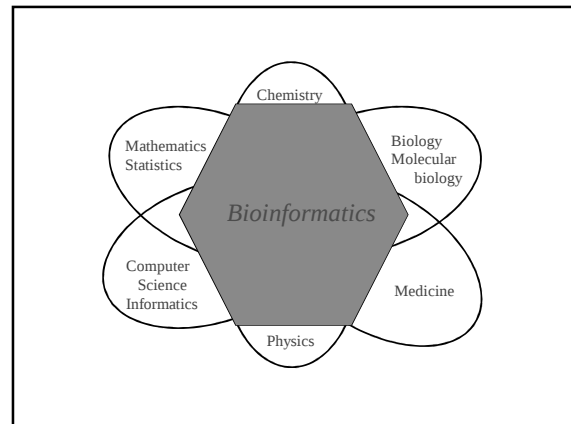


**1-Month Practical Master Course
Genome Analysis (Integrative
Bioinformatics & Genomics)**

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Biological Sequence Analysis

Pair-wise sequence alignment
Residue exchange matrices
Multiple sequence alignment
Phylogeny

DNA sequence

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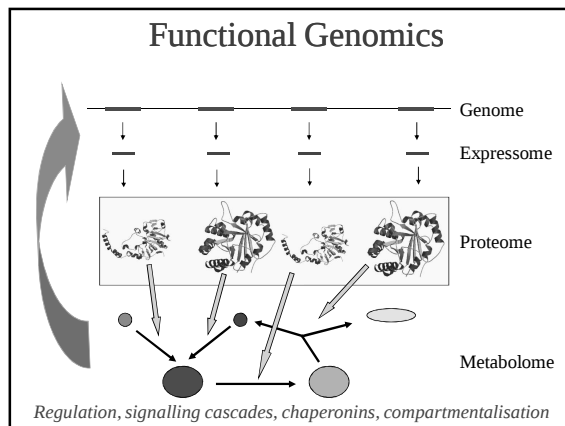
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caaatcttgt gacacacctc ccccggtccc acggtgccca
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cgcttccat gctggaatcc gacgctctc tcttctcta
cagcaagctc accgtggaca agacaggtg gcagcagggg
aacctctct catgctcgt gatcatgag gctctgcaca
accgtcac gcagaagacc ctctc....

```

Genome size	
Organism	Number of base pairs
φX-174 virus	5,386
Epstein Bar Virus	172,282
Mycoplasma genitalium	580,000
Hemophilus Influenza	1.8×10^6
Yeast (<i>S. Cerevisiae</i>)	12.1×10^6
Human	3.2×10^9
Wheat	16×10^9
Lilium longiflorum	90×10^9
Salamander	100×10^9
Amoeba dubia	670×10^9

Three main principles

- DNA makes RNA makes Protein
- Structure more conserved than sequence
- Sequence → Structure → Function



How to go from DNA to protein sequence

A piece of double stranded DNA:

5' attcgttggcaaatcgcccctatccggc 3'
