Getting statistical significance and Bayesian confidence limits for your hidden Markov model results, with pairwise alignment of nucleotide sequences as an example

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Not just hidden Markov models:

```
GCGAA--CGACGTCAGGCAGA---TCTAGA
CCGAAGCCGA-GCCGGG--AAGCGTGTTGA
```

$m = 25$, $n = 27$

You can do #1, but want to do #2 and #3:

Example: Sequence Alignment

1. For two sequences, of lengths m and n, what is the optimal alignment A and what is its score S?
2. Is S statistically significant given m and n? — is it unlikely to arise with random sequences?
3. Is A credible? — are other plausible alignments of these sequences substantially the same?

You can do #1, but want to do #2 and #3:

Example: Word Wrapping Text

1. For a paragraph of words, what is the optimal way to divide them into lines A, and how pretty is it S?
   E.g., $S = -\sum w_i^2$, where $w_i =$ spaces added to line i
2. Is S unusual? — Is this paragraph of words particularly hard (or easy) to wrap?
3. Is A special? — are other reasonable word wrappings of these words similar?

You can do #1, but want to do #2 and #3:

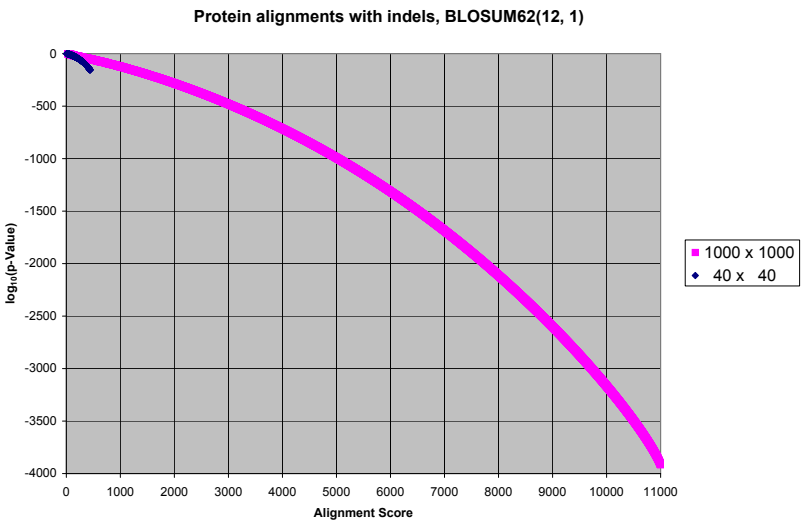
Problem Statement

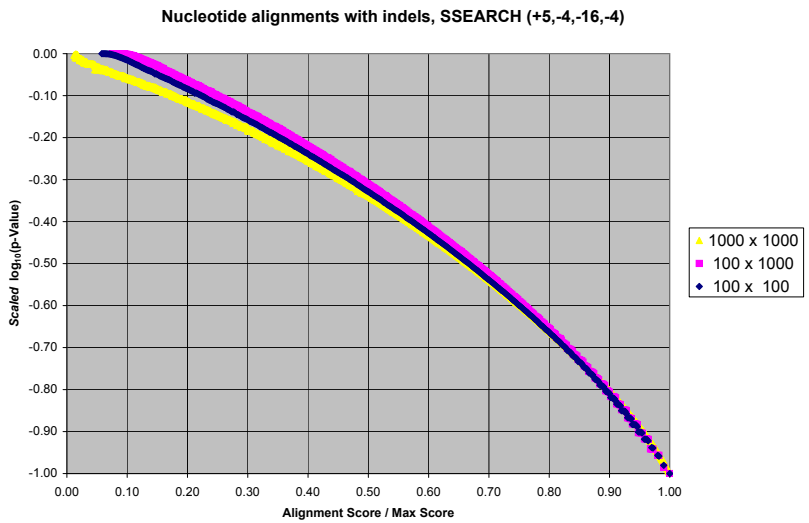
1. Optimization: Find and evaluate an optimum using a dynamic programming algorithm, hidden Markov model, or partition function calculation.
2. Hypothesis Testing: What is the probability that random inputs would score as well? Null distribution. p-value.
3. Bayesian Confidence Limits (a.k.a. Credibility Limits):
   - What fraction of solution space has exactly d differences from the optimum, for $d = 0, \ldots, d_{\max}$. Difference distribution.
   - How many differences must be allowed to capture 95% of solution space? 95% credibility limit.

