Estimating Statistical Significance for Reverse-sequence Null Models

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Outline of Talk

- What is a null model?
- Why use the reverse-sequence null?
- Two approaches to statistical significance.
- What distribution do we expect for scores?
- Fitting the distribution.
- Does calibrating the E-values help?

Scoring HMMs and Bayes Rule

- The *model* M is a computable function that assigns a probability $\text{Prob}\left(A \mid M\right)$ to each string A.
- When given a string A, we want to know how likely the model is. That is, we want to compute something like $\text{Prob}\left(M \mid A\right)$.
- Bayes Rule:

$$\text{Prob}\left(M \mid A\right) = \text{Prob}\left(A \mid M\right) \frac{\text{Prob}(M)}{\text{Prob}(A)} \; .$$

- Problem: $\text{Prob}(A)$ and $\text{Prob}(M)$ are inherently unknowable.

Null models

- Standard solution: ask how much more likely M is than some *null hypothesis* (represented by a *null model*).

$$\frac{\text{Prob}\,(M \mid A)}{\text{Prob}\,(N \mid A)} = \frac{\text{Prob}\,(A \mid M)}{\text{Prob}\,(A \mid N)} \, \frac{\text{Prob}(M)}{\text{Prob}(N)} \; .$$

- $\frac{\text{Prob}(M)}{\text{Prob}(N)}$ is the *prior odds ratio*, and represents our belief in the likelihood of the model before seeing any data.
- $\frac{\text{Prob}\left(M|A\right)}{\text{Prob}\left(N|A\right)}$ is the *posterior odds ratio*, and represents our belief in the likelihood of the model after seeing the data.
- We can generalize to a forced choice among many models ($M_1, \ldots, M_n$)

$$\frac{\text{Prob}\,(M_i \mid A)}{\sum_j \text{Prob}\,(M_j \mid A)} = \frac{\text{Prob}\,(A \mid M_i)\,\text{Prob}(M_i)}{\sum_j \text{Prob}\,(A \mid M_j)\,\text{Prob}(M_j)} \; .$$

  The Prob(M_j) values can be scaled arbitrarily without affecting the ratio.

Standard Null Model

- Null model is an i.i.d (independent, identically distributed) model, that is, each letter is treated as being independently drawn from the background distribution.
- $$\text{Prob}\left(A \mid N, \text{len}\left(A\right)\right) = \prod_{i=1}^{\text{len}(A)} \text{Prob}(A_i)\ .$$
- $$\text{Prob}\left(A \mid N\right) = \text{Prob}(\text{string of length len}\left(A\right)) \prod_{i=1}^{\text{len}(A)} \text{Prob}(A_i)\ .$$
- The length modeling is often omitted, but one must be careful then to normalize the probabilities correctly.

Reversed model for null

- When using the standard null model, certain sequences and HMMs have anomalous behavior. Many of the problems are due to unusual composition—a large number of some usually rare amino acid.
- For example, metallothionein, with 24 cysteines in only 61 total amino acids, scores well on any model with multiple highly conserved cysteines.
- We avoid this (and several other problems) by using a reversed model M^r as the null model.
- The probability of a sequence in M^r is exactly the same as the probability of the reversal of the sequence given M.
- If we assume that M and M^r are equally likely, then

$$\frac{\text{Prob}\,(M \mid S)}{\text{Prob}\,(M^r \mid S)} = \frac{\text{Prob}\,(S \mid M)}{\text{Prob}\,(S \mid M^r)} \ .$$

- This method corrects for composition biases, length biases, and several subtler biases.

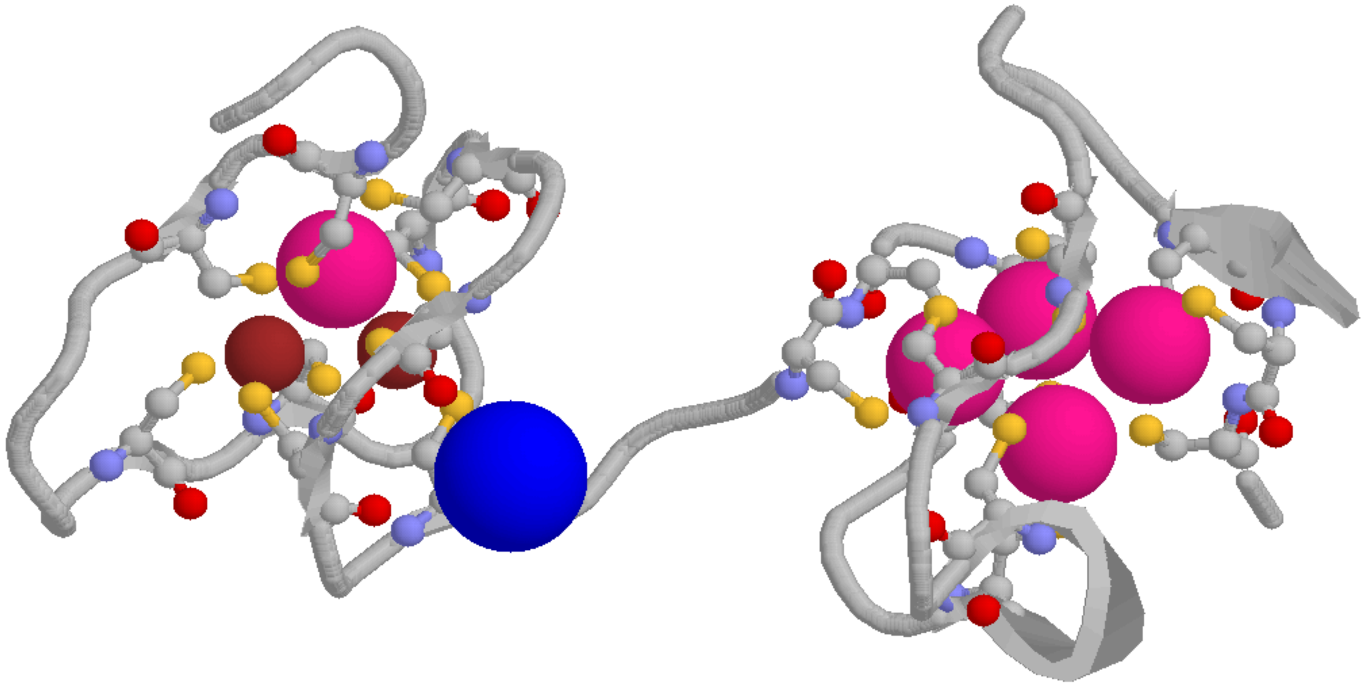
Composition as source of error

A cysteine-rich protein, such as metallothionein, can match any HMM that has several highly-conserved cysteines, even if they have quite different structures:

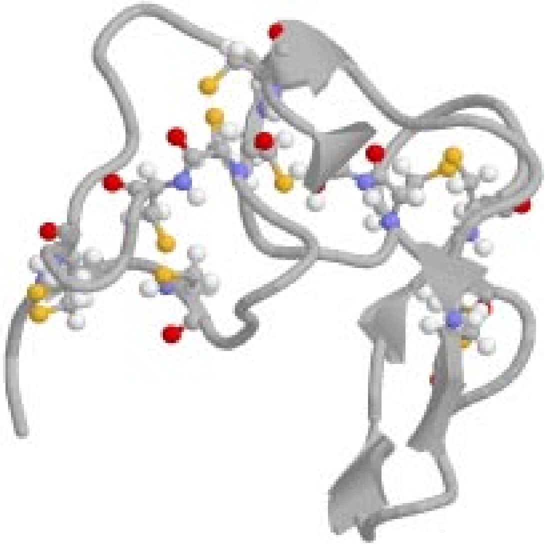
		cost in nats	
		model –	model –
HMM	sequence	standard null	reversed-model
1kst	4mt2	-21.15	0.01
1kst	1tabI	-15.04	-0.93
4mt2	1kst	-15.14	-0.10
4mt2	1tabI	-21.44	-1.44
1tabI	1kst	-17.79	-7.72
1tabI	4mt2	-19.63	-1.79

