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| <b>Exercise 13</b> | <b>Phylogeny of Prokaryotes based on 16S rRNA genes: Introduction to Bio-Informatics</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Advisor</b>     | Thomi Horath, <a href="mailto:horath@botinst.unizh.ch">horath@botinst.unizh.ch</a> , 01 634 82 27                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Reading</b>     | Chapters in BBOM 9 <sup>th</sup> : 12.4 - 12.7<br>Chapters in BBOM 10 <sup>th</sup> : 11.4-11.8<br>BBOM: Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9 <sup>th</sup> edition, 1999. 10 <sup>th</sup> edition, 2003. Prentice Hall.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Objectives</b>  | <ul style="list-style-type: none"> <li>• Which are the most common sequence analysis databases available on the internet?</li> <li>• What is the most common computer-based resource for sequence analysis?</li> <li>• Why did the 16S rRNA molecule become so famous?</li> <li>• Why will sequence analyses conducted on different databases yield different results ?</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Background</b>  | <p>Originally, the taxonomic <b>classification of prokaryotes</b> depended exclusively on <b>phenotypic traits</b> such as</p> <ul style="list-style-type: none"> <li>• <b>shape</b>: coccus, rod, spirillum, vibrio, etc. (BBOM 9<sup>th</sup>, 3.4; BBOM 10<sup>th</sup>, 4.4),</li> <li>• <b>motility</b>: not motile, movement by gliding, with flagellum or flagella (monotrich, polytrich, peritrich), etc. (BBOM 9<sup>th</sup>, 3.11, BBOM 10<sup>th</sup>, 4.10-4.11)</li> <li>• <b>behavior</b>: chemotactic, phototactic (BBOM 9<sup>th</sup>, 3.12; BBOM 10<sup>th</sup>, 4.12)</li> <li>• <b>membrane structure</b>: e.g. ester-lipids vs. ether-lipids (BBOM 9<sup>th</sup>, 3.5; BBOM 10<sup>th</sup>, 4.5),</li> <li>• <b>cell inclusions and surface structures</b>: slime layers, capsules, glycogen, sulfur, magnetosomes, spores etc. (BBOM 9<sup>th</sup>, 3.13-3.15; BBOM 10<sup>th</sup>, 4.13-4.15)</li> <li>• <b>metabolism</b>: phototrophic, chemotrophic, lithotrophic, organotrophic, autotrophic, heterotrophic (BBOM 9<sup>th</sup>, 4.1; BBOM 10<sup>th</sup>, not available)</li> <li>• <b>resistance</b> to antibiotics (BBOM 9<sup>th</sup>, 18.12; BBOM 10<sup>th</sup>, 20.12)</li> <li>• <b>cell wall</b>: Gram-positive or Gram-negative, LPS (BBOM 9<sup>th</sup>, 3.7, 3.8; BBOM 10<sup>th</sup>, 4.8-4.9)</li> <li>• <b>pathogenicity, virulence</b> etc. (BBOM 9<sup>th</sup>, 1.7, 1.8; BBOM 10<sup>th</sup>, 1.4, 1.5)</li> <li>• <b>and other characters</b>: Pigments, temperature tolerance, ecotype, etc.</li> </ul> <p>Some of these properties turned out to be good distinguishing characteristics (e.g., the Gram stain), while others were not (e.g., cell shape).</p> <p>Recently, <b>genotypic classification</b> based on nucleotide sequence comparison of <b>16S ribosomal RiboNucleicAcid (16S rRNA)</b> genes has become available as an additional taxonomic tool (BBOM 9<sup>th</sup> chapters 12.4 &amp; 12.5; BBOM 10<sup>th</sup>, 11.4, 11.5). 16S rRNA, along with the 23S rRNA, has properties which predestine it as a <b>universal phylogenetic marker</b>. Every living organism, prokaryotes as well as eukaryotes, contains it either as a 16S or an 18S molecule (23S or 28S, respectively), and it has always the same function (BBOM Fig. 12.7 BBOM 10<sup>th</sup>, Fig. 11.8). Ribosomal RNA must have been present since very early in the development of life forms, because it is essential for protein synthesis. Mutations in the 16S rRNA gene affect the proper functioning of the ribosome which quickly leads to the elimination of less efficient cells through selection. One may assume, therefore, that the 16S rRNA genes contain a large number of <b>highly conserved sequence patterns</b>. There are a number of sequence differences which did not impair on the functioning of the ribosome.</p> |

