

- FILOGENESI

- Scaricare da GenBank le sequenze del gene della citocromo ossidasi I (COI)
- delle seguenti specie in formato fasta

Xiphias gladius

Xenopus laevis

Crocodylus niloticus

Serinus canaria

Canis lupus

- Effettuare una ricerca in BLAST con tali entry di riferimento per ottenere
- altre sequenze di organismi evolutivamente vicini e inserirle nel file fasta.
- Fare un multiallineamento con il software ClustalW o ClustalX.
- Preparare un file in formato meg utilizzando il software DNAsp.
- Caricare e far correre il file .meg con il software MEGA e calcolare la matrice
- delle distanze genetiche.
- Costruire l'albero filogenetico utilizzando il metodo del Neighbor-joining e il
- modello Kimura-2-parametri.
- Fare il test di Bootstrap settando 1000 repliche.
- Ripetere la stessa analisi utilizzando le sequenze del gene del citocromo b e confrontare gli alberi ottenuti.

save the alignment with a different name. Redo the Neighbor-Joining tree, again using the bootstrap. How do the tree topologies compare (are there any differences in tree topology?). How do the bootstrap values that you have calculated compare to the ones in you calculated earlier? Why do they differ in this way?

10. You can also use Mega to infer trees by maximum parsimony. Infer a tree using maximum parsimony and compare to the Neighbor-Joining tree. Try out different search parameters and compare results and how long the searches take. The differences may be particularly noticeable when you bootstrap.

Exercise 2

1. Use the file named “Thunnus COI” and choose the outgroup.
2. Construct an alignment from these sequences using ClustalW within BioEdit and save it in fasta format.
3. Start Mega. On the file menu select 'convert to mega format' and select the fasta file you saved in BioEdit.
4. Save the mega format file and then open the mega file (using 'open' on the file menu).
5. Infer pairwise genetic distances from the sequences using the default options. Notice that there are lots of options under the 'Models' menu. What pair of sequences are the most distantly related? The numbers in the lower left of the matrix are the distances; the numbers in the upper right part are standard errors of the distances.
6. Construct phylogenetic trees using the Neighbour Joining method (you will find this option under 'construct phylogeny' on the phylogeny menu). It's usually a good idea to view the unrooted tree first (the radiation tree under the view menu in Mega). Root the tree using the human sequence and again look carefully at the result.
7. Investigate the effect of radical changes in the nucleotide substitution model on the inferred tree topology. You can change the model using the options you are given when you select the neighbor-joining tree option on the phylogeny menu.
8. Construct phylogenetic trees again, this time using the bootstrapping re-sampling technique (you will find this option under the phylogeny menu - select bootstrap test of phylogeny and then neighbor-joining).
9. Open the sequence alignment in BioEdit . In the top left corner change the 'Mode' to 'Edit'. Delete most of the sequence alignment - keeping just the first 100 nucleotides and

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>gi|269848004|gb|GQ414572.1| Thunnus thynnus isolate TT567 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial
GGCACGGCCTTAAGCTTGCTCATCCGAGCTGAAC TAANCCAACCAGGTGCCCTTCTTGGGGACGACCAGA
TCTACAATGTAATCGTTACGGCCCATGCCTTCGTAATGATTTTCTTTATAGTAATACCAATTATGATTGG
AGGATTTGGAAACTGACTTATTCCCTCTAATGATCGGAGCCCCGACATGGCATTCCACGAATGAACAAC
ATGAGCTTCTGACTCCTTCCCTCCCTCTTTCCCTTCTGCTCCTAGCTTCTTCAGGAGTTGAGGCTGGGGCCG
GAACCGGTTGAACAGTCTACCC TCCCCCTTGCCGGCAACCTAGCCCACGCAGGGGCATCAGTTGACCTAAC
TATTTTCTCACTTCACTTAGCAGGGGTTCCCTCAATTCTTGGGGCAATTAACCTTCATCACAACAATTATC
AATATGAAACCTGCAGCTATCTCTCAATATCAAACACCACCTGTTTGTATGAGCTGTACTAATTACAGCTG
TTCTTCTTCTACTTTCCCTTCCAGTCCCTTGCCGCTGGTATTACAATGCTCCTTACAGACC GAAACCTAAA
TACAACCTTCTTCGACCCTGCAGGAGGGGGAGACCCAATCCTTTACCAGCATCTATTC
>gi|269848002|gb|GQ414571.1| Thunnus alalunga isolate TA4 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial
GGCACGGCCTTAAGCTTGCTCATCCGAGCTGAAC TAAGCCAACCAGGTGCCCTTCTTGGGGACGACCAGA
TCTACAATGTAATCGTTACGGCCCATGCCTTCGTAATGATTTTCTTTATAGTAATACCAATTATGATTGG
AGGATTTGGAAACTGACTTATTCCCTCTAATGATCGGAGCCCCGACATGGCATTCCACGAATAAACAAC
ATGAGCTTCTGACTCCTTCCCTCCCTCTTTCCCTTCTGCTCCTAGCTTCTTCAGGAGTTGAGGCTGGGGCCG
GAACCGGTTGAACAGTCTACCC TCCCCCTTGCCGGCAACCTAGCCCACGCAGGGGCATCAGTTGACCTAAC
TATTTTCTCACTTCACTTAGCAGGGGTTCCCTCAATTCTTGGGGCAATTAACCTTCATCACAACAATTATC
AATATGAAACCTGCAGCTATCTCTCAATATCAAACACCACCTGTTTGTATGAGCTGTACTAATTACAGCTG
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