3' taagcaaccgtttagcggggataggccg 5'

DNA direction is from 5' to 3'

How to go from DNA to protein sequence

6-frame translation using the codon table (last lecture):

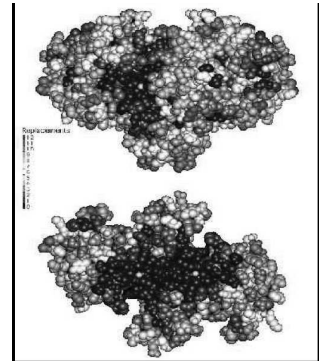
5' attcgttggcaaatcgcccctatccggc 3'
[] [] [] [] [] [] [] [] [] []
[] [] [] [] [] [] [] [] [] []
3' taagcaaccgtttagcggggataggccg 5'
[] [] [] [] [] [] [] [] [] []
[] [] [] [] [] [] [] [] [] []

Evolution and three-dimensional protein structure information

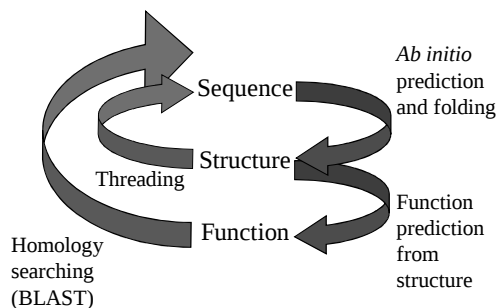
Isocitrate dehydrogenase:

The distance from the active site (in yellow) determines the rate of evolution (red = fast evolution, blue = slow evolution)

Dean, A. M. and G. B. Golding: *Pacific Symposium on Bioinformatics 2000*

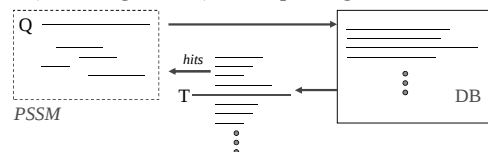


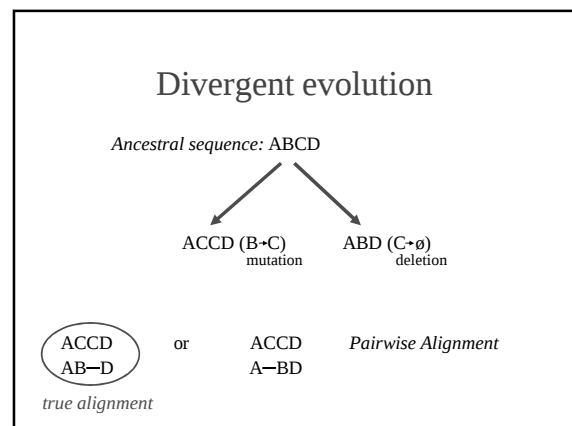
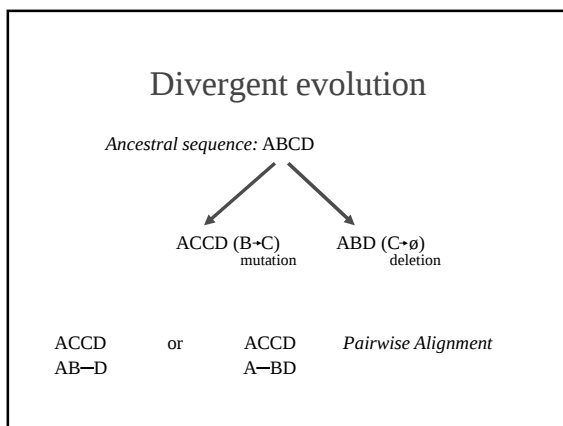
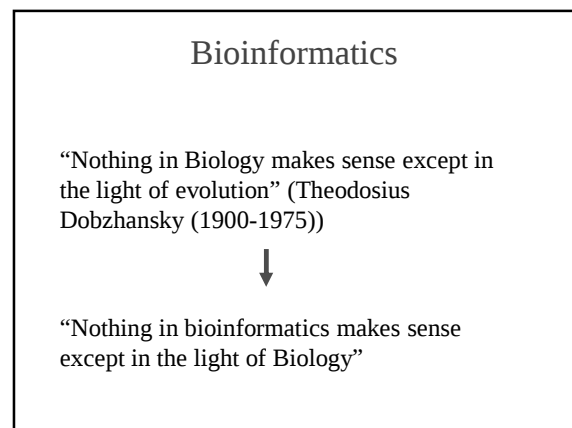
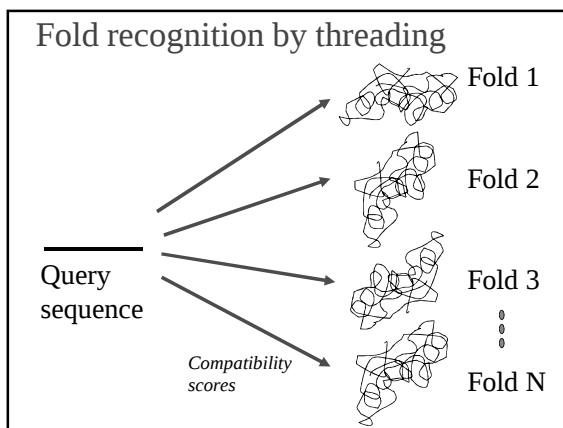
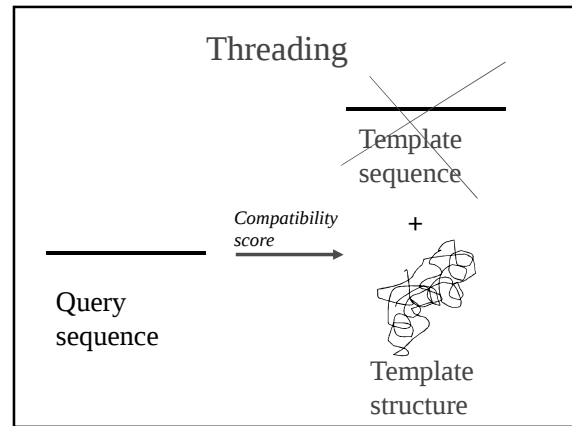
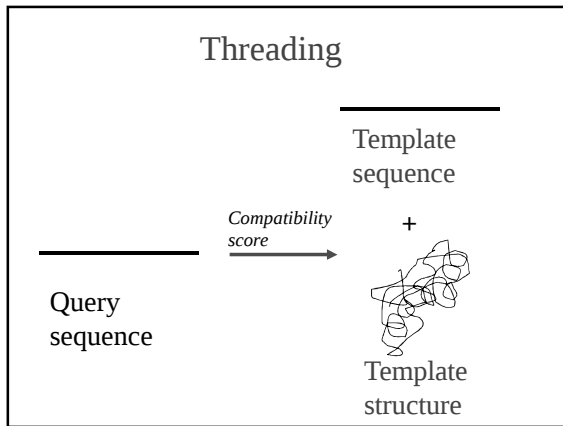
Protein Sequence-Structure-Function



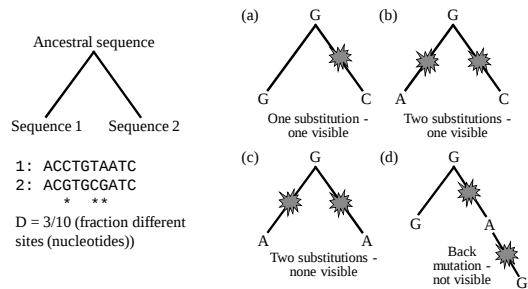
Widely used tool for homology detection: PSI-BLAST

- Heuristic tool to cut down computations required for database searching (~1M sequences in DB)
- Sensitivity gained by iteratively finding hits (local alignments) and repeating search





Mutations under divergent evolution



Convergent evolution

- Often with shorter motifs (e.g. active sites)
- Motif (function) has evolved more than once independently, e.g. starting with two very different sequences adopting different folds
- Sequences and associated structures remain different, but (functional) motif can become identical
- Classical example: serine proteinase and chymotrypsin

Serine proteinase (subtilisin) and chymotrypsin

- **Different evolutionary origins, no sequence similarity**
- Similarities in the reaction mechanisms. Chymotrypsin, subtilisin and carboxypeptidase C have a catalytic triad of serine, aspartate and histidine in common: serine acts as a nucleophile, aspartate as an electrophile, and histidine as a base.
- The geometric orientations of the catalytic residues are similar between families, despite **different protein folds**.
- The linear arrangements of the catalytic residues reflect different family relationships. For example the catalytic triad in the chymotrypsin clan (SA) is ordered HDS, but is ordered DHS in the subtilisin clan (SB) and SDH in the carboxypeptidase clan (SC).

A protein sequence alignment

