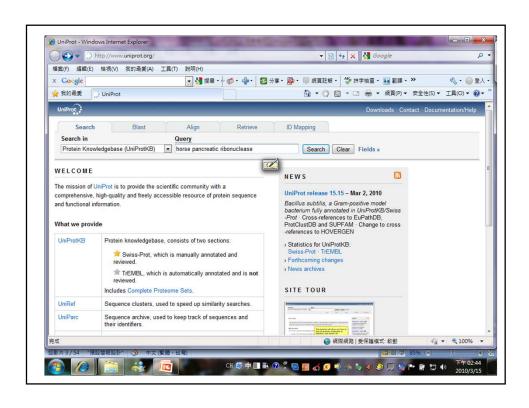
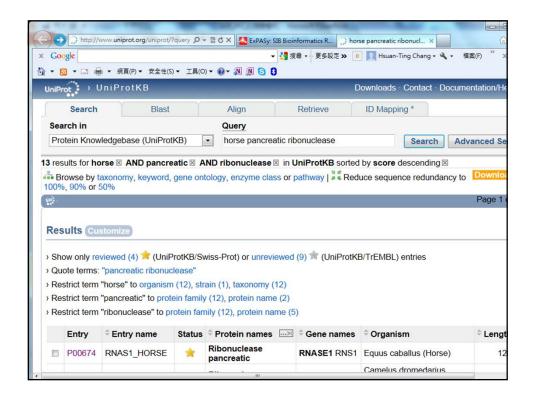
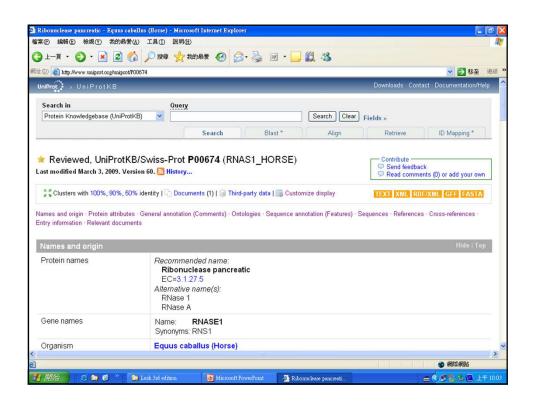
Use of sequences to determine phylogenetic relationships

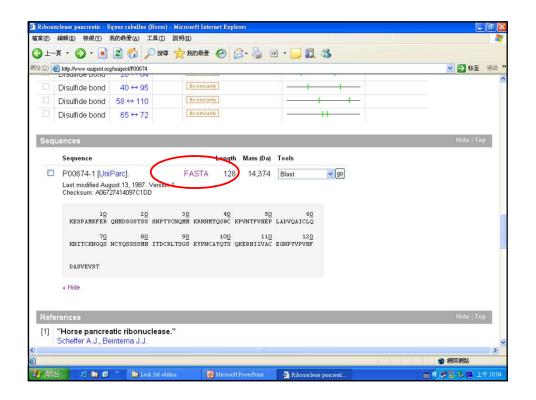
- Example 1.1 Retrieve the amino acid sequence of horse pancreatic ribonuclease
- ExPASy server: http://www.expasy.org/
- NCBI server: http://www.ncbi.nlm.nih.gov/
- Type the keywords: <u>horse pancreatic</u> ribonuclease (胰臟,核醣核酸脢)
- Select RNAS1_HORSE and then find FASTA format









