6.047 Computational Biology, Problem Set 1 Writeup: Aligning and Modeling Genomes

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1 Evolutionary distances of orthologs and paralogs

a. Needleman-Wunsch

```
for i in range(1, len(seq1) + 1):
    for j in range(1, len(seq2) + 1):
        b1 = seq1[i - 1]
        b2 = seq2[j - 1]
        # Option 1:
        # i, j align
        opt1 = F[i - 1][j - 1] + \
               subst_matrix[base_idx[b1]][base_idx[b2]]
        # Option 2:
        # i aligns with gap, so we want to align remainder of seq1
        opt2 = F[i - 1][j] - gap_pen
        # Option 3:
        # j aligns with gap, so we want to align remainder of seq2
        opt3 = F[i][j - 1] - gap_pen
        F[i][j], TB[i][j] = max((opt1, PTR_BASE),
                                (opt2, PTR_GAP2),
                                (opt3, PTR_GAP1), key=lambda x: x[0])
```

The code above generates the following for `CTAAGTACT` and `CATTA`:

```
Score:  -6
CTAAGTACT
C--ATTA--
```

```
F(i, j) with traceback:
  0    -4    -8   -12   -16   -20
    `-.
 -4     3    -1    -5    -9   -13
        |
 -8    -1     1     2    -2    -6
        |
-12    -5     2    -1     0     1
          `-.
-16    -9    -2     0    -3     3
             `-.
-20   -13    -6    -4    -2    -1
                `-.
-24   -17   -10    -3    -1    -4
                     `-.
-28   -21   -14    -7    -5     2
                           |
-32   -25   -18   -11    -8    -2
                           |
-36   -29   -22   -15    -8    -6
```

Using the provided similarity matrix, the alignment score between the human and mouse HoxA13 gene is 2971.

b. Distance metric

```
F[i][j], TB[i][j] = min((opt1, PTR_BASE),
                        (opt2, PTR_GAP2),
                        (opt3, PTR_GAP1), key=lambda x: x[0])

S = [
   # A  G  C  T
    [0, 1, 2, 2], # A
    [1, 0, 2, 2], # G
    [2, 2, 0, 1], # C
    [2, 2, 1, 0]  # T
]

gap_pen = -4 # add penalty instead of subtract
```

In order to convert the standard similarity matrix into a matrix that would generate alignment scores that could be used as distances, I had to change two things in the alignment program:

1. First, if two symbols match, then they should have a distance of 0; Furthermore, the more dissimilar two symbols are, the greater (instead of smaller) the value they should have in matrix S. Additionally, since we subtract the gap penalty, the gap penalty must now be negative instead of positive. To achieve all these changes, I changed the values along the main diagonal of S to 0, and flipped the signs of all other values.

2. Second, because we are now dealing with finding the minimum distance instead of the maximum score, I had to change the aggregating function in the dynamic programming loop from `max` to `min`.

c. Distance between human and mouse HoxA13

Using the modified "similarity" (since now we are in effect measuring differences) matrix S defined in part (b), the distance between the human and mouse HoxA13 gene is 197.

d. Dating HoxA13 and HoxD13

Again, using the modified "similarity" matrix S defined in part (b), the distance between the human HoxA13 and human HoxD13 gene is 1145, and the distance between the mouse HoxA13 and mouse HoxD13 gene is 1095.

If we assume that the alignment score can be used as a distance metric, that the distance metric is consistent across mutations and species, and that it is linear in that $c \times \text{dist}(a, b)$ implies that the evolutionary age between a and b is c times older, then we can estimate the date that whole-genome duplication gave rise to HoxA13 and HoxD13. Concretely, since 197 corresponds to a date 70 million years ago, then $\frac{1145}{197} \times 70 = 290.6$ million years ago for the divergence of human HoxA13 and HoxD13, and $\frac{1095}{197} \times 70 = 277.9$ million years ago for mouse HoxA13 and HoxD13 divergence.