Composition examples

Metallothionein Isoform II (4mt2)

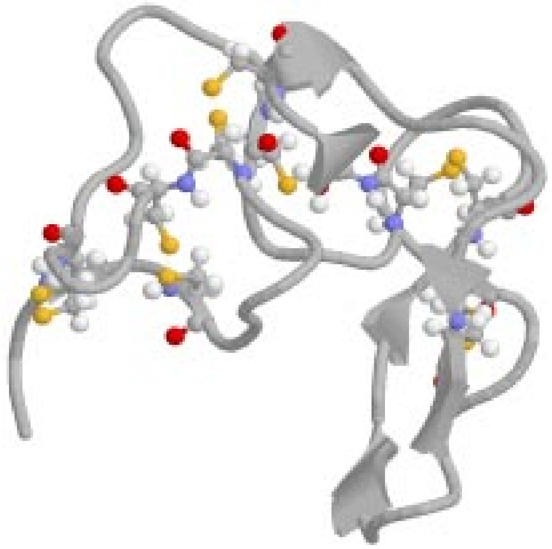


Kistrin (1kst)

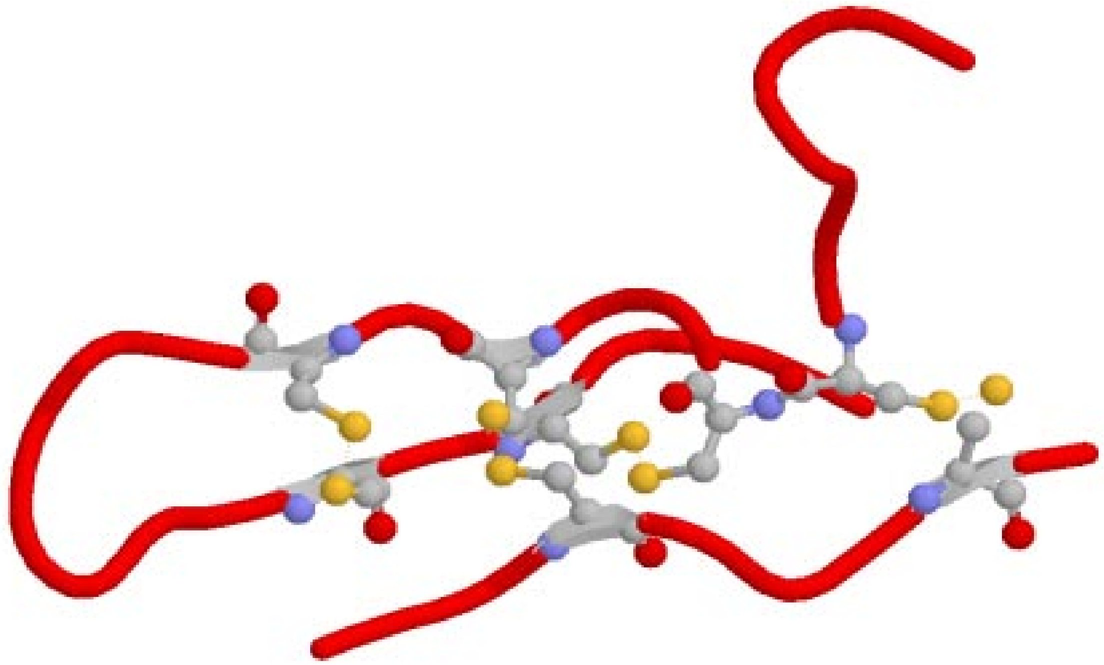


Composition examples

Kistrin (1kst)



Trypsin-binding domain of Bowman-Birk Inhibitor (1tabI)



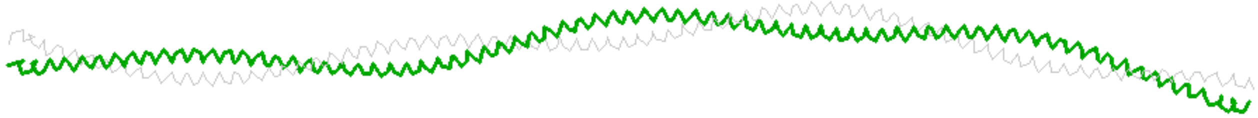
Long helices as source of error

Long helices can provide strong similarity signals from the periodic hydrophobicity, even when the overall folds are quite different:

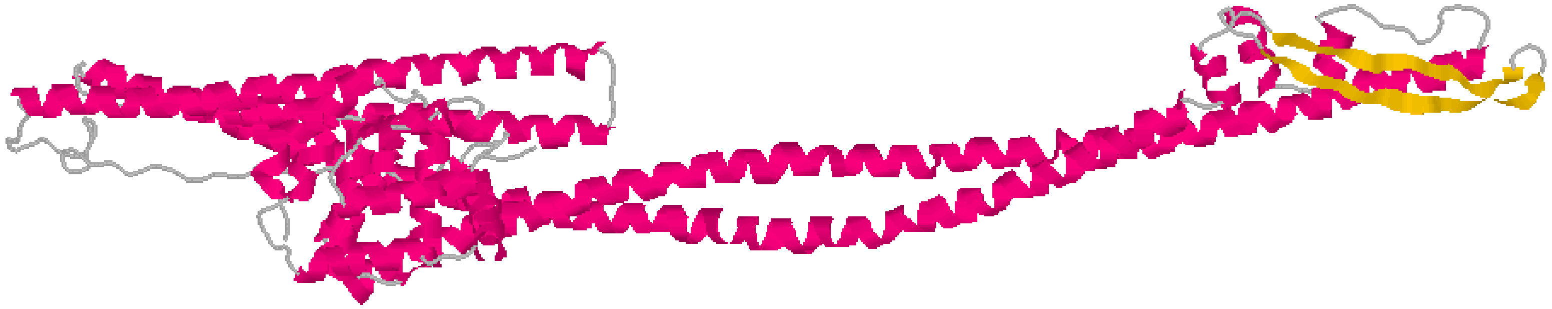
		cost in nats, normalized using	
HMM	sequence	Null model	reversed-model
1av1A	2tmaA	-22.06	2.13
1av1A	1aep	-21.25	1.03
1av1A	1cii	-13.67	-1.75
1av1A	1vsgA	-7.89	-0.51
2tmaA	1cii	-20.62	0.46
2tmaA	1av1A	-17.96	1.01
2tmaA	1aep	-12.01	0.78
2tmaA	1vsgA	-8.25	0.08
1vsgA	2tmaA	-14.82	-1.20
1vsgA	1av1A	-13.04	-2.68
1vsgA	1aep	-13.02	-3.52
1vsgA	1cii	-11.12	0.28
1aep	1av1A	-11.30	1.79
1aep	2tmaA	-10.73	1.06
1aep	1cii	-8.35	1.38
1aep	1vsgA	-6.87	0.53
1cii	2tmaA	-23.24	-1.48
1cii	1av1A	-19.49	-5.62
1cii	1aep	-12.85	-1.77
1cii	1vsgA	-10.20	-1.57