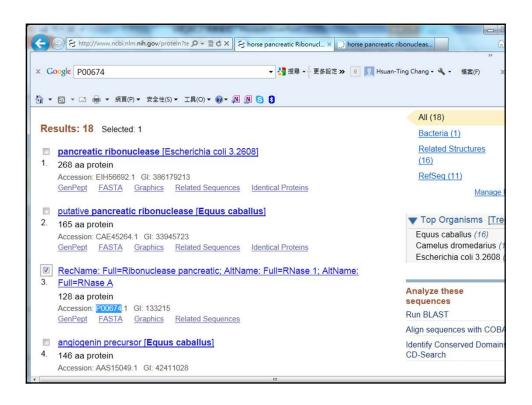


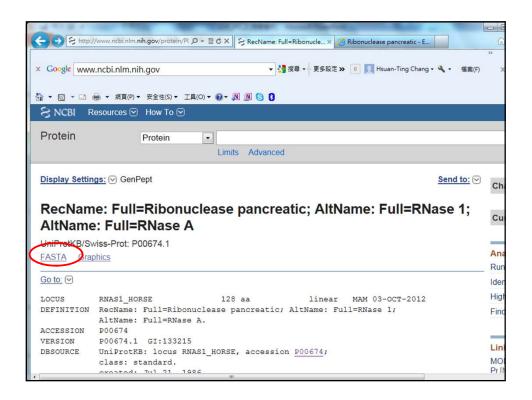
FASTA format

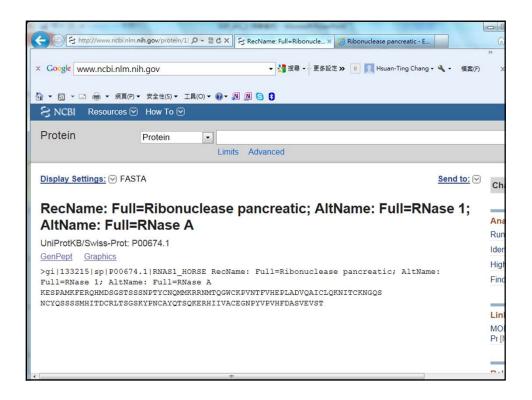
>sp|P00674|RNAS1_HORSE Ribonuclease pancreatic (EC 3.1.27.5) (RNase 1) (RNase A) - Equus caballus (Horse).

KESPAMKFERQHMDSGSTSSSNPTYCNQMMKRRNMT QGWCKPVNTFVHEPLADVQAICLQKNITCKNGQSN CYQSSSSMHITDCRLTSGSKYPNCAYQTSQKERHIIV ACEGNPYVPVHFDASVEVST





