# Exercises - Week 5 - solutions

Genomics and bioinformatics

## 1 Hidden Markov Model

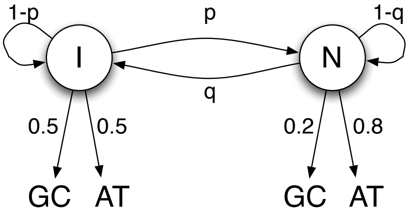
1. We observe a sequence of bases A, T, G and C. For this exercise, one can group G and C in one variable "GC", and similarly A and T in "AT". So there are two states, $I$ (isochore) and $N$ (normal), that emit variables AT and GC. From each state, the outgoing probabilities must sum to 1.

2. 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 $x = P(I) = 7'000/23'000'000 = 3 \cdot 10^{-4}$.

3. In state $I$ (isochore), the probability to see GC is 0.5, same for AT. In state $N$, the probabilities are 0.2 for GC and 0.8 for AT. This is if we consider the isochore to be small with respect to the normal region and not contribute to the 20%. Otherwise, using Bayes, one must isolate $y = P(GC|N)$ in

$$0.2 = P(GC) = P(GC|I) \cdot P(I) + P(GC|N) \cdot P(N) = 0.5 \cdot \frac{7'000}{23'000'000} + y \cdot \frac{23'000'000 - 7'000}{23'000'000}$$

One finds $y = 0.19990866785543426$. From now on let us assume $P(GC|N) = 20\%$.



4. From Baye's Theorem, one has

$$P(I|N) = \frac{P(N|I)P(I)}{P(N)} \quad \Leftrightarrow \quad q = \frac{x}{1-x} \cdot p$$

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

$$P(X = k) = (1-p)^k p.$$

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

[1] http://en.wikipedia.org/wiki/Geometric_distribution

6. If the isochore sequence $IIIIII \cdots IIII$ is generated from a geometric process as given in point 5, its length is most probably the mean of the distribution. So $7000 = E[X] = \frac{1-p}{p} \Rightarrow p = \frac{1}{7001}$, or $p = \frac{1}{7000}$ with the alternative formulation, confirming what one could expect intuitively. Taking $p = \frac{1}{7000}$, one deduces from point 4 that $q = \frac{x}{1-x} \cdot p = \frac{1}{22993000}$. One may also compute $q$ as follows: Exchanging the role of $I$ and $N$ in point 5, writing $Y$ for the corresponding random variable and $L$ for the average length of the normal region, one obtains $L = 23000000 - 7000 = 22993000$, $L = E(Y) = \frac{1}{q}$, so $q = \frac{1}{22993000}$ as before.

   Now we have all the parameters of the HMM that will most probably generate, in average, isochore regions such as the ones observed in Falciparum.

## 2 Reading frame

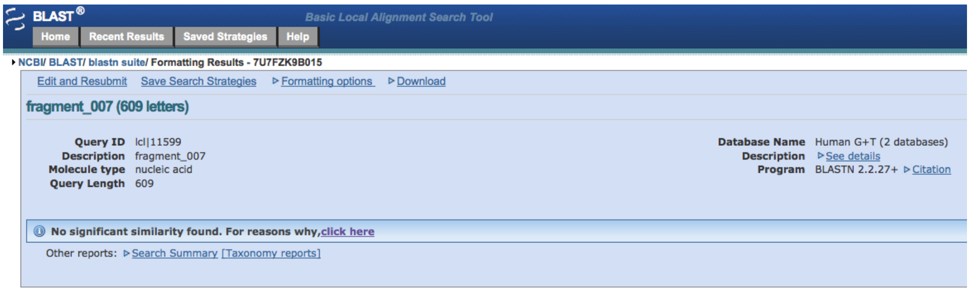
See `series4_solution.py`.

## 3 BLAST

(Results here may change with the evolution of sequencing databases).

### 3.1 Nucleotide BLAST

1. In general, the default parameters will lead to zero matches. Possible reasons are: the selected species is not correct, the alignment optimization criterium is too stringent.