2 Sequence hashing and dotplot visualization

a. Exact 30-mers

```
$ python ps1-dotplot.py human-hoxa-region.fa \
> mouse-hoxa-region.fa human-mouse-hoxa-30-mer.png
62829 hits found
24.70197% hits on diagonal
```

The off diagonal hits formed a grid like shape in the graph, which means that hits were found in a steady, repeated pattern. These hits that were not along the diagonal could possibly be promoter sequences and oriC locations, which appear repeatedly in a DNA sequence.

Matches along the diagonal are more likely to be "correct", or orthologous alignments because we reason that the two sequences we want to align are reasonably related, and so differences between the two should not span a large distance, i.e. pieces that were related previously should still be in close proximity to their origin, and if both sequences come from the same origin, the hits should be near the same place, which appears as "along the diagonal" in our graph.

b. Variations

i. Exact 100-mers

```
$ python ps1-dotplot.py human-hoxa-region.fa \
> mouse-hoxa-region.fa human-mouse-hoxa-100-mer.png
```

```
1198 hits found
100.00000% hits on diagonal
```

Many fewer hits were found, so the graph looked particularly sparse, but all the hits that were found were along the diagonal.

ii. 60-mers

```
$ python ps1-dotplot.py human-hoxa-region.fa \
> mouse-hoxa-region.fa human-mouse-hoxa-60-mer.png
23933 hits found
38.74149% hits on diagonal
```

Accordingly, 60-mers had more hits than 100-mers and fewer hits than exact 30-mers because of the increased length, but had many more than the 100-mers because of the flexibility of only every other symbol needing to match.

iii. 90-mers

```
$ python ps1-dotplot.py human-hoxa-region.fa \
> mouse-hoxa-region.fa human-mouse-hoxa-90-mer.png
8887 hits found
93.85619% hits on diagonal
```

As a length of 90 is large, the number of hits decreased correspondingly; however, we had many more hits than the exact 100-mer again because of the requirement that only every third base match, rather than every base.

iv. 120-mers

```
$ python ps1-dotplot.py human-hoxa-region.fa \
> mouse-hoxa-region.fa human-mouse-hoxa-120-mer.png
6044 hits found
82.13104% hits on diagonal
```

The number of hits decreased once again, and the specificity decreased as well, since we were checking too few bases along the entire k-mer.

v. 100-mers with mismatches

In order to implement this, I would first hash every sixth base in a length of 100 monomers, and find the hits based on the simple every sixth base heuristic. Afterwards, however, I would filter each hit in linear time to determine if the hit satisfies the constraint that only two mismatch for every contiguous block of six. Checking for this additional constraint takes 100 operations per hit, and with fewer than $10,000,000$ hits, this is computationally feasible on a modern computer.

c. Specificity to the diagonal

Having the 90-mer that matched every third base was the most specific to the diagonal, having 93.86% of the hits be located along the diagonal. This is likely due to the right balance of the length of the k-mer we are trying to hit and the spacing between each monomer we actually check for equivalence. For a 120-mer, there were many more options in between the 30 bases that we checked to match, and so fewer were specific to the diagonal. With the 60-mers, we still only checked quite a short length of sequence for alignment, which allowed for many more substrings to match, which also resulted in a lower specificity.

d. Sensitivity vs. Specificity

If the length of the k-mer we are hashing is low, then we get many more hits (sensitivity increases), but a fewer percentage of those fall along the diagonal, because we are too lenient with our matching. Likewise, if the length of the k-mer is too long, then most matches we have are along the diagonal, but we have much fewer matches (specificity is much higher). Hashing only every nth symbol in a sequence allows us to match more hits; the larger n is, the more hits we are likely to find (because we allow for many more single symbol mismatches). In other words, increasing n trades specificity for sensitivity.