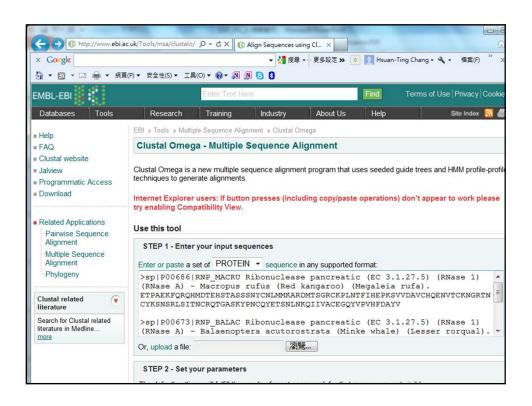


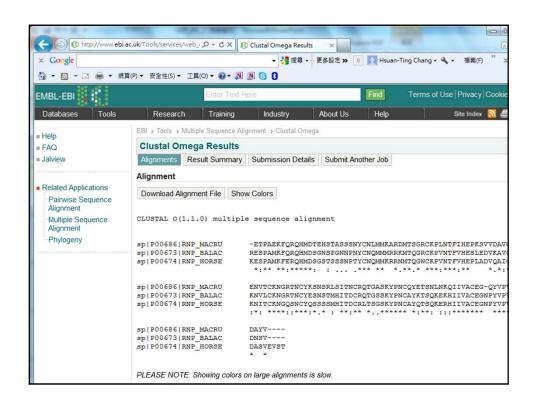


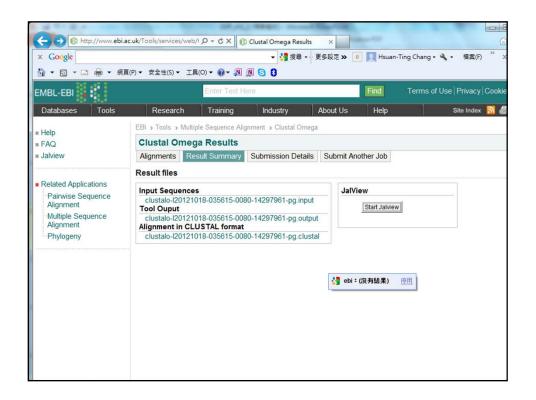
Example 1.2

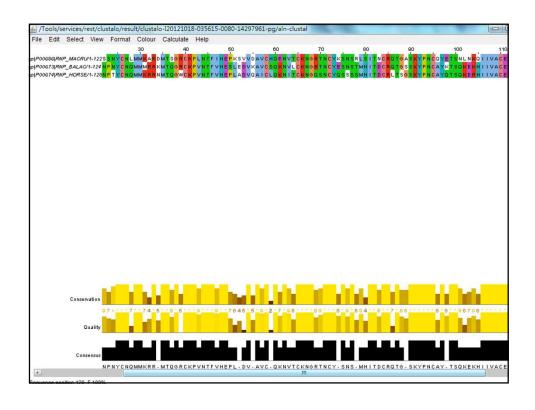
- Determine, from the sequences of pancreatic ribonuclease from *horse*, *minke whale* (小鬚鯨) and *red kangaroo*, which two of these species are most closely related.
- Retrieve the amino acid sequences from the database and use the multiple sequence alignment (MSA) tool CLUSTAL-Omega
 - http://www.ebi.ac.uk/Tools/msa/
 - New MSA tool that uses seeded guide trees and HMM profileprofile techniques to generate alignments. Suitable for mediumlarge alignments
- Clustal Omega is a multiple sequence alignment program for proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. volutionary relationships can be seen via viewing Cladograms or Phylograms.

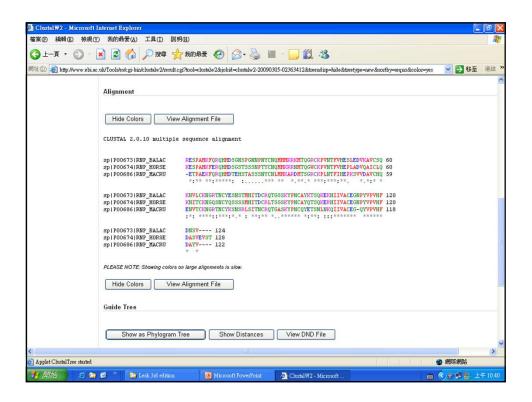
- >sp|P00686|RNP_MACRU *Ribonuclease pancreatic* (EC 3.1.27.5) (RNase 1) (RNase A) Macropus rufus (Red kangaroo) (Megaleia rufa).
- ETPAEKFQRQHMDTEHSTASSSNYCNLMMKARDMTSGRCKPLNTFI HEPKSVVDAVCHQENVTCKNGRTNCYKSNSRLSITNCRQTGASKYP NCQYETSNLNKQIIVACEGQYVPVHFDAYV
- >sp|P00673|RNP_BALAC *Ribonuclease pancreatic* (EC 3.1.27.5) (RNase 1) (RNase A) Balaenoptera acutorostrata (Minke whale) (Lesser rorqual).
- RESPAMKFQRQHMDSGNSPGNNPNYCNQMMMRRKMTQGRCKPV NTFVHESLEDVKAVCSQKNVLCKNGRTNCYESNSTMHITDCRQTGS SKYPNCAYKTSQKEKHIIVACEGNPYVPVHFDNSV
- >sp|P00674|RNP_HORSE *Ribonuclease pancreatic* (EC 3.1.27.5) (RNase 1) (RNase A) Equus caballus (Horse).
- KESPAMKFERQHMDSGSTSSSNPTYCNQMMKRRNMTQGWCKPVN TFVHEPLADVQAICLQKNITCKNGQSNCYQSSSSMHITDCRLTSGSK YPNCAYQTSQKERHIIVACEGNPYVPVHFDASVEVST

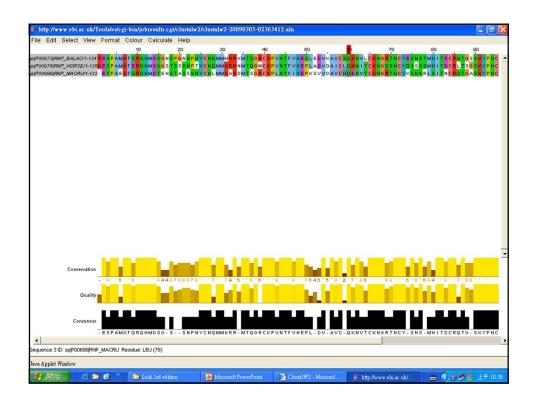


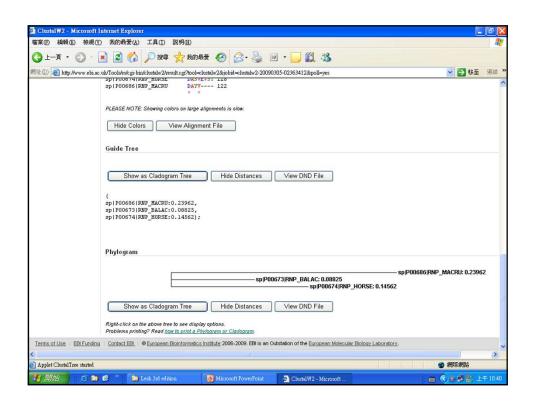












Alignment Result

- Horse and whale share the most identical residues.
 The result appears significant, and therefore confirm our expectation.
 - Knowing that horse and whale are placental (有 胎盤的) mammals and kangaroo is a marsupial (有袋動物), we expect horse and whale to be closer pair.