Results: Statistical Significance vs. Score

For Smith & Waterman (1981) sequence alignment, score and statistical significance are related, but . . .

- relationship is non-trivial and depends upon input size.





Protein-protein alignment

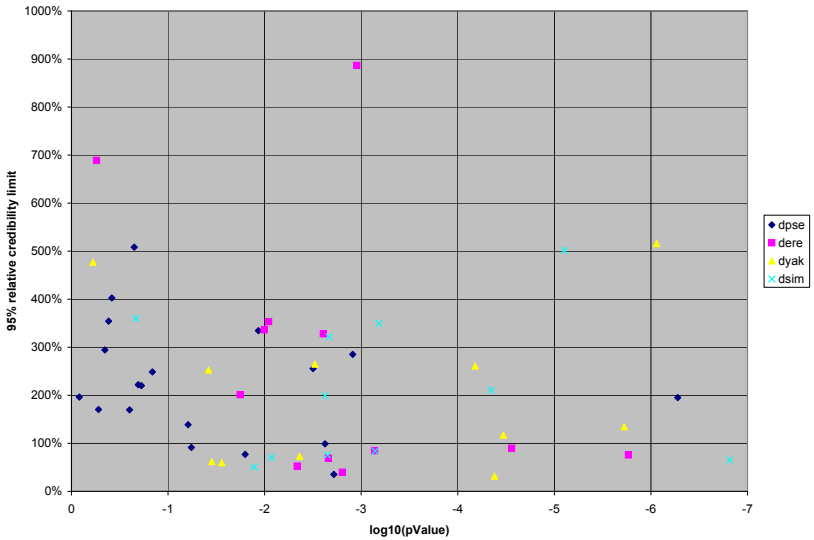
Nucleotide-nucleotide alignment

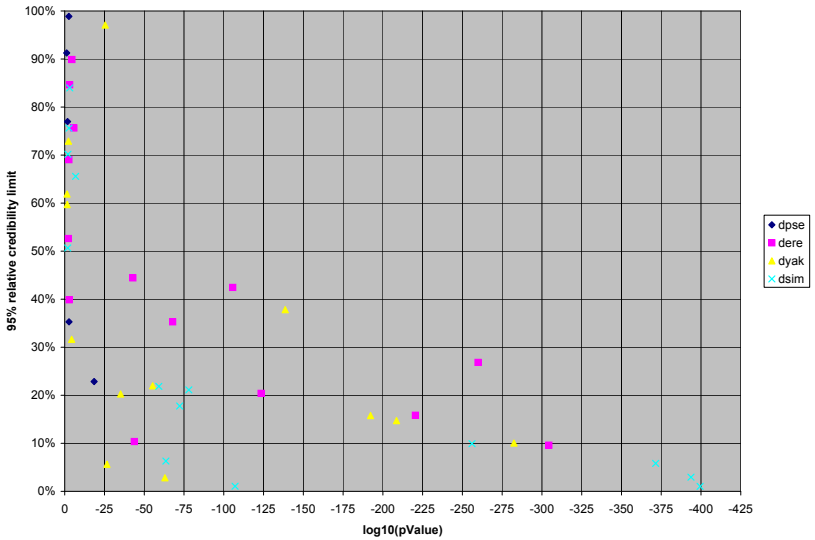
Compare: Karlin & Altschul (1990)

Results: Credibility vs. Statistical Significance

Significance and Bayesian confidence are related, but . . .

- poor credibility exists even at superb *p*-values.