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|                   | <p>however, and which were maintained over evolutionary times. These can be used to distinguish <b>phylogenetically different organisms</b>. There are regions on the 16S rRNA which are quite conserved and others which are variable. Comparing the differences in the <b>base sequence of 16S rRNA genes</b> is, therefore, an excellent means to study evolutionary changes and phylogenetic relatedness of organisms. The question remains, however, of how many point mutations might have occurred at one base, from A to G and back to A for instance; they cannot be accounted for in the 16S rRNA <b>evolutionary clock</b>.</p> <p>One branch of <b>bio-informatics</b> is studying the relatedness between organisms based on sequence comparison. It makes use of <b>statistical methods</b> such as "maximum likelihood", "maximum parsimony" or "distance matrix". The molecular sequence data obtained worldwide from genomic sequencing projects are collected in <b>databases</b> and made publicly available. With this information, one can test novelty and possibly function of new sequences, search for homologous patterns and regulatory domains and create hypothetical phylogenetic relationships which allow one to build evolutionary trees. The information which is encoded in sequences needs to be analyzed by comparing against existing sequences whose functions are already known. <b>The computer uses algorithms to find similarities between the query sequence and every sequence in the database and scores them according to the degree of relative similarity</b>. Different databases might lead to differences in scoring depending on the algorithm used and the dataset which is available for comparison. It is best, therefore, to conduct searches on a number of different database libraries in order to obtain the highest possible number of homologous sequences.</p> <p>As a course exercise, we will contact one of the gene databases (NCBI) and apply the <b>BLAST algorithm</b> to the query nucleotide sequences given below (BLAST = Basic Local Alignment Search Tool). The BLAST algorithm searches for patches of similarity, and it is based on ungapped sequence alignments, i.e. the alignments created by BLAST do not allow for gaps, but BLAST does allow multiple hits on the same sequence. With this type of statistical model one increases search speed but one reduces sensitivity, i.e. BLAST might miss certain matches.</p> <p>The basic concept underlying all <b>comparing and tree building methods</b> is this: Take two sequences, make them the same length and divide the number of identical nucleotides by the number of all nucleotides in one sequence (and gaps if there are any). What you get is the basic similarity percentage between two sequences (BBOM 9<sup>th</sup> Fig. 12.9; BBOM 10<sup>th</sup>, 11.10). Presently, similarities of less than 95% define different genera.</p> |