FASTA format description

A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (">") symbol in the first column. It is recommended that all lines of text be shorter than 80 characters in length. An example sequence in FASTA format is:

>gi|532319|pir|TVFV2E|TVFV2E envelope protein
ELRLRYCAPAGFALLKCNDADYDGFKTNCSNVSVVHCTNLMNTTVTTGLLLNGSYSENRT
QIWQKHRTSNDSALILLNKHYNLTVTCKRPGNKTVLPVTIMAGLVFHSQKYNLRLRQAWC
HFPSNWKGAWKEVKEEIVNLPKERYRGTNDPKRIFFQRQWGDPETANLWFNCHGEFFYCK
MDWFLNYLNNLTVDADHNECKNTSGTKSGNKRAPGPCVQRTYVACHIRSVIIWLETISKK
TYAPPREGHLECTSTVTGMTVELNYIPKNRTNVTLSPQIESIWAAELDRYKLVEITPIGF
APTEVRRYTGGHERQKRVPFVXXXXXXXXXXXXXXXXXXXXXXXVQSQHLLAGILQQQK
NL LAAVEAQQQMLKLTIWGVK

In <u>bioinformatics</u>, a **sequence alignment** is a way of arranging the sequences of <u>DNA</u>, <u>RNA</u>, or <u>protein</u> to identify regions of similarity that may be a consequence of functional, <u>structural</u>, or <u>evolutionary</u> relationships between the sequences. Aligned sequences of <u>nucleotide</u> or <u>amino acid</u> residues are typically represented as rows within a <u>matrix</u>. Gaps are inserted between the <u>residues</u> so that identical or similar characters are aligned in successive columns.

Global and local alignments

Global FTFTALILLAVAV F--TAL-LLA-AV

Local FTFTALILL-AVAV
--FTAL-LLAAV--

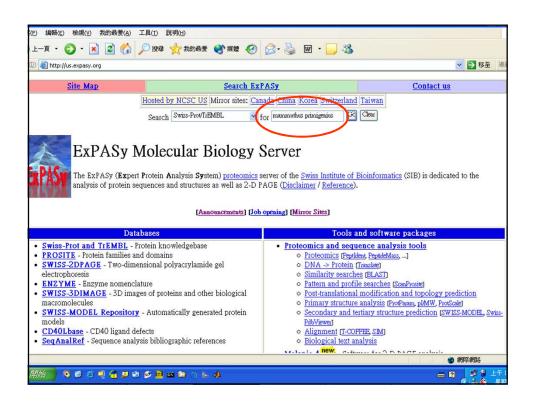
Homework #1

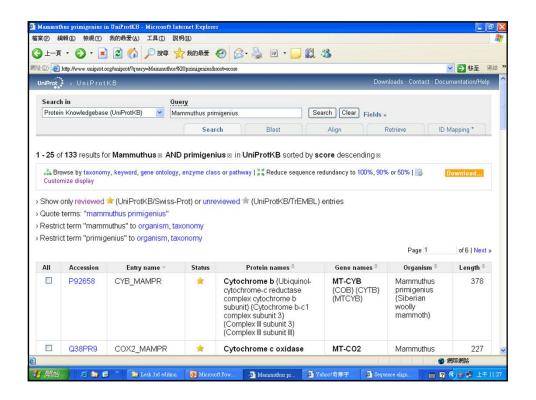
- Find the pancreatic ribonuclease of any other three species and perform following operations:
- 1. Perform multiple sequence alignment by use of CLUSTAL-W software
- 2. Find the corresponding DNA sequences.
- 3. Perform multiple sequence alignment for retrieved DNA sequences.