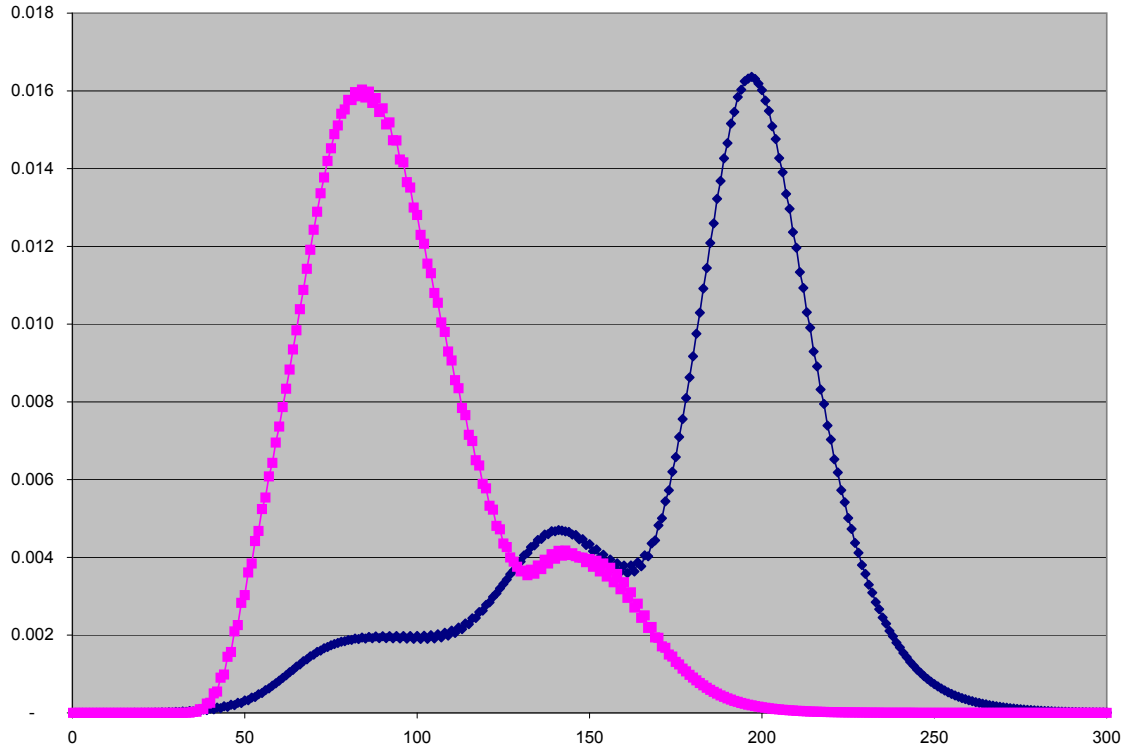




20 gene promoters of *Drosophila melanogaster* aligned to orthologous regions in four other fly genomes.

Results: Distribution of Differences

For Smith-Waterman sequence alignment, the distribution of differences can have a rich structure.



Orthologous Human (1677 nt) vs. Mouse (1666 nt).
Viterbi(Dark) = 450 bp, Centroid(Light) = 438 bp.

Algorithms for Discrete High-Dimensional Inference

Many problems are tackled with dynamic programming:

Hidden Markov Model

- Sequence alignment: HMMER
- Protein folding: HMMSTR / ROSETTA

Partition Function Computation / Markov Random Field

- RNA secondary structure: Sfold

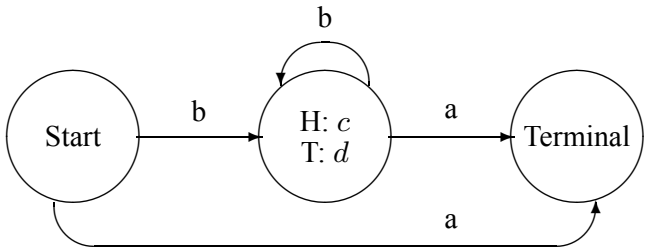
Maximum Probability / Maximum Score / Minimum Energy

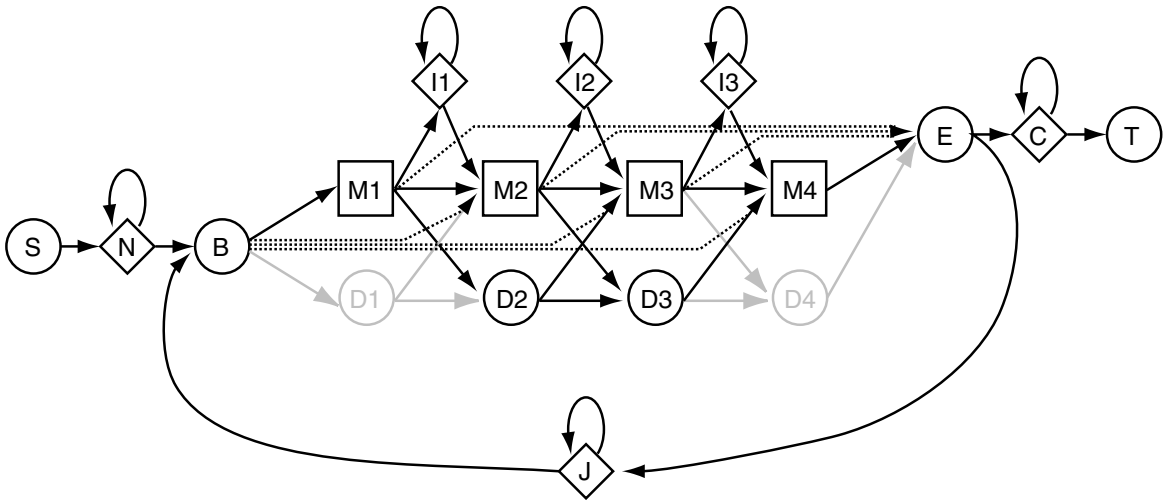
- Viterbi (1967) algorithm
- Seq. Alignment: Smith-Waterman, Needleman-Wunch
- RNA secondary structure: Mfold

