

Series 5 - solutions

Genomics and bioinformatics - Week 5

October 18, 2011

1 Hidden Markov Model

1. There are two states, say I (isochore) and N (normal). We observe sequences of bases A, T, G and C. For this exercise one can group G and C in one variable “GC”, and similarly A and T in “AT”. Note that from each state, probabilities of incoming arrows must sum to 1, as well as outgoing arrows.
2. In state I (isochore), the probability to see GC is 0.5, same for AT. From state N, probabilities are 0.2 for GC and 0.8 for AT.
3. The isochore is 7000 bases long, the genome 23'000'000, so the probability for a random base in the genome to belong to the isochore is $x = P(I) = 7'000/23'000'000 = 3 \cdot 10^{-4}$.
4. Bayes' formula says that

$$P(I) = P(I|N)P(N) + P(I|I)P(I)$$

$$P(N) = P(N|I)P(I) + P(N|N)P(N)$$

so in terms of x , p and q :

$$x = q(1 - x) + (1 - p)x$$

$$1 - x = px + (1 - q)(1 - x)$$

Note that the two equations are equivalent, so one cannot use them to find p and q .

5. From state I, one can consider the event “staying in I” as a fail, with probability $q = 1 - p$, and “leaving to N” as a success, with probability p . The number X of failures before the first success is given by a geometric distribution:

$$P(X = k) = (1 - p)^k p, \quad P(X < k) = 1 - (1 - p)^{k+1},$$

which mean is $E[X] = \frac{1-p}{p}$ (another formulation, taking X as the time of the first success, leads to $E[X] = \frac{1}{p}$).

6. If the isochore is generated from a geometric process as in the previous point, its length is most probably the mean of the distribution. So $7000 = E[X] = \frac{1-p}{p} \Rightarrow p = \frac{1}{7001}$ (or $\frac{1}{7000}$ with the alternative formulation), confirming what one could expect intuitively.

2 BLAST

1. Do you get any matches to **fragment_007**? Which parameters did you use? Record the alignment statistics for the top hits.

Using the Nucleotide Collection (nr/nt) Database and optimizing for “more dissimilar sequences” (discontiguous megablast)

```
>[emb|CR954246.1] [D] Pseudoalteromonas haloplanktis str. TAC125 chromosome I, complete
sequence
Length=3214944

Features in this part of subject sequence:
  Serine protease precursor

Score = 59.0 bits (64), Expect = 4e-05
Identities = 55/70 (79%), Gaps = 0/70 (0%)
Strand=Plus/Plus

Query  5          GGCACGGGACGCATGTAGCTGGAACAGTGGCAGCCGTAAATAATAATGGTATCGGAGTTG  64
      |||
Sbjct  2803550 GGCACGGTACACATGTAGCGGGTACTGTTGCTGCAGTTACTAATAATGGTGAGGGTGTG  2803609

Query  65          CCGGGGTTGC  74
      |||
Sbjct  2803610 CTGGGGTTGC  2803619

>[emb|FP565814.1] [D] Salinibacter ruber M8 chromosome, complete genome
Length=3619447

Features in this part of subject sequence:
  peptidase families S8 and S53 domain protein

Score = 51.8 bits (56), Expect = 0.005
Identities = 63/86 (73%), Gaps = 0/86 (0%)
Strand=Plus/Minus

Query  1          AACGGGCACGGGACGCATGTAGCTGGAACAGTGGCAGCCGTAAATAATAATGGTATCGGA  60
      |||
Sbjct  2941385 AACGGTCACGGGACGCATGTGACCGGAACGGTGGCTGCCGTCACCAACAACGCCTTCGGC  2941326

Query  61          GTTGCCGGGGTTGCAGGAGGAAACGG  86
      |||
Sbjct  2941325 GTAGCGGGCACTGCCGGTGGAAATGG  2941300
```

2. Extract the sequence of the hit with the highest query coverage (this may not necessarily be the top hit) and perform another nucleotide BLAST, using the same parameters. Record the alignment statistics for the top hits.

```
>[emb|CR954246.1|] [D] Pseudoalteromonas haloplanktis str. TAC125 chromosome I, complete
sequence
Length=3214944
```