Helix examples

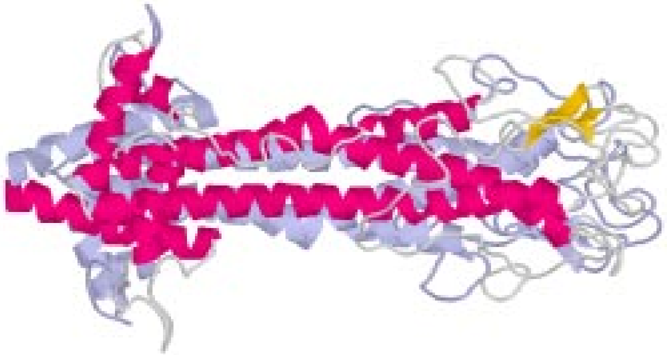
Tropomyosin (2tmaA)



Colicin Ia (1cii)

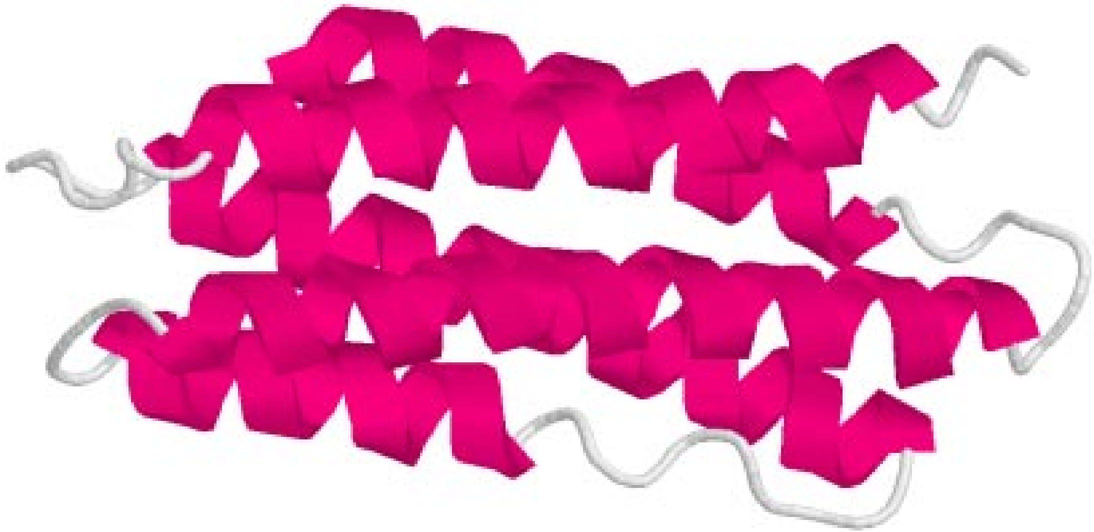


Flavodoxin mutant (1vsgA)

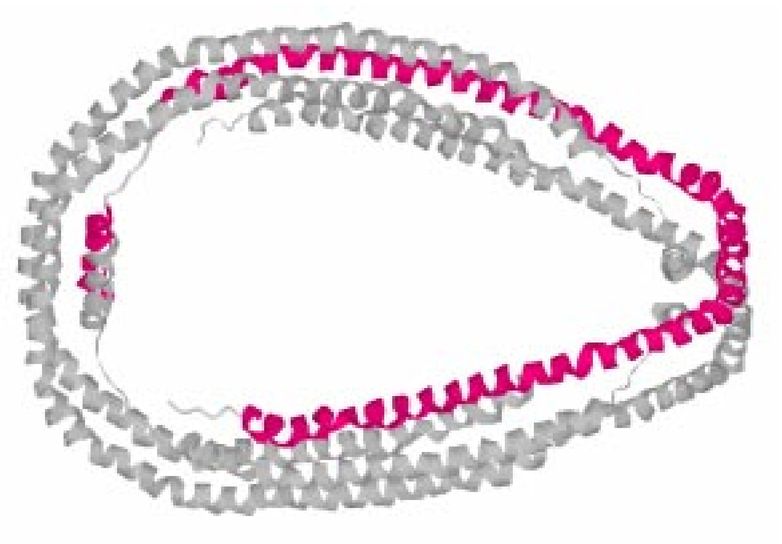


Helix examples

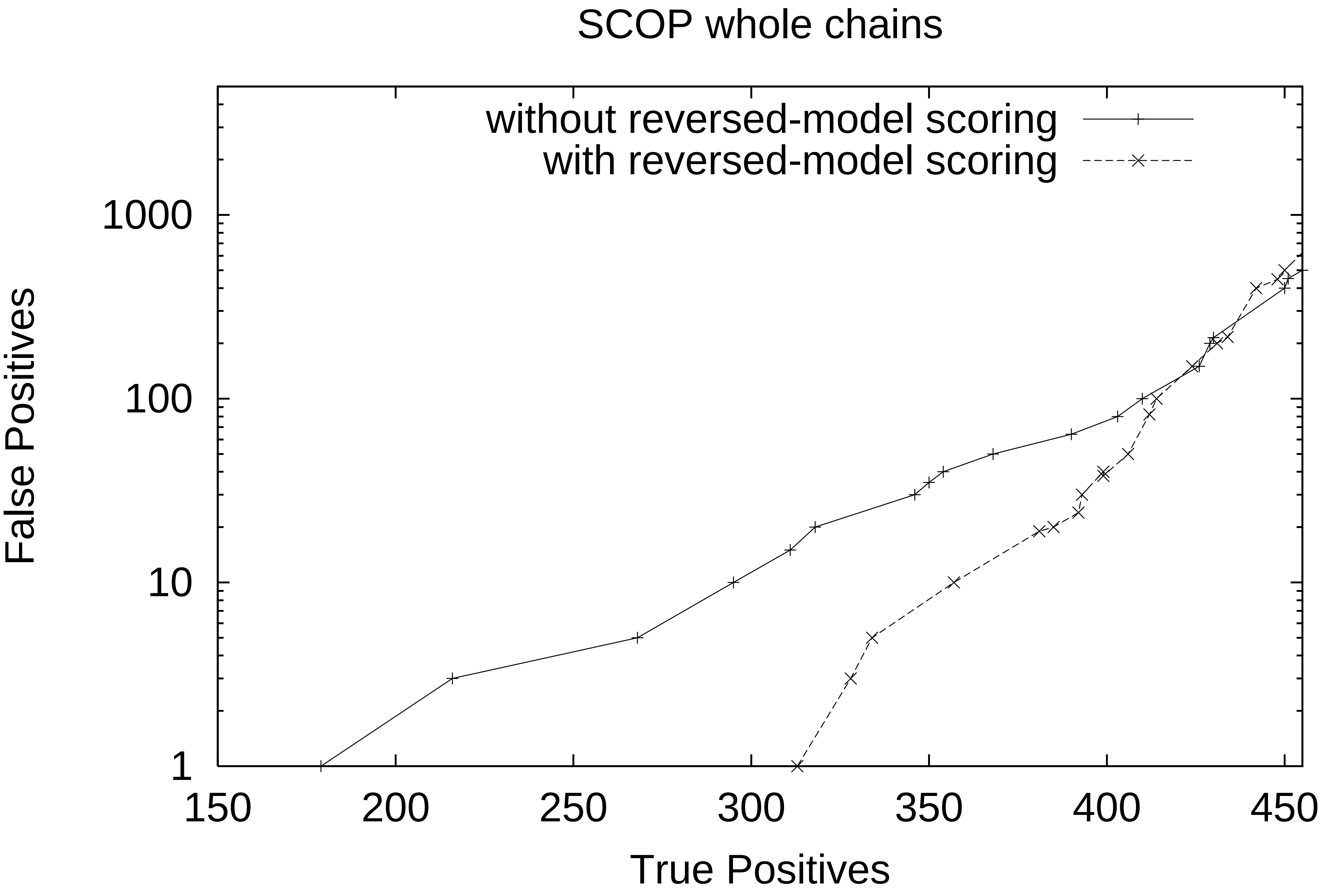
Apolipophorin III (1aep)



Apolipoprotein A-I (1av1A)



Discrimination Performance as a Function of Null Model



What is Statistical Significance?