Collectively, *Hidden Boltzmann Models*

Hidden Boltzmann Models

Flipping a biased coin





A Plan7 Profile-HMM (Eddy, 2003)

Emission vs. Evaluation. Also, Viterbi vs. Forward

Estimating Statistical Significance

Naïve Sampling

1. Generate some random examples from the null.
2. Observe the fraction that score as well as your result.

Need $\mathcal{O}(1/p)$ samples for a small p-value. ☹

Importance Sampling

Similar to simulated annealing.

0. Establish a probability model, if absent.
1. Choose a temperature.
2. Generate random samples at the new temperature.
3. Compute temperature-corrected fraction $\geq$ your result.

Need 100–10,000 samples, even for $p = 10^{-4000}$. ☺

Newberg (2008, 2009) Warning: 3 pages of math follow

0. Establish a Probability Model

An *emission path* through the computation has a . . .

Dynamic programming algorithm: score, computed by addition of encountered transition and emission scores.

HMM (or Partition function): (unnormalized) probability or odds ratio, computed by multiplication.

Convert a Dynamic Programming Algorithm To Multiplications

- For each score s, instead use an unnormalized probability

$$Z = \exp(\lambda s) \; .$$

  E.g., $\lambda = \ln(10)/5$ gives $Z \mapsto 10Z$ when $s \mapsto s + 5$.
- Addition of scores $\rightarrow$ multiplication of Zs.
- Maximum of scores $\rightarrow$ addition of Zs.

1. Choose a Temperature

Use a reasonable *ad hoc* procedure to obtain T.

- Generally, want 20-60% of instances $\geq$ your result.

2. Generate Samples

Goal: Instead of from the null, generate input instances from a temperature-biased distribution. *E.g.*, generate a pair of sequences (x, y) for alignment, with a bias towards higher scoring pairs.

Outline of approach:

1. Raise each transition and emission probability to power $1/T$. (Like thermodynamics.)
2. Compute partition function (*i.e.*, sum over all emission paths) that also sums out over all possible emissions.

3. Compute Temperature-Corrected Fraction

Defining $\Theta(\text{true}) = 1$ and $\Theta(\text{false}) = 0$:

Importance Sampling

$$p(Z_0) = \sum_{\text{all } (x,y)} \Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0)$$