NCBI/ BLAST/ blastn suite/ Formatting Results - 7U7FZK9B015

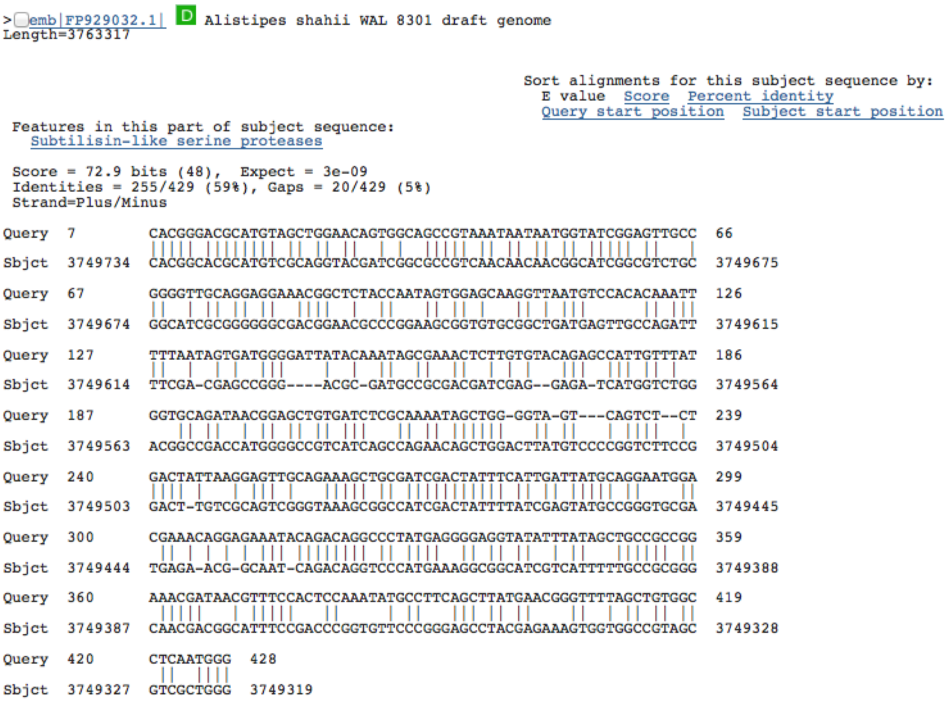
fragment_007 (609 letters)

Query ID lcl|11599
Description fragment_007
Molecule type nucleic acid
Query Length 609

Database Name Human G+T (2 databases)
Description See details
Program BLASTN 2.2.27+ Citation

No significant similarity found. For reasons why,click here

2. Using the *Nucleotide Collection (nr/nt)* database and optimizing for *More dissimilar sequences (discontiguous megablast)*, setting *Match/Mismatch Scores* to *(1,-1)* and *Gap Costs* to *(Existence: 1 Extension: 2)*, one finds **Alistipes shahii WAL 8301 draft genome** as the top hit (maximum score).