- The statistical significance of a hit, P_1, is the probability of getting a score as good as the hit “by chance,” when scoring a single “random” sequence.
- When searching a database of N sequences, the significance is best reported as an E-value—the expected number of sequences that would score that well by chance: $E = P_1 N$.
- Some people prefer the p-value: $P_N = 1 - (1 - P_1)^N$, For large N, $P_N \approx 1 - e^{-E}$, so P_N is essentially the same as E for small E-values.
- I prefer to use E-values, because our best scores are often not significant, and it is easier to distinguish between E-values of 10, 100, and 1000 than between p-values of 0.999955, $1 -$ 4E-44, and $1 -$ 5E-435

Two Approaches to Statistical Significance

- (Markov's inequality) For any scoring scheme that uses
  $$\ln \frac{\text{Prob}\,(\text{seq} \mid M_1)}{\text{Prob}\,(\text{seq} \mid M_2)}$$
  the probability of a score better than T is less than e^{-T} for sequences distributed according to M_2. This method is independent of the actual probability distributions. We have had good results with this method.

- (Classical parameter fitting) If the "random" sequences are not drawn from the distribution M_2, but from some other distribution, then we can try to fit some parameterized family of distributions to scores from a random sample, and use the parameters to compute P_1 and E values for scores of real sequences.

  This calibration needs to be done for each model—which includes each setting of parameters, such as alignment style.

What family should we use for reverse-sequence null?

Bad assumption 1: The scores with a standard null model are distributed according to an extreme-value distribution:

$$P\left(\ln \text{Prob}\left(\text{seq} \mid M\right) > T\right) \approx G_{k,\lambda}(T) = 1 - \exp(-ke^{\lambda T})\ .$$

Bad assumption 2: The scores with the model and the reverse-model are independent of each other.

Result: The scores using a reverse-sequence null model are distributed according to a sigmoidal function:

$$P(\text{score} > T) = (1 - e^{\lambda T})^{-1}\ .$$

Derivation of sigmoidal distribution

(Derivation for *costs*, not *scores*, so more negative is better.)

$$\begin{aligned}
P(\text{cost} < T) &= \int_{-\infty}^{\infty} P(c_M = x) \int_{x-T}^{\infty} P(c_{M'} = y) dy dx \\
&= \int_{-\infty}^{\infty} P(c_M = x) P(c_{M'} > x - T) dx \\
&= \int_{-\infty}^{\infty} k\lambda \exp(-ke^{\lambda x}) e^{\lambda x} \exp(-ke^{\lambda(x-T)}) dx \\
&= \int_{-\infty}^{\infty} k\lambda e^{\lambda x} \exp(-k(1+e^{-\lambda T})e^{\lambda x}) dx
\end{aligned}$$

If we introduce a temporary variable to simplify the formulas: $K_T = k(1 + \exp(-\lambda T))$, then

$$\begin{aligned}
P(\text{cost} < T) &= \int_{-\infty}^{\infty} (1+e^{-\lambda T})^{-1} K_T \lambda e^{\lambda x} \exp(-K_T e^{\lambda x}) dx \\
&= (1+e^{-\lambda T})^{-1} \int_{-\infty}^{\infty} K_T \lambda e^{\lambda x} \exp(-K_T e^{\lambda x}) dx \\
&= (1+e^{-\lambda T})^{-1} \int_{-\infty}^{\infty} g_{K_T,\lambda}(x) dx \\
&= (1+e^{-\lambda T})^{-1}
\end{aligned}$$

Fitting λ

- The λ parameter simply scales the scores (or costs) before the sigmoidal distribution, so λ can be set by matching the observed variance to the theoretically expected variance.
- The mean is theoretically (and experimentally) zero.
- The variance is easily computed, though derivation is messy:

$$E(c^2) = (\pi^2/3)\lambda^{-2} \ .$$

- λ is easily fit by matching the variance:

$$\lambda \approx \pi \sqrt{N/(3 \sum_{i=0}^{N-1} c_i^2)} \ .$$

Two-parameter family

- We made two dangerous assumptions: extreme-value and independence.
- To give ourselves some room to compensate for deviations from these assumptions, we can add another parameter to the family.
- We can replace $-\lambda T$ with any strictly decreasing odd function of T with range $[-\infty, +\infty]$, and still get a probability distribution.
- Somewhat arbitrarily, we chose

  $$-\operatorname{sign}(T)|\lambda T|^{\tau}$$

  so that we could match a “stretched exponential” tail.

Fitting a two-parameter family

- For our two-parameter symmetric distribution, we can fit using 2nd and 4th moments:

$$\begin{aligned} E(c^2) &= \lambda^{-2/\tau} K_{2/\tau} \\ E(c^4) &= \lambda^{-4/\tau} K_{4/\tau} \end{aligned}$$

  where K_x is a constant:

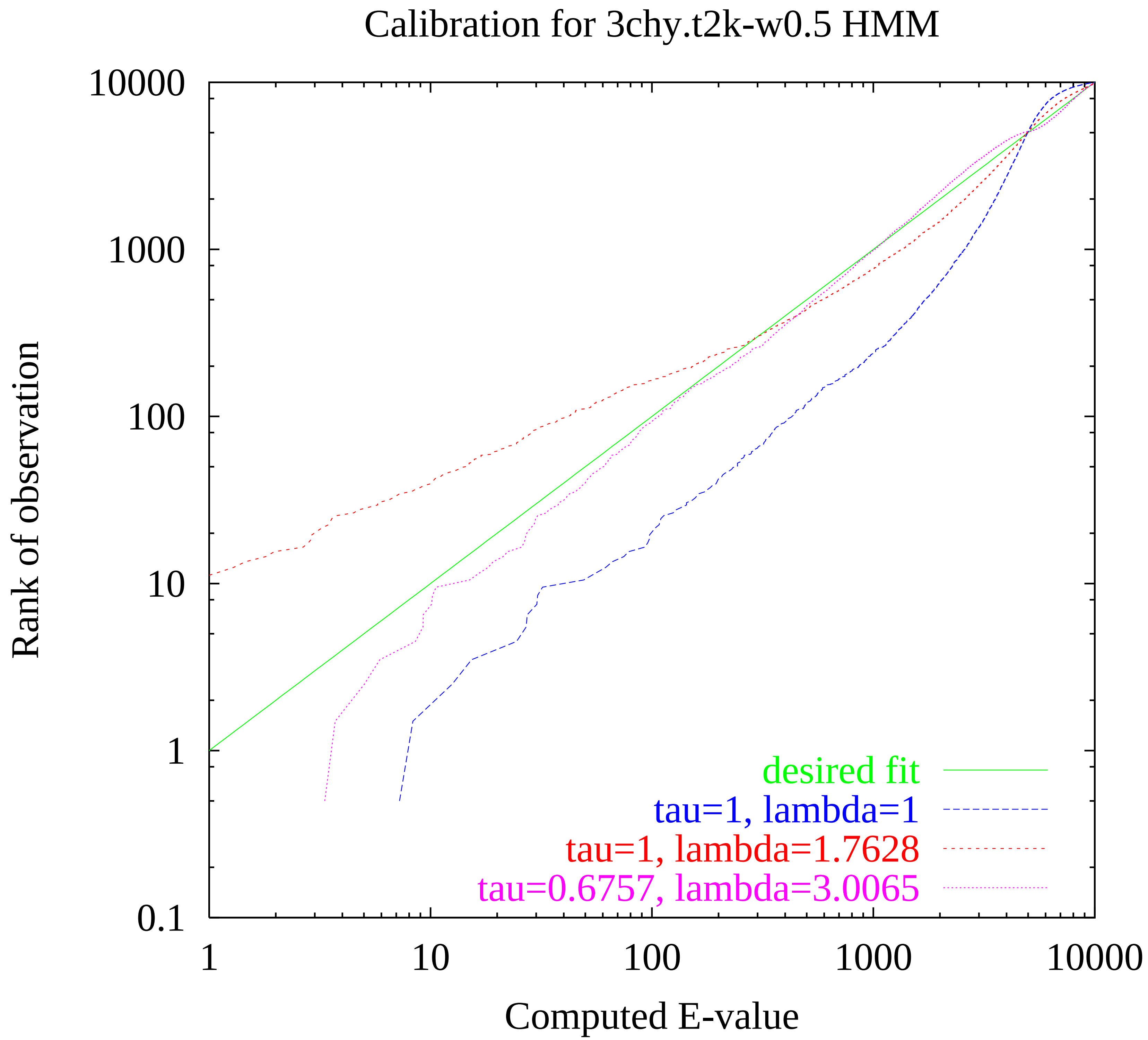
$$\begin{aligned} K_x &= \int_{-\infty}^{\infty} y^x (1+e^y)^{-1}(1+e^{-y})^{-1} dy \\ &= -\Gamma(x+1) \sum_{k=1}^{\infty} (-1)^k / k^x \ . \end{aligned}$$