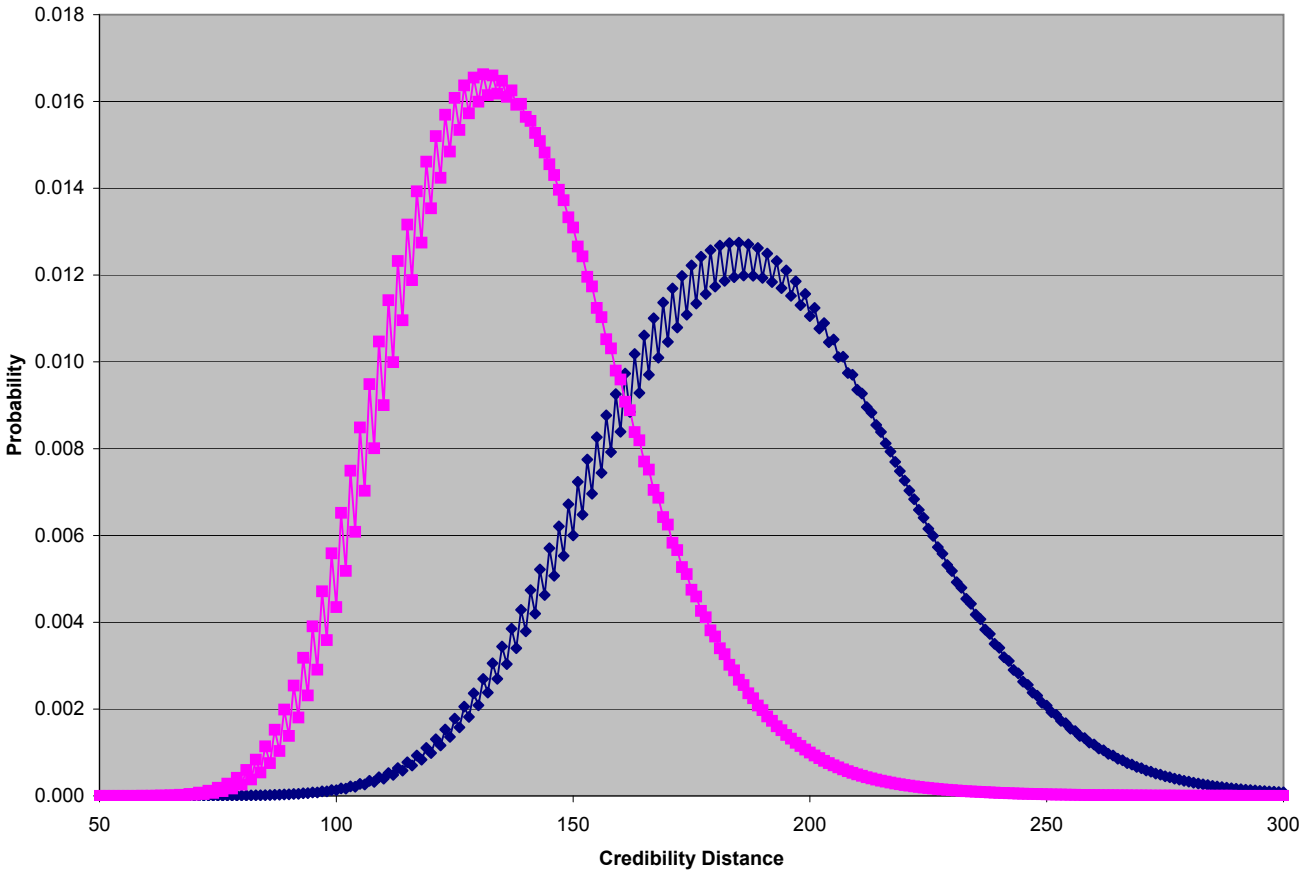
$$p(Z_0) = \sum_{\text{all } (x,y)} \Pr_T(x,y)\frac{\Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0)}{\Pr_T(x,y)}$$

$$\widehat{p(Z_0)} = \frac{1}{N}\sum_{(x,y)\sim\Pr_T} \frac{\Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0)}{\Pr_T(x,y)}$$

Done with statistical significance!

Computing Bayesian Confidence Limits

How do we efficiently compute this (or its cumulative form)?



Newberg & Lawrence (2009)

Bayesian Confidence Limits

0. Establish a probability model, if absent.
1. Choose an integer difference measure.

Use *Sampling Approach* (Webb-Robertson *et al.*, 2008), *Direct Approach*, *Polynomial Approach*, or

Fourier Transform Approach

2. Choose an integer (with only small factors) that is a little larger than the maximum number of differences.
3. Run modified *forward algorithm* to compute each Fourier transform coefficient (in parallel).
4. Fourier transform the coefficients.

Direct Approach

4 pages of math begin here, but don't tune out yet.

Unaltered Sequence Alignment Algorithm (Simplified)

Algorithm's typical step looks something like:

$$\begin{aligned} Z[i,j] = \; & Z[i-1,j-1]\,Z_M(x_i,y_j) + \\ & Z[i-1,j]\,Z_D(x_i) + \\ & Z[i,j-1]\,Z_I(y_j) \end{aligned}$$

Goal is $Z[m,n]$, where m and n are input strings' lengths.