```
MSTGAVLIY--TSILIKECHAMPAGNE-----
--GGILLFHRTHELIKESHAMANDEGGSNNS
*  *      *  *  *  *
```

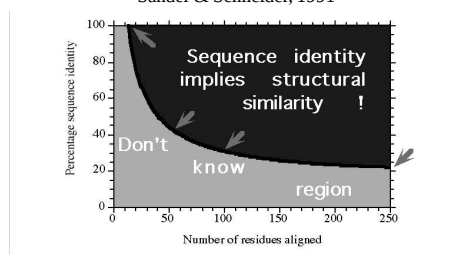
A DNA sequence alignment

```
attcgttggcaaatacgcccctatcgggccttaa
att---tggcggatcg-cctctacggggc-----
***      ***      ***      *
```

What can sequence tell us about structure

(HSP)

Sander & Schneider, 1991



Searching for similarities

What is the function of the new gene?

The "lazy" investigation (i.e., no biological experiments, just bioinformatics techniques):

- Find a set of similar protein sequences to the unknown sequence
- Identify similarities and differences
- For long proteins: identify domains first

Evolutionary and functional relationships

Reconstruct evolutionary relation:

- Based on sequence
 - Identity (simplest method)
 - Similarity
- Homology (common ancestry: the ultimate goal)
- Other (e.g., 3D structure)

Functional relation:

Sequence → Structure → Function

Searching for similarities

Common ancestry is more interesting:

Makes it more likely that genes share the same function

Homology: sharing a common ancestor

– a binary property (yes/no)

– it is a very useful property:

When (an unknown) gene X is *homologous* to (a known) gene G it means that we gain a lot of information on X: what we know about G can be transferred to X as a good suggestion.

Biological definitions for related sequences

- q **Homologues** are similar sequences in two different organisms that have been derived from a common ancestor sequence. Homologues can be described as either orthologues or paralogues.
- q **Orthologues** are similar sequences in two different organisms that have arisen due to a speciation event. Orthologues typically retain identical or similar functionality throughout evolution.
- q **Paralogues** are similar sequences within a single organism that have arisen due to a gene duplication event.
- q **Xenologues** are similar sequences that do not share the same evolutionary origin, but rather have arisen out of horizontal transfer events through symbiosis, viruses, etc.

How to evolve

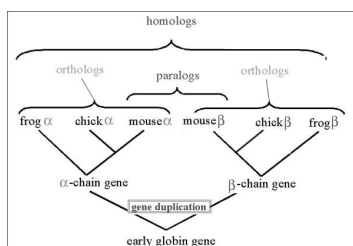
Important distinction:

- **Orthologues:** homologous proteins in different species (all deriving from same ancestor)
- **Paralogues:** homologous proteins in same species (internal gene duplication)



- In practice: to recognise orthology, *bi-directional best hit* is used in conjunction with database search program (this is called an operational definition)

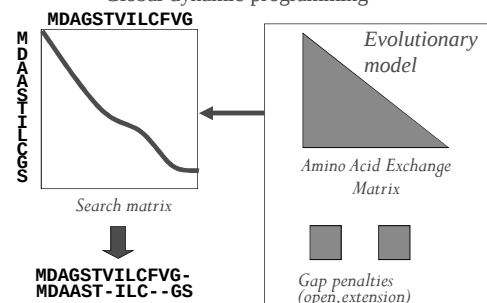
So this means ...



Source: <http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/Orthology.html>

Pairwise sequence alignment needs sense of evolution

Global dynamic programming



How to determine similarity

Frequent evolutionary events at the DNA level:

1. Substitution
2. Insertion, deletion
3. Duplication
4. Inversion

We will restrict ourselves to these events

A DNA sequence alignment

attcgttggcaaatcggccctatccggccttaa
 att---tggcggatcg-cctctacgggcc-----
 *** ***** ** *****

nucleotide one-letter code

A protein sequence alignment

MSTGAVLIY--TSILIKECHAMPAGNE-----
 ---GGILLFHRTHELIKESHAMANDEGGSNNS
 * * * ***** *

amino acid one-letter code