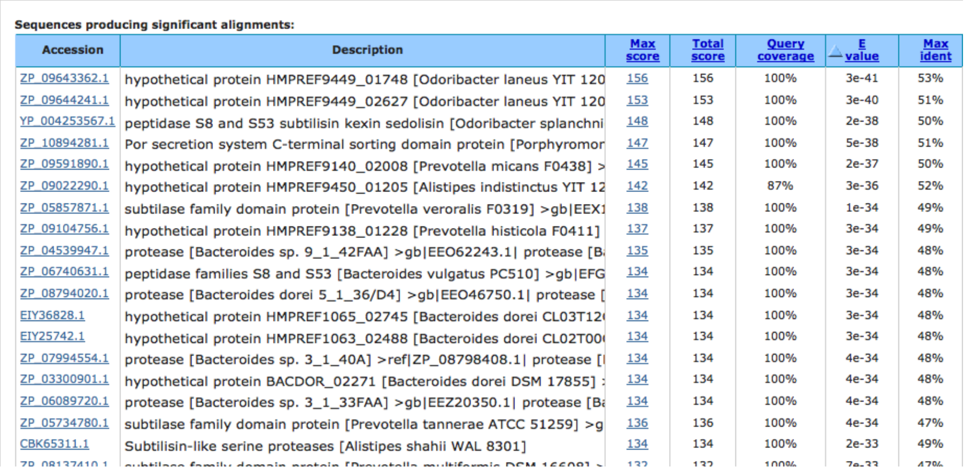
```
>□emb|FP929032.1| D Alistipes shahii WAL 8301 draft genome
Length=3763317

                                        Sort alignments for this subject sequence by:
                                          E value  Score  Percent identity
                                          Query start position  Subject start position
 Features in this part of subject sequence:
   Subtilisin-like serine proteases

 Score = 72.9 bits (48),  Expect = 3e-09
 Identities = 255/429 (59%), Gaps = 20/429 (5%)
 Strand=Plus/Minus

Query  7        CACGGGACGCATGTAGCTGGAACAGTGGCAGCCGTAAATAATAATGGTATCGGAGTTGCC  66
                ||||| |||||||| || || ||  | |  ||||| || || || || ||||| ||   |
Sbjct  3749734  CACGGCACGCATGTCGCAGGTACGATCGGCGCCGTCAACAACAACGGCATCGGCGTCTGC  3749675

Query  67       GGGGTTGCAGGAGGAAACGGCTCTACCAATAGTGGAGCAAGGTTAATGTCCACACAAATT  126
                ||  | || || ||  ||||  |  ||   || || |   || | |||      || |||
Sbjct  3749674  GGCATCGCGGGGGGCGACGGAACGCCCGGAAGCGGTGTGCGGCTGATGAGTTGCCAGATT  3749615

Query  127      TTTAATAGTGATGGGGATTATACAAATAGCGAAACTCTTGTGTACAGAGCCATTGTTTAT  186
                ||  |  | |  |||    |  |  ||  ||  ||  | | |   |||  ||| || |
Sbjct  3749614  TTCGA-CGAGCCGGG----ACGC-GATGCCGCGACGATCGAG--GAGA-TCATGGTCTGG  3749564

Query  187      GGTGCAGATAACGGAGCTGTGATCTCGCAAAATAGCTGG-GGTA-GT---CAGTCT--CT  239
                   || ||  | || || || |||   || || ||||||   || ||   | ||||  |
Sbjct  3749563  ACGGCCGACCATGGGGCCGTCATCAGCCAGAACAGCTGGACTTATGTCCCCGGTCTTCCG  3749504

Query  240      GACTATTAAGGAGTTGCAGAAAGCTGCGATCGACTATTTCATTGATTATGCAGGAATGGA  299
                |||| |   | ||| |   ||||| || ||||||||||| || || ||||| ||    ||
Sbjct  3749503  GACT-TGTCGCAGTCGGGTAAAGCGGCCATCGACTATTTTATCGAGTATGCCGGGTGCGA  3749445

Query  300      CGAAACAGGAGAAATACAGACAGGCCCTATGAGGGGAGGTATATTTATAGCTGCCGCCGG  359
                 || | | | | ||| |||||||| || ||||  || || ||  | ||   |||||| ||
Sbjct  3749444  TGAGA-ACG-GCAAT-CAGACAGGTCCCATGAAAGGCGGCATCGTCATTTTTGCCGCGGG  3749388

Query  360      AAACGATAACGTTTTCCACTCCAAATATGCCTTCAGCTTATGAACGGGTTTTAGCTGTGGC  419
                 |||||   | |||||   ||     | ||   ||| || ||    ||  | || || ||
Sbjct  3749387  CAACGACGGCATTTCCGACCCGGTGTTCCCGGGAGCCTACGAGAAAGTGGTGGCCGTAGC  3749328

Query  420      CTCAATGGG  428
                 ||  ||||
Sbjct  3749327  GTCGCTGGG  3749319
```

3. Depending on your interests, the following parameters may be used: The **max score** and **total score** specify the quality of the largest and total local alignment, respectively. The **query coverage** specifies the proportion of the query sequence that have been used during the alignment. The **E-value** specifies the nomber of alignments in a random database giving a score larger or equal to the one obtained.