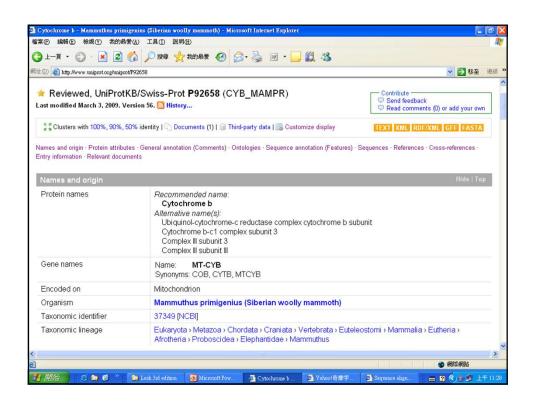
Case Study 1.5 - A harder one

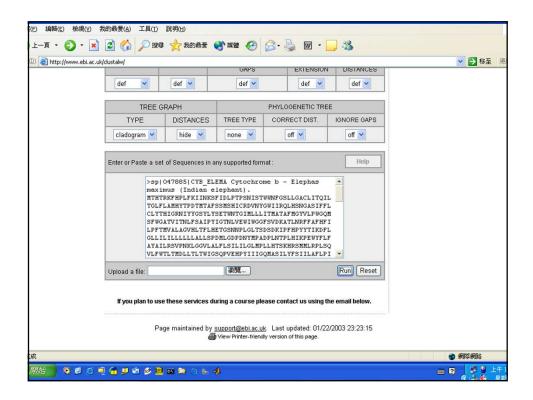
- Two living genera of elephant: the African elephant and the Indian
- 藉由北極長毛象(Mammuthus primigenius)化石之粒腺體 細胞色素b (mitochondrial cytochrome b), 界定出哪一種 現代大象(Loxodonta africana and Elephas maximus)與遠古之長毛象有較近之血緣關係。
- From the results, it appears that mammoth is more closely related to African elephant. However, there are few differences. Are they significant?
- Questions:
 - Could we tell from these sequences alone that they are from closely related species?
 - Given the differences are small, do they represent evolutionary divergence arising from selection, or merely random noise or drift?

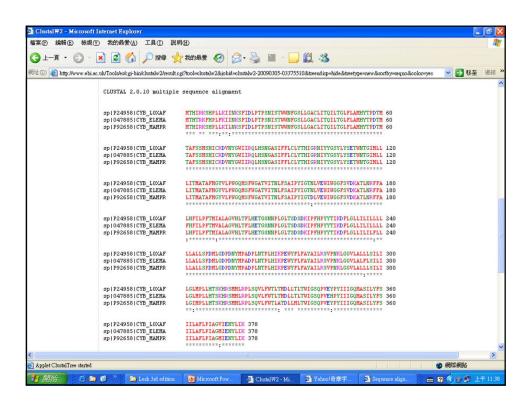
- >sp|P92658|CYB_MAMPR Cytochrome b OS=Mammuthus primigenius GN=MT-CYB PE=3 SV=3
- MTHIRKSHPLLKILNKSFIDLPTPSNISTWWNFGSLLGACLITQILTGLFLAMHYTPDTM
 TAFSSMSHICRDVNYGWIIRQLHSNGASIFFLCLYTHIGRNIYYGSYLYSETWNTGIMLL
 LITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTDLVEWIWGGFSVDKATLNRFFA
 LHFILPFTMIALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLGLLILILFLL
 LLALLSPDMLGDPDNYMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALLSILI
 LGIMPLLHTSKHRSMMLRPLSQVLFWTLATDLLMLTWIGSQPVEYPYIIIGQMASILYFS
 IILAFLPIAGMIENYLIK
- >sp|P24958|CYB_LOXAF Cytochrome b OS=Loxodonta africana GN=MT-CYB PE=3 SV=2
- MTHIRKSHPLLKIINKSFIDLPTPSNISTWWNFGSLLGACLITQILTGLFLAMHYTPDTM
 TAFSSMSHICRDVNYGWIIRQLHSNGASIFFLCLYTHIGRNIYYGSYLYSETWNTGIMLL
 LITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLVEWIWGGFSVDKATLNRFFA
 LHFILPFTMIALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLGLLILLLLL
 LLALLSPDMLGDPDNYMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALLLSILI
 LGLMPLLHTSKHRSMMLRPLSQVLFWTLTMDLLTLTWIGSQPVEYPYIIIGQMASILYFS
 IILAFLPIAGVIENYLIK
- >sp|O47885|CYB_ELEMA Cytochrome b OS=Elephas maximus GN=MT-CYB PE=3 SV=1
- MTHTRKFHPLFKIINKSFIDLPTPSNISTWWNFGSLLGACLITQILTGLFLAMHYTPDTM
 TAFSSMSHICRDVNYGWIIRQLHSNGASIFFLCLYTHIGRNIYYGSYLYSETWNTGIMLL
 LITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLVEWIWGGFSVDKATLNRFFA
 FHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLGLLILILLL
 LLALLSPDMLGDPDNYMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSILI
 LGLMPLLHTSKHRSMMLRPLSQVLFWTLTMDLLTLTWIGSQPVEHPYIIIGQMASILYFS
 IILAFLPIAGMIENYLIK