- The ratio $E(c^4)/(E(c^2))^2$ is independent of λ and monotonic in τ, so we can fit τ by binary search.
- Once τ is chosen we can fit λ using $E(c^2)$.

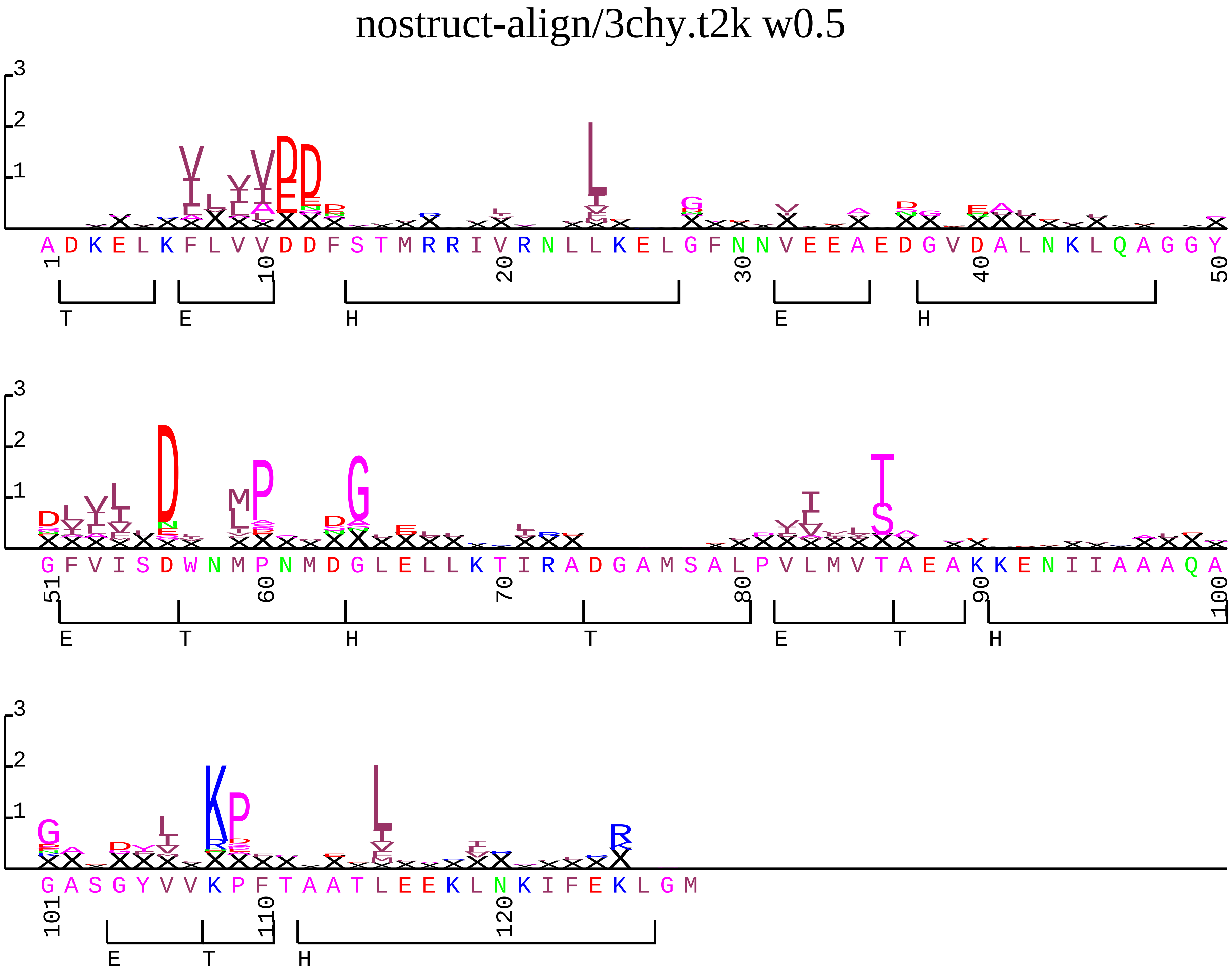
Student’s t-distribution

- On the advice of statistician David Draper, we tried maximum-likelihood fits of Student’s t-distribution to our heavy-tailed symmetric data.
- We couldn’t do moment matching, because the degrees of freedom parameter for the best fits turned out to be less than 4, where the 4th moment of Student’s t is infinite.
- The maximum-likelihood fit of Student’s t seemed to produce too heavy a tail for our data.
- We plan to investigate other heavy-tailed distributions.