Features in this part of subject sequence:
Serine protease precursor

Score = 59.0 bits (64), Expect = 3e-06
 Identities = 55/70 (79%), Gaps = 0/70 (0%)
 Strand=Plus/Plus

```
Query 5      GGCACGGGACGCATGTAGCTGGAACAGTGGCAGCCGTAAATAAATGGTATCGGAGTTG 64
Sbjct 2803550 GGCACGGTACACATGTAGCGGGTACTGTTGCTGCAGTTACTAATAATGGTGAGGGTGTG 2803609

Query 65     CCGGGGTTGC 74
Sbjct 2803610 CTGGGGTTGC 2803619
```

```
>[emb|FP565814.1|] [D] Salinibacter ruber M8 chromosome, complete genome
Length=3619447
```

Features in this part of subject sequence:
peptidase families S8 and S53 domain protein

Score = 51.8 bits (56), Expect = 5e-04
 Identities = 63/86 (73%), Gaps = 0/86 (0%)
 Strand=Plus/Minus

```
Query 1      AACGGGCACGGGACGCATGTAGCTGGAACAGTGGCAGCCGTAAATAAATGGTATCGGA 60
Sbjct 2941385 AACGGTCACGGGACGCATGTGACCGGAACGGTGGCTGCCGTCACCAACAACGCCTTCGGC 2941326

Query 61     GTTGCCGGGGTTGCAGGAGGAAACGG 86
Sbjct 2941325 GTAGCGGGCACTGCCGGTGGAAATGG 2941300
```

- What changes do you observe in the E-values? To which parameter could you attribute the these changes?

Improvement in the E-values. Parameter - Query coverage.

- What is the default threshold for the E-value on NCBI BLAST?

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- Do you have any significant hits suggesting a possible function for fragment_007?

The E-values are not significant.

2.1 Protein BLAST

- Are any well-known protein domains found?

Peptidases S8 S53 superfamily

- Do you get any significant hits? Record the alignment statistics for the top hits.

Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value |
|----------------|--|-----------|-------------|----------------|---------|
| ZP_05857871.1 | subtilase family domain protein [Prevotella veroralis F0319] >gb EEX18266.1 subtila | 194 | 194 | 99% | 8e-55 |
| YP_004253567.1 | peptidase S8 and S53 subtilisin kexin sedolisin [Odoribacter splanchnicus DSM 20712 | 192 | 192 | 99% | 2e-54 |
| ZP_06407789.1 | subtilase family domain protein [Prevotella melaninogenica D18] >gb EFC73751.1 su | 187 | 187 | 99% | 2e-52 |
| YP_003815043.1 | peptidase families S8 and S53 [Prevotella melaninogenica ATCC 25845] >gb ADK974 | 187 | 187 | 99% | 2e-52 |
| CBK65311.1 | Subtilisin-like serine proteases [Alistipes shahii WAL 8301] | 186 | 186 | 99% | 3e-52 |
| ZP_08836625.1 | hypothetical protein HMPREF0666_02801 [Prevotella sp. C561] >gb EGW49352.1 hy | 185 | 185 | 99% | 9e-52 |
| ZP_08172701.1 | peptidase, S8/S53 family [Prevotella denticola CRIS 18C-A] >gb EGC85848.1 peptid | 185 | 185 | 100% | 1e-51 |
| ZP_08137410.1 | subtilase family domain protein [Prevotella multiformis DSM 16608] >gb EGC18824.1 | 184 | 184 | 100% | 2e-51 |
| YP_004329881.1 | peptidase, S8/S53 family [Prevotella denticola F0289] >gb AEA22070.1 peptidase, S | 184 | 184 | 100% | 4e-51 |

3. What is the possible function of the protein encoded by **fragment_007**?

fragment_007 encodes for a subtilase family domain protein. It is a member of the peptidases S8 (subtilisin and kexin) and S53 (sedolisin) family. These include endopeptidases and exopeptidases.

4. Which species is most predominant in your BLAST output?

Prevotella species

5. Could you have obtained the same results using another BLAST program, without having to translate the nucleotide sequence of **fragment_007**?

Yes, using blastx

6. How do results from Protein BLAST compare with the results from Nucleotide BLAST?

Amino acid sequences are more conserved than nucleotide sequences. Often even the highest-scoring subject sequences retrieved using the nucleotide sequence will cover only small regions of the query sequence, while quite often the corresponding sequences retrieved using the amino acid sequence will cover more of the gene.

3 Finding orthologs

Putative ortholog of *Saccharomyces cerevisiae* Pho2p in *Candida glabrata*?

CAGL0L07436g

Are the findings of the paper consistent with your observations?

Yes