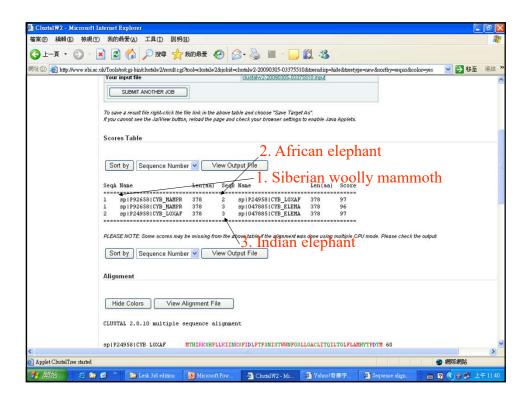


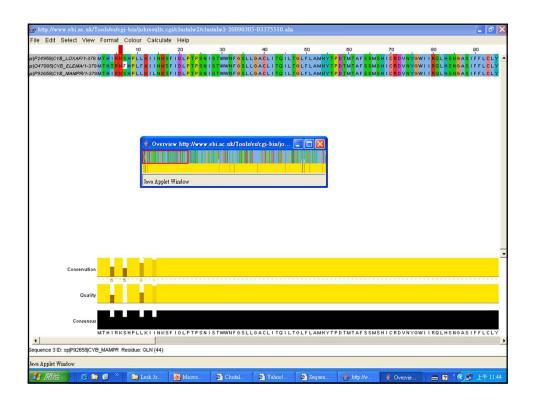












Background: Similarity & Homology

- *Similarity*: the observation or measurement of resemblance (相似點) and difference, independent of the source of the resemblance.
- *Homology*: the sequences and the organisms in which they occur are descended from a common ancestor, with the implication that the similarities are shared ancestral characteristics.
 - Homology must be an *inference* from observations of similarity.
 Only a few special cases is homology directly observables.
- The need for thoughtful scientific judgment!

- Does the very high degree of similarity of the sequences justify the conclusion that they are homologous; or are there other explanations?
- A functional cytochrome b *requires* so many conserved residues
- It starts out from independent ancestors and that common selective pressures forced them to become similar??
- Indian elephant has evolved faster than that of the African elephant or the mammoth, accumulating more mutations
- They gained a common gene by transfer from an unrelated organism via a virus.

SINES & LINES

- Short and long interspersed nuclear elements repetitive non-coding sequences that form large fractions of eukaryotic genomes. 30% of human chromosomal DNA, and over 50% of some higher plant genomes.
- Typically,
 - SINES are $\sim 70 500$ bps long, up to 10^6 copies may appear.
 - $-\,$ LINES may be up to 7000 bps long, up to 10^5 copies may appear.
- To derive the phylogenetic relationship
- Features of SINES that make them useful for phylogenetic studies include:
 - A SINE is either present or absent.

- SINES are inserted at random in the non-coding portion of a genome.
 - Appearance of similar SINES at the same locus in two species implies that the species share a common ancestor in which the insertion event occurred.
- SINE insertion appears to be irreversible: no mechanism for loss of SINES is known, other than rare large-scale deletions that include SINE.
- Not only do SINES shown relationships, they imply which species came first. The last common ancestor of species containing a common SINE must have come after the last common ancestor linking these species and another that lacks this SINE.

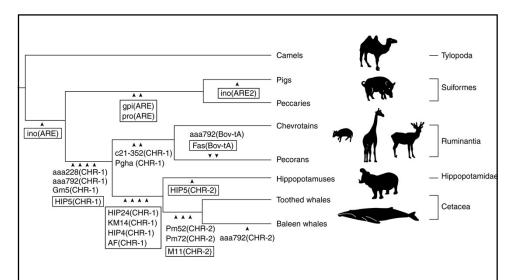


Fig. 1.5 Phylogenetic relationships among cetaceans and other artiodactyl subgroups, derived from analysis of SINE sequences.

Searching for similar sequences in databases: PSI-BLAST

- The search of a database for items similar to a probe.
- The ideal method is both sensitive and selective:
 - Sensitive: it picks up even very distant relationships
 - Selective: all the relationships that it reports are true
- A powerful tool from NCBI USA: PSI-BLAST
 - Position Specific Iterated basic linear alignment sequence tool
- Example 1.4. Homologues of the human PAX-6 gene.
- Example 1.5. What species contain homologues of human PAX-6 detectable by PSI-BLAST?