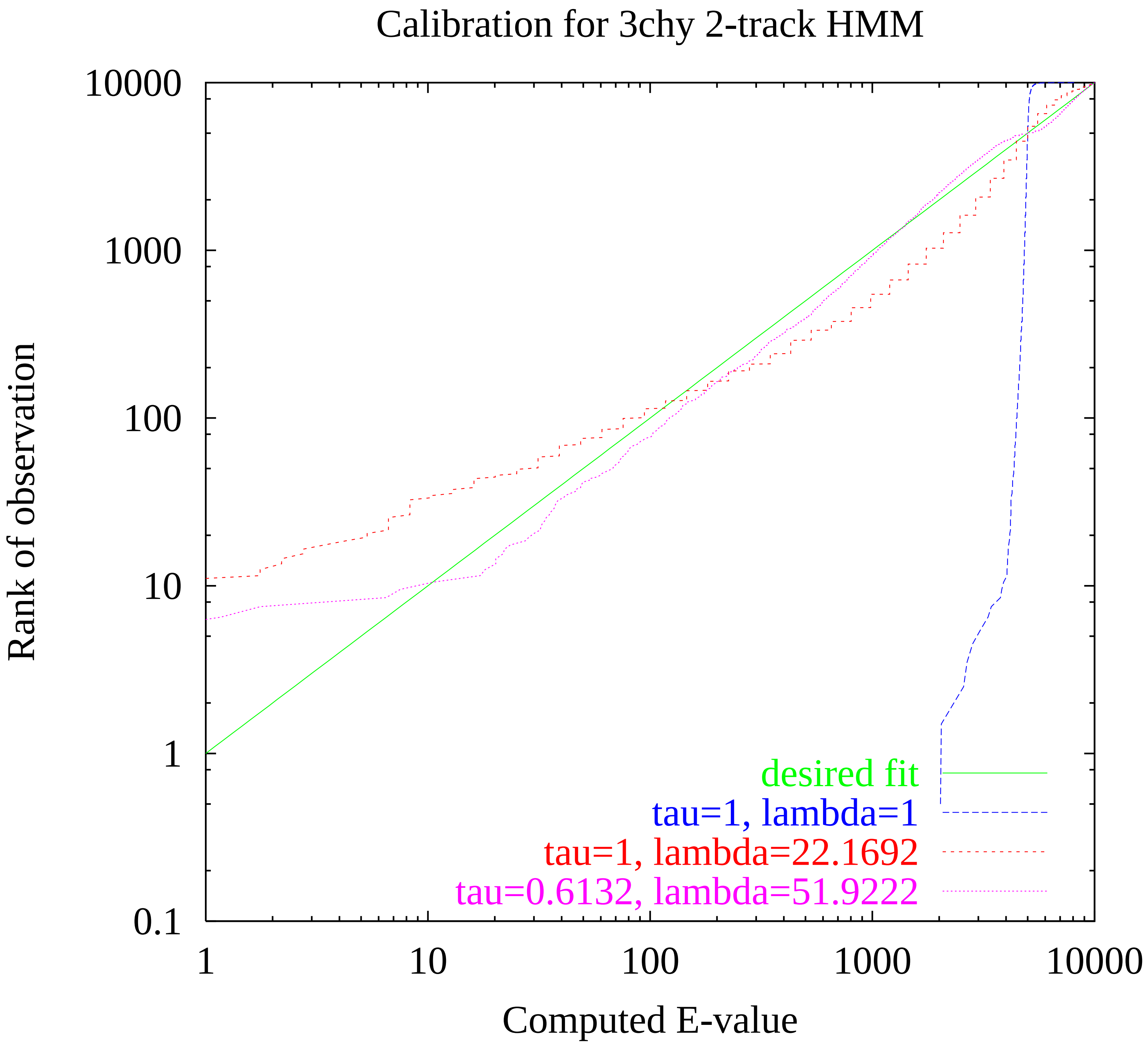
Example for single-track HMM



What is single-track HMM looking for?