| <b>Literature</b> | <ul style="list-style-type: none"> <li>• Stackebrandt, Erko and Michael Goodfellow (eds.) 1991. "Nucleic Acid Techniques in Bacterial Systematics", John Wiley &amp; Sons; Chichester, New York, Brisbane, Toronto, Singapore. ISBN: 0-471-92906-9</li> <li>• Peruski Jr., Leonard F. and Anne Harwood Peruski. 1997. "The Internet and the New Biology - Tools for Genomic and Molecular Research". American Society for Microbiology, Washington DC. ISBN 1-55581-119-1</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>www. Links</b> | <ul style="list-style-type: none"> <li>• U.S. National Library of Medicine and National Institutes of Health (NIH); <b>National Center for Biotechnology Information (NCBI)</b>: <a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a></li> <li>• The <b>Ribosomal Database Project (RDP)</b>: <a href="http://rdp.cme.msu.edu/html/index.html">http://rdp.cme.msu.edu/html/index.html</a></li> <li>• The <b>European Molecular Biology Laboratory</b>: <a href="http://www.embl-heidelberg.de/">http://www.embl-heidelberg.de/</a></li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

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|                  | <ul style="list-style-type: none"> <li>• EMBL's <b>European Bioinformatics Institute (EBI)</b>:<br/><a href="http://www.ebi.ac.uk/index.html">http://www.ebi.ac.uk/index.html</a></li> <li>• The <b>Oligonucleotide Probe Base</b>:<br/><a href="http://www.microbial-ecology.de/probebase/index2.html">http://www.microbial-ecology.de/probebase/index2.html</a></li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Exercises</b> | <p>Below you will find four 16S rDNA nucleotide sequences. Try to find out the following, applying the NCBI internet resources:</p> <ol style="list-style-type: none"> <li>1. What are the sequences available in BLAST that share the highest relative level of similarity with the sequences given?</li> <li>2. What kind of information is NCBI sending back to you?</li> <li>3. To which organisms do sequences 1 to 4 probably belong?</li> <li>4. What is the minimal length of nucleotides to be typed into the BLAST window to still get the correct nearest organism?</li> <li>5. Is this length always the same or does it depend on the fragment location which you choose from the entire sequence?</li> <li>6. Take a small fragment of your favorite sequence and exchange one or more nucleotides in it. Does this new sequence still have the same nearest relatives?</li> </ol> <p><b>Genomic sequences to be analyzed with BLAST:</b></p> <p>Ask your advisor for the meaning of designations which are different from the common letter designation used for the four bases G, C, T and A.</p> <ol style="list-style-type: none"> <li>1. <b>A famous inhabitant of lake Cadagno:</b><br/> CGTGGCGGTATGCTTAACACATGCAAGTCGAACGTCAAAGGTCTTCGGATTGAGTAG<br/> CGTGGCGGACGGGTGAGTAAAGCGTGGGAATCTGCCTTCGAGTGGGGGATAACCCG<br/> GGGAACTCGGGCTAATACCGCATACGCCCTACGGGGGAAAGGGGGCTTTGGCTCT<br/> CGTTGCAAGATGAGCCACGTCCGATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAG<br/> GCGACGATSGGTGCTGGTCTGAGAGGATGACCAGCCACACTGGGACTGAGACACG<br/> GCCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGGACAATGGGGGAAACCCCTG<br/> ATCCAGCAATACCGCGTGTGTGAAGAAGCCTGCGGGTTGTAAAGCACTTTCAGTGG<br/> GAAAGAAAACCTGGTGGTTAATACCCATCGGCTTTGACGTTACTCACAAAAGAAGCAC<br/> CGGCTAACTCCGTGCCAGCAGCGCGGTAATACGGAGGTGCAAGCTGTAATCTCGGA<br/> ATTACTGGGCGTAAAGCGCACGTAGGCGGCGCCGTGAGTCCGATGTGAAAGCCCTG<br/> GGCTTAACCTGGGAAGTGCATTGGATACTGCGGCGCTAGAAATGTGAAAGAGGGGAGT<br/> GGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAACACCAGTGGCGAA<br/> GGCGGCTCCCTGGTTCAACATTGACGCTGAGGTGCGGAAGNGTGGGTAGCAACAG<br/> GNTTAGATACCCTGGTAGTCCACGCGNGTAAACGATGTCGACTAGCCGTTGGGTCCAT<br/> TTAAGGGCTTAGTGGCGCATAAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCG<br/> CAAGGTTAAACTCAAAGGAATTGACGGGGGCCCCGACAAAGCGGTGGAGCATGTGGT<br/> TTAATTGATGCAACGCGAAAAACCTTACCAGCCCTTGACATCTCGGAATCTTGAG<br/> AGATGTGAGAGTGCCCTTCGGGAACCGAGAGACAGGTGCTGCATGGCTGTCGTCAGC<br/> TCGTGTCGTGAGATGTTGGGTAAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGTT<br/> GCCAGCGCGTCAAGGCGGGAAGTCTAAGGAGACTGCCGGTGATAAACCGGAGGAAG<br/> GTGGGGATGACGTCAAGTCAATGAGGCTTATGGCTGGTACACACAGTGCATACA<br/> ATGGCCGGTACAGAGCGTTGCGACCCGCGAGGGTGAGCCAATCGCAGAAAACCGG<br/> TCGTAGTCCGGATCGAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAAT<br/> CGCGAATCAGCATGTCGSGGTGAATACGTTCCCGGGCCTTGTACACACCGCCSGTC<br/> ACACCATGGGAGTTGGTTGCACCAGAAGTAGATCGCTTAACCGCAAGAMGGGCGTTT<br/> ACCACGGTGTGTACACTGACTGGGGTGAAGTCGTACAAGG </li> <li>2. <b>A wetland colonizer of the Cadagno di Fuori swamp:</b><br/> AGAGTTTGATCCTGGCTCAGAGCGAACGCTGGCGGCAGGCTTAACACATGCAAGTCG<br/> AGCGCCCGTAGCAATACGGGAGCGGCAGACGGGTGAGTAAACGTTGGGAACGTACC<br/> CTTTGGTTCGGAACAACCCAGGGAACTTGGGCTAATACCGGATAAGTCCGTGAGGA<br/> GAAAGATTTATCGCCAAAGGATCGGCCCGCGTCTGATTAGCTAGTTGGTGTGTAAC<br/> GGCGCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGG<br/> ACTGAGACACGGGCCGAGTCTACGGGAGGCGAGCATGGGGAAATATTGGACAATG<br/> GGGGAAACCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAG<br/> CTCTTTCACTGGGGAAGATAATGACGTACCCACAGAAGAGCCCCGGCTACACTTCG<br/> TGCcagcagccggtataacGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGCG<br/> CACGTAGGCGCTTTCTAAGTCNGGGGTGAAATCCCGGAGCTCaACTCCGGAAGTGC<br/> CCTTGATACTGGArAGCTCGAGTCCGGGAGAGGtGRRTGGAAGTSCGAGTGTAGARGT<br/> GAAATTCGTAGATATTCGCAAGAACACCAATGGCGAAGGCGGCYCaCTGmCCkGTA<br/> CTGACGCTSAkGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCCTGGTAGTCC<br/> ACGCCSTAAACGATGkATRCATAGCCGTTGGGYMKCTTGCTCAGTGCGCGCAgCTA<br/> ACGCTTTAAGYATCCCGCCTGGGGAgtACGGTCGCAARRTTAaaactcaaaggaattgacggGG<br/> GCCCnCACAAAGCnGTGGAGCATGtKGTTCAATTcGAAGTCAACGCGCAGAACTTACCA </li> </ol> |

GCCCTTGACATGTACGACGNTTTCGGAGAMGGACTCCTCCCCGCAAGGGGCKTG  
AACaCAGKTGCTGCATGGCTGTTSTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCC  
gcaaNgagcgcaacccTCGCCCTTAGTTGCCATmATTyAGTTGGGCAmTTTAAGGGGAmTG  
CCGGTGATAArCCGCGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGG  
GCTGGGCTACACACGTGmTACAATGGCGGTGACAGTGGGACGCGAAGGGGCAACCC  
TTGGCAAATCTCAAAAAGCCGTCTCAGTTCGGATTkGGGTCTGCAACTCGACCCCAT  
GArGTckGAATckCTAGTAATckCAGATCAGCACGCTGCGGtGAATACGTTCCCGGGC  
CTTgTACACACCGCCCGTCACACCATGGGAGTTGGYTTTACCTGAAGGCGCTGCGCT  
ANCCCCGAAGGGAGGCAGGCCANcwCGGTAGGgTyAGCGACNNGGGgTGGC

3. **Something present in humans?:**

GCTAAACCTAGCCCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCA  
TTTACCCAAATAAGTATAGGCGATAGAAATTGAAACCTGGCGCAATAGATATAGTACC  
GCAAGGGAAAGATGAAAAATTATAACCAAGCATAATATAGCAAGGACTAACCCCTATAC  
CTTCTGCATAATGAATTAAGTAACTAGAAATAACTTTGCAAGGAGAGCCAAAGCTAAGACCC  
CCGAAACCAGACGAGCTACCTAAGAACAGCTAAAAGAGCACACCCGTCTATGTAGCA  
AAATAGTGGGAAGATTTATAGGTAGAGGCGACAAACCTACCGACCGCTGGTGATAGCTG  
GTTGTCCAAGATAGAATCTTAGTTCAACTTTAAATTTGCCACAGAACTAACTAAATCC  
CCTTGTAATTTAACTGTTAGTCCAAAGAGGAACAGCTCTTTGGACACTAGGAAAAAAC  
CTTGATAGAGAGTAAAAAATTTAACACCCATAGTAGGCCTAAAAGCAGCCACCAATTA  
AGAAAGCGTTCAAGCTCAACACCCACTACCTAAAAAATCCCAACATATAACTGAACT  
CCTCACACCCAAATTGGACCAATCTATCACCCCTATAGAAGAACTAATGTTAGTAAAGTA  
ACATGAAACATTCTCCTCCGCATAAGCCTGCGTCAGATTAAGAACTGAAGTACAA  
TTAACAGCCCAATATCTACAATCAACCAACAAAGTCATTATTACCTCACTGTCAACCC  
AACACAGGCATGCTCATAAGGAAAGGTTAAAAAAGTAAAGGAAGTACGGCAATCTTA  
CCCCGCCTGTTTACCAAAAACATCACCTCTAGCATCACCTATTAGAGCGACCGCC  
TGCCAGTGACACATGTTTAAACGGCCGCGGTACCCTAACCGTGCAAAGGTAGCATAA  
TCACTTGTTCTTAAATAGGGACCTGTATGAATGGCTCCACGAGGGTTCAGCTGTCT  
CTTACTTTTAAACAGTGAAATTGACCTGCCCGTGAAGAGGCGGGCATAACACAGCAA  
GACGAGAAGACCCCTATGGAGCTTTAATTTATTAATGACCACTAATCAACAAACCCA  
CAGGTCTTAACTACCAACCTGCATTAATAATTTTCGGTTGGGGCGACCTCGGAGCA  
GAACCCAACCTCCGAGCAGTACATGCTAAGACTTCACCAGTCAAAGCGAACTACTATA  
CTCAATTGATCCAATAACTTGACCAACGGAACAAGTTACCTTAGGGATAACAGCGCAA  
TCCTATTCTAGAGTCCATATCAACAATAGGGTTTACGACCTGATGTTGGATGCGGAC  
ATCCCGATGGTGCAGCCGCTATTAAAGGTTTCGTTTGTCAACGATTAAGTCTACGT  
GATCTGAGTTCAGACCGGAGTAATCCAGGTCGGTTTCTATCTACCTTCAAATTCCTCC  
CTGTACGAAAGGACAAGAGAAATAAGGCCTACTTCACAAAGCGCCTTCCCCCGTAAAT  
GATATCATCTCAACTTAGTATTATACCCACACCCACCCAAGAACAGGGTTT

4. **This one also lives in the human gut:**

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG  
CAAGTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGT  
GAGTAATGTCTGGGAACTGCCTGATGGAGGGGGATAACTACTGGAACGGTAGCTA  
ATACCGCATAAACGTCGCAAGACCAAGAGGGGGACCTTCGGGCCTCTTGCCATCGG  
ATGTGCCAGATGGGATTAGCTAGTAKGTGGGGTAACGGCTCACCTAGGCGACGATC  
CCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGAAGACGGTCCAGACT  
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAKCC  
ATGCCGCGTGTATGAARAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAG  
GGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCGAGAAGAAGCACCGGCTAAC  
TCCGTGCCAGCAGCCGCGTAATACGGAGGGGTGCAAGCGTTAATCGGAATTACTGG  
GCGTAAAGCGCACGCAGGCGGTTTGTAAAGTCAGATGTGAAATCCCCGGGCTCAACC  
TGGGAAGTGCATCTGATACTGGCAAGCTTGAGTCTCGTARAGGGGGGTAGAATTTCCA  
GGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCC  
CCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA  
CCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGARGCGT  
GGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTA  
AAACTCAAATGAATTGACGGGGGGCCCGCACAAAGCGGTGGAGATTTGGTTAATTCC  
ATGCAACGCGAAGAACCCTTACCTGGTCTTGACATCCACGAGGCTTTCAGATGAGA  
ATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTG  
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCCTTTGTTGCCAGCGG  
TCCGGCCGGGAAGTCAAAGGAGACTGCCAGTGATAAAGTGGAGGAAGGTGGGGATGA  
CGTCAAGTCATCATGGCCCTTACGACCAAGGGCTACACACGTGTACAAATGGCGCATA  
CAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCCTCGTAGTCCG  
GATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGTAGTAATCGTGGATCAG  
AATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG  
AGTGRGTTGCAAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACCTTGT  
GATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGA  
TCACCTCCTTA

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| <b>Equipment</b>                | Internet work stations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Rules &amp; Precautions</b>  | No microbial risks, only computer viruses ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Experiences gained</b>       | <ul style="list-style-type: none"> <li>• Familiarize yourself with a few theoretical and practical aspects of computer-aided molecular sequence analysis techniques</li> <li>• Experience how the internet can be used to get phylogenetic information from a nucleotide sequence</li> <li>• Perform simple sequence database searches</li> <li>• Practice to download and understand sequence records from international data bases</li> <li>• Learn about the usefulness and limitations of some computer algorithms for sequence analysis</li> </ul>                                                                                                                                            |
| <b>Timing</b>                   | 90 minutes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Reporting</b>                | Take notes on the exercise and present the results in your report and to the class.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Questions to be answered</b> | <ol style="list-style-type: none"> <li>1. What is the RDP?</li> <li>2. What is an evolutionary distance (ED)?</li> <li>3. How old is Earth?</li> <li>4. How old are the oldest known microfossils?</li> <li>5. Why are ribosomal RNA genes good evolutionary chronometers?</li> <li>6. How does the universal tree support the idea that early Earth was very hot?</li> <li>7. Describe why the results of phylogenetic community analyses of microbial habitats have been rather surprising.</li> <li>8. Why are we using rRNA genes for studying phylogenetic relationships?</li> <li>9. What effect do slightly different sequences have on the positioning in a phylogenetic group?</li> </ol> |
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