4. From the top hit, one cannot deduce any particular function for `fragment_007`. However, looking at the next hits one finds out that "protease" is a good candidate for the function of `fragment_007`.

## 3.2 Protein BLAST

1. Using your custom function from exercise 2, one can extract the following nucleotides sequence from the translation of the forward strand with shift 0 (must start with 'M'; incomplete):
`MSTQIFNSDGDYTNSETLVYRAIVYGADNGAVISQNSWGSQSLTIKELQKAAIDYFIDYAGMDETGEIQT`
`GPMRGGIFIAAAGNDNVSTPNMPSAYERVLAVASMGPDFTKASYSTFGTWTDITAPGGDIDKFDLSEYGV`
`LSTYADNYYAYGEGTSMACPHVAGAA`.
Copy it into a file `aa_007.fasta`, or directly into the BLASTp interface, and run the alignment. After a few seconds, you get the following matches of the peptidases S8 S53 superfamily:



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident |
|---|---|---|---|---|---|---|
| ZP_09643362.1 | hypothetical protein HMPREF9449_01748 [Odoribacter laneus YIT 120 | 156 | 156 | 100% | 3e-41 | 53% |
| ZP_09644241.1 | hypothetical protein HMPREF9449_02627 [Odoribacter laneus YIT 120 | 153 | 153 | 100% | 3e-40 | 51% |
| YP_004253567.1 | peptidase S8 and S53 subtilisin kexin sedolisin [Odoribacter splanchni | 148 | 148 | 100% | 2e-38 | 50% |
| ZP_10894281.1 | Por secretion system C-terminal sorting domain protein [Porphyromor | 147 | 147 | 100% | 5e-38 | 51% |
| ZP_09591890.1 | hypothetical protein HMPREF9140_02008 [Prevotella micans F0438] > | 145 | 145 | 100% | 2e-37 | 50% |
| ZP_09022290.1 | hypothetical protein HMPREF9450_01205 [Alistipes indistinctus YIT 12 | 142 | 142 | 87% | 3e-36 | 52% |
| ZP_05857871.1 | subtilase family domain protein [Prevotella veroralis F0319] >gb\|EEX1 | 138 | 138 | 100% | 1e-34 | 49% |
| ZP_09104756.1 | hypothetical protein HMPREF9138_01228 [Prevotella histicola F0411] | 137 | 137 | 100% | 3e-34 | 49% |
| ZP_04539947.1 | protease [Bacteroides sp. 9_1_42FAA] >gb\|EEO62243.1\| protease [B | 135 | 135 | 100% | 3e-34 | 48% |
| ZP_06740631.1 | peptidase families S8 and S53 [Bacteroides vulgatus PC510] >gb\|EFG | 134 | 134 | 100% | 3e-34 | 48% |
| ZP_08794020.1 | protease [Bacteroides dorei 5_1_36/D4] >gb\|EEO46750.1\| protease [ | 134 | 134 | 100% | 3e-34 | 48% |
| EIY36828.1 | hypothetical protein HMPREF1065_02745 [Bacteroides dorei CL03T12 | 134 | 134 | 100% | 3e-34 | 48% |
| EIY25742.1 | hypothetical protein HMPREF1063_02488 [Bacteroides dorei CL02T00 | 134 | 134 | 100% | 3e-34 | 48% |
| ZP_07994554.1 | protease [Bacteroides sp. 3_1_40A] >ref\|ZP_08798408.1\| protease [ | 134 | 134 | 100% | 4e-34 | 48% |
| ZP_03300901.1 | hypothetical protein BACDOR_02271 [Bacteroides dorei DSM 17855] : | 134 | 134 | 100% | 4e-34 | 48% |
| ZP_06089720.1 | protease [Bacteroides sp. 3_1_33FAA] >gb\|EEZ20350.1\| protease [B | 134 | 134 | 100% | 4e-34 | 48% |
| ZP_05734780.1 | subtilase family domain protein [Prevotella tannerae ATCC 51259] >g | 136 | 136 | 100% | 4e-34 | 47% |
| CBK65311.1 | Subtilisin-like serine proteases [Alistipes shahii WAL 8301] | 134 | 134 | 100% | 2e-33 | 49% |
| ZP_08137410.1 | subtilase family domain protein [Prevotella multiformis DSM 16608] > | 132 | 132 | 100% | 7e-33 | 47% |

2. `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.