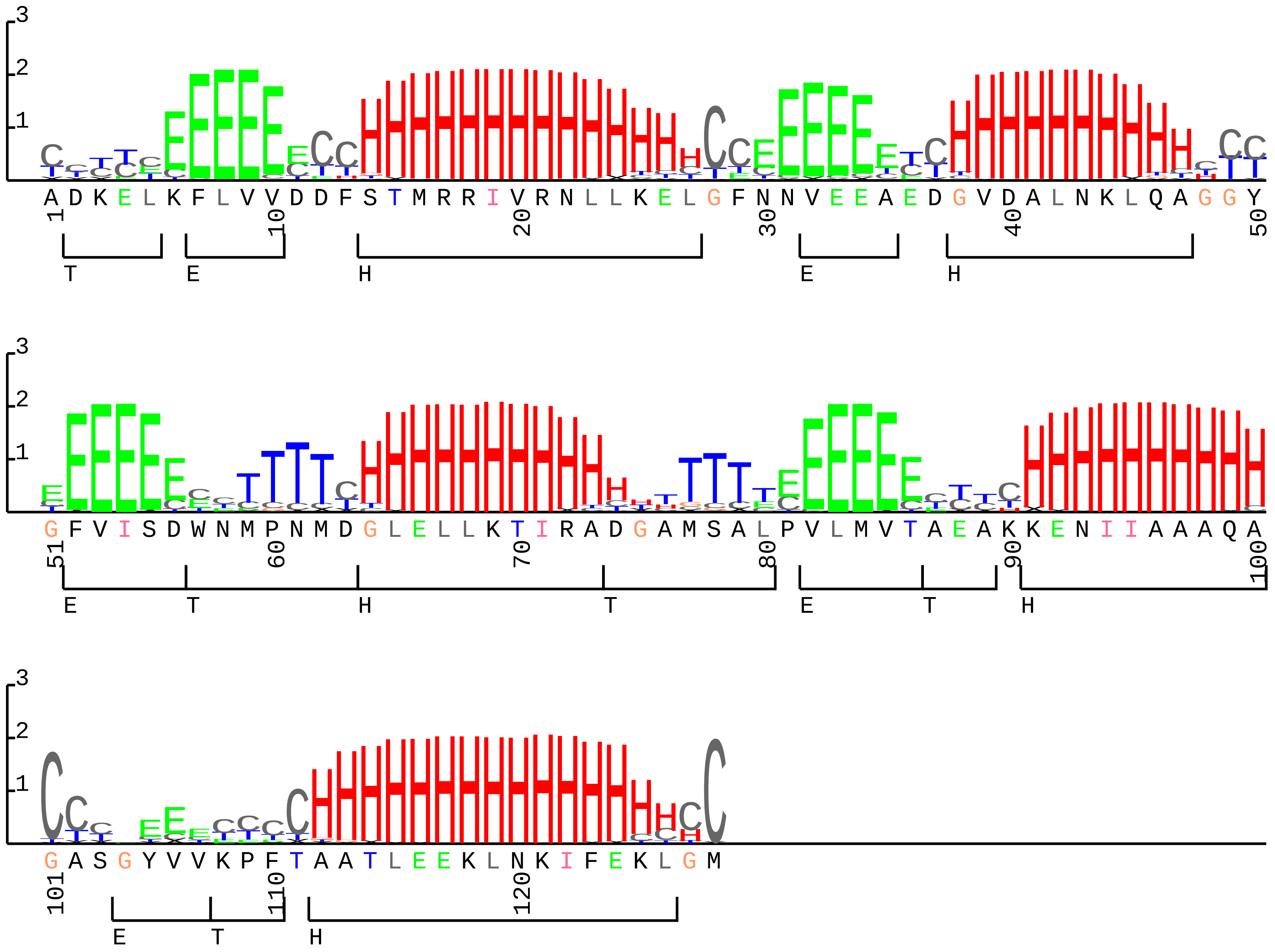


Example for two-track HMM

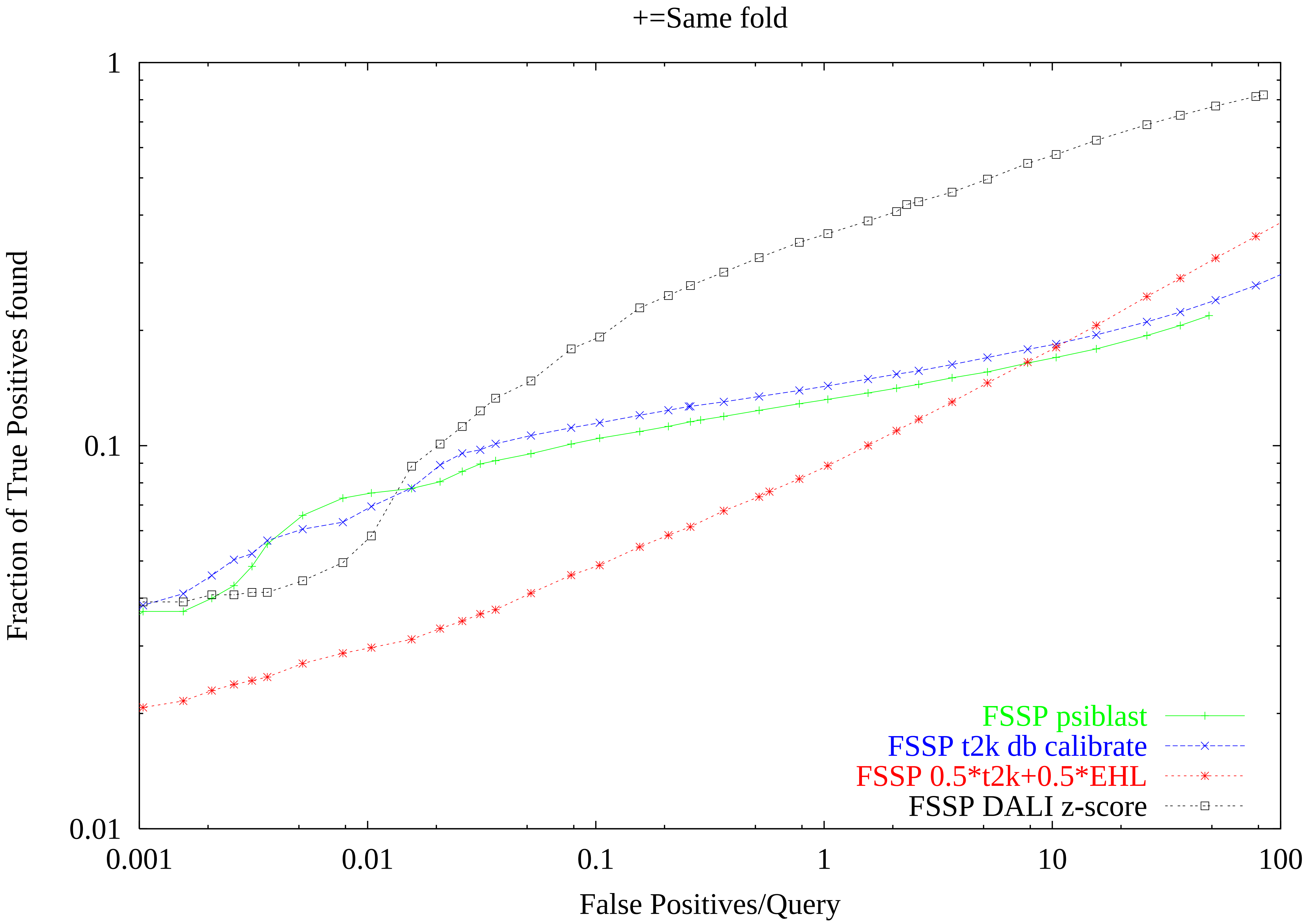


What is second track of HMM looking for?

