
UniProt	472581860	274510105	72%
UniProt-VS	506796094	275391669	84%
MSDB	481919777	276523755	74%
NRP	495502241	283160529	75%
NCBI-nr	619132252	378721915	63%
UnionNR	674700840	385369671	75%
Union	2157353500	385369671	460%

Minimum Size C^3 Sequence Database

Sequence Database	C^3 30-mer Enumeration	Overhead	Compression	Compression Bound
IPI-HUMAN	13854679	14.35%	68.05%	59.51%
IPI	37961385	27.52%	70.11%	54.98%
Swiss-Prot	52662145	18.68%	93.28%	78.60%
Swiss-Prot-VS	54534356	20.36%	60.90%	50.60%
UniProt	337119564	22.81%	71.34%	58.09%
UniProt-VS	338890778	23.06%	66.87%	54.34%
MSDB	342924164	24.01%	71.16%	57.38%
NRP	351600578	24.17%	70.96%	57.15%
NCBI-nr	463517034	22.39%	74.87%	61.17%
UnionNR	473665310	22.91%	70.20%	57.12%
Union	473665310	22.91%	21.96%	17.86%

Implementation

- Suitable for use by Mascot, SEQUEST, …
  - FASTA format
- All connection to protein context is lost
  - Must do exact string search to find peptides in original database

Extensions

- Drop compactness constraint!
  - Reuse edges rather than starting a new path
  - Similar to the "Chinese Postman Problem"
  - Solvable to optimality using a network flow formulation.

Other Ideas

- We can drop correctness too!
  - Equivalent to shortest substring on the set of 30-mers
- 30-mer subsets
  - …containing two tryptic sites?
  - …containing Cysteine?
- Smaller suffix-tree oracles for short queries