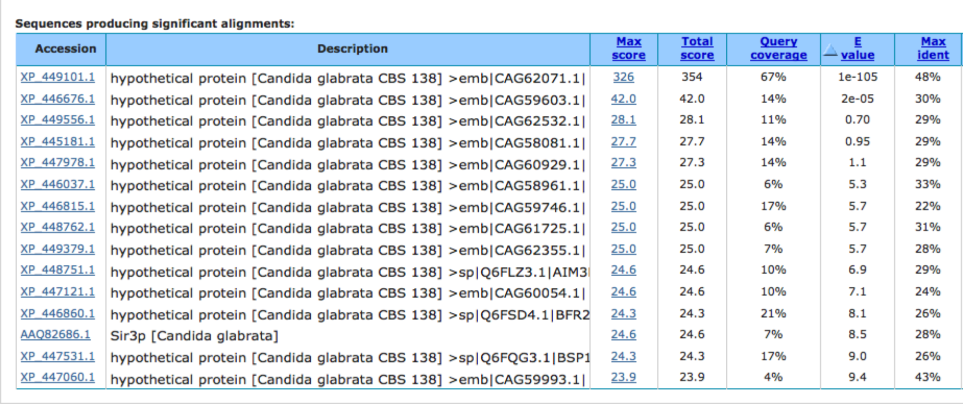
3. *Odoribacter, Prevotella, Porphyromonas* and *Alistipes* species are predominant. Note that *Alistipes* is the one you found with the nucleotide BLAST, and it is not the top match.

4. BLASTx

5. 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.

### 3.3 Finding orthologs

Specify in the *Organism* section of the BLASTp interface that you want to align on species *Candida glabrata*. Consistently with the publication, the best match indicates
`GENE ID: 2890989 CAGL0L07436g`:



Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Max ident |
|---|---|---|---|---|---|---|
| XP_449101.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG62071.1\| | 326 | 354 | 67% | 1e-105 | 48% |
| XP_446676.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG59603.1\| | 42.0 | 42.0 | 14% | 2e-05 | 30% |
| XP_449556.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG62532.1\| | 28.1 | 28.1 | 11% | 0.70 | 29% |
| XP_445181.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG58081.1\| | 27.7 | 27.7 | 14% | 0.95 | 29% |
| XP_447978.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG60929.1\| | 27.3 | 27.3 | 14% | 1.1 | 29% |
| XP_446037.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG58961.1\| | 25.0 | 25.0 | 6% | 5.3 | 33% |
| XP_446815.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG59746.1\| | 25.0 | 25.0 | 17% | 5.7 | 22% |
| XP_448762.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG61725.1\| | 25.0 | 25.0 | 6% | 5.7 | 31% |
| XP_449379.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG62355.1\| | 25.0 | 25.0 | 7% | 5.7 | 28% |
| XP_448751.1 | hypothetical protein [Candida glabrata CBS 138] >sp\|Q6FLZ3.1\|AIM3 | 24.6 | 24.6 | 10% | 6.9 | 29% |
| XP_447121.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG60054.1\| | 24.6 | 24.6 | 10% | 7.1 | 24% |
| XP_446860.1 | hypothetical protein [Candida glabrata CBS 138] >sp\|Q6FSD4.1\|BFR2 | 24.3 | 24.3 | 21% | 8.1 | 26% |
| AAQ82686.1 | Sir3p [Candida glabrata] | 24.6 | 24.6 | 7% | 8.5 | 28% |
| XP_447531.1 | hypothetical protein [Candida glabrata CBS 138] >sp\|Q6FQG3.1\|BSP1 | 24.3 | 24.3 | 17% | 9.0 | 26% |
| XP_447060.1 | hypothetical protein [Candida glabrata CBS 138] >emb\|CAG59993.1\| | 23.9 | 23.9 | 4% | 9.4 | 43% |