nostruct-align/3chy.t2k EBGHTL



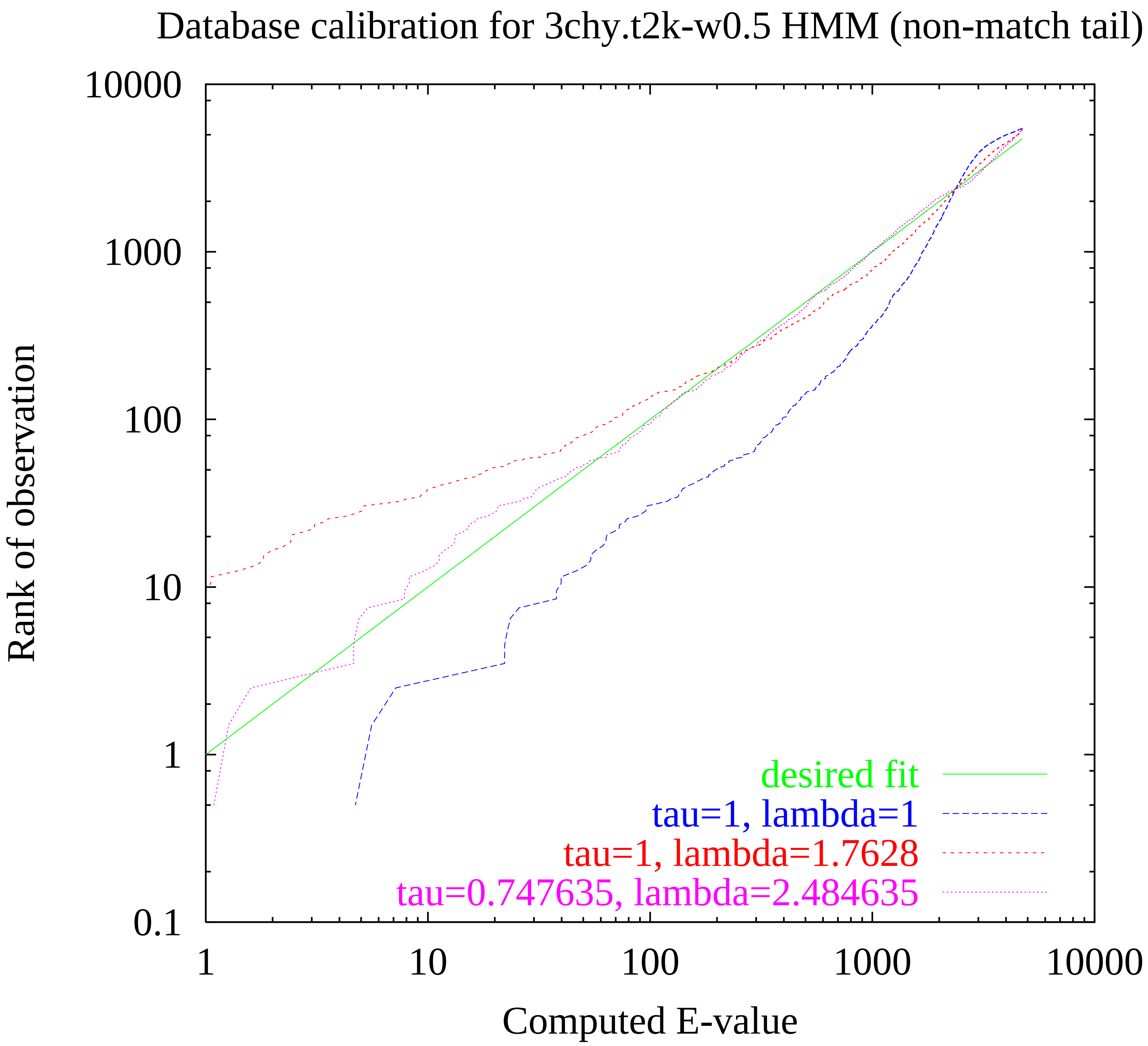
Fold recognition results



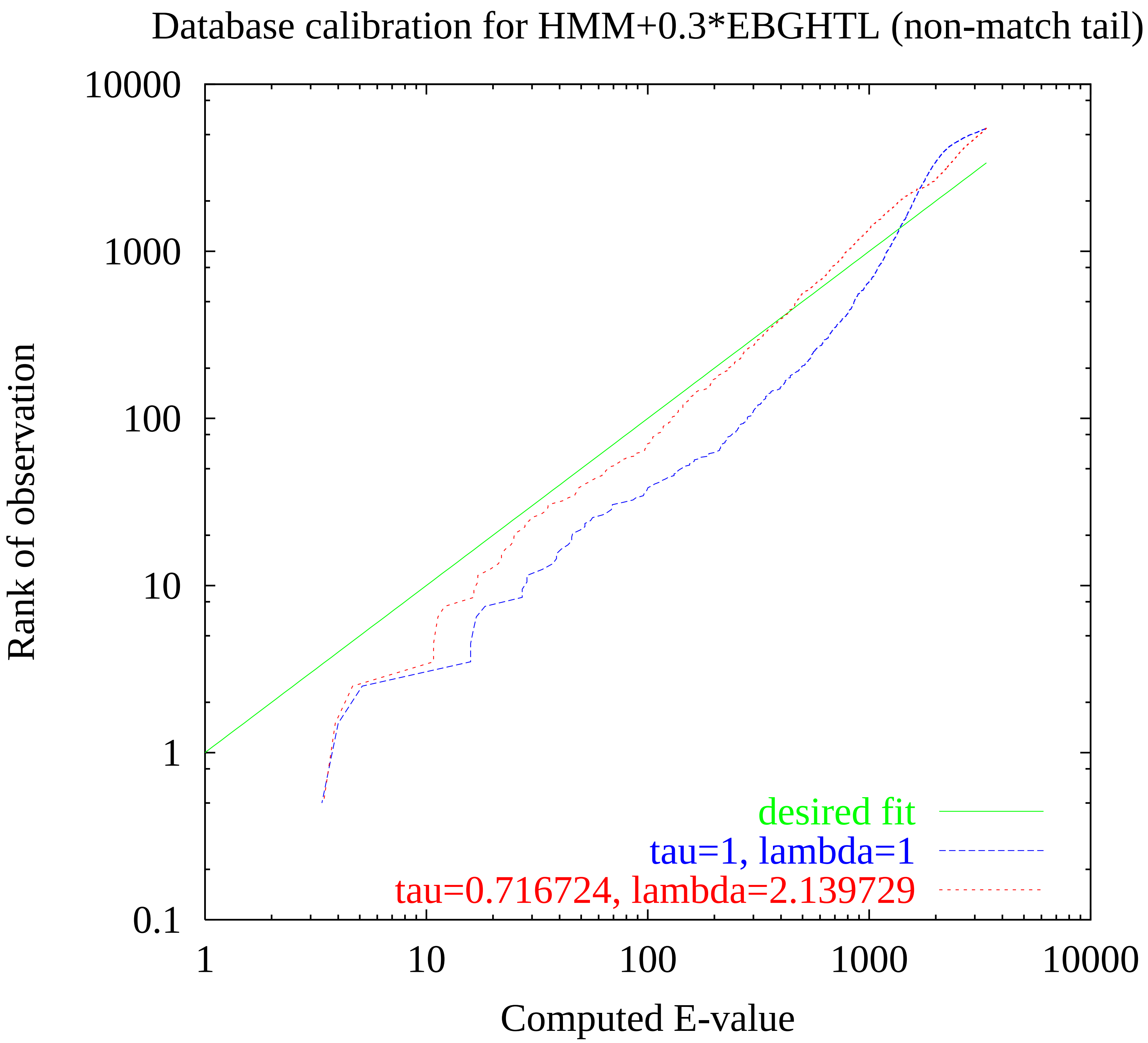
Wave hands and say "but we can fix that"

- Why did calibrated fold recognition fail for 2-track HMMs?
- "Random" secondary structure sequences (i.i.d. model) are **not** representative of real sequences. Almost any real protein (which has runs of helix or strand), will score much better than an i.i.d. random sequence.
- Fixes:
  - Better secondary structure decoy generator.
  - Use real database, but avoid problems with contamination by true positives by taking only costs > 0 to get estimate of $E(\text{cost}^2)$ and $E(\text{cost}^4)$.

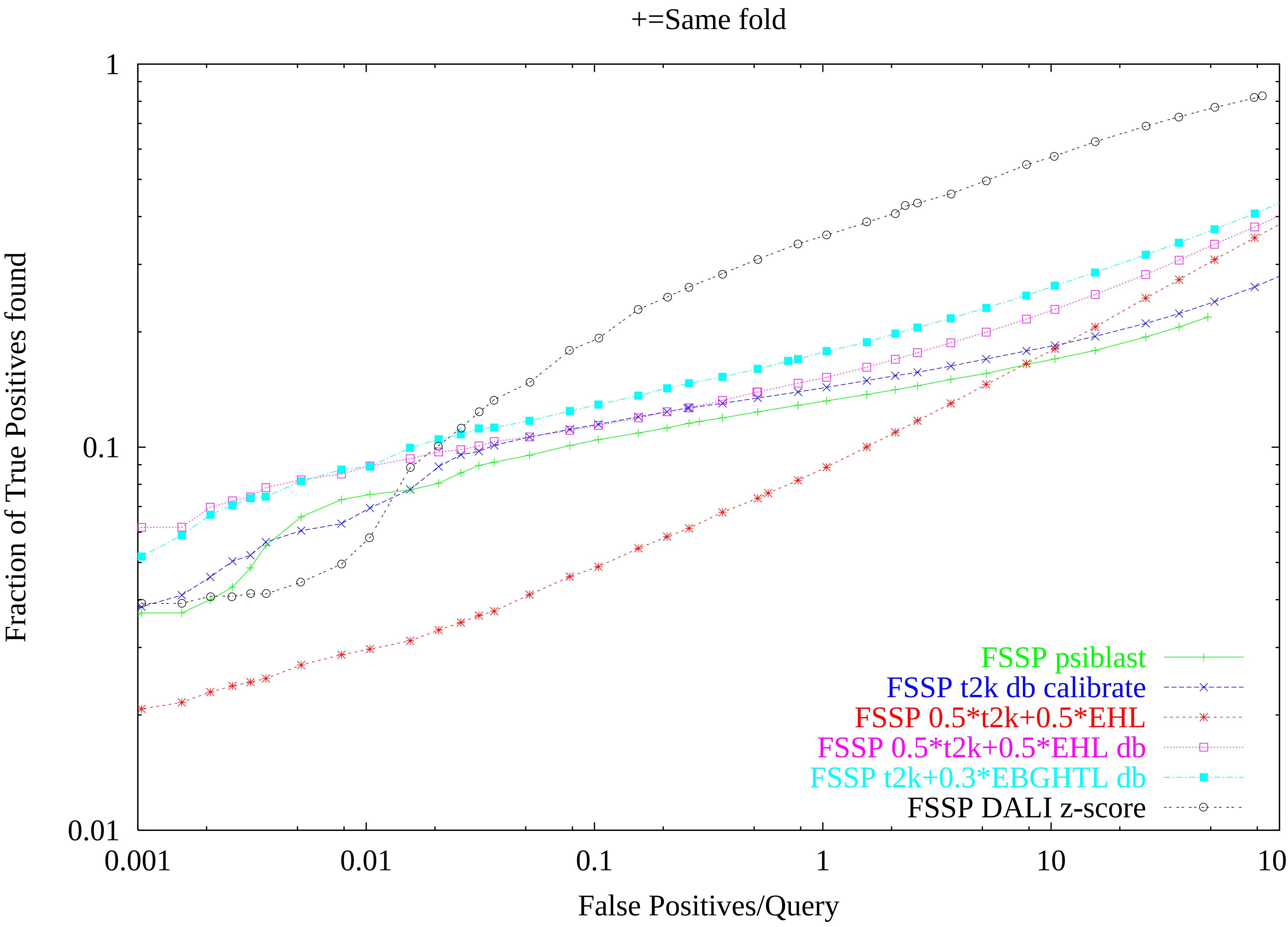
Database fit: one-track



Database fit: two-track



Fold recognition results with database fit



Web sites

UCSC bioinformatics info: `http://www.cse.ucsc.edu/research/compbio/`

SAM tool suite info: `http://www.cse.ucsc.edu/research/compbio/sam.html`

HMM **servers:** `http://www.cse.ucsc.edu/research/compbio/hmm-apps/`

SAM-T99 prediction server: `http://www.cse.ucsc.edu/research/compbio/`
`hmm-apps/T99-query.html`

These slides: `http://www.cse.ucsc.edu/~karplus/papers/mm2001.pdf`