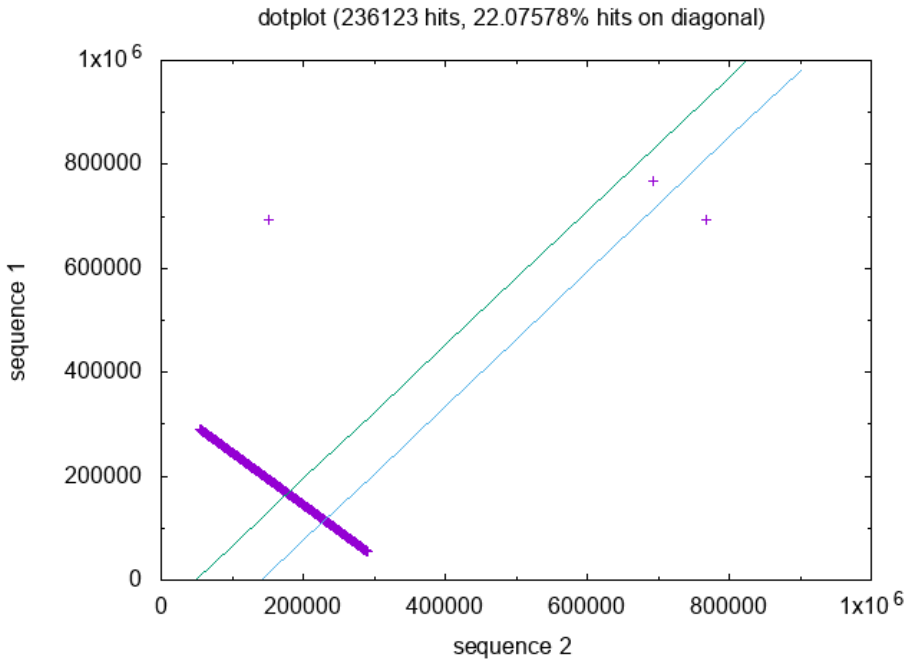
e. Inversions

The inversion ranges from indices `[54549, 290751)`. The inversion can be found by checking the reverse of every 200-mer against the original order 200-mer hashes, and look for a large section of matches. We can then look at the list of hits and find the first in the large chunk, and find the last as well.

```
# to find the last in the large chunk of hits
for i in xrange(len(seq2) - 1, -1, -1):
    key = seq2[i:i + kmerlen]
    key = key[::kmerskip]
    key = invert(key)

    # store hits to hits list
    any_hits = lookup.get(key, [])
    if len(any_hits) != 0:
        chain.append(i)
        if len(chain) > 50:
            print(max(chain))
            quit()
    else:
        chain = []

# to find the first in the large chunk of hits
for i in xrange(0, len(seq2) - kmerlen + 1):
    key = seq2[i:i + kmerlen]
    key = key[::kmerskip]
    key = invert(key)
```




```
# store hits to hits list
any_hits = lookup.get(key, [])
if len(any_hits) != 0:
    chain.append(i)
    if len(chain) > 50:
        print(min(chain))
        quit()
else:
    chain = []
```

3 HMMs for GC-rich regions: State durations and limitations

a. State durations

Let D_k represent the duration of staying in state k. Then the distribution of state durations D_k is a random variable that follows a **geometric distribution**, defined as the number of failures before the first success, if we consider a *success* as transitioning out of state k.

More concretely, the probability distribution function of D_k is

$$p_{D_k}(d) = \mathbb{P}(D_k = d) = (1-p)^{d-1}p$$

where p is the probability of transitioning out of state k (i.e. from state k to

another state $k' \neq k$). The expected value of state duration D_k is

$$\mathbb{E}[D_k] = \frac{1}{p} - 1$$

where again, p is the probability of transitioning out of state k.

b. Viterbi algorithm

Based on the transition probabilities hardcoded into the program, the expected duration for both high and low GC regions should be 99 (since we don't count the transitioning out state).

c. Mystery sequences

Mystery 1

```
Authoritative annotation statistics
-----------------------------------
High-GC mean region length:  100
High-GC base composition:  A=19.94% G=29.87% C=30.20% T=20.00%
Low-GC mean region length:  101
Low-GC base composition:  A=29.87% G=20.27% C=19.73% T=30.13%

Viterbi annotation statistics
-----------------------------
High-GC mean region length:  234
High-GC base composition:  A=20.51% G=29.18% C=29.40% T=20.91%
Low-GC mean region length:  220
Low-GC base composition:  A=29.62% G=20.66% C=20.20% T=29.52%

>> Accuracy:  71.96%
```

In mystery 1, the distribution of authoritative state durations was more or less uniform from lengths of 40 to 140 for both high and low GC content. However, the Viterbi decoding found regions with duration ranging from 50 to 900, with a mode around 100 and a long right tail.