Direct Approach

Recap: Unaltered Algorithm

$$\begin{aligned} Z[i,j] = \; & Z[i-1,j-1]\,Z_M(x_i,y_j) + \\ & Z[i-1,j]\,Z_D(x_i) + \\ & Z[i,j-1]\,Z_I(y_j) \end{aligned}$$

Difference Distribution via the Direct Approach

Number of ways to get differences d. Typical step:

$$\begin{aligned} Z[i,j,d] = \; & Z[i-1,j-1,d-\Delta_M(i,j)]\,Z_M(x_i,y_j) + \\ & Z[i-1,j,d-\Delta_D(i)]\,Z_D(x_i) + \\ & Z[i,j-1,d-\Delta_I(j)]\,Z_I(y_j)\ , \end{aligned}$$

where Δ is the number of new differences.
Goal is $Z[m,n,d]$ for all possible total differences d.
Requires increased runtime and memory. ☹

Polynomial Approach

Recap — Difference Distribution via the Direct Approach:
$Z[m,n,d]$ is number of ways to get score d.

$$\begin{aligned} Z[i,j,d] = \; & Z[i-1,j-1,d-\Delta_M(i,j)]\,Z_M(x_i,y_j) + \\ & Z[i-1,j,d-\Delta_D(i)]\,Z_D(x_i) + \\ & Z[i,j-1,d-\Delta_I(j)]\,Z_I(y_j)\ . \end{aligned}$$

Difference Distribution via the Polynomial Approach

$P[i,j]$ is a polynomial in indeterminant ω that "packs" the $Z[i,j,d]$ values. Define $P[i,j] = \sum_d Z[i,j,d]\omega^d$. Typical step:

$$\begin{aligned} P[i,j] = \; & P[i-1,j-1]\,Z_M(x_i,y_j)\,\omega^{\Delta_M(i,j)} + \\ & P[i-1,j]\,Z_D(x_i)\,\omega^{\Delta_D(i)} + \\ & P[i,j-1]\,Z_I(y_j)\,\omega^{\Delta_I(j)}\ . \end{aligned}$$

Seeking $P[m,n]$ polynomial.
Still increased runtime and memory. ☹

Fourier Transform Approach

Recap — Difference Distribution via the Polynomial Approach: $P[m,n]$ is a polynomial that packs the difference distribution.

$$\begin{aligned} P[i,j] = \; & P[i-1,j-1]\, Z_M(x_i,y_j)\, \omega^{\Delta_M(i,j)} + \\ & P[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(i)} + \\ & P[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(j)} \; . \end{aligned}$$

Difference Distribution via the Fourier Transform Approach

Can recover coefficients of $P[m,n]$ with via its valuation at sufficiently many points. Its value for a fixed ω is from:

$$\begin{aligned} C[i,j] = \; & C[i-1,j-1]\, Z_M(x_i,y_j)\, \omega^{\Delta_M(i,j)} + \\ & C[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(i)} + \\ & C[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(j)} \; . \end{aligned}$$

Coefficients recovery is efficient via Discrete Fourier Transform, so let $\{\omega_0,\ldots,\omega_{r-1}\}$ be the rth complex roots of unity.

function ComputeScoreDistribution
for $k \in \{0, \ldots, r-1\}$
    $\omega = \cos(2\pi k/r) + i \sin(2\pi k/r)$
    $f(k) =$ BackgroundExec(CalcFourier(ω))
WaitForBackgroundProcesses
return DiscreteFourierTransform(f)

function CalcFourier(ComplexNumber ω)
for $i \in \{0, \ldots, m\}$
    for $j \in \{0, \ldots, n\}$
        $C[i,j] = C[i-1,j-1]\, Z_M(x_i, y_j)\, \omega^{\Delta_M(x_i,y_j)} + C[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(x_i)}$
            $+ C[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(y_j)}$
return $C[m,n]$

- Serial algorithm has original memory requirement. ☺
- Parallel algorithm has (nearly) original runtime. ☺

Concluding Observations

For dynamic programming algorithms, hidden Markov models, and partition function calculations

- . . . optimum score, statistical significance (*p*-value), and credibility / Bayesian confidence limits are not fungible.

Solutions

In many cases, if you can optimize score then you can

- . . . estimate even a very extreme *p*-value.
- . . . calculate the difference distribution and credibility limits.

Links `http://www.rpi.edu/~newbel/publications/`

Statistical Significance of sequence alignments: Newberg (2008)
Statistical Significance of hidden Boltzmann models: Newberg (2009)

Credibility: Newberg & Lawrence (2009)