Mystery 2

```
Authoritative annotation statistics
-----------------------------------
High-GC mean region length:  100
High-GC base composition:  A=19.85% G=29.78% C=30.07% T=20.30%
Low-GC mean region length:  99
Low-GC base composition:  A=29.84% G=19.86% C=19.99% T=30.31%

Viterbi annotation statistics
-----------------------------
High-GC mean region length:  214
High-GC base composition:  A=20.56% G=29.15% C=29.46% T=20.83%
```

```
Low-GC mean region length:  212
Low-GC base composition:  A=29.16% G=20.45% C=20.56% T=29.83%

>> Accuracy:  68.80%
```

In mystery 2, the distribution of authoritative state durations for high GC content was normally distributed with mean of 100 and standard deviation of 9.75; for low GC content, the distribution was normal with mean 99 and standard deviation of 10.7. Again, however, the Viterbi decoding found regions with lengths ranging from 50 to 1000, with a mode around 100 but a very long right tail. In other words, the Viterbi decoding found a geometric-like distribution for the state durations, rather than the true, normal distribution.

Mystery 3

```
Authoritative annotation statistics
-----------------------------------
High-GC mean region length:  100
High-GC base composition:  A=19.81% G=29.71% C=30.56% T=19.91%
Low-GC mean region length:  100
Low-GC base composition:  A=29.56% G=20.09% C=20.11% T=30.24%

Viterbi annotation statistics
-----------------------------
High-GC mean region length:  221
High-GC base composition:  A=20.56% G=29.05% C=29.84% T=20.55%
Low-GC mean region length:  207
Low-GC base composition:  A=29.10% G=20.46% C=20.53% T=29.91%

>> Accuracy:  67.72%
```

In mystery 3, the distribution of authoritative state durations was a constant length of 100 for both high and low GC content. However, the Viterbi decoding, using the provided parameters, still found region durations in the bimodally around durations of 80 and 230 with a long right tail, causing the mean to be skewed to 221 and 207 for high and low GC regions, respectively. Because the state durations were not accurately modeled by the topology and parameters of the HMM, the HMM only achieved an accuracy of 67.72%.

In all three of the mystery sequences, the authoritative state durations for both high and low GC content never exceeded 200; however, the Viterbi decoding consistently determined sequences with durations greater than 200.

d. Retraining the HMM parameters

Even if we had correctly annotated sequences for the mystery sequences, I do not believe that performance of the Viterbi algorithm, using the supervised learning training procedure discussed in class, would improve. The reason is that all the authoritative sequences had a mean duration of around 100; to get the MLE for this mean duration, the transition probabilities a_{kl} would be assigned the same (or very similar) values to they are now, because the expected value of the

transition probability is the mean value of the actual durations. The problem steps from our topological assumption that durations depend only on the single previous state, which leads to an assumed geometric or exponential distribution, rather than the true distribution.

e. GENSCAN

GENSCAN overcomes the inherent assumption of Markov Chains that duration distributions are geometric by *allowing the probability of transition to depend on the amount of time that the model has been in a certain state*; in other words, GENSCAN takes advantage of a semi-Markov property, also known as a hidden semi-Markov model. Hidden semi-Markov models allow for any state duration distribution to be modeled, rather than be confined to geometric distributions. We can implement HSMMs by defining transition and emission probabilities as functions of time rather than constants, and keep a record of the current duration when computing the parameters.

4 Final project preparation

The responses to this question are in `FengMatthew_